UNIVERSIDAD DE GRANADA

DEPARTAMENTO DE FISIOLOGÍA

INSTITUTO DE NUTRICIÓN Y TECNOLOGÍA DE LOS ALIMENTOS
"JOSÉ MATAIX VERDÚ"

PROGRAMA DE DOCTORADO NUTRICIÓN HUMANA Y CIENCIA DE LOS ALIMENTOS

FUNCTIONAL AND STRUCTURAL ASPECTS OF AGED PANCREAS RELATED TO THE NATURE OF DIETARY FAT SUPPLEMENTED OR NOT WITH COENZYME Q_{10}

TESIS DOCTORAL

ADRIÁN GONZÁLEZ ALONSO

2016

Editor: Universidad de Granada. Tesis doctorales
Autor: Adrián González Alonso
ISBN: 978-84-9125-948-0
URI: http://hdl.handle.net/10481/44065

Este trabajo de Tesis Doctoral ha sido realizado en el Instituto de Nutrición y Tecnología de los Alimentos "José Mataix Verdú" situado en el Centro de Investigación Biomédica de la Universidad de Granada como miembro del Grupo de investigación "Fisiología y Bioquímica Nutricional del Estrés Oxidativo" (CTS-627).

Durante la realización del siguiente trabajo, el Ldo. Adrián González Alonso fué financiado por las siguientes fuentes:

Ayudas para contratos predoctorales de Formación del Profesorado Universitario (FPU) (Diciembre 2010 – Noviembre 2014)

Ayudas para la realización de estancias breves en el extranjero para beneficiarios de FPU. Ministerio de Educación, Cultura y Deporte (Febrero 2014 – Abril 2014)

Proyectos de investigación que han financiado este estudio:

Título del proyecto: Análisis de expresión génica y causas de muerte en ratas con diferente longevidad y envejecimiento por ingesta de diferentes fuentes grasas (oliva, girasol y pescado) suplementadas o no con coenzima Q. Entidad financiadora: Ministerio de Ciencia e Innovación. Referencia: AGL2008-01057/ALI. Investigador responsable: Dr. José Luis Quiles Morales. Duración: de 01/01/2009 a 31/12/2011.

Título del proyecto: Estudio multidisciplinar del papel de los aceites de oliva virgen, girasol y pescado y del coenzima Q10 en el proceso de envejecimiento. Entidad financiadora: Consejería de Innovación, Ciencia y Empresa. Junta de Andalucía (Referencia P05-AGR 832). Duración: de 2006 a 2009. Investigador responsable: Dr. José Luis Quiles Morales.

D. José Luis Quiles Morales. Catedrático de la Universidad de Granada, Departamento de Fisiología.

Dª. María del Carmen Ramírez Tortosa. Catedrática de la Universidad de Granada, Departamento de Bioquímica y Biología Molecular II.

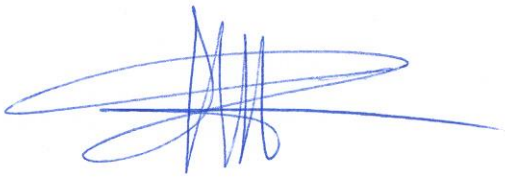
CERTIFICAN:

Que los trabajos de investigación que se exponen en la Memoria de Tesis Doctoral: **"Functional and structural aspects of aged pancreas related to the nature of dietary fat supplemented or not with coenzyme Q_{10}",** han sido realizados bajo nuestra dirección por la licenciada en Ciencia y Tecnología de los Alimentos D. Adrián González Alonso y la encontramos conforme para ser presentada y aspirar al Grado de Doctor por la Universidad de Granada con el Tribunal que en su día se designe.

Y para que conste, en cumplimiento de las disposiciones vigentes, extendemos el presente en Granada a cinco de Marzo de dos mil catorce.

Dr. D. José Luis Quiles Morales

Dra. Dª. María del Carmen Ramírez Tortosa



El doctorando Adrián González Alonso y los directores de la tesis Dr. D. José Luis Quiles Morales, y la Dra. Dª. María del Carmen Ramírez Tortosa, garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por la doctoranda bajo la dirección de los directores de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

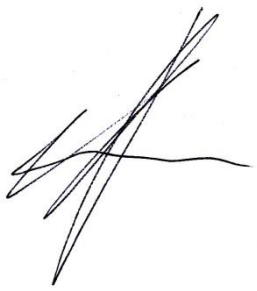
En Granada, a uno de Julio de dos mil dieciséis

Director/es de la Tesis

JOSÉ LUIS QUILES MORALES

MARÍA DEL CARMEN RAMÍREZ TORTOSA

Doctorando



ADRIÁN GONZÁLEZ ALONSO

MEMORIA QUE PRESENTA EL LDO. ADRIÁN GONZÁLEZ ALONSO PARA ASPIRAR AL GRADO DE DOCTOR POR LA UNIVERSIDAD DE GRANADA

ESTA TESIS DOCTORAL HA SIDO REALIZADA BAJO LA DIRECCIÓN DE:

Dr. D. José Luis Quiles Morales

Dra. Dª. María del Carmen Ramírez Tortosa

Ldo. D. Adrián González Alonso

Granada, 2016

AGRADECIMIENTOS

Si alguien me hubiera dicho durante me etapa de niñez o desde mi etapa de adolescente que me iba a encontrar en la situación en la que me encuentro, le habría dicho que "tú tienes mucha fe", que es lo que se suele decir en mi tierra a alguien que tiene expectativas muy improbables, pero uno no sabe a dónde le va a llevar la vida, pero si empiezo a recapitular y echo la mirada atrás, puedo ver que terminé mis estudios obligatorios, el Bachiller de ciencias de la salud, comencé la Universidad estudiando Biología y la termine con Ciencia y Tecnología de los Alimentos y finalmente los estudios de posgrado, ¡Vaya! Aquella persona tenía razón, yo pensaba en ese momento que a estas alturas estaría… pues no me acuerdo muy bien cuales eran mis expectativas, era solo un chaval de barrio cuyas preocupaciones eran cumplir mis obligaciones estudiantiles, jugar al futbol, balonmano, incluso atletismo y pasármelo bien, sin preocuparme mucho en lo que vendría después, y pasado los años esa es una de las cosas en las que no he cambiado, el no pensar en lo que puede venir después, sino en lo que tengo en el presente, lo que en términos futbolísticos se conoce como "partido a partido".

Evidentemente el camino hasta llegar hasta aquí no ha salido como se suele decir un camino de rosas, ha sido un camino lleno de sacrificios, pero también de satisfacciones y aprendizaje. Quien me conozca bien, sabrá que soy una persona que le cuesta mostrar incluso en privado mis sentimientos más profundos, pues en público ya ni os digo, de hecho en algún momento pensé en no escribir esta nota de agradecimientos con la excusa de que me parecía una "cursilada ", pero reflexioné y pensé que quizás sea el momento para mostrar mis agradecimientos, no solo a aquellas personas que han formado y forman de mi entorno o de mi vida, es de justicia acordarse de las instituciones que me han ayudado a conseguir lo que tengo.

Empezando cronológicamente, he de agradecer al colegio Los Pinos de Algeciras por la educación recibida en mi etapa desde los 4 a 15 años de edad, en especial a los muchos profesores que tuve durante esa etapa, los cuales me inculcaron valores muy positivos que sigo conservando hoy, y a mis compañeros de clase de entonces, con algunos de los cuales sigo manteniendo el contacto aunque sea por Facebook.

Agradecer también al I.E.S Levante de Algeciras, sus profesores y mis compañeros de entonces, por los 2 años que estudié allí, sin duda uno de los institutos con mayor tasa de aprobados en la prueba de selectividad de la comarca, además de haberme preparado para los primeros años de mi etapa universitaria.

Como no, mostrar mi profundo agradecimiento a todas las instituciones relacionadas con la Universidad de Granada, como la Facultad de Ciencias y de Farmacia y a todo el profesorado y personal de administración y conserjería que me facilitaron mi labor, y en general a la propia universidad, ya que no sólo me ha aportado los conocimientos que debo tener, sino lo que es más importante,

que me ha guiado para utilizar los recursos y herramientas que una persona de mi formación ha de saber manejar, además de instruirme en valores como la solidaridad, el bien común, la justicia social, la reflexión y otros tantos valores positivos que difícilmente habría podido adquirir en cualquier otro lugar, y todo junto me ha hecho ser no sólo una persona apta y útil para la sociedad, sino una persona que se siente realizada para mi vida de ahora en adelante.

Otra institución a la que le debo gran parte de lo conseguido es el Ministerio de Educación, Cultura y Deporte, por facilitarme las ayudas económicas necesarias para poder seguir formándome y que sin duda me han facilitado muchísimo mi labor y mi realización personal, que al fin y al cabo es una apuesta que hace la sociedad española para invertir en gente como yo, para que este País siga mejorando en todo los aspectos, y que por lo tanto me siento en deuda con el pueblo de España y haré todo lo posible por devolverlo durante mi etapa laboral.

Agradezco enormemente a la Universidad de Reading por facilitarme la realización de las estancias en el extranjero en sus instituciones y edificios, en especial a la Profesora Julie Lovegrove que aceptó mi solicitud sin apenas tener referencias mías, a Jane y Salisu que estaban siempre atentos a mí, facilitándome mi adaptación al "Department of Food and Nutritional Sciences" y siendo mi guía durante dicha estancia. Allí también conocí a gente que me ayudaron mucho a sentirme lo mejor posible fuera de casa, muchas gracias Luis, Isidro, Yianna y especialmente Natalia, que durante los primeros días de mi estancia fue esencial con su ayuda y predisposición a lo que hiciera falta, mil gracias.

Como no, agradecer al Departamento de Fisiología de la Facultad de Farmacia que me acogió desde el primer momento y me hizo sentirme parte suya, especialmente por facilitarme una experiencia de la que me siento muy realizado, que es la de impartir clases universitarias. Muchas gracias a todo el equipo de profesorado, a los compañeros becarios, pero en especial a Elisa y Encarna, 2 piezas claves que facilitan enormemente el trabajo docente y de investigación de los que estamos ahí.

Agradecimientos también para el Centro de Investigaciones Biomédicas, lugar de mi rutina durante este tiempo, para los conserjes del edificio, el equipo de administración, el equipo de limpieza y las distintas unidades que nos allanaban nuestra labor de manera notable. En especial me siento agradecido al Instituto de Nutrición y Tecnología de los Alimentos "José Mataix Verdú", institución de la que formo parte de manera orgullosa, en especial a algunos con los que han estado a mi lado el día a día y con los que me he sentido muy a gusto, como Mari Ángeles, Laura, Elenita, Alicia, Silvia, Jesús, Tomás y Paco. Mención especial para Javi, un talento que desborda humor, alegría, una capacidad de trabajo fuera de lo común, buen rollo y sobretodo su ayuda incondicional y desinteresada, gracias por todo ello Javi. Mis compañeros de grupo y laboratorio también son de muy especial mención, con todos ellos he vivido momentos muy bueno, que perdurarán en mi memoria, por la buena sintonía que hemos tenido, por el compañerismo tan profundo que vivimos en el 120, por las mañanas y tardes de risas que hemos vivido a pesar de las jornadas intensas de laboratorio que nos hemos metido, muchas gracias Sergio, fuiste un gran apoyo durante mi primera etapa en el grupo y alguien del que aprender, muchas gracias Patri, aparte de ser una gran

compañera, admiro tu capacidad de superar las adversidades que la vida pone, muchas gracias Nuri, por tu nobleza y bondad, muchas gracias Naroa, por darle alegría y buen rollo al grupo. Muchas gracias Mario, una persona a la que admiro por su enorme talento, su compañerismo incondicional y su humor a todas horas del día; muchas gracias Alfonso, por tu humildad y modestia a pesar de las enormes virtudes que posees, y gracias Julio que has sido como un compañero más a pesar de que dabas consejos de director de tesis, gracias a todos, me he sentido como si estuviera dentro de una familia. Me siento enormemente agradecido a mis directores de tesis, Pepe, Mayca y César, por darme la oportunidad de emprender esta aventura y apostar por mí frente a otros, a pesar de mis defectos y mis debilidades, me siento un privilegiado porque lo que aquí he aprendido, no habría podido aprenderlo en otro lugar, os estaré eternamente agradecido.

Tengo que agradecer también a mis amigos, por ser como son, por sentirme querido y aceptado, porque de ellos he aprendido valores, enseñanzas, otros puntos de vista, a valorar la amistad entre otras cosas. Muchas gracias a mis amigos de la carrera Juanma, Iván, Chema, Jorge, Susana, Marga, Irene, Diego, Jaime, Worty, Alejandra, Omar, Rocío, María, Javi, Javito, Inma, Fran, Ginés, Alejandro, Rubén, Azahara y Miriam, ellas 2 en especial porque eran compañeras de sufrimiento y les agradezco la enorme ayuda que me dieron a la hora de realizar los trámites burocráticos que supone el pedir una beca FPU o las estancias en el extranjero. Del mismo modo también me siento agradecido por otros amigos que forman parte de mi vida y mi ser, Rafa, Roberto, Lechera, Alberto, Cangrejo, Javi, Fran, Nazaret, y Manolo, sé que me falta más gente pero también se lo agradezco.

Durante estos últimos años tengo que agradecer a cierta familia que me han aceptado como a uno más de ellos, gracias Miguel, Eleuteria, Antonia, Eleuterio, Rocío, Javi, Miguel Angel, María José y los pequeños Javi y Alba, no os imagináis lo afortunado que me siento al haberos conocido, os siento como a mi propia familia porque vosotros me lo habéis transmitido a mí, además de ser unas personas con una honestidad, cariño y saber estar fuera de lo común, mil gracias.

Las lágrimas me inundan, porque solo pensar en lo que mi familia es para mí lo es todo, la emoción que emerge sobresale más allá de lo común, y en lo profundamente agradecido y afortunado que me siento de teneros a mi lado, muchas gracias a mis tíos y tías, Berto, Jaime, Mari, Carlos, Fabi, Ali, Paco, Pepe, muchas gracias a mis primos y primas, Hugo, Maite, Guillermo, Fernando, Esther y Pablo, Miriam, Mariola y Ester. Abuela, no sabes lo que te quiero y admiro, todo el enorme sacrificio que has vivido durante tu vida no tiene nombre, criando a 7 hijos y nietos y nietas, como yo, pasando por momentos muy difíciles, los que la vida te pone, estoy muy orgulloso de ti abuela y cada día que estés con nosotros es un regalo. Irene, Hermana, estoy muy orgulloso de ti, todavía recuerdo el día que naciste y cuando te cogí en brazos por primera vez, fue maravilloso, y el verte crecer hasta el día de hoy, en el que ya veo a mi hermana pequeña como una mujer madura, con una capacidad de trabajo admirable y con unas ideas y valores excepcionales me hace sentir tremendamente orgulloso. No me puedo olvidar de los que hoy ya no están, mis abuelos Jaime, Pepe y Rosario que se fueron cuando yo era muy pequeño, pero que todavía tengo grabado momentos de inmensa felicidad cuando estuve a su lado y que daría lo que fuera porque me estuvieran

observando desde allá donde quiera que estén y que se sintieran orgullosos de mí, de todos los pasos que he dado en mi vida, tanto de los aciertos como los fracasos. Papá, Mamá, que puedo decir de vosotros, en primer lugar gracias por haberme dado la vida, por haber tenido una infancia muy feliz, por inculcarme valores tan positivos que me hacen ser la persona que soy, por inculcarme lo importante que es la educación, la formación y sobretodo el valor de ser honesto y buena persona, el tremendo sacrificio que ha supuesto el irme a otra ciudad a iniciar mis estudios universitarios, esos son los mejores regalos que unos padres pueden dar a sus hijos, lo máximo que un hijo puede aspirar, y vosotros me lo habéis dado, os estaré eternamente agradecido. Y a ti Mar, mi Mar, son sólo 4 años, 3 meses y 11 días desde que te conocí, pero ya no puedo vivir sin ti, sin tus besos, sin tus caricias, sin tu mirada de complicidad, sin tu sonrisa ni tus abrazos, especialmente los mañaneros que hacen que vea que esto no es un sueño sino una realidad, una bendita realidad. Te agradezco todo lo que haces por mí en el día a día, soportar mis defectos, el sentirme tremendamente querido, tus enseñanzas y ayudas, gracias por todo amor y por todo lo que venga, te quiero.

TABLE OF CONTENTS

ABBREVIATIONS

4-AP: 4-aminophenazone

AC: alcohol consumption

ADP: adenosine diphosphate

AIDS: acquired immune deficiency syndrome

Akt: protein kinase B

ALA: α-linolenic acid

ALT: Alanine Tranferase

AMP: adenosine monophosphate

ANOVA: analysis of variance

AP: acute pancreatitis

AR: antigen retrieval

ATP: adenosine triphosphate

BMI: body mass index

BRCA2: breast cancer type 2 susceptibility protein

cAMP: cyclic adenosine monophosphate

CCK: cholecystokinin

CD4: cluster of differentiation 4

CD8: cluster of differentiation 8

CDH1: cadherin 1 type 1

CDKN2A: cyclin-dependent kinase inhibitor 2A

cDNA: complementary DNA

CF: cardiac failure

CFTR: cystic fibrosis transmembrane conductance regulator

CHE: cholesterol esterase

CHOD: cholesterol oxidase

CK: creatin kinase

CMV: cytomegalovirus

c-myc: myelocytomatosis viral oncogene homolog

CoQ: coenzima Q

CP: chronic pancreatitis

CR: caloric restriction

CS: cigarette smoking

CT: computed tomography

CTLA-4: cytotoxic T-lymphocyte antigen 4

CV: cardiovascular

CVB: coxsackievirus B

CVD: cardiovascular disease

DAB: 3,3'-diaminobenzidine

DGLA: dihomo-Gamma-Linolenic Acid

DHA: docosahexaenoic acid

DM: diabetes mellitus

DMBA: 7,12-dimethylbenz[a]anthracene

DNA: deoxyribonucleic acid

DNPH: 2,4-dinitrophenylhydrazine

EDTA: ethylenediaminetetraacetic acid

EGFR: epidermal growth factor receptor

ELISA: enzyme-linked immunoSorbent assay

EPA: eicosapentaenoic acid

EPHA3: EPH receptor A3

ER: endoplasmic reticulum

ERCP: endoscopic retrograde cholangiopancreatography

EVOO: extra virgin olive oil

FAMMM: familial atypical mole melanoma

FBXW7: F-box and WD repeat domain containing 7

FFA: free fatty acid

FOF: fish oil fed

G6PDH: glucose-6P-dehydrogenase

GAD65: glutamic acid decarboxylase

GH: growth hormone

GK: glycerol-kinase

GLUT: glucose transporter

GOD: glucose oxidase

GOT: glutamyl oxaloacetic transaminase

GPO: glycerol phosphate oxidase

GPT: glutamic pyruvic transaminase

GTP: guanosine triphosphate

H_2O_2: hydrogen peroxide

HCl: chlorhydric acid

HFD: high fat diet

HDL: high density lipoprotein

HLA: human leukocyte antigen

HK: hexoquinase

HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A

HOMA: homeostasis model assessment index

HPLC: high-performance liquid chromatography

HPOO: high phenolic olive oil

Hsp70: 70 kilodalton heat shock protein

HT: hydroxytyrosol

IA-2: islet antigen 2

IA-2 β : islet antigen 2 β

I-CAM1: Intercellular Adhesion Molecule 1

IDH1: isocitrate dehydrogenase 1

IFAPA: Institute for Agricultural and Fisheries of Andalusia

IL-2: interleukine 2

IL-6: interleukine 6

IL2RA: interleukin 2 receptor alpha

IPMNs: intraductal papillary mucinous neoplasms

IR: insulin resistance

IRS: insulin receptor substrate

JNKs: c-Jun N-terminal kinases

KRAS: Kirsten rat sarcoma viral oncogene homolog

LA: linoleic acid

LDH: lactate dehydrogenase

LDL: low density lipoprotein

LO: lactate oxidase

LPL: lipoprotein-lipase

MCNs: mucinous cystic neoplasms

MCP-1: monocyte chemoattractant protein–1

MD: mediterranean diet

MDH: malate dehydrogenase

MEN1: multiple endocrine neoplasia type 1

METC: mitochondrial electronic transport chain

MLH1: mutL homolog 1

MRC: mitochondrial respiratory chain

mRNA: messenger ribonucleic acid

mROS: mitochondrial reactive oxygen species

mtDNA: mitochondrial Deoxyribonucleic acid

MUC1: mucin 1 cell surface associated

MUC2: mucin 2 cell surface associated

MUFA: monounsaturated fatty acid

NAD^+: nicotinamide adenine dinucleotide (oxidizes form)

NADH: nicotinamide adenine dinucleotide (reduced form)

NADPH: nicotinamide adenine dinucleotide phosphate

NEFA: non-esterified fatty acid

NF1: neurofibromin 1

NHL: non-Hodgkin lymphoma

NYHA: New York Heart Association

OD: optical density

p-NPP: p-nitrophenylphosphate

p27Kip1: cyclin-dependent kinase inhibitor 1B

PA: physical activity

PAL: phosphatase alkaline

PanIn: pancreatic intraepithelial neoplasia

PaSC: pancreatic stellate cell

PC: pancreatic cancer

PDAC: pancreatic ductal adenocarcinoma

PDK1: phosphoinositide-dependent kinase type1

PGE2: prostaglandin E2

PGI3: prostaglandin I3

PI3K: phosphoinositide 3-kinase

PLA2: phospholipase A2

PNETs: pancreatic neuroendocrine tumors

POD: peroxidase

PP: pancreatic polypeptide

PPAR: peroxisome proliferator-activated receptor

PSTI: pancreatic secretory trypsin inhibitor

PTPN22: Protein tyrosine phosphatase non-receptor type 22

PUFA: polyunsaturated fatty acid

PYY: peptide tyrosine-tyrosine

RBP4: retinol-binding protein 4

RER: rough endoplasmic reticulum

RIA: radio-immune assay

RNA: ribonucleic acid

ROS: reactive oxygen species

SAP: severe acute pancreatitis

SCN: serous cystic neoplasm

SEM: standard error of the mean

SFA: saturated fatty acids

Sfrp4: secreted frizzled-related protein 4

SIRS: systemic Inflammatory response syndrome

SMAD4: SMAD family member 4

SMARCA4: SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4

SOD: superoxide dismutase

SOF: sunflower oil fed

SPINK1: serine protease inhibitor Kazal-type 1

STK11: serine/threonine kinase 11

T1D: type 1 diabetes

T2D: type 2 diabetes

TAG: triacylglyceride

TBP: tributylphosphine

TFA: trans-fatty acid

TNF-α: tumor necrosis factor

TP53: tumor protein 53

TXA2: thromboxane A2

TXA3: thromboxane A3

UFA: unsaturated fatty acid

US: ultrasonography

V-CAM1: Vascular cell adhesion protein 1

VHL: von Hippel–Lindau syndrome

VIP: vasoactive intestinal peptide

VLDL: very low-density lipoprotein

VOOF: virgin olive oil fed

WDHA: watery diarrhea, hypokalemia, and achlorhydria

WHO: world health organization

WHR: waist-hips ratio

Wnt: wingless/integrated

WT-2: Wilms tumor 2

SUMMARY

Today, aging entails a challenge for scientific community, as longevity increases generation after generation, leading to the appearance of new causes of death to which society faces. Aging can be defined from biological point of view as a common physiologic process of multicellular organisms, which the individual suffers over time a progressive and endogen decline of the efficacy of physiologic processes after reproductive phase, so that sooner or later it becomes non-viable keeping the stability of biochemical reactions as well as body functions which depends on metabolism.

Although many theories have been proposed in order to explain this phenomenon, only a couple of them have been considered as feasible, among them the free-radical theory of aging proposed by Harman in 1956, whereby free radicals produced through aerobic respiration generate damages on biomolecules of organism, which accumulate over time, triggering in a gradual loss of maintenance of homeostatic functions.

Aging process can be aggressive or moderate depending on genetic and environmental factor. It is calculated that genetic factor comprises around 25% of aging process, whilst the remaining 75% corresponds to environmental factors, which means that it is quite more relevant lifestyle or pollution on aging than genetic. Within these moldable factors, nutrition is getting much relevance about its role on aging. The first evidence why nutrition was related to such process was thanked to caloric restriction, which is the decrease of caloric ingestion without nutrient deficit. Many studies performed on experimentation animals reached a conclusion which those subjects underwent caloric restriction were observed a significantly increased longevity in respect to those which were fed with a balanced caloric ingestion. In addition to caloric restriction, antioxidant supplementation was researched in order to verify if it possessed a similar effect to caloric restriction, obtaining positive results in some cases but negative in other ones.

Over last years, dietary fat is getting attention as feasible modulator of aging process, as body fat has not a predetermined genetic component like proteins, but it must be contributed through diet. In fact it was observed that lipid component of cellular membranes from tissues and organs have fatty acid profile similar to which is contributed by dietary fat, what means that there is an adaptation process.

On other hand, many studies have searched for a correlation between longevity and unsaturation degree of fatty acids of cellular membranes from tissues and organs, observing that the lesser degree of fatty acids unsaturation, the most longevity of such specie was observed. An explanation of this phenomenon is that there is a greater susceptibility to be oxidized by free radicals as the unsaturation degree is higher in a fatty acid. So, it may be concluded that depending on nature of dietary fat source throughout subject's life, it influences on aging process of a determined tissue or organ, understood as the damage accumulated by its biomolecules, and therefore it may be accelerated or braked the dysfunction of itself due to consequence of aging.

Thus, many experiments have been performed in which animals were fed with the same diet but differed in nature of fatty component, either olive oil, fish oil,

sunflower oil, coconut oil, lard among others. So that it was pretended observing the differential effect that a determined dietary fat source may exert on longevity, and moreover how it could affect to functionality and structure of determined tissue or organ.

With this background it was disposed to investigate which role dietary fat could possess about age-related signs at pancreatic level. For this purpose, *Wistar* rats were used as experimental model. Rats were fed with a standard diet differing in the fat component: virgin olive oil, fish oil or sunflower oil, resulting in 3 experimental groups. Moreover, half of the animals from each group, were supplemented with 0.7mg/kg·day of coenzyme Q (CoQ) in order to ascertain which effects may be obtained on age-related process together which a specific dietary fat. Animals were sacrificed at 6 or 24 months of age by decapitation followed to cervical dislocation. Blood plasma was obtained in order to analyze the fatty acid profile by gas chromatography and for determination of several biochemical parameters. Moreover, pancreatic tissue was collected in order to evaluate total proteins content, an histopathological and immunohistochemical analysis, as well as several markers of pancreas health evaluated by Enzyme-Linked ImmunoSorbent Assay (ELISA), and RT-PCR techniques.

As results, it can be outlined that there are signs by which it may exist a differential affectation in either, endocrine or exocrine compartment depending on dietary fat source, so that being the group fed with virgin olive oil that was offered the best results. On one hand, it was observed in non-supplemented groups that sunflower group showed the most signs of age-related alterations in the endocrine compartment, as it was found a high number of β-cells joined by high levels of insulin and leptin, typical of pre-pathologic states. Meanwhile in the exocrine compartment, fish oil group showed the most relevant alterations, such as acinar atrophy, fibrosis signs, fatty infiltration or hyperplasia among others. Concerning CoQ supplemented groups, it was observed that sunflower group achieved better results compared with its counterpart, ranking as results obtained by virgin olive oil group globally. However this did not happen in fish oil group, as there were no significant changes in respect to its counterpart.

Overall, from this study, it can be extracted that depending on nature of ingested dietary fat source throughout life the age-related effects suffer by pancreas may be different depending on it is considered endocrine or exocrine pancreas. So that, sunflower oil led to more evident age-related sign in endocrine pancreas, and fish oil did in exocrine pancreas. Coenzyme Q supplementation in sunflower oil group was able to prevent the age-related signs observed in its counterpart in the endocrine pancreas but it was not able to do it in the fish oil group in the exocrine pancreas. Anyway it must be ascertained the mechanism by which coenzyme Q may be beneficial about structure and function of both pancreatic compartments.

RESUMEN

A día de hoy, el envejecimiento supone un desafío para la comunidad científica, ya que generación tras generación la longevidad aumenta, y ello trae consigo la aparición de nuevas causas de mortalidad a las que la sociedad se enfrenta. El envejecimiento puede definirse desde el punto de vista biológico como un proceso fisiológico común a todos los organismos multicelulares donde el sujeto en cuestión sufre con el tiempo un descenso progresivo y endógeno de la eficacia de los procesos fisiológicos tras la fase reproductiva, de manera que llega un punto en el que es inviable mantener la estabilidad de las reacciones bioquímicas y las funciones corporales dependientes del metabolismo.

Aunque se han propuesto diversas teorías para explicar los mecanismos del envejecimiento, sólo unas pocas han sido consideradas como factibles, entre ellas la teoría del envejecimiento por radicales libres enunciada por Harman en 1956, por la que los radicales libres generados durante la respiración aeróbica producen daños en las biomoléculas del organismo, daño que se va acumulando con el paso del tiempo, produciendo esa pérdida gradual del mantenimiento de las funciones homeostáticas.

El que el proceso de envejecimiento sea más agresivo o más moderado va a depender de factores genéticos y de factores externos o ambientales. La genética se considera que es responsable entorno a un 25% de dicho proceso, mientras que el ambiente sería resonsab le del 75% restante. Esto implica que son mucho más relevantes factores como el estilo de vida o la contaminación ambiental que la genética del individuo desde el punto de vista del envejecimiento. Dentro de los factores modulables del envejecimiento, en las últimas décadas la nutrición está adquiriendo gran relevancia en el ámbito de la investigación. La primera evidencia por la que la nutrición se relacionaba con dicho proceso fue gracias a la restricción calórica, es decir, la disminución de la ingesta calórica sin déficit de nutrientes. Diversos estudios realizados en animales de experimentación llegaron a la conclusión de que aquellos sujetos sometidos a restricción calórica tenían una longevidad significativamente mayor con respecto a aquellos que tenían un aporte calórico equilibrado. Además de la restricción calórica, se estudió si la suplementación con antioxidantes podría tener un efecto parecido al de la restricción calórica, obteniendo resultados positivos sólo en algunos casos.

En los últimos años, nuestro grupo de investigación ha trabajado consistentemente desde el punto de vista de la grasa de la dieta como posible moduladora del proceso de envejecimiento. La grasa corporal no tiene un componente genético predeterminado como las proteínas, sino que tiene que ser aportado a través de la dieta. Así, se ha observado que el componente lipídico de las membranas celulares de los tejidos y órganos tiene un perfil de ácidos grasos que refleja el aportado por el componente graso de la dieta, es decir, existe un proceso de adaptación.

Por otra parte, diversos estudios han buscado una correlación entre la longevidad y el grado de insaturación de los ácidos grasos presentes en las membranas celulares de tejidos y órganos, observándose cómo a menor grado de insaturación de los ácidos grasos presente en dichas membranas, mayor era la longevidad de la especie en

cuestión, dándose como explicación la mayor susceptibilidad a ser oxidado por radicales libres cuanto menos insaturado se encuentra un ácido graso. Por lo que se podría concluir que la naturaleza de la fuente grasa de la dieta, ingerida a lo largo de la vida de un sujeto, tendría efecto sobre el proceso de envejecimiento de un tejido u órgano. Dicho efecto podría entenderse, al menos en parte, como el daño acumulado que sufren sus estructuras, lo cual podría acelerar o frenar sus pérdidas funcionales asociadas al envejecimiento. Esto ha motivado la realización de diversos estudios en los cuales animales de experimentación eran alimentados con dietas similares en las que se cambiaba la naturaleza del componente graso de la misma, tales como aceite de oliva, aceite de pescado, aceite de girasol, aceite de coco, manteca de cerdo etc. De esta manera se ha pretendido ver el efecto diferencial que una fuente grasa podría ejercer sobre la longevidad por una parte, y por otra como podría afectar a la funcionalidad y estructura de un tejido u órgano en concreto.

Con todos estos antecedentes, en el presente estudio se dispuso investigar qué papel podría tener la grasa de la dieta sobre signos relacionados con el envejecimiento a nivel pancreático, para ello se usó como modelo experimental ratas *Wistar* que fueron alimentadas con una dieta estándar, pero que variaban en la fuente grasa, pudiendo ser aceite de oliva virgen, aceite de girasol o aceite de pescado. Además, dichos grupos se dividieron en función de que estuviesen suplementados o no con coenzima Q en una dosis de 0.7 mg/kg·día, para averiguar qué efectos se podrían obtener con dicha suplementación al complementarse con una determinada fuente grasa en lo que concierne a procesos relacionados con la edad. Los animales se sacrificaron a los 6 o 24 meses de edad, extrayendo sangre para la obtención de plasma, el cual sirvió para determinar el perfil lipídico por cromatografía de gases y para la determinación de varios parámetros bioquímicos, y por otra lado el páncreas del animal, usado para la determinación de proteínas totales, análisis histopatológico, análisis inmunohistoquímico, técnicas de radioinmunoensayo y técnicas de RT-PCR. Con respecto a los resultados obtenidos, se observó que la grasa de la dieta afectaba de forma diferenciada al páncreas exocrino y al endocrino. En relación a los grupos sin suplementación con coenzima Q, los mejores resultados se obtuvieron en aquellos animales alimentados con aceite de oliva virgen. Por su parte, los animales alimentados con aceite de girasol fueron los que mostraron mayores signos de alteración en el páncreas endocrino, ya que se halló un alto número de β-células acompañado de altos niveles de insulina y leptina, propios de estados prepatológicos. Mientras que en el páncreas exocrino, el grupo que mostró alteraciones más relevantes fue el alimentado con aceite de pescado, hallándose atrofia acinar, fibrosis, infiltración grasa, hiperplasia y otras alteraciones. La suplementación con coenzima Q obtuvo una mejora de los parámetros del páncreas endocrino en el grupo alimentado con aceite de girasol con respecto a su homólogo sin suplementar, equiparándolo a los resultados que se obtuvieron con los grupos alimentados con aceite de oliva virgen. Sin embargo, en el páncreas exocrino, el grupo alimentado con aceite de pescado, que fue el que más alteraciones sufrió, la suplementación con coenzima Q no hizo surgir la mejora esperada. Como conclusión global, se puede extraer de este estudio que en función de la naturaleza de la grasa ingerida en la dieta a largo de toda la vida, los efectos que sufre el

páncreas son distintos en función de que hablemos del compartimento exocrino o endocrino, de manera que el aceite de girasol genera indicios de alteraciones en el compartimento endocrino, mientras que el aceite de pescado lo fue en el compartimento exocrino. La suplementación de los mismos grupos con coenzima Q solo revirtió los efectos en el grupo alimentado con aceite de girasol, pero no en el grupo alimentado con aceite de pescado. En cualquier caso, se requieren más estudios para tratar de esclarecer en su totalidad los mecanismos por los que el coenzima Q podría ser beneficioso en la estructura y función de ambos compartimentos pancreáticos durante el envejecimiento.

INTRODUCTION

I DEFINITION AND BEGINNINGS OF AGING

Aging is an endogenous, progressive, unique and individual process which occurs in each person. It is necessarily related to social, cultured and historical factors that possess a relevant social impact, since it historically involves seniors of several generations. Due to its multi-faceted character, aging comprises many approaches, such as physical, emotional, political, social, economic, ideological, cultural, and historical among others. The body of theses aspects guides the different ways of living and knowing aging, as well as, the cultural determination take differentiated form in time and space. Its social relevance firstly due to a large number of individuals over 65 (about 20%), the remarkable increase of individuals over 80, in addition to the rise of chronic aging-related illness (1). Another differential one concerns to the social status which individuals take place. People who live in locals with proper sanitation, in clean residences which they can reach a balanced nutrition, efficient health services, nets of collective transport that serve to population demands and net of competent teaching in all grades. All these premises favor seniors so as to live and age healthier and more dignified than those ones excluded from above mentioned services.

Finding an accurate and exhaustive definition of aging could be a hard job, but biologically it can be defined as a physiological process which is common to all multicellular organisms, wherein the person, over time, suffers an endogenous and progressive descent on the efficacy of physiological processes after reproductive phase of life, so that it becomes unable in order to maintain both the stability of biochemical reactions and the body functions depending on metabolism against alterations from external environment (2). It should be considered that aging process has an added difficulty, which it consists in the separation of the process itself from the illness which derivate from aging (1).

All the variety of living beings have a parameter named maximum life, which is defined as the tangible maximum age at which is able to reach the individuals of each species. In human beings case, this parameter was set at 110-120 years old. Currently, a tiny part of human beings are able to reach the maximum age, mostly because of infectious illness and a poor and inadequate nutrition (1).

From the Industrial Revolution began in the eighteenth century, the society underwent an improvement from the social and technological point of view, which completely changed the life expectancy of the population. The so-called industrialized countries suffered an annual increase of 3 months in life expectancy. This increase is sustained even in present days. However, it is important to consider the large increase in the cost of health as well as the emergence of previously unknown diseases, such as Alzheimer disease, cancer, and cardiovascular diseases, among others. Therefore, it could be said that the half-life increased to a greater instance, in the most advanced societies in comparison with primitive, even though the maximum life is the same (2).

Aging, therefore, is a process that involves a number of constants that determine its intensity. Inhibition of the process could make clear by suppressing the cause that

generates oxidative damage at the genetic level. When a reduction of aging is given by single mutations that extend life, it is observed a decrease in fertility. Food also plays a main role. This is a source of damage, once many of the genes that are involved in the reduction of the aging process, are also involved in the response to changes in nutrient levels. It has been shown that the reduction of food intake decreases aging in a variety of organisms ranging from yeast to mammals (1).

II AGING THEORIES

The study of aging, multidisciplinary in nature, has been characterized by a large variety of theories, by a wide literature of phenomenological character and the absence of a firm establishment of the primary causes that produce it. Numerous theories have been proposed in order to explain how and why the aging phenomenon occurs, although many of them lack of sufficient empirical support to be considered as such. In general, any theory which claims to be credible, it must satisfy a number of conditions (3): it must be able to explain how an organism loses its ability to maintain homeostasis in the latter part of his life; it must be able to clarify the basis for the wide variations in the length of life cohort genetic strains and species; it must be able to identify the factors responsible for life extension by single mutations or through experimental regimens such as caloric restriction (CR) in rodents and changes on environmental temperature in poikilotherms organism; must demonstrate that the degree of aging can be manipulated by varying the factors that are suspected of causing senescence.

In 1928 Pearl proposed "The rate of living theory" based on the observation that species with high metabolic rate had often shorter lives, and therefore the life expectancy is inversely rational to the metabolic rate of the species according to this theory. Although initially the link between metabolism and longevity was unknown, in 1956 Harman proposed biochemical explanation for this relationship postulating the "free radical theory of aging". The theory suggests that normal aging results from random tissue damage mediated by endogenous oxidants. Although the concept of endogenous oxidant initially resulted in a dispute, the discovery in 1969 of the enzyme known as superoxide dismutase (SOD) (4) provided base to this theory, demonstrating the first evidence of generation "*in vivo*" of superoxide radical and the subsequence requirement by cell of creating an antioxidant defense (5). According to this theory, a high metabolism leads to high free radical generation, accelerating aging and shortening longevity, the link between Pearl's hypothesis and Harman's theory. Over time Harman was focusing his theory on mitochondria as the main source of production of free radicals damage, being the mitochondria the target of damage caused by free radicals (6). Use of SOD as tool for locating the subcellular compartments which produce superoxide radical ($O_2^{\cdot -}$) led to the conclusion that mitochondria is the main source on production of endogenous oxidants (7). Thus, from the idea that the mitochondrion produces most of the energy consumed in the cell and accordingly consumes a large volume of intracellular oxygen, the theory of free radicals was bonded to the rate of

living theory early in the century (8). The study of oxidant production, oxidative stress and antioxidant systems led to the confirmation of the theory proposed by Harman: "free radicals produced during aerobic respiration cause oxidative damage accumulation accelerating the process of aging and cell death."

Later, Miquel and coworkers (9) proposed the “theory of mitochondrial aging” which enounces that the progressive damage to mitochondrial Deoxyribonucleic acid (DNA) is performed by reactive oxygen species (ROS). Being as it is currently known that some ROS are not free radicals, today it is known as “Aging theory of oxidative stress”.

In addition to previous theories mentioned, there is other group of theories which have been proposed and own certain similarities with the theory of oxidative stress and that somehow could be explained by it. Together they form the group of “theories of damage accumulation” (2) where it could be included on it the “theory of crossover” (the random crossover between proteins and DNA alters cellular function); “theory of catastrophic failure” (an accumulation of random damage on protein biosynthesis); “theory of glycosylation” (production of glycosylated proteins leads to a severe disruption of cellular functions); “theory of longevity determinants” (aging is caused by metabolism products and aging degree is due to the ability of discarding waste products); “theory of membrane hypothesis” (damage on cellular membrane lead to a decrease on ability for discarding waste product, to decreased protean biosynthesis and to loss of water from cytoplasm, triggering a decrease of enzymatic activity).

It could be set a group of mathematical and physical-mathematical theories. Normal or young state is characterized by the existence of a large number of regulatory factors interacting chaotically, and aging is characterized by loss of complexity and tendency to move towards non-chaotic, simpler and dynamic systems, leading in ultimately to a loss of the adaptive capacity of body. More than an explicative theory, the enounce tallies to a model based on chaos theory. The “theory of entropy” enounces that some mechanism such as caloric restriction, reduce the degree of entropy production, delivering energy slower and delaying molecular deterioration (10). In so-called evolutionist theories, such as “theory of immediate surviving”, aging happens because nature selects the gens which possess value of immediate surviving, but in long-term they have deleterious consequences (11). It is emphasized that the theories mentioned are not mutually exclusive and it seems clear that the intrinsic and extrinsic processes that lead to aging have been established, through evolution, to provide maximum lifespan same for all of a species. Evolution by natural selection has led to human being to adapt to live in different conditions to which today forms the lifestyle of most humans. So that certain elements of intrinsic aging process may be considered resulting from a lack of adaptation, maybe this explains that the problems as vascular diseases caused by the diets of modern power and osteoarthritic joints that have never adapted to the standing.

More than 300 hypothesis have been proposed in order to explain the processes that occur in senescence (12), within these ones exists other group of theories called "immunological theories" where aging is originated by decrease of the ability of immune system for creating antibodies: as the immune response decreases, the ability of the system to discriminate between their constituents and others is also reduced, with an increase of autoimmune reactions. This theory has the disadvantage that only can be applied to the immune system and would not rule out the possibility that these changes are secondary to others earlier, for example, hormone-like. "The neuroendocrine theory" is based on the fact that there is no any part of the body which can act aisled from endocrine and nervous system, therefore if any of them is disturbed, the remaining systems will be affected one way or another. However, as the immunological theory, this one lacks of universality because not all organisms own a neuroendocrine system, and in spite of it, they age (10). Besides, many other theories have been proposed so as to explain aging, among them, the "genetic theories of aging" (13). Genetic theories are varied and generally propose that aging is continuing the process of development and differentiation, being a sequence of events encoded in the genome. These theories are based on a fact which the aging would be the late consequence in the expression of genes selected by evolution because they increase reproductive success. The power that favors the selection of beneficial genes are displayed more in young people because these are the ones that are responsible for reproduction and genetic transmission, so that no one considers what happens to these genes in mature age. Thus, a gene that promotes reproduction but detrimental in the long-term will not be selected for deletion.

Within this group of theories is found the "hypothesis of disposable soma", which enounces that energy throughout life must be used preferentially for reproduction, at the expense of repairing mechanism itself, which capacity would be exceeded soon at the time of overpassing reproductive age. This theory proposes that the main candidates which determine the life expectancy of a species from the genetic point of view are those genes that regulate the repair and maintenance of somatic cells (14).The study of gene expression in rodents related to aging, reveals genes that alter their expression with age, or which expression is altered by interventions such as calorie restriction, affecting the rate of aging (15). So, it is not surprising that the fact which the most of these genes are involved in both cellular response to stress or oxidative damage pathways.

According to the hypothesis of disposable soma, natural selection favors those genes that act in the early stages of life allowing the reproduction of the species, compared to those genes that are responsible for preserving the non-germ cell or disposable soma (11, 16). Therefore, somatic lineages of all animals (unlike germ cells), are which decline and degenerate with age causing the phenotypic changes which is recognized as aging (17).

At the cellular and molecular level, disposable soma theory predicts that the effort for cellular maintenance and repairing processes vary in direct ratio to longevity. There are numerous studies which support this idea, for instance, it is found that the

long-life rodent species which ROS production is low, oxidative damage levels in proteins is lower and cellular concentration of some antioxidant enzymes is higher in regard to short-life ones. Likewise, it was also observed a direct correlation between life expectancy and repairing ability in mammals (18). Hence, it could be concluded that quality of repairing and cellular maintenance mechanism is determined by ability for facing to any oxidative stress. Comparisons between the functional capacity of cell cultures in response to a variety of stressors, demonstrate that cells taken from long-life species are more resistant to oxidative stress than those made of short-life species (8).

In general, it could be affirmed that aging genetic has 2 main ways:

The first one proposes that aging is programmed under control of genes which sort smartly a number of changes in cell that ultimately get it to fall and auto-destruction. Its mechanism of action is based on activation of pernicious genes and/or deactivation of others beneficial for life. Theories based on the genome suggest that aging is genetically programmed, either because the original program ("somatic mutation theory") is altered or because cellular changes associated with aging are included within the incorporated instructions in DNA from birth, that is to say, they are part of normal develop. Other implicit possibility in these theories is that those genes which encode the production of a protein with useful function, adaptive to a certain age, they become harmful to an elderly (pleiotrophy) (19). Aging is, on this as well, a multifactorial phenomenal.

The second one is based on changes or damage suffered by DNA by a more or less random process. The cells present these repair mechanisms but are not 100% efficient. The damage will occur due to the effect of free radicals on the genetic material either by DNA demethylation or fragments telomere losses.

Some scientists think that the keys to aging must be sought in the process of cell division, an idea that leads to the "telomere theory of aging" (20). According to the hypothesis formulated by Olovnikovk (1971), the telomere shortening in each of cellular division cycle is the responsible of the limitation on the proliferation of culture cells, what is denominated “Hayflick’s limit” found by Hayflick and Moorhead (1961). Studies on *in vitro* cells have shown that certain cultures loss overtime the division ability. It is possible because such cells specialize in each cellular division cycle until they loss its replication ability, thus reaching what is known as replicative senescence.

For this group, the biological timing that controls the life of all cells is precisely the telomere, a long DNA fragment consisting of the repetition of a series of 6 or 7 bases in high Guanine, located at the ends of all eukaryotic chromosomes. The telomeres function is protecting the chromosomal ends, preventing that fusions occur between them or that the genetic material could recombine improperly. Every time cell divides, the telomere progressively shorten to until nearly disappear, reaching cell the state of senescence in which the division capacity and other metabolic functions are lost.

In 1998, Hayflick enounced a newfangled hypothesis based on telomere shortening and subsequent loss of cellular replicative capacity, which exclusively determines extension of the life of each specie, However aging process is determined by the accumulation over time of cell damage. This hypothesis is based on the followings observations:

• The length of telomeres in cells characteristic of patients with premature aging (such as Hutchinson-Gilford´s progeria and Werner's syndrome), is initially shortened in correlation with a Hayflick limit visibly diminished. Patients show remarkable characteristics of accelerated aging at younger ages compared to healthy individuals.

• Telomere cells from individuals extremely old never reach critical length, which indicates that telomere shortening has not got a direct link to aging

In order to establish a correlation between aging and this theory, Hayflick propose that senescence and subsequent demise do not need to reach Hayflick limit in all organism tissues. Depletion of proliferative potential in certain areas of some organs may be sufficient to promote the emergence of certain diseases. The blend of these disorders increases gradually with age, leading to aging. Apparently, telomere shortening and the blocking of cellular replication occur irregularly in different tissues and organs and at different speed in each person. This fact leads to corresponding organ dysfunction as in the case of neuroendocrine glands, heart and hematopoietic tissue, increasing susceptibility to suffer various diseases which rate of appearance and severity increases with age, eventually leading to death. There are evidences about telomere shortening in certain tissues promotes the appearance of disorders such as hypertension, diabetes mellitus non-insulin dependent, atherosclerosis and cancer (21).

According to this theory, aging could be defeated by obstructing and blocking the process which telomeres are shortened. There is a gen which controls production of enzyme named as telomerase, a ribonucleoprotein which catalyzes the addition of DNA fragments to telomeres, thus the preservation of its length remains assured, this fact allows infinite cellular replication, conferring it immortality. Nevertheless, the telomerase is not innocuous, because if it was not allowed to cell reproducing, that could trigger the expansion of a carcinogenic tumor. At the moment it has been demonstrated *in vitro* that cells may live longer if telomeres from chromosomes are artificially extended; but still to be seen if an entire organism may enjoy a longer life through telomeres using, without exhibiting the risk involved.

III NUTRITION AND AGING

III.1 CALORIC RESTRICTION AND DELAY OF AGING PROCESS

Some observations demonstrate that reduction of caloric intake positively influence the longevity of several species. Limiting food intake was first proposed in 1935 by Macey *et al*. From this point it was made a description of increased longevity or lifetime in several species and rodents. It was also observed that a delay of emergence of some diseases related to age was promoted (1). The most accepted hypothesis so as to explain such phenomenon is based on the possible reduction of oxidative stress. It was observed through experiments that mice fed by a restricted caloric diet generated less oxidative stress than its homologues fed *ad libitum*, besides exhibiting less incidence on lipid, protein and DNA oxidation (22). Many of the changes on gene expression during aging, including the increase of heat shock proteins or amelioration on expression of protein induced by stress of Hsp70 (70 kilodalton heat shock protein), are observed. In addition to this it was observed that caloric restriction confers to rodents a higher ability to resist the different types of physiological stress, improves thermotolerance and reduces damage induced by heat in aged rats (1).

Caloric restriction, therefore, can be considered as an efficient and valuable mechanism in order to be used in therapies against aging and, consequently against diseases bound to it (23). However its unviability becomes evident because there are some ethic obstacles besides being impracticable in order to implement in society (1).

III.2 EFFECTS OF ANTIOXIDANTS SUPPLEMENTATION

The feasible anti-aging therapy through use of antioxidant is one of the alternatives so as to obstruct aging process. Among the first studies described it is highlighted Miquel and Ecónomos's about the properties of thiazolidine carboxylate, which increases vitality and life expectancy in mice (24). Oral glutathione intake *versus* descent of immune activity associated with aging was described by Fukurawa (25). Furthermore, throughout scientific development is was observed the use of numerous antioxidants in experiments which aimed countering aging process, as the case of vitamins C and E, coenzyme Q, some herbal extracts rich in flavonoids and polyphenols, among others (2, 26). Results about these antioxidants were positive in regards to decrease of oxidative stress associated with aging. Nevertheless, regarding to longevity increase, only some cases were observed positive effects (27). Knowledge of the pharmacological properties of the substances used in the studies would be important for greater effectiveness in terms of therapy based on antioxidants, especially regarding its absorption, tissue distribution and metabolism. It must be also considered the role which ROS perform on cellular signaling, so antioxidant doses must be adjusted in order to avoid changes on redox state that could lead to alteration of cellular function. The use of synthetic antioxidant substances which are mimetic of SOD and catalase are solving part of propounded problems. These substances are being tested successfully in part, and they are been demonstrated to be effective on longevity increase in mice and nematode *Caenorabditis elegans* (28).

IV BIOLOGICAL MEMBRANES RELATED TO DIETARY FAT

Some substances, such as proteins, have its cellular content defined in part by a genetic component. In fat case it does not occur, so some factors such as age, physiological state, cellular type, antioxidant capacity, metabolic activity and especially diet, are factors which affect to the type and amount of fat at corporal level. According to mentioned above, it seems evident that ingested fat through diet affects preponderantly to fatty acids composition of tissue lipids. Thus, fat type will affect at every level the fatty acid production, where its composition, genetic factors, regulation factors and adaptation to environment will be which lead to the organism to alter cellular content of different fatty acids in different organs and corporal tissues (29, 30).

According to Quiles *et al*. (30), dietary fat relatively determines fatty acid composition of membrane, even desaturase system also contribute for such event through inhibition of different components of various fatty acids series. However, it is relevant to mention that the tissues are distinct, so this phenomenon is observed distinctly in the several biological membranes presented in the organism (31).

Some fatty acids are converted in animal tissues into polyunsaturated fatty acids (PUFA). Among fatty acids, it is observed different series (table IV.1) the division into series is done according to position of last double bound observed in each member or group. Linoleic, linolenic and oleic acid initiate 3 relevant series, n-6, n-3 and n-9, respectively, once the double bound is on the 6, 3 and 9 atoms of each formed fatty acid.

Table IV.1. different components of fatty acid series n-6; n-3; and n-9

n-6 Series		**n-3 Series**		**n-9 Series**	
Linoleic	C18:2n-6	Linolenic	C18:3n-3	Oleic	C18:1n-9
Eicosatrienoic	C18:3n-6	Eicosatrienoic	C20:4n-3	Eicosadienoic	C20:2n-9
Eicosatetranoic	C20:4n-6	Eicosapentanoic	C20:5n-3	Eicosatrienoic	C20:3n-9
Docosatetranoic	C22:4n-6	Docosapentanoic	C22:5n-3	Docosatrienoic	C22:3n-9
Docosapentanoic	C22:5n-6	Docosahexaenoic	C22:6n-3	Docosatetraenoic	C22:4n-9

Linoleic acid is Dihomo-Gamma-Linolenic Acid (DGLA-C20:3n-6) precursor, which in turn comes from the formation of type1 prostaglandins and arachidonic acid (C20:4n-6), which in turn precedes type2 prostaglandins formation.

Linoneic acid precedes eicosapentaenoic (EPA-C20:5n-3) acid which in turn, come from type 3 prostagladines. Other n-3 series member is docosahexaenoic acid (DHA-C22:6n-3), relevant for brain and retina development.

Oleic acid in standard conditions is presented in low ratio compared to other lipid tissues. But its importance and rate markedly increase when it is observed a decrease of essential fatty acids levels. This fact can be tested, as example, observing a tissue increase of 5,8,11 eicosaenoic acid (C20:3n-9) in substitution of arachidonic acid in such circumstances. From palmitic and stearic acid, by Δ9 desaturase action, is formed in the body palmitoleic (C16:1n-9) and oleic (C18:1n-9) acid, and thanks to elongation process, remaining monoeoic fatty acids will be produced, which relevance is linked to production of cerebral myelin (32).

Several experiments demonstrated that olive oil stimulates membrane formations where presence of n-3 PUFA is plentiful compared to rich diets in sunflower oil (mainly formed on n-6 PUFA) as fat source (33-35). It was also observed a decrease of palmitic and stearic acid, as well as a significant decrease of linoleic and arachidonic acid in plasma and other organs (36). However, dietary olive oil stimulates monounsaturated fatty acids contents in membranes and also increases Δ9 desaturase activity.

Lower unsaturated fatty acids levels are observed in rich diet in monounsaturated fatty acids (MUFA), such as oleic acid, if it is compared to diets with significant amounts of n-6 PUFA and saturated fatty acids –SFA- (37-39).

Some studies could determinate there is no difference between unsaturated fatty acids (UFA) and SFA ratios in membrane phospholipids from rats fed with three different fat sources, that lead us to guess that this variation is not related to the amount of UFA molecules, but it is related to saturation degree of itself (40).

It was also determined through specific studies that the dietary effect on fatty acids tissue composition is possibly caused by a competition among PUFA, MUFA and SFA for enzymatic systems, both elongation, as in the desaturation, without underestimating other factors such susceptibility to peroxidation in membranes, the organ in question, membrane fluidity or specified receptors. It have been shown that there is a Δ9 desaturase specific affinity for fatty acids which own a greater number of double bonds (C18:1n-9<C18:2n-6<C18:3n-3), that explains partially the lower n-9 derived levels in eukaryotic cells (32).

About rich diets in n-3 PUFA it was observed a displacement in the ratio of n-6 fatty acids series, including polyunsaturated ones. There is a ratio between 2 series (n-9/n-6) which suggests n-9 fatty acids series have more facility for being incorporated to the detriment of the n-6 ones. Diets based on SFA increase levels of MUFA and those diets based on n-3 PUFA increase the n-3/n-6 ratio (41).

Maintenance of membrane fluidity is achieved in mammals by a homeostatic mechanism of control and compensation which keeps an optimal relation between

unsaturated and saturated fatty acids ratio (30, 32, 42). This argument is demonstrated once several studies which diet was shifted in relation to saturation degree of ingested fat were not observed variations as expressive as on saturation of membrane lipids as an increase in n-6 series provoked a decreased in n-3 series and vice versa (30, 40).

Quiles *et al* (30) also observed in an experiment which rats fed during eight weeks with a fat amount (olive oil) equivalent to 8% overall, obtaining variations in stearic acid levels. MUFA levels were higher in rats fed with olive oil compared to those ones fed with sunflower oil. Specimens fed with sunflower oil showed higher n-6 PUFA proportions in mitochondrial membranes, whilst those fed with olive virgin oil had reported higher levels of n-3 PUFA. Both results were justified based on competence among fatty acids series.

Lipid oxidation at mitochondrial membrane level of rats is modulated in accordance to dietary fat, so MUFA induce to a minor susceptibility to such peroxidation. MUFA seem also to exert an important role in peroxidation. It tried to find out the different organs adaptability to different diets regarding fat source. It seems that some organs such liver and muscles, adapted better to lipid profile than heart. Free radicals production depended mainly on type of tissue and secondarily on the diet in liver and muscle. In heart did not occur such phenomenon. α-tocopherol had an antioxidant action significantly smaller than MUFA against lipid peroxidation (31).

Antioxidant action against lipid peroxidation seems to own a great importance in heart when specimens are fed with diet rich in monounsaturated exhibits higher amounts of such fatty acids (40).

If it is attempted to be more specific in regard to the action of MUFA like modulators of membrane and specific tissues. It was observed in several studies that C14:0 levels are equivalent in all rat groups, C16:0 varies according to diet and C18:0 have a higher dependence on tissue adaptability. Thus, those animal which are fed with diet which the fat source presents higher amounts of C16:0 increases MUFA levels, what implies stimulation on Δ9 desaturase activity. It was observed in muscle that oleic acid is no displaced by neither n-3 or n-6 PUFA due to a reduced ability to synthesize and/or incorporation by muscle. Linolenic acid levels depend on what was ingested by diet, however, araquidonic acid levels depend on tissue requirements, and therefore, although linolenic acid is rich, this does not convert into araquidonic acid (43).

If it is considered some beginnings, it could be said that fatty acid composition of biological membranes varies according to type of fat source administered in diet. Taking into account some directives: 1) it is observed there is a tendency which ratio of SFA remains stable, like C18:0, whose levels depend mostly on requirement of each tissue. Concerning to C16:0, it is observed an adaptability according to diet composition, having a special ability to activate Δ9 desaturase. 2) diets rich in n-9 fatty acids series have been observed a leaning to the increase of MUFA, especially when oleic acid is administered. 3) for n-6 series, its membrane levels only seem to increase when a great dose of its own agents are administered. 4) n-3 series is noted when a great

dose of its own agents are administered, when there is a n-6 deficiency, once both compete for Δ6 desaturase, conditioning n3/n-6 ratio (44).

V RELEVANCE OF DIETARY FAT ON HEALTH MAINTENANCE

It is widely demonstrated that dietary fat contributes to the health maintenance outcome, however it will be focused on health aspects which are related directly or indirectly to the pancreas.

V.1 EFFECTS ON CARDIOVASCULAR SYSTEM

The studies which relate dietary fat composition with cardiovascular disease possess a great relevance because it is known that diets rich in animal fats (those which contains great amounts of saturated fatty acids), generate problems on lipid metabolism, which lead ultimately to atherosclerosis problems (36, 45, 46). On the other hand, UFA, especially those derived from fish (rich in n-3 PUFA) seem to own antiatherosclerotic effects. Many studies have revealed a lower ratio of cardiovascular diseases occurrence in countries which assume a Mediterranean diet pattern, whose main dietary fat source is olive oil, rich in MUFA. Thus, if a saturated diet is replaced by a monounsaturated or a polyunsaturated diet, this results in a decrease of plasma cholesterol, as well as low density lipoprotein (LDL) cholesterol levels. A diet rich in MUFA prevents oxidative impairment to lipoprotein in a greater extent that does a polyunsaturated one. Dietary fatty acids have a great influence on lipoprotein oxidation, exerting a great effect on adhesion molecules and others inflammatory factors. Thus, dietary oleic acid decreases genetic expression of endothelium adhesion protein (V-CAM1) (47).

When n-3 PUFA are ingested, a higher proportion of ecosanoids derived from EPA are formed. That may be a thrombogenesis preventive factor, because allow prostaglandins I3 (PGI3) formation, which works as a vasodilator and platelet antiaggregating and it also allows thromboxane A3 (TXA3) formation, hardly with vasoconstrictor and proagreggant effect. Likewise, EPA and DHA suppress prostaglandins I2 (PGI2) formation from arachidonic acid, which owns similar PGI3 and thromboxane A2 (TXA2) effects, due to its vasoconstrictor ability and platelet aggregating. Thereby they own an antithrombogenic character and therefore it is justified in part that population eating higher amounts of fish have less cardiovascular diseases occurrence. Oleic acid from olive oil shows a more obvious effect than EPA derived from fish, since oleic acid produce additionally an increase of PGI3 and TXA3 levels, and decreases simultaneously PGI2 and TXA2 synthesis (45, 48).

V.2 EFFECTS ON DIGESTIVE SYSTEM

Experiments performed on animals and humans, related to digestive secretions (gastric, pancreatic and bile), both under fasting and in response to food intake, showed after an adaptation or in response to diets rich in olive oil certain positive effects. Among those positive effects, authors found a decrease of intragastric acidity after

intake of mixture of diets containing virgin olive oil in opposition to PUFA rich diets (49). It has been described variations on fatty acids profile related to eicosanoid biosynthesis and inflammatory response. It has been observed that such changes may have a beneficial effect on intestinal inflammatory disease after altering the lipid environment required in order to the generation of mediators related to anti-inflammatory response, because of being more resisting to lipid peroxidation process. Virgin olive oil alters basal and postprandial levels of several gastrointestinal peptides such as colecystoquinine (CCK), polypeptide Y (PPY), gastrin and Polipeptide P (PP), which are related to this disease. It is possible that this beneficial effect is due to the standardization of the gastrointestinal peptides pattern (50). It also reduces cytokine productions, the expression of adhesion molecules and levels of other endothelial markers on this pathology. Monounsaturated fatty acids and phenolic compounds from olive oil are aspirants to increase the apoptotic threshold of intestinal cells, thus decreasing necrosis and exerting a protective role (51). Studies about the role of dietary fat on pancreas will be discussed in subsequent sections.

V.3 EFFECTS ON DIABETES

Several studies in recent years have showed that virgin olive oil intake reduces glucose levels in blood, due to a better using of glucose and likewise, through a decrease in insulin requirements. Garg in 1998 performed a meta-analysis of ten studies performed on type 2 diabetics that compared effect of a diet rich in MUFA and other rich in carbohydrates. It was demonstrated that the first one reduced fasting and postprandial glucose levels, without changing insulin levels in fasting. Furthermore, diets rich in virgin olive oil reduced glucose and insulin profile in 24 hours and it was also found a reduction in the daly requirements of the hormone (52).

V.4 EFFECTS ON CANCER

When talking about fat and cancer, it should be considered both, amount and quality of fat and fatty acids, and the list of fatty acids displayed in the type of fat source (53). In this sense, there are experimental evidences that point the possible existence of certain level of n-6 PUFA, which can define the difference in the etiologic ability of different fat types concerning cancer genesis (54). It seems that a 4% of diet weight as linoleic acid (corresponding to 8% of overall energy) define a limit, below which, certain fatty acids have a different carcinogenetic ability, and whilst over such limit, everyone have the same ability.

Virgin olive oil seems to be a fat with palpable advantages in relation to cancer. From the point of view of tumor initiation, the role of the fat is characterized by oxidative factors, such as free radical production conditioned by fat unsaturation. In relation to cancer growing and progression, it is required an increase of vascularization and parallel the immune system must be altered, in this process certain metabolites from arachidonic acid are essential, among it is emphasized prostaglandin E_2 (PGE_2), which is generated by derivatives from linoleic acid. Virgin olive oil would ameliorate growing by a minor production of this one. In metastasis, genetic expression of

carcinogenetic adhesion factor cells (E-cadherines) is diminished. Other group of adhesion molecules to vascular endothelium such as Intercellular Adhesion Molecule 1 (I-CAM1), V-CAM1 and E-selectine, are overexpressed. It is unknown the precise role of fatty acids, but it set up a clear correlation between oxidative state and free radical role on this overexpression (2, 47, 55-57).

Breast cancer has been the subject of many studies in relation to dietary fat intake, specifically concerning virgin olive oil intake, achieving very interesting results. Recently, Moral *et al*. (58) have carried out an experiment by feeding female Sprague-Dawleys rats on a low-fat, high-corn oil, or high extra-virgin olive oil (EVOO) diet from weaning, and with further induction of mammary tumours by administration of 7,12 dimethylbenz[a]anthracene (DMBA). These authors observed that those rats fed with high-corn oil reported a higher proliferation/apoptosis ratio and modifications in the expression profile, which help in order to create a microenvironment to produce malignant cells. Previously, Escrich *et al*. had analyzed data from 16 experimental studies about the effects of dietary lipids on mammary carcinogenesis in an animal model. They concluded that EVOO, when is mildly ingested throughout life, is the choice that seems to be the most healthy, and positively takes influence on breast cancer prevention, especially on tumor progression (59).

Granados *et al*. also approached the benefits of virgin olive oil components on breast cancer. These authors did not focused on the fatty component of the oil, but on a particular compound of the minor fraction of EVOO named hydroxytyrosol (HT). They also induced breast cancer by DMBA administration to female Sprague-Dawley rats. Results showed that those rats treated with HT inhibited the tumour growth and cell proliferation. In addition to this, HT modified the expression of some genes related to cell proliferation, apoptosis and the wingless/integrated (Wnt) signaling pathway, which resulted in a high expression of secreted frizzled-related protein 4 (Sfrp4) (60). In the same study, Granados *et al*. (61) observed that the negative effects of doxorubicin (a common chemotherapeutic agent administered in patients of breast cancer), such as cardiac alterations at the cellular and mitochondrial level, was ameliorated by administration of HT in female Sprague-Dawleys rats which previously were induced mammary tumors and subsequently treated with doxorubicin.

Virgin olive oil has not been only tested on animal models of breast cancer. Hashim *et al.* have tested possible positive effects on colon cancer cells. They had shown that the phenolic extract of virgin olive could inhibit invasion of HT115 colon cancer cells *in vitro*. Later they tested this phenolic extract on Balb-c mouse model, observing similar results as in vitro model (62).

V.5 EFFECTS ON AGING PROCESS

More and more studies have proved that the aging process has a modifiable factor which depends on our lifestyle. One of the most relevant factor which researchers are increasingly studying is diet, where dietary fat is getting attention in the last years so that according to the intake of a specific fatty acids or group of lipids in diet, either as a

supplement or long-life, it could be exerted a different outcome on aging process, and therefore on health.

When it is talked about dietary fat and aging, it must be considered either quantity as well as quality. It seems to be clear, through numerous studies, that a high-fat diet prolonged in time can trigger in obesity, which could early lead to age-related diseases such as diabetes, atherosclerosis, hypertension or neurodegenerative diseases among others (63, 64).On the other hand, a low-fat diet prolonged in time may be more feasible to be a low-caloric diet, or commonly known as caloric restriction (CR), which in turn, it was previously said that CR has proved to be a way in order to extend longevity as studies show (65, 66).

Obesity is usually observed in individuals who intake more calories than they do spend over time. This fact provokes that adipose tissue increases. Recent studies have observed that a change of the adipokine profile according to the obesity degree, so that the most amount of adipose tissue, the most delivery of pro-inflammatory adipokines is done from adipose tissue, which results in a modification of preadipocyte number and function and a macrophages infiltration in adipose tissue. This is believed to be a mechanism that produces a major prevalence of age-related diseases at early age such as diabetes, atherosclerosis, hypertension or neurodegenerative diseases (67).

On the other hand, in non-obesity conditions or normal weight, the quality of dietary fat over time may involve a different susceptibility of suffering age-related diseases, and therefore that could lead to a modification of life span. It was previously mentioned that oxidative stress can take part in the aging process, what it is known as "free radical theory of aging", where ROS have a pivotal role in order to oxidize biological molecules and therefore that could be the way which ROS are involved. It was also mentioned that biological membranes can adapt its fatty acid profile from dietary fat at long-term. These two premises are linked so that according to the degree of unsaturation of biological membranes, it could be modified the susceptibility of such membranes to oxidative alterations, and thus affecting the production of reactive species such as ROS.

Experiments found that the most unsaturated is a fatty acid, the most susceptible it is to oxygen radical damage because double bonds (68). Therefore, decreasing the degree of fatty acid unsaturation in biological membranes, it might decrease its sensitivity to lipid peroxidation, which in turn, might protect even other molecules against lipid-derived damage (31). Supporting what recently mentioned, some studies showed that the degree of fatty acid unsaturation of mammalian tissues is negatively correlated with maximum longevity (69, 70). In fact, several studies support this premise, because it has been found that long-lived animals, such as birds and humans, have a lower degree of total tissue and mitochondrial fatty acid unsaturation than short-lived ones (71-75). In addition to this, it was experimentally observed that when the degree of fatty acid unsaturation decreased in liver and brain, the oxidative damage in mitochondrial deoxyribonucleic acid (mtDNA) also was lower (76). Furthermore, this

decrease in the degree of fatty acid unsaturation in long-lived animals was accompanied by a low sensitivity to lipid peroxidation in cells from another tissues like skeletal muscle, heart, liver and brain (70, 77).

A comparative study of dietary fat and longevity fulfilled the expectation (78) about the studies previously mentioned, in which C57BL/6J mice were fed different fat sources, soybean oil, fish oil or lard, rich in n-6 PUFA, n-3 PUFA or MUFA/SFA, respectively at CR conditions. It was observed an increase in life span in those mice whose diet was low in n3-PUFA and those ones that were fed on a high MUFA or SFA diet. However, another comparative study of dietary fat performed on SAMP8 mice that were fed with different fat sources, namely lard, soybean oil or fish oil, an opposite result was obtained, as those mice which were fed with a diet rich in PUFA achieved a greater longevity (79). Likewise, a recent review (80) recruited studies which focused on dietary lipids and longevity, showing that dietary PUFA may affect to longevity. According to this review, arachidonic acid (n-6 PUFA) may extend longevity by inducing apoptosis of cancer cells. Moreover, In *C. elegans*, a diet rich n-6 PUFA enhance longevity and resistance against nutrient deprivation by inducing autophagy.

Other way through which dietary fat might take influence on longevity, or at least on healthy aging, is through its involvement on age-related dysfunctions such as cardiovascular diseases (CVD), bone health, immunological decline, mental and cognitive diseases and muscle function.

In regards to mental health, fish oil consumption has proved to be a feasible tool in order to improve this aspect in the elderly. Most of the studies and reviews show a positive association between regular fish oil consumption or supplementation and reduction of age-related decline of mental and cognitive functions (81). This association improves when the intervention begins as soon as possible (82). A potential mechanism which could explain this fact may be because DHA is involved in regulation of synapsis and neuroinflammation (83). However some studies did not found such evidences. In that sense, a study carried out by Sergeant *et al*. (84) found that fish oil supplementation did not improved the age-related declines in memory or in brain inflammatory status. A systematic review pointed out that these controversial results may be explained by a different methodology, concluding that n-3 PUFA consumption may trigger in a decreased risk of cognitive problem in the elderly (85). Meanwhile virgin olive oil consumption has been under study concerning mental health, either by itself or by adherence to Mediterranean diet. Specifically this edible oil, rich in polyphenols was administered to C57Bl/6J mice in a long-term study in order to assess cognitive, motor, and emotional behavior. Authors observed that virgin olive oil reversed age-related dysfunctions in motor coordination and contextual memory by differentially affecting several brain areas (86). Other study tested virgin olive oil in order to assess cognitive function in old people during 6 and a half years of treatment, observing an improvement of cognitive function due to the virgin olive oil treatment.

Cardiovascular diseases are one of the leading causes of premature death in developed societies. Diverse parameters affecting the prevalence of these diseases, such as cholesterol levels, arterial stiffness, blood pressure or diabetes, have been analyzed with respect to dietary fat type. An approach these aspects have been recently reviewed (80). This review has focused the antetion on that those diets enriched in UFA had high levels of high density lipoprotein (HDL) cholesterol, reduced levels of LDL cholesterol, lower blood pressure, and better insulin sensitivity, and therefore a reduced CVD risk. On the other hand, dietary trans-fats triggered inflammatory responses, which lead to a higher cardiovascular (CV) risk. However, they did not found consensus about dietary SFA.

Fish oil has been widely studied in relation to CV health thank to its high content in n-3 PUFA, either as supplementation or as fish consumption. Supplementation in fish oil during 4 weeks is observed to improve lipid metabolism and endothelial function in postmenopausal women with type 2 diabetes (T2D) (87). Furthermore supplementation in fish oil administered in late-life during 4 months ameliorated the aged-related declines in diastolic function in aged rats (84). Retrospective surveys have also approached consumption of fish, fish oil or liver fish oil and CV function. Concerning previously mentioned, it was observed a reduced risk of coronary diseases in old women who consume liver fish oil at elderly stage, and this protective role is improved at this stage if consumption is done throughout life (88). Other similar study performed on volunteers around 59 years on average concluded that consumption of fish more than once per month is associated with a lower risk of heart failure (89). However when it was assessed consumption of fish oil long-life in respect to arterial stiffness, it was not observed an association between both, although an association was found between high levels of plasmatic n-3 PUFA and lower arterial stiffness (90).

Reviews have also involved in analyzing studies about ingestion of n-3 PUFA and CV health. Most of them match positive CV effects in relation to n-3 PUFA consumption, either lowering heart failure risk (91), reducing triacylglyceride (TAG) levels, improving endothelial function and control of inflammatory processes or exerting an antiarrhythmic action (92). Nevertheless, Patel *et al*. pointed that these positive effects of n-3 PUFA on CV health are limited to cultures with high ingestion of n-3 PUFA and the ethnicity could also control availability from dietary n-3PUFA (93). In addition, those possible positive effects could be highly influenced by an ingestion of a proper dose (92).

Although fish oil is the most studied fat source in respect to CV health, virgin olive oil has been also considered about this aspect. Most of those studies highlight the protective role either olive oil itself or its phenolic fraction. A review recruited the main latest results from epidemiological studies and dietary interventions in large cohort which were mainly performed in Southern European countries (94). From them, they conclude that diets rich in virgin olive oil have a cardiovascular protective effect and observing a decreased risk of mortality close to 45% among the highest consumers. Moreover, virgin olive oil consumption is related to lower T2D risk in women, which in

turn, is a risk factor for CVD (95). This positive effect was enhanced by replacing other dietary fats such mayonnaise, margarine or butter contain by virgin olive oil. Studies are pointed to the phenolic fraction of olive oil as additional CV benefits; in fact, they are associated with a wide spectrum of bioactive properties such as improvement of lipid profile, anti-inflammatory, vasodilation, anti-platelet aggregation and antioxidant effects (94). Accordingly with recently said, individuals at high CV risk who ingest virgin olive oil, are associated with lower CV risk, specially its extra-virgin variety. Similarly, when dietary fat from a typical American diet was replaced by this edible oil in overweight aged people, there were a widely decreased of systolic pressure and a strong trend toward incremented levels of HDL (96). Moreover a supplementation in 25 ml/day of virgin olive oil in a wide range of aged population during 12 weeks improved the anti-atherogenic activity of HDL through regulating or maintaining cholesterol homeostasis (97). A very similar study to the previous one showed that consumption of the same dose of virgin olive oil increased the anti-inflammatory effects of HDL cholesterol, reducing the age-relate decline in anti-atherogenic activity (98). Furthermore, there is an "artificial" variety of olive oil with higher fraction of phenolic compound than the virgin olive oil, namely high phenolic olive oil (HPOO), which a review focused on it regarding its beneficial properties on CV health, observing that the consumption of this variety of olive oil produces small benefits on systolic pressure, so it may be considered as a nutraceutical in CV prevention (99).

Other aspect which declines with age is the immune system. It is observed a worse lymphocyte functioning and proliferation, a decreased antibody formation among other effects. That could lead to be more susceptible to infections which tend to have more severe consequences. A recent review, analyzed five studies which aimed the effect of n-3 PUFA on immune function in the elderly, concluding that those fatty acids even at low doses could be enough to improve the immune response of aged people (85). Virgin olive oil could improve this function at elderly according to a study which observed that the replacement of American dietary fat by virgin olive oil during three months led to a moderate enhancement in T cell proliferation, besides a positive correlation between increased production of T cell cytokine interleukine 2 (IL-2) and the magnitude of increase plasma oleic acid (96).

Other essential age-related regard in the elderly is muscular function. It seems that quality of fat type might take influence on age-related loss of skeletal muscle mass. A study compared the effect of several type fat and dietary percentage of fat energy in relation to skeletal muscle mass in women aged 18-79. They concluded that a diet with a high PUFA/SFA index corresponded to a lower loss of skeletal muscle mass, but when MUFAs, trans-fatty acids (TFAs) and SFAs index were high corresponded to a worse maintenance of skeletal muscle mass (100). Accordingly, a recent review (85) affirms that supplementation with n-3 PUFA seems to be very useful and promising in order to promote the protein turnover during healthy aging. However, a study which investigated associations between plasma PUFA and muscle parameters such muscle

size, intermuscular adipose tissue and muscle strength, did not achieve consistent relations among each other (101).

Bone health is a subject which concerns in the elderly, as a good bone health is essential for a better quality of life at this stage of life. Although there are no much researches about this, it seems that a regularly great intake of fish oil in the elderly may be related to lower osteoporotic fracture risk, particularly in men (102), suggesting that critical time periods for n-3 PUFA consumption may differ by sex. But Farina *et al*. observed that long-term fatty fish supplementation contributed to the maintenance of femoral neck bone mineral density in both sex (103). Of interest is the study conducted by Bullón *et al*. which concerned about the role of different type of dietary fat in relation to age-related alveolar bone loss (a feature of periodontal disease). The mechanisms involved in this fact are related to an ablation of the cell capacity to adapt to aging, where MUFA, as virgin olive oil, as well as n-3 PUFA might allow mitochondrial maintaining turnover through biogenesis or autophagy (104).

VI COENZYME Q

VI.1 DISCOVERY OF COQ

Within mitochondrial respiratory chain is allocated a component chemically defined as representative of liposoluble quinones, which performs a central role on adenosine triphosphate (ATP) synthesis during oxidative phosphorylation process. It was discovered in 1995 by Fernenstein *et al*., and was named as ubiquinone. However, in 1957, was discovered a compound which was named as coenzyme Q_{10} (CoQ_{10}) (105). Folkers *et al.* determined that ubiquinone and CoQ_{10} structure was similar (Figure VI.1). Regardless of the fact of its discovery in the 50's, the knowledge and understanding of its action mechanism in biological systems and diseases is still limited, so its therapeutic use is not fully assessed. Therapeutically, this molecule is used in the treatment of neuropathies, myopathies, immunological perturbations, in certain diseases with a cardiovascular origin and in chronic fatigue syndrome.

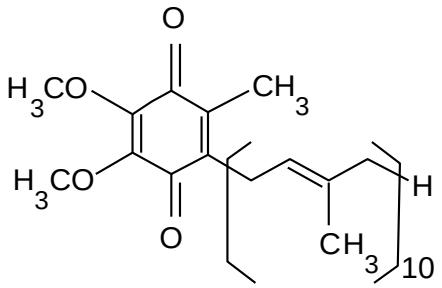


Figure VI.1. Coenzyme Q_{10} Structure

VI.2 SYNTHESIS OF COQ

Ubiquinone is the only lipid endogenously synthetized by mammals which presents a redox function. It is presented in a wide set of tissues which exhibit a specific intracellular distribution in each of them. Two functions are attributed to CoQ, as electron transporter in the mitochondrial electronic transport chain (mETC), as well as liposoluble antioxidant (106). In the last decade the scientific interest for CoQ was increased, especially since numerous diseases have been related to alterations of mitochondrial function and oxidative stress. The decrease of its tissue concentrations was observed in several studies about aging in different organs that raised the idea that the dietary supplementation could potentially provide physiological advances, leading to positive benefits on health.

The ubiquinone or CoQ is one of the representative ones from a group of homologues which have as common characteristic a lateral chain, different at size, on the sixth position, which in addition, is constituted by a variable number of isoprenoid units. Chemically is defined by 2,3-dimethoxy,5-methyl,6 parabenzoquinone polyisoprene. The CoQ_{10} which has been found in humans has a polyisoprene chain comprised of 10 isoprene units (with 5 carbons each one) and 50 carbons overall. Methoxy and methyl groups contribute the specificity of its enzymatic action. The quinonic rings do not permit that additional reaction occur to thiol groups in cells, presents in glutathion, thioredoxine or thiotic acid. The functional group is the quinonic ring. The isoprenoid chain is responsible for the lipophilic nature of the molecule and its ubication inside biological membranes. Through the reduction from quinone to quinol it is generated the electronic transport (107).

The percentage of compound as ubiquinol form varies between 30-90%, depending on the tissue and on its cellular metabolic state (108). Ubiquinol is more hydrophilic, so that its head is pressed on the membrane surface. The change of position, which depends on the oxide-reduction, alters the structural and enzymatic properties in the membrane. Phospholipase activity in the membrane can be determined by redox state (109).

It is about one of the final product from mevalonate pathway that in turn, it is one of the products from farnesyl pyrophosphate (FPP) metabolic pathway, a common substrate of dolichyll-P (110). In contrast to other isoprenoids, the ubiquinone is formed by a monoisoprenoid half, where is observed the presence of a benzoate ring, from tyrosine, which in turn comes from phenylalanine. The aromatic ring derives from diet, once mammals are not able to produce such substances (111). After the condensation between the ring and the isoprenoid lateral chain, several substitutions and modifications on the benzoate ring occur. These steps of the enzymatic sequence are not fully understood in mammals, nevertheless it is known that it is included on it 1 decarboxylation, 3 hidroxilation, 2 O-methylations and 1 C-methylation in order to obtain the final product.

Most of studies which researched clarifying the full ubiquinone synthesis were performed on bacteria and yeast. The enzymes implicated were not isolated, neither purified or cloned yet from mammals. *In vitro*, the reconstitutions of metabolic pathways are not totally identified yet, due to the difficulty of detection and determination on involved enzymatic reactions. In contrast to bacteria, mammalian transphenyltranferase has more affinity to geranyl-PP rather than FPP as substrate (112). An only enzyme, FPP synthetase, is responsible for formation of FPP from dimethylalil-PP and isopenthenyl pyrophosphate (113). It is not certainly known yet if there is any other intermediary or if FPP synthetase is the responsible to geranyl pyrophosphate formation.

In humans and the great part of mammals, a predominance of CoQ_{10} compared to CoQ_9 is observed (114). CoQ_9 levels correspond to 2-7% of overall in humans. However, in rats, the percentage of CoQ_{10} is about 10-30% of overall. Because ubiquinone has structural characteristics similar to liposoluble vitamins, a possibility admitted was that they have similar properties, being therefore, a substance not produced by mammals. However, the nutritional restriction of ubiquinone did not lead to a decrease on its levels in rats, what determined a very possible biosynthesis by mammals. The ubiquinone is also absorbed through feed, therefore it may be assimilated at intestinal level. Kalen *et al*. demonstrated that that CoQ is produced by hepatic microsomals from rats, presenting relevant concentrations in golgi apparatus and lysosomes (115). The incubation of bacteria under controlled conditions in mammal tissues, in the presence of radioactive 4-hydroxibenzoate, demonstrated a polypropenyl-4-hydroxibenzoate accumulation (116). It was suggested that de next step (hydroxylation or decarboxylation) could be limiting. Nevertheless, there are 2 possibilities, the production of polypropenyl-4-hydroxibenzoate as final product or 4-hydroxibenzoyl-CoA, which would be the required substrate for polypropenyl-4-hydroxibenzoate formation that in turn would be later converted for the next step of CoQ formation. In case of mammals, 4-hydroxibenzoate could be not the formed substrate and instead, it is observed the production of more completed benzoates for catecholamine catabolism, such as vinyl acid (117). It is important to reiterate that mammals, in contrast to bacteria and yeast, are not able to synthetize aromatic rings, turning into a limiting factor to CoQ production, therefore it must be correct by one way or another.

Its transport, when is analyzed in the blood circulation, reveals that about 60% of this molecule is transported by LDL and less than 30% by HDL_2, however, it has been observed that the impossibility of achieving the ubiquinone from a exogenous source triggers that the corporal tissues have to synthetize it, because they are ready to do it. It is noteworthy that endogenous origin of CoQ is not transported or reallocated in the organism. As exogenous source, it is noted meat and poultry as its main sources, taking into account that for human being the most important production is the endogenous source. In relation to oral supplementation, it is observed an increase of its plasma levels, noticing concentrations peaks between the fifth and the tenth hour after

ingestion. Its high molecular weight and low hydrosolubility triggers that its absorption by intestinal tract is slow. 4 days after ingestion is obtained stable a 90% of levels. The level of plasmatic clearance is low, and its expectancy in blood is even 34±5 hours. Expectancy in different tissues varies between 49-125 hours, similar to cholesterol, dolychol and phospholipids expectancy. Regarding brain, the expectancy is about 90 hours, whilst dolychol and cholesterol expectancy are not much higher, what shows the importance of terminal regular of this parameter. When ubiquinone and dolychol are marked by radioactive H-mevalonate, hepatic levels and its daily turnover cannot be limited because bile secretion takes care of 86% of ubiquinone and 60% of dolychol. Therefore, its excretion is mostly carried out through bile tract (118). It argued that CoQ fate when is ingested orally, it is processed in subcellular organelles, especially at mitochondrial matrix and inner membranes level after approximately 72 hours. CoQ tissue levels vary substantially among different organs, these levels seem to be higher in high metabolic aerobic tissues, which in turn are those tissues with higher ability to produce free radicals (119). Compounds identified in urine and feces displayed intact the short chain (5-7 carbon atoms) quinonic ring, with a carboxyl group on ω-ending. The great most was at conjugated form, disulfate form or glucoronidase.

VI.3 DISTRIBUTION AND BIOAVAILABILITY OF COQ

Ubiquinone bioavailability in blood is about 2-3% (120). The increase from twice to four times on this concentration can be achieved by administration of high doses. Bioavailability in rats for liver and kidney is increase when it is exogenously administered (121).

Low increase in organs is due to the physic situation of ubiquinone, which is located at the membrane hydrophobic side, between two phospholipid parts (122). It was observed the presence of other isoprenoids (dolichol and dolichyll-phosphate) which also surround it. These three isoprenoids determine and limit the space between them, and definitely the membrane fluidity and permeability. In standard conditions, membrane functional structure is already saturated by these compounds. Concentration of such long chain isoprenoids in subcellular organelles present a variation of 0, 4-1.5 μg/mg of protein, excluding Golgi and lysosomes complex, which present between 4 and 7 μg/mg (123).

The subcellular fraction of rat liver, points that most of endogenous CoQ concentration is presented in the mitochondria (124). When supplementation happens, the higher extramitochondrial levels are found at golgi complex and lysosomes (125).

Great part of benefit effects are observed by an increase at blood level, thus altogether with α-tocopherol can confer an additional protection against lipid peroxidation, microangiopathies, temporal monoischemies and inflammatory reactions (126).

Genetic mutations, aging, cancer and some specific drugs can reduce serum and tissue ubiquinone concentrations. The amount of CoQ so as to vary serum concentrations must be an equal administration or upper 100mg/day in humans.

VI.4 MECHANISM OF COQ ACTION AND ITS RELATION TO AGING

Within mitochondrial respiratory chain, CoQ performs an electron transport role. Its activity let processing reversible interactions among nicotinamide adenine dinucleotide (NADH), succinate dehydrogenase and the cytochrome systems. When it acts, do it as proton transference agent (redox) coming in a oxide-reduction cycle at the mitochondrial transport chain, where it is presented as semiquinone, in addition to its absolute reduced form (ubiquinol $CoQ_{10}H_2$) or oxidized form (ubiquinone CoQ_{10}) (119). Other dehydrogenases, donate equivalent reducers to cytochrome systems, toward CoQ.

CoQ is an essential part of ATP producer machinery, which provides energy to muscle for its contraction and maintaining of vital cellular functions. Most of the ATP production is processed inside of mitochondrial membrane, where CoQ is found. CoQ transfers electrons from primary substrates to oxidase system while it transfers protons toward outer side of mitochondrial membrane. This transference promotes a protonic gradient through the membrane, whilst protons back to inner by ATP producer enzymatic machinery. CoQ is destined to guide protein enzymatic complexes and it externally delivers protons to the membrane, besides capturing electrons and protons within mitochondrial membrane (127).

There are two protein complexes at membrane where electrons and protons are transferred by CoQ intervention. The first one is a primary reductase which reduces CoQ by NADH assistance (Complex I). During reduction process, four protons are transported through membrane by action of any CoQ reduced (127). Details of this process are not still totally clear, but it was proposed that CoQ reduces and reoxides twice before electronic transference into CoQ in quinol form, where the electron travels around the liposoluble membrane toward a second complex, which promotes the quinol oxidation one more time (Complex III) (128). Meanwhile at complex I there is a cyclic oxidation-reduction-reoxidation, including a protonic delivering to the outer membrane side, which it will be correctly redirected within this process. Newly four protons are transported by membrane in each oxidation of quinonic cycle.

A simple form of energetic conversion based on CoQ oxidation and a reduction event is found in lysosomes (129). In this case, protonic transference through lysosomal membrane acidifies the inner part of the cell, performing work against protonic gradient. It is not generated ATP because lysosomes do not possess ATP sintetase. Lysosome acidification actives some hydrolytic enzymes for cellular digestion. Enzymatic complex involves the reduction of CoQ by NADH action in cytoplasm with reoxidation of quinol by oxygen action.

As mentioned above, CoQ_{10} is transported by lipoproteins in circulation as ubiquinol (reduced form). Such compound acts as antioxidant agent because it reduces

the oxidative level of the fatty acids transported on lipoproteins, what allows during those processes, its transformation into ubiquinone.

In oxidative stress situations, where the plasma is subjected to oxidative stress, it is observed that the first antioxidant substance to be expended is ubiquinone. An important role is assigned to CoQ regarding preventing initiation or propagation of lipid peroxidation in plasma and membrane lipoproteins. Moreover, when it is referred to damage on nucleic bases of DNA, it is confirmed its protective action during such oxidation.

The oxidative stress mediated by ischemia/reperfusion phenomenon seems to suffer an important antioxidant action by CoQ. Some studies, which experimented with animal models, proved that ubiquinone is able to promote a protective action against coronary hyperactivity of ischemia/reperfusion. It was affirmed that the previous administration of CoQ prevents regular function of cardiac endothelium, simultaneously the free radical level decreased as the reperfusion beginning. In a canine model it was observed that the malondialdehyde production is totally eliminated, besides reducing oxidative damage in an ischemia/reperfusion situation on dogs treated with CoQ_{10} (130).

CoQ administration *in vivo* in rabbits improves their ability to produce ATP, besides warranting the maintaining of these phosphate levels, due to a better preservation of myocardial contractible function, together with a decrease of creatine phosphoquinase delivering on ischemia/reperfusion phenomenon. There are also studies which prove by CoQ_{10} treatment, in aisled heart models, an improvement on diastolic function during reperfusion, the maintenance of high ATP levels, preservation of the coronary vasodilation produced by sodium nitroprusside and increase of coronary flow. The improved cardiac mechanic and metabolic recovering thanks to CoQ_{10}, has proved that it ameliorates the intracellular charge of calcium, which suppresses the phosphorylation in the mitochondria (119). Analysis of electronic microscopy about heart treated with ubiquinone have observed low calcium phosphate levels in the intramitochondrial matrix, what proves a prevention related to calcium overload (131).

VI.5 AGING AND PHYSIOPATHOLOGY IN RELATION TO COENZYME Q

Concentration of ubiquinone varies according to age and depends on the organ in question (132). It is observed an increase on its concentration, between 20-30 years, and a gradual decrease thereafter, so this reduction provokes that an individual 80 years old has the same concentration as one's which is 1 year old. In experimental animals are not always produced an age-depending reduction which it observed in humans. Other native component from mevalonate pathway such as cholesterol, keeps its concentration stable throughout life, but in disease, and concentrations of dolichol are continuously increased.

With the exception of skeletal muscle, concentration of ubiquinone as protein base in mitochondria does not decrease so pronouncedly with age. Consequently,

variations of CoQ contents are reflected by variation of mitochondrial content or by extra-mitochondrial variations. Therefore, it is premature to affirm that the decrease on its concentration is only related to age. It could be a deficit syndrome or a general simple adaptation of organism created by amelioration of metabolism with age. It would be necessary to develop a model for determining the partial deficiency, enumerating consequences of such reduction in the tissues, in the mitochondrial function and in its antioxidant status. In situations which ubiquinone blood levels are modified through diets or drugs, which alters its biosynthesis, cellular proportion does not vary (133).

Tissues have efficient mechanisms for keeping reduced CoQ, which is a prerequisite for maintaining its antioxidant function. During the carcinogenic process in rats and humans, there are important variations in concentrations of ubiquinone (134). In models of induction to carcinogenic through use of acetyl aminofluoren, precarcinogenic changes can be identified. The nodules which sometimes are developed, they are converted into hepatocarcinomes. In pre-cancer state, concentrations of ubiquinone is double than usual, whilst when disease is started, levels in rats are 25% lower than regular levels. In humans this decrease is about 50%. Theorically, the changes observed in concentration of ubiquinone reflect a cellular adaptation to the oxidative increase. That was experimented during the pre-carcinogenic period, whilst in carcinogenic state, developed below, it is observed an adaptation to the new metabolic conditions.

In some neurodegenerative diseases lipid changes on mevalonate pathway products have been observed (135). Surprisingly, the lipid changes keep the same pattern in spite of etiologist differences in question. It was promoted a decrease of dolichol concentrations and an increase of dolichyll-P and ubiquinone concentrations. Cholesterol levels do not suffer alterations. The finding that α-tocopherol concentrations increase *in situ*, confirms the hypothesis of the increase of liposoluble antioxidant levels as cellular adaptive response due to the increase of free radicals. Oxidative stress is involved in numerous neurodegenerative diseases (136). Such vents may be interpreted as a ubiquinone deficiency; in this case the supplementation might be valid. In experimental models about Huntigton´s disease, Parkinson and lateral amyotrophic sclerosis, point the beneficial effects of supplementation with CoQ10 (137).

An infrequent mioencephalopathy was diagnosed in three brothers in which severe neural and muscle symptoms were observed. It was observed *in vitro* that mitochondrial respiration was obstructed. An analysis of fibroblast and lymphoblast revealed the absence of CoQ. The resulting products derived from mevalonate, cholesterol and dolichol, had its parameters unaltered in spite of the punctual mutation which limited ubiquinone production. Supplementation with CoQ led to a dramatic change on quality life of the brothers, allowing that they did not need their wheelchairs and recovering their ability for moving, besides recovering utterance, writing and logic reasoning abilities. It is clear that administrated ubiquinone blocked initial deficiency, distributing in subcellular organelles and membranes which suffered CoQ deficit (138, 139).

Aside from being involved in energetic preservation cycle of mETC, it has been suggested that CoQ delivers electrons to other electrolytic pathway considered important within physiopathologic process which contributes for establishment of age-depending oxidative stress. It is talked about ubisemiquinones redox cycle (140).

Ubisemiquinones redox cycle can be determined by spectrophotometry in oxygen absence and its presence. The ubisemiquinones identified did not suffer redox alterations caused by oxygen in untouched mitochondria. However, under some circumstances, especially when the mitochondrial membrane is not untouched, could generate superoxide anion (O_2^-) due to arising of an electronic escape via, which make suffer an autoxidation process to ubisemiquinones (141).

Those specific conditions are observed when the redox potential values transfer an electron to O_2. It was observed a change in ubiquinone redox cycle which remains as the deprotonated form once it losses one electron. Redox cycle associated with the cytosolic side of complex III interacts with cytochrome b566 redox center. Electrostatic interaction with hemo center of cytochrome b566, promotes a heavy changes on negatives values, which allows transferring an electron to oxygen (142). Nevertheless, the prevention of autoxidation is presented in the untouched mitochondrial membrane. In mitochondrial membranes which suffer molecular alterations in its phospholipidic bases induced by accumulation of toluene, it is more common than in the ubiquinone redox cycle (143). $O_2^{\cdot-}$ radical is lineally increased in the cytosol aqueous side, in the ubisemiquinone redox cycle at the inner mitochondrial membrane. Structural disorders were induced due to toluene administration.

The great variation of CoQ concentration in different organ, besides variation which depends on the cells belonging to the same organ, shows that it is talked about a substance produced (observing its amounts) according to requirements and metabolic efficiency, besides cellular type in question (144).

One of the regulator enzymes of the mevalonate pathway is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase which basically takes control of cholesterol production. In hypercholesterolemia treatments are used HMG-CoA reductase inhibitors (145). Use of these medicines for long times, such novastatine, besides a decrease of cholesterol, it is also observed a decrease of ubiquinone only in plasma. In case of rats, it is observed that such decrease can be processed in several tissues (146). However use of esculistaline1, inhibitor of squalene synthase, increases biosynthesis and in turn, ubiquinone levels as well (147).

Other condition where is observed the increase in ubiquinone synthesis, is during body adaptation to cold process (148). Mediator would be thyroxine, a thyroid hormone. This hormone increases the metabolism, promoting a change at cellular metabolic level (149). That leads to promote a change in respiratory activity, increasing ubiquinone synthesis.

VI.6 ANTIOXIDANT FUNCTIONS AND COQ DEFICIENCY

CoQ is located at the membranes which are intimately related to unsaturated lipid chain, and it acts inactivating those free radicals presented as quinol form (150). Quantitatively it has an occurrence in tissues from 3 to 30 times higher than tocopherol amount (121). More important is the presence of many enzymes located at the cellular membranes which reduce any quinone CoQ radical generated by reaction with any lipid or ROS.

Three known enzymes perform the role of keeping CoQ in its reduced form in plasma and endogenous membranes(151): NADH cytochrome b5 reductase; NADH/nicotinamide adenine dinucleotide phosphate (NADPH) oxide reductase (DT-diaphorase) and NADPH CoQ reductase (152). In mitochondria, NADH and succinate dehydrogenate can keep CoQ partially reduced. Reductases I and III presented in membranes can be especially important concerning the transference of an electron for reducing any semiquinone regenerated by reaction between a quinol and a radical. The DT-diaforase reduces through transference of two electrons to any formed intermediary quinone, without intermediary formation of semiquinone.

In oxidative stress conditions induced by nutritional lacks of selenium and α-tocopherol, it is observed a great increase of CoQ in membranes (153). Similar decreases of α-tocopherol induced by peroxisomal proliferation also widely increase amounts of CoQ (121). Some mutant yeasts which suffer a CoQ deficiency, exhibit higher levels of lipid peroxidation compared to regular yeasts (154).

Other clear demonstration of free radicals elimination is observed in the treatment which through CoQ usage of skin creams in old people. Free radical luminescence is eliminated at the time which cream is applied on skin (155). Quinol can act recovering topheryl radicals generated in lipid reactions or in ROS, reducing newly to tocopherol (156). Being CoQ absent in membrane, this reconversion is slowly promoted. There are some evidences which electron-CoQ-depending transported through membrane can be used for regenerating ascorbate from ascorbate radical (monodihydroxiascobate) (157). This is processed at the ascorbate locate out of cells.

In healthy individuals, CoQ is synthesized in cells from tyrosine (phenylalanine) and mevalonate pathway (158). Only four registered cases report fails in the genetic types on its synthesis (159). Apart from decrease of its biosynthesis, other factors affect to CoQ levels and functions (160). This includes increase of its degradation or changes in lipid membranes which impede the quinonic movement (161). Change of CoQ content in some tissues and membranes during aging is variable. During aging in rats is observed a selective decrease in CoQ levels in skeletal muscle mitochondria (162), and however an increase in brain (163). Changes in other membranes need more clarification regarding its gradual loss of function and antioxidant capacity.

Normal levels of CoQ in blood are about 1 μg/ml (164). The increment up to 2 μg/ml is feasible due to supplementation above 100mg/day. This increment could be profitable in conditions which some tissues require an increment of CoQ.

VI.7 CARDIAC FAILURE AND ITS RELATION TO COQ USAGE

CoQ_{10} levels in both, blood and myocardia tissue, in patients with cardiac failure are lowers when are compared to individuals free of such disease. In a pioneering experiment, Langsjoen found a symptomatic improvement on 19 patients in CF (cardiac failure) III and IV of the NYHA (New York Heart Association), due to the daily administration of 100 mg of CoQ_{10} by oral via. In the protocol it was established a first period of four weeks of patient stabilization who after they were selected randomly for the administration of CoQ_{10} or placebo, during 12 weeks. At the end of this lapse, it was newly crossed treatments during another 12 additional weeks (119). This study let observing an improvement of cardiac failure symptomatology once the ventricular function improved markedly, what let to patients an improvement in their life quality. Those patients also improved, in lesser extent, in relation to exercise tolerance, assessed by cycloergometre.

VII THE PANCREAS

The pancreas has a central role both in the digestive process as well as the consumption process. This dual function can be performed due to the presence of 2 compartments, the exocrine pancreas and the endocrine one. The exocrine compartment is the most abundant, corresponding to 98% of pancreatic mass and is formed by interstitial mesenchymal, ductal and acinar cells, the latter producing enzymes responsible for food digestion in the duodenum. The endocrine compartment is formed by aggregates cells (also called islets of Langerhans) disseminated throughout the exocrine structure and compound of different cell types specialized in the biosynthesis and secretion of specific hormones: insulin-producing β-cells (around 60% of islet mass), glucagon-producing α-cells, pancreatic polypeptide-producing δ-cells, and finally, ghrelin-synthesizing E-cells (only present embryogenesis) (165).

VII.1 ANATOMY AND HISTOLOGY OF PANCREAS

The pancreas is a pinkish white organ which lies in the upper abdomen behind the stomach. It is part of the gastrointestinal system that makes and secretes digestive enzymes into the intestine, and also an endocrine organ that makes and secretes hormones into the blood to control energy metabolism and storage throughout the body, therefore, according to the specific function, the pancreas can be sorted as exocrine and endocrine pancreas. The exocrine pancreas is the portion of the pancreas that makes and secretes digestive enzymes into the duodenum. This includes acinar and duct cells with associated connective tissue, vessels, and nerves connected by stroma. The exocrine components comprise more than 95% of the pancreatic mass. Meanwhile, the endocrine

pancreas comprises the portions of the pancreas (the islets) that make and secrete insulin, glucagon, somatostatin and pancreatic polypeptide into the blood. Islets correspond to 1-2% of pancreatic mass.

In order to go deep into the microscopic structure of the pancreas, it is required to stain tissue sections, the most used staining is the Hematoxylin and Eosin, where it can be observed that genetic material (DNA and ribonucleic acid (RNA)) stains blue, and most protein and carbohydrate stain pink to red (mainly used for exocrine pancreas); fat is extracted by organic solvents used in processing leaving unstained spaces. However when it is wanted to differentiate specific hormones separately such glucagon, insulin or somatostatin which are in α, β and δ-cells respectively (in pancreatic islet), it is used the immunoperoxidase staining, with the proper antigen in each case; there, the hormone in question stains brown. But when it is wanted to observe a section where those three hormones are simultaneously detected in the pancreatic islet, it is appealed to the triple-immmunolabeling staining, where β-cells stain pink, α-cells brown and δ-cells blue; it can be also observed the predominance of β-cells and the typical location of the δ-cells at the peripheria of clusters β-cells (166). Sections for light microscopy are most often made from formalin-fixed paraffin-embedded tissue and the sections are usually 4 or 5 micrometers (μm) thick. Thinner (1 μm) sections of plastic embedded tissues (prepared for electron microscopy) may also be used for light microscopy.

As previously said, the exocrine pancreas can be stained by hematoxilyn and eosin, showing that it is a complex tubular network where pancreatic acini are not arranged in clusters like grapes at the ends of a branching duct system but rather as an anastomosing tubular network that at some termini form classic acini. Centroacinar cells are typically located at the junction of an acinus or acinar tubule with a small ductule, but they may be interspersed within an acinar tubule. Acinar cells stain blue at their base because of the high content of RNA and the presence of nuclei. They are pink at their apex (lumenal aspect) where there is a high content of zymogen proteins (digestive enzymes). The nuclei of centroacinar cells are sometimes seen within an acinus. If it is performed a toluidine staining, it could be observed the presence of numerous round empty capillaries in the interstitial space which would indicate that the pancreas was perfused with fixative and blue granules in the acinar cells represent zymogen granules, a digestive enzyme (166).

The components of the duct system are the main pancreatic duct (duct of Wirsung), interlobular ducts that drain into the main duct throughout the pancreas and intralobular ducts (sometimes called intercalated ductules) that link acinar tubules to the interlobular ducts. The intralobular ducts and ductues are ordinarily seen only at the level of light and electron microscopy. Enzymes from acinar cells are released into a bicarbonate-rich solution that is secreted by the centroacinar and ductal cells and flows from the acini and acinar tubules to the intralobular ducts, then into the interlobular ducts and main duct, and finally into the duodenum at the major or minor papillae. The integrity of the duct system is of key importance in preventing entry of the exocrine

enzymes into the interstitial space where they may be activated and cause tissue damage manifest as pancreatitis. The main and interlobular ducts have thick dense collagenous walls. The connective tissue component of the duct wall becomes progressively thinner as the ducts branch and become narrower. Intercellular tight junctions, also called zonula occludens, between duct cells, centroacinar cells and acinar cells play a major role in preventing leakage of the duct system (167).

Regarding endocrine pancreas, most islets (islets of Langerhans) that collectively comprise the endocrine pancreas are too small to be seen by gross examination. Islets vary greatly in size, ~70% are in the size range of 50-250 μm in diameter in humans with an average in the range of 100-150 μm (168). Smaller islets are dispersed throughout the acinar lobules and largest islets lie along the main and interlobular ducts of the pancreas. Most islets are spherical or ellipsoid, but they can be irregular in shape, sometimes reflecting the pressure of an adjacent structure, often a duct, or limitation by a tissue plane. Several reports provide support for the presence of a higher population density of islets in the tail of the pancreas than in the head and body (169-171) although another finds no difference (172). In adult humans the number of islets is calculated to be 500,000-1 million whereas there are far fewer in smaller animals (167, 173). Islets comprise 1-2% of the pancreas in adults of most mammalian species. In addition to the islets, isolated islet cells may be found dispersed in the acinar lobules or in association with ducts.

VII.2 ROLES OF PANCREAS

As previously mentioned, pancreas has a dual function in the digestive process as well as the consumption of the resulting nutrients from the digestive process. These roles are performed by exocrine pancreas and endocrine pancreas respectively.

VII.2.1 Role of exocrine pancreas

The main function of exocrine pancreas is the secretion of pancreatic juice, which contains the enzymes responsible for digestion of the most part of total macronutrient from diet. This secretion not only happens after an intake of a meal, known as digestive phase, but it also does during fasting, which is known as interdigestive phase, and concur when the upper intestinal tract is empty of food.

The functional unit of the exocrine pancreas consists of an acinus and its draining ductule. The ductal system extends from the lumen of the acinus to the duodenum, briefly the smaller ducts transport the product from the acini, which converge into bigger ducts (intercalated ducts) successively, going by pancreatic ductal system. As result, all the secretion from acini converges into duodenum. The acinar cells of the acinus undertake synthesizing, storing, and secreting digestive enzymes. Thanks to the receptors for hormones and neurotransmitters located at the basolateral membrane, it is stimulated the secretion of these enzymes (174), which are stored in zymogen granules at the apical region of the cell, and when is required the release of

these granules, the microvilli of the apical surface and filamentous actin meshwork triggers the exocytosis of the content zymogen granules (175).

In brief, the digestive enzymes can be sorted according to the substrate nature (protein, lipid, carbohydrate and genetic material), namely proteolytic, amylolytic, lipolytic and nuclease digestive enzymes respectively. Within amylolytic enzymes, amylase is the most abundant enzyme in the pancreatic secretion, that is involved in hydrolyzing complex carbohydrates such as starch by rupture of 1,4 glycoside linkages at any other attachment point between carbon 1 and oxygen. As product of such reaction it is obtained maltose and maltotriose and separately more complex glucides such as α-dextrins, which contain 1,6 glycosidic linkages which cannot be hydrolyzed by amylase. For this reason the brushborder enzymes conclude the hydrolysis of the products of amylase digestion to glucose, which finally is transported across the intestinal absorptive epithelial cell by a Na^+ coupled transport (176, 177).

Regarding lipolytic enzymes included in the pancreatic secretion, the two most representative enzymes are lipase and phospholipase. The pancreatic lipase takes charge of hydrolyzing any triglyceride molecule from diet to a monoglyceride with a fatty acid esterified to glycerol at carbon level 2 and separately two fatty acids cleaved from carbon 1 and 3 (178). Meanwhile pancreatic phospholipase hydrolyzes the phosphatidylcholine molecule by cleavage of the fatty acid ester linkage at carbon 2 level, resulting in a free fatty acid and lysophosphatidylcholine (179).

The catalysis of proteins is performed by proteases, which are divided into two groups, the endopeptidases and the exopeptidases. Both are found as inactive precursors in the pancreas, but when they are released into the duodenum, trypsin takes charge of activating it. Within endopeptidases are found the trypsin itself, chymotrypsin and elastase, which act cleaving internal peptide bonds within a peptide chain. Meanwhile exopeptidases such as carboxypeptidases act cleaving peptide bonds located at the terminal carboxyl of a peptide chain.

All digestive enzymes of pancreatic secretion are susceptible to damage the pancreatic tissues itself, for avoiding that, there are mechanisms which prevent the self-digestion of the pancreas. One of them includes storage and packing in acidic zymogen granules to inhibit activity, another one includes synthesis and storage as inactive precursor forms. In order to prevent intracellular activation of trypsin before being secreted into duodenum, it is required a trypsin inhibitor, known as pancreatic secretory trypsin inhibitor (PSTI). It is a 56-aminoacid which operating mechanism forming a relatively stable form complex with the enzyme close to its catalytic site, so that makes that trypsin remains inactive (180), avoiding the pancreatic autodigestion which triggers in disorders such as pancreatitis (179, 181).

Another cell that is becoming important because of its role in pathologic states is the pancreatic stellate cell (PaSC) (182-187). This is a very slender star shaped (hence the name stellate) cell that drapes itself around the acinar and ductular structures as well as the islets of Langerhans. The role of PaSCs in normal function is probably to lay

down the basement membrane to direct proper formation of the epithelial structures. Their role in pathologic states, such as chronic pancreatitis (CP) and pancreatic cancer, has been of considerable interest. In these diseases, the PaSC is transformed into a proliferating myofibroblastic cell type that synthesizes and secretes extracellular matrix proteins, proinflammatory cytokines and growth factors. These actions of the transformed PaSCs are central to the inflammatory and fibrosing pathologic processes of chronic pancreatitis and are procarcinogenic for pancreatic cancer. In fact, the myofibroblastic transformed state of the PaSC is emerging as a key participant in both the rate of growth of the cancer and the development of resistance to chemotherapy.

VII.2.2 Role of endocrine Pancreas

As previously mentioned, the endocrine pancreas houses 4 cellular types, β-cell α-cell, δ-cell and E-cell, which synthetize, store and secrete specific hormones (insulin, glucagon, pancreatic polypeptide and ghrelin respectively) in order to facilitate storage of foodstuffs (by release of insulin following a meal), and to provide a mechanism for mobilization of foodstuffs (by release of glucagon during periods of fasting), therefore the pancreatic hormones regulate the lipid, carbohydrate and protein metabolism.

VII.2.2.1 *Endocrine pancreas and carbohydrate metabolism*

When it is talked about pancreas and carbohydrate, it is mainly referred to glucose which is a small, polar and, thus, water-soluble monosaccharide. Its physiological importance greatly outweighs its size for two reasons. The first one is that it has multiple metabolic pathways. The second is that neurons have an absolute nutritional requirement for a continuous supply from it, in its absence they would die. Thus, homoeostatic regulation of the concentration of extracellular fluid glucose is vital. It is achieved primarily by the actions of the hormones secreted by the pancreas, insulin and glucagon, although other hormones are also involved. Loss of insulin secretion or resistance to its actions (termed insulin resistance) causes an increase in circulating glucose concentrations and the disease diabetes mellitus (DM). As it would be expected from the multiple metabolic pathways involving glucose, DM is a multifaceted metabolic disease although it is defined solely in terms of an elevated blood glucose concentration (188).

Glucose is present in polysaccharides (such as starch), disaccharides (such as sucrose, maltose and lactose) or alone as monosaccharide. The more complex carbohydrate the more digestion is required in order to convert into simple carbohydrates such as maltose, lactose, glucose, or fructose which are absorbed across the hydrophobic cell membrane of the gut involves specialized transport protein know as glucose transporter (GLUT).

The major targets for the anabolic actions of insulin are the liver, adipose tissue and muscle. At the cellular level, such target cells possess specific insulin receptors.

Within the liver and insulin, it stimulates glycogen synthetase and moreover inhibits glycogen phosphorylase, all this makes trigger in a glycogen synthesis, but it is not observed an increase of glucose uptaking into hepatocytes, which is corroborated by the not observed changes on GLUT2 tranporters. However, insulin is able to stimulate the synthesis of intracellular GLUT4 transporters in muscle and adipose tissue, resulting in an increase of its cell-surface expression, so that induces a quick uptake of glucose. This glucose will be used for producing glycogen in muscle, and fatty acids for its storage as triglyceride in adipose tissue. In addition to this, insulin promotes the synthesis of muscle tissue from amino acids. Simultaneously, insulin inhibits the catabolic metabolism suppressing the breakdown of glycogen in the liver or the release of amino acids from muscle or free fatty acids from adipose tissue (188).

Glucagon has opposing actions to those of insulin, promoting mobilization of fuels, particularly glucose. Its primary action is on the liver where it binds to a seven transmembrane G-protein-linked glycoprotein receptor and stimulates the production of cyclic adenosine monophosphate (cAMP). It may also activate the phosphatidylinositol signaling pathway. Through a subsequent cascade of intracellular events, glucagon ultimately stimulates the breakdown of glycogen to glucose and the production of glucose from amino acids (gluconeogenesis). In addition, it stimulates the release of free fatty acids from adipose tissue and, when these penetrate the liver, glucagon directs their metabolic fate. Rather than being used for the synthesis of triglycerides, they are shunted towards β-oxidation and the formation of ketoacids (see below). Thus, glucagon is both a hyperglycemic and a ketogenic hormone (188).

By and large, it is the molar ratio of insulin to glucagon in the portal blood that governs the metabolic state of the liver. As insulin concentrations fall during the postabsorptive state, its inhibitory effect on glycogenolysis is removed whilst the increasing concentration of serum glucagon reduces glycogen synthesis by inactivating (by phosphorylation) glycogen synthetase. In the same way that glycogen synthesis and breakdown is controlled by the molar ratio of insulin to glucagon, so too is gluconeogenesis. Thus, in the post-absorptive state, glycolysis is reduced and gluconeogenesis increases. In the early post-absorptive state, glycogenolysis and gluconeogenesis occur simultaneously but, as the glycogen stores become exhausted, gluconeogenesis becomes the sole source of glucose. Other hormones that also play major roles in the regulation of blood glucose concentrations include cortisol, adrenaline and growth hormone, all of which act to raise blood glucose concentrations and are, thus, considered to be counter-regulatory and in excess diabetogenic (188).

VII.2.2.2 *Endocrine pancreas and lipid metabolism*

Lipids are stored as triglycerides (three fatty acids attached to a glycerol molecule) and transported around the body associated with proteins such as lipoproteins. The overall action of insulin on the adipocyte is to stimulate fat storage and inhibit mobilization. The effects of locally injected insulin on the accumulation of triglyceride into adipocytes are remarkable. Whilst this was likely to have been caused

by de novo lipogenesis from glucose, it is generally believed that on a Western diet, triglycerides are usually accumulated in adipocytes by uptaking from plasma. This process is also stimulated by insulin-mediated activation of key enzymes inducing hydrolysis of very low-density lipoproteins (VLDL) and chylomicron triglycerides into nonesterified or free fatty acids (FFAs), thus making them available for uptaking into adipocytes. As insulin concentrations fall, FFAs are released from adipocytes into the circulation. Taken up by the liver, they can be esterified to form triacylglycerol stored in the liver and used either for hepatic energy needs or as the basis of VLDL formation. Alternatively, the FFAs may undergo β-oxidation that also produces energy for hepatic metabolism together with acetoacetate and 3β-hydroxybutyrate. These ketones are exported into the blood. The relative concentrations of insulin and glucagon regulate the rates of esterification and β-oxidation (188).

The uptake of FFAs into mitochondria, where β-oxidation occurs, is facilitated by the enzyme carnitine O-palmitoyltransferase-1. The activity of this enzyme is inhibited by malonyl-coenzyme A, and when insulin concentrations are high so too are the concentrations of malonyl-CoA. Thus, β-oxidation is inhibited and ketone body formation is low. The reverse occurs when the insulin:glucagon ratio is reduced and ketone body formation increases. This, together with a reduction in insulin suppressive actions on the release of fatty acids from adipocytes and increased gluconeogenesis, fuels ketone body formation. The presence and degree of ketosis is a sensitive indicator of circulating insulin concentrations (188).

The interactions between glucose metabolism and the fatty acid cycle were noted more than 30 years ago. Elevated concentrations of free or non-esterified fatty acids (NEFA) increase lipid oxidation and decrease the activity of pyruvate dehydrogenase and phosphofructokinase, enzymes involved in glycolysis. The resulting gain in glucose 6-phosphate leads to a decrease in glucose consumption and oxidation. There are also evidences that increased concentrations of FFAs decrease non-oxidative glucose metabolism, for instance, glucose to lactate (188).

The changes in the concentrations of FFAs are translated into changes in the expression of genes by a family of nuclear transcription factors, the peroxisome proliferator-activated receptors (PPAR). After binding FFAs, these heterodimerize with the retinoid X receptor and interact with specific response elements in DNA. There are three PPARs (α, β, γ) in the family and they share strong structural similarities with other nuclear receptors such as those for steroids and tri-iodothyronine. PPARγ plays an important role in the adipocyte.

VII.2.2.3 *Endocrine Pancreas and protein metabolism*

The importance of proteins (or, more correctly, their constituent amino acids) in the control of blood glucose concentrations is that they can be converted to glucose by gluconeogensis or form ketoacids by ketogenesis. Alternatively they can be degraded and the released of ammonium incorporated into urea in the liver via a biochemical pathway known as the urea cycle. In turn, this cycle is linked to the energy-producing

citric acid cycle. Thus, whatever the catabolic fate of proteins, they are all energy producing pathways. Insulin promotes the uptake of amino acids (specially the essential amino acids leucine, valine, isoleucine, tyrosine and phenylalanine) into muscle and stimulates protein synthesis. Simultaneously, it prevents protein breakdown and the release of certain amino acids from muscle. Glucagon predominantly acts on the liver, stimulating the extraction of amino acids from the circulation and increasing the activity of the gluconeogenic enzymes whilst decreasing the activity of the glycolytic enzymes (188).

VIII NON-NEOPLASTIC DISORDERS OF THE PANCREAS

In brief, non-neoplastic disorders of the pancreas include congenital abnormalities of the pancreas such as aplasia, hypoplasia, ductal abnormalities, pancreas divisum, annular pancreas and congenital cyst. It also includes hereditary diseases such as cystic fibrosis, infectious diseases, enzymatic deficiencies or diabetes mellitus among others. Inflammatory processes take part in non-neoplastic disorders of the pancreas, which acute and chronic pancreatitis are the most representative non-neoplastic lesions of the pancreas. Finally, it could be included among non-neoplastic disorders of the pancreas, acquired lesions/disease such as heterotopic pancreas, pseudocyst, true non-neoplastic cyst and age-related alteration among others.

In this section it is focused on pancreatitis and diabetes because of its high incidence in population. Age-related alterations will be mentioned in subsequent sections.

VIII.1 PANCREATITIS

Pancreatitis is an inflammation of the pancreas due to a leakage of pancreatic enzymes such as trypsin which attacks pancreatic tissue that in turn provokes release of inflammatory mediator, responsible for such inflammation in the tissue which can endanger the patient´s health. The most common pancreatitis is chronic and acute pancreatitis, furthermore other type of pancreatitis less common is groove and autoimmune pancreatitis.

VIII.1.1 Acute Pancreatitis

Acute pancreatitis (AP) is an infrequent acute inflammatory disorder of the pancreas in which the common characteristics are abdominal pain and abnormal levels of pancreatic enzymes observed in biological fluids such as blood and urine. Its presentation ranges from a self-limiting mild disorder to a more severe and fulminant disease. Gallstones and alcohol misuse are the main risk factors for acute pancreatitis (189). Other factors, possibly genetic, could likely play a part, including smoking, which might increase the risk of acute pancreatitis (190-192). Anatomical/functional disorders (e.g. pancreas divisum or sphincter of Oddi dysfunction), autoimmune conditions (systemic lupus erythematosus), hypercalcemia/hyperparathyroidism,

pancreatic infections, and accidental/iatrogenic trauma can also cause AP (193, 194). Gallstones as well as alcohol abuse represent about 75-85% of pancreatitis causes, due to the obstruction of the common bile duct (195).

In case of gallstone-induced pancreatitis, the obstruction responsible for the disease can be located at the bile duct, the pancreatic duct, or both. Such obstruction provokes a higher ductal pressure which in turn, affects the regulation on activation of digestive enzymes (196). A useful method which may help us to predict biliary origin of pancreatitis is the measurement of serum pancreatic enzyme concentration, being that some of them such as ALT (Alanine transferase), is found three time at least over normal upper limit (197). Furthermore, excessive alcohol consumption, is considered the second most frequent cause of AP, however the correlation between them is not enough clear yet, although Gorelick (198) through experimental models which were observed that ethanol may provoke that acinar cells are more sensitive to cholecystokinin stimulation. However, AP has also a genetic component (199) which falls on two genes, SPINK1, whose absence or mutation lead to a fail in the inhibition of trypsin activity, and beside the gene mutation with dysfunction of the cystic fibrosis transmembrane conductance gene (CFTR). Both alterations might promote alcoholic pancreatitis (196).

In respect to acute pancreatitis pathophysiology, although there is no a consensus about, most researchers believe that AP is caused by the unregulated activation of trypsin within pancreatic acinar cells (196). Histopathological changes in AP include edema of the pancreatic lobules, peripancreatic fat necrosis, and neutrophil infiltration of interstitial spaces, while necrotic and hemorrhagic areas are seen in severe acute pancreatitis (SAP) with necrosis (200). Because pancreatic biopsy is difficult and the late presentation in patients, changes at cellular level are determined from animal studies. Inflammatory changes in AP begin in acinar cells (201). The pathophysiology of AP comprises a complex cascade of events. Trypsinogen activation within the pancreas is the critical event in AP. Trypsin activates the complement system and the kallikrein-kinin cascade as well as coagulation and fibrinolysis (202). Inflammatory cytokines have been long considered responsible for the systemic manifestations and complications of systemic inflammatory response syndrome (SIRS) in AP (203). Cytokines cause local and distant upregulation of adhesion molecules that further trigger inflammatory cascades via leukocyte migration, complement activation, neutrophil degranulation, and production of phospholipase A2 (PLA2), nitric oxide, and oxygen radicals (202). These events together cause the characteristic clinical picture of SIRS in AP (204).

Regarding microscopic features of AP, all the alterations observed have as common origin the release of digestive enzymes from acinar cells. The lipase released provokes fat necrosis in peripancreatic tissues as well as subcutaneous fat. It is also observed the presence of pseudocysts, resulting from duct rupture. The formation of abscesses have been observed, whose origin comes from the necrotisation of connective tissue as a consequence of the autodigestion and activity of mixed bacterial flora. If AP

is interstitial, it may be detected by the presence of a dispersed acute inflammatory cell infiltrate where are found polymorphonuclear leukocytes within interstitial tissue, edema, and fibrinous exudates. Secondary metaplastic changes, such as mucous cell metaplasia, hyperplasia, and squamous metaplasia might be observed as well. However, if the AP is hemorrhagic, the essential feature is the presence of necrosis signs involving all elements of the pancreas, including acini, ducts, interstitial tissue, vascular structures, nerves, islets of Langerhans, and adipose tissue (205).

VIII.1.2 Chronic pancreatitis

Whatever its etiology, CP is characterized by typical signs of alteration including irregular fibrosis, destruction, and distortion of the pancreatic ducts with loss of exocrine and endocrine parenchyma. Alcohol abuse is the main reason of its emergence, meanwhile other groups of pancreatitis disease such as hereditary, tropical, autoimmune, and idiopathic pancreatitis comprise the remaining causes, highlighting idiopathic pancreatitis, which accounts for 10% to 30% of cases (206). About 20% of patients are observed endocrine or exocrine dysfunction and the absence of abdominal pain (207). Exocrine dysfunction leads to maldigestion causing patients to present diarrhea, steatorrhoea, and weight loss.

CP is a multifactorial disorder where two probably incident are the responsible for the beginning of this disorder. The first incident is related to a decrement of bicarbonate secretion due to a functional impairment which, and the second one, may be caused by genetics factors, where the mutation of cystic fibrosis transmembrane conductance regulator and serine protease inhibitor kazal type 1could take part, and additionally, the mentioned functional impairment might be related to tumor, strictures and sphincter of Oddi dysfunction (208). The second incident involves the premature activation of enzymes within pancreatic juice within pancreatic gland, observing in there alterations such as fat necrosis and hemorrhage, which trigger fibrosis events, duct alterations and an altered pancreatic secretion. This second incident has been also related to a genetic component. All these damages, altogether, ultimately triggers an irreversible harm to the organ (209-211).

At the microscopic level, the histologic features of CP include preservation of the normal lobular architecture of the gland, irregular loss of acinar and ductal tissue combined with various types of duct alterations (dilatation and ectasia, cyst formation, and the presence of inspissated secretions, calculi and duct epithelial alterations such as hyperplasia or metaplasia), and a degree of chronic inflammation and fibrosis. However the lobular architecture keeps intact, what makes us distinguish from duct-type adenocarcinoma, although it may contain various degree of atrophy according to disease progression. Inflammatory processes are observed at this level, being that it is observed the existence of a marked eosinophil infiltration, inflammation at perineural and intraneural level, and perineural fibrosis, all them typical features of CP. It is also barely observed hyperplasia and hypertrophy process on nervous tissue (205).

It can be appreciated that in spite of all the alterations suffered by pancreas, the islets of Langerhans seem to be resistant to the effects of CP, at most it has been observed slight morphologic alterations, however as the diseases progresses, it suffers a reduction in number and progressive atrophy, its β-cells in number remains diminished, even sometimes it could be seen islet cell hyperplasia. Concerning the affectation to the other hormone-producer cells in endocrine pancreas, α-cells and PP-cells are increased as well, but Δ-cells keep uniform its numbers. As the degree of acinar atrophy augments, it is also observed potential signs of endocrine neoplasm due to the apparition of hyperplasia symptoms along with a higher number in the islets. Finally in the later stage of the disease, the islets of Langerhans may disappear as well (205).

VIII.2 DIABETES MELLITUS

DM has become in one of the diseases with greater occurrence worldwide. It is about a metabolic disease which common thread is the deregulation of blood glucose, leading to abnormal higher levels. If it is maintained over time, the risk of suffering damage to any tissues or organs, in some case irreversible, increases considerably. Some of the consequences of not intercepting this abnormality are related to loss of vision, renal failure, amputations of parts of the body, cardiovascular risks, sexual dysfunction, cerebrovascular disease, hypertension and abnormalities of lipoprotein metabolism among others. The cause of this hyperglycemia is related to 2 events, which are able to trigger the disease lonely, although sometimes both simultaneously concur to trigger. The first one results from a defect about insulin secretion by β-cells in order to satisfy the demand. The second one is related to insulin insensitivity from the insulin receptor in tissues such as liver, muscle and adipose tissues, which are insulin-accepters, affecting to carbohydrate, fat, and protein metabolism. These events lead to early symptoms such as polyuria, polydipsia, weight loss or blurred vision which warn us to remedy (212).

The vast majority of cases of diabetes can be classified into two broad etiopathogenetic categories, type 1 diabetes (T1D) and T2D, which differ in that type 1 cause is due to an absolute deficiency of insulin secretion whilst type 2 is due to a combination of resistance and an inadequate compensatory insulin secretory response. Epidemiologically, nowadays about 6-7% of adult population suffer diabetes that results in 285 million people worldwide, of which no more than 10% correspond to T1D and about 90% of cases correspond to T2D (213).

VIII.2.1 Type 1 diabetes

As previously said, the main cause of T1D is an autoimmune destruction of β-cells from endocrine pancreas. So people who are affected by this disease are insulin-dependent. For this reason, this kind of diabetes it characterized by affecting to young and non-obese people although it may happen at any stage of life and at any physical stage. Apparently the autoimmune disorder that involves the destruction of the β-cells activated by lymphocytes T CD4+ and CD8+ and macrophages infiltrating the pancreatic islets (214). For this reason, we help us of markers which point the existence

of immune destruction of β-cell by antibodies related to islet cell, insulin, GAD65 (glutamic acid decarboxylase) or tyrosine phosphatase islet antigens (IA-2 and IA-2β) (212). Other markers not related to immune destruction such as low or very low levels of C-peptide in plasma which is indicative of ketoacidosis provoked by a poor insulin secretion, may suggest the existence of the disease, especially in children and adolescent (212).

The susceptibility of suffering T1D depends on a genetic predisposition in the individual in combination to environmental factors. Regarding genetic factors, there are few genetic explanations which lead to T1D, such as rare monogenic forms (mutations in Foxp3 transcription factor) (215) or allelic expressions in certain chromosomal region, where the human leukocyte antigen (HLA) region on chromosome p21 is a critical susceptibility locus for many human autoimmune diseases, including T1D (216, 217). The HLA proteins are located on the cell surfaces that help the immune system to distinguish body's normal cells from foreign infectious and non-infectious agents. In T1D, an abnormality in the HLA proteins leads to an autoimmune reaction against the β-cells (213). Other alleles confirmed could lead to T1D such as PTPN22, CTLA-4, or IL2RA (Protein tyrosine phosphatase non-receptor type 22, Cytotoxic T-Lymphocyte Antigen 4 and interleukin 2 receptor alpha respectively) (213). An individual, who possesses any genetic predisposition, requires of an environmental trigger which finally culminates in a T-cell-mediated destruction of pancreatic β-cell, that includes viral infection and cow´s milk protein.

Studies in humans and animal models clearly suggest that viral infections can modulate autoimmune responses (218-220). Evidence from epidemiological studies and animal models demonstrate a dual role of viruses as inducers or accelerators of disease (221), as well as a protective role supporting the so-called 'hygiene hypothesis.' Several viral infections such as coxsackievirus B, varicella-zoster, enterovirus, rotavirus, cytomegalovirus, rubella and mumps suggest putative triggering agents. In particular, studies into enteroviruses that target β-cells support the hypothesis that certain viral infections can trigger β-cell autoimmunity (222, 223). Enteroviruses have been shown to target and destroy islet β-cells *in vitro* (224) and anti-enterovirus antibodies (as well as enterovirus RNA) are more frequently found in newly-diagnosed cases of T1D as compared to healthy individuals (225-228). Ultimately, epidemiological and experimental evidence supports the hypothesis that enteroviruses play a role in the development of T1D by altering β-cell function and viability (223). Nevertheless, a better understanding of the cellular and molecular mechanisms underlying the interaction between virus and host is required to better understand its role in T1D development.

Concerning cow´s milk protein, due to it contains bovine insulin and it is often an infant's most common source of foreign complex proteins in most developed countries, and its potential role as a trigger for T1D has been studied. Bovine insulin is very similar to human insulin, differing only by three amino acids. It is possible that in young patients exhibiting β-cell autoimmunity, the initial immune response to bovine

insulin may change in nature to become a response to endogenous insulin. Nevertheless, further studies are needed to make a conclusion on the role of cow's milk in T1D pathogenesis (229).

There is another group of T1D which the autoimmune destruction of β-cell is not the common cause, the idiopathic T1D. It is characterized by no known etiologies. It is curious that the most of patients which suffers this disease are African or Asian ancestry, this fact corroborates that it is strongly inherited. Although these patients are not detected signs of autoimmunity, they have permanent insulipenia and therefore liable to ketoacidosis, so they continuously require insulin replacement (212).

VIII.2.2 Type 2 diabetes

T2D, formerly known as non-insulin-dependent diabetes mellitus or adult-onset diabetes, is the most prevalent diabetes accounting for approximately 90% of all diabetes worldwide (212). Although the incidence and prevalence increases with age (230), it is increasingly suffering T2D population under 40 years old (231), as lifestyle and genetic factors influence on triggering the disease (232, 233). This tendency increase the morbidity and mortality and increments the risk of development premature microvascular and macrovascular disesase. For this reason changes on lifestyle as diet (234) or physical exercise are considered the best therapy to defeat the T2D.

The primary event to trigger the disease is the insulin resistance (prediabetes state), it means that human tissues do not respond appropriately when insulin is present because insulin receptors in target cells of specific tissues do not act properly, that leads to glucose plasma levels are increased (hyperglycemia). So as to compensate insulin resistance, pancreatic islets are forced to raise its cell mass and insulin secretion, but there is a point which β-cells of endocrine pancreas became unable to compensate the insulin resistance which may lead to development T2D.

There are many risk factors so as to trigger the insulin resistance (IR) such as obesity, aging, β-cell dysfunction, tissue lipid accumulation, oxidative stress, endoplasmic reticulum (ER) stress in β-cell, tissue inflammation and physical inactivity (235). Response to insulin concerns to signaling pathway which includes the molecular complexes involved in this issue, such as insulin receptor substrate (IRS), Phosphoinositide 3-kinase (PI3K), phosphoinositide-dependent kinase 1(PDK1) or protein kinaseB (Akt). All of them play a pivotal role about metabolic actions of insulin in many cell types, according to several studies revealed (236). At the systemic insulin resistance level, a study suggested that it exists an interstitial communication (237), which in addition to the traditional hormonal and nervous mediation, it seems that it could be also mediated by the generation and secretion of bioactive molecules. One of the most studied bioactive molecules is adipokine, which is biosynthesized by adipocyte. It is known several types of adipokines such as leptin, tumor necrosis factor alpha(TNF-α), resistin, adiponectin and RBP4 (retinol-binding protein 4) among others. All of them are related to inflammatory processes, so that it is found that some adipokines can be pro-inflammatory or anti-inflammatory. Apparently, the most degree

of obesity, the most pro- inflammatory pattern is released from adipocytes, and therefore, it is more likely the development of insulin resistance. Monocyte chemoattractant protein–1, (MCP1) a pro-inflammatory adipokine, had an increased secretion in obese mice. This one may trigger a macrophage recruitment which in turn, may promote the secretion of more pro-inflammatory adipokines, which ultimately might affect the gene expression in adipocytes, leading to systemic insulin resistance (236). FFA is a common feature of insuline resistance as well, so FFA are also potential mediators of insulin resistance associated with obesity because release of FFA is related to adipocyte size, which in turn, is related to obesity grade. It is suggested that diet-induced obesity and insulin resistance could be determined by the transport of FFAs into cells and its intracellular availability (238).

As consequence of the initial insulin resistance, it is observed high levels of plasma glucose (hyperglycemia) as well as high FFA plasma levels, which in turn, are related to the β-cell dysfunction (239). Both levels increased in T2D are named as glucolipotoxicity. This glucolipotoxicity in turn may cause ER-stress within pancreatic islets (240). It is known that ER takes care of the most protein synthesis including pro-insulin in β-pancreatic cells, which is massively produced during chronically high glucose levels, exerting extra stress on ER. But if this high protein production does not decrease up to regular levels, ER-stress might end up with cell apoptosis (241). In addition to this, hyperglycemia may also lead to generation of ROS, promoting oxidative stress in β-cell, which origin is related to alteration of the mitochondrial activity of mETC by mROS generation (involving complex I-IV) (242). This oxidative stress leads to a synthesis and releasing of pro-inflammatory mediators (cytokines and chemokines) which cause β-cell dysfunction leading to insulin resistance and inflammation attributed to T2D. Other studies on diabetic mice hypothesize that the reduced pancreatic β-cell mass apparent with insulin resistance is because of an alteration in cell cycle progression (243) due to an accumulation of p27Kip1 (Cyclin-dependent kinase inhibitor 1B) in β-cell nucleus, which is an inhibitor of cyclin, thereby preventing β-cell regeneration.

VIII.2.3 Other diabetes types

Type 1 and 2 diabetes comprise the 95% of diabetes disease, which etiological causes have been explained. However the remaining 5% of the disease are due to other etiological causes.

Genetic causes: within this group it is found an autosomal dominant mode of inheritance. Mutations in certain genes involved in the regulation of insulin secretion have been shown to be the cause of hyperglycemia and, insulin resistance is very rare (244). Additionally, diabetes can be a cause of genetic defects of insulin action due to a disruption between receptor binding of insulin and the subsequent cascade of event.

Disease of pancreas: hyperglycemia may occur during an episode of acute pancreatitis, cystic fibrosis or due to excessive production of some hormones that

antagonize insulin action such as cortisol, GH (growth hormone), glucagon and catecholamines due to a tumor in the pancreas.

Finally, without inquiring more, other diabetes are induced by chemical, drug or infections.

IX NEOPLASTIC DISORDERS OF THE PANCREAS

Neoplasm is an alteration in proliferation, and often, in cellular differentiation that is manifested by the generation of a mass named as tumor or cyst. Neoplasm is commonly known as cancer. These tumors are grossly manifested, located and delimited, altering the organ architecture. Sometimes these tumors are only visible microscopically. Pancreas, like any other organ, is susceptible to suffer a neoplastic process in its tissues. It can be located at exocrine pancreas with 98-99% of occurrence or at endocrine pancreas with 1-2% of occurrence. It is noted that both occurrences are directly proportional to the percentage which each one takes place in the pancreas.

IX.1 TUMORS IN THE EXOCRINE PANCREAS

Tumors in the exocrine pancreas include those tumors that are related to the pancreatic ductal and acinar cells and their stem cells. As previously said, almost all pancreatic tumors have an exocrine origin, and almost all tumors of exocrine pancreas emerge from the ductal epithelium (>90%), leading to pancreatic ductal adenocarcinoma (PDAC), or commonly known as pancreatic cancer (PC) (245). PC has a high mortality due to most patients do not present with symptoms until after the cancer has spread to other organs and due to the vast majority of pancreatic cancers do not respond to existing chemo- and radiation therapies (246). Risk factor for PC can be classified into non-genetics and genetics. Regarding non-genetic risk factors, the most significant which have been studied are age, smoking, physical habits, diet, obesity, race and alcohol consumption. PC is mainly a disease of the elderly, so it is a rare disease under 40, being the median age at diagnosis 73 years (247). Smoking is by the far the leading preventable case of PC, doubling the risk of PC and it is believed that it is responsible for at least one in four cases of PC (248-250). Diets high in meat, sedentary and abdominal obesity are other modifiable risk factors which increased the PC occurrence (247). In alcohol consumption case, studies on it had several controversial results (251). Other risk factors which were studied and increase risk of PC are low serum folate levels, long-standing diabetes Mellitus and CP (248). However genetic risk factors are no modifiable. Within this group it is found a family history of the disease where it is more probably developing the disease providing there is a strong family history of PC (252). The reason of this fact is the existence of determined germ-lines which harbor an autosomal dominant inheritance of a rare allele which are denominated as genetic syndrome of cancers. Among the most studied germ-lines which have been studied to increase PC risk they are found germ-line *BRCA2* (breast cancer type 2 susceptibility protein), *STK11* (serine/threonine kinase 11) for Peutz-Jeghers syndrome,

CDKN2A (cyclin-dependent kinase inhibitor 2A) for familial atypical mole melanoma (FAMMM) syndrome, *PRSS1* (protease serine 1) for hereditary pancreatitis or *MLH1* (mutL homolog 1) for Lynch syndrome (245, 247). Family history comprises 7-10% of total PC, so the sporadic PCs comprise remaining, that is mutations in cancer-causing genes. A study carried out by Jones *et al.* (253) achieved revealing the sequencing of 23,219 transcripts representing 20,661 protein-coding genes in 24 ductal adenocarcinomas of the pancreas, where the most significant mutated genes were *KRAS*, presented in more than 95% of PC which encodes a membrane-bound guanosine triphosphate (GTP) binding protein; *CDKN2A* which encodes 2 tumor suppressor proteins (*P16INK4A* and *P14ARF*) and it is inactivated in most of the PDAC cases; *TP53* (tumor protein 53) is inactivated in about 75% or more of PC cases and it is a stress-inducible transcription factor that exerts this protective impact by the induction of cell cycle arrest as well as apoptosis in damaged cells. Functional loss of the TP53 protein activates cellular survival and division when DNA damage is presented, facilitating the accumulation. *SMAD4* is inactivated in more than half pancreatic cancers; loss of *SMAD4* function cancels the *SMAD4*-dependent TGF-β (transforming growth factor beta) pathway resulting to unregulated cellular proliferation. Those were the 4 most frequent mutations in PC, but they found numerous hills involving *SMARCA4* (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4), *CDH1* (cadherin 1 type 1), *EPHA3* (EPH receptor A3), *FBXW7* (F-box and WD repeat domain containing 7), *EGFR* (epidermal growth factor receptor), *IDH1* (isocitrate dehydrogenase 1), and *NF1* (neurofibromin 1). All these mutations alter 12 core signaling pathways: K-Ras, TGFβ, JNKs, Integrin, Wnt/Notch, Hedgehog, small GTPase pathways, control of G1/S phase transition, apoptosis, DNA damage control, invasion and hemophilic cell adhesion (254).

Pathologically, 3 histologically defined precursors to invasive adenocarcinoma of the pancreas have been identified. These include microscopic, non-cystic "pancreatic intraepithelial neoplasia" (PanIn), cystic "intraductal papillary mucinous neoplasms" (IPMNs) and "mucinous cystic neoplasms" (MCNs) (246).

PanINs are microscopic lesions in the smaller (<5 mm) pancreatic ductal cells. It is found papillary as well as flat appearance, with columnar to cuboidal cells and presenting a wide range of amounts of mucin. However PanINs are sorted according to the degree of cytologic and architectural atypia, so that they are established 3 subgroups (PanIN-1, PanIN-2, and PanIN-3) from no dysplasia, to high-dysplasia up to invasive carcinoma (255, 256). These PanINs lesions harbor many of the genetic alterations for PC, for instance PanIN-1early low-grade lesions occur typically because of mutations of *KRAS2* genes. PanIN-2 intermediate lesions are related to inactivating mutations in the *p16/CDKN2A* genes. Finally PanIN-3 late lesions are related to inactivating mutations of some genes such as *SMAD4*, *TP53*, and *BRCA2* (257). Likewise, telomere shortening is an event that was related to the contribution to accumulation of chromosomal abnormalities in PanIN-1 lesions (258).

In IPMNs case, they are considered epithelial neoplasms which produce mucine with papillary structure. They emerge from inside the main pancreatic duct or any of its branches (255), so they are distinguished from PanINs by the diameter of the ducts in which they arise, typically greater than 10 mm in size. Other ways to distinguish in respect to PanINs are, the phenotypic expression of one kind or another mucin, namely *MUC1* (mucin 1 cell surface associated) in most PanINs lesions and *MUC2* (mucin 2 cell surface associated) in IPMNs lesions, and moreover, the gene product from *STK11* is significantly more common on IPMNs lesions (259), whereas it is less common on those lesions the inactivation of *SMAD4* gene (247).

The third histologically distinct precursor lesion in the pancreas is MCN, which as a curiosity the most part emerge in women. Unlike IPMNs, MCNs have no communication with larger pancreatic ducts, but its distinctive characteristic is an ovarian-type stroma (260). Similarly to IPMNs, they are defined as cystic neoplasms composed of mucin-producing columnar epithelial cells that may be arranged to form a single layer of benign epithelium or may present gross papillary projections that harbor the full spectrum of cellular modifications from low-grade atypia to carcinoma *in situ* and associated with invasive carcinoma (261). Despite not having been many genetic studies on MCNs, apparently they possess similar alterations found in PDAC but at lower frequency (258).

It seems to be clarified that some noninvasive IPMNs and MCNs could become invasive adenocarcinoma over time. Likewise, it is estimated that patients enduring an invasive lesion are usually around 3-5 years older that its noninvasive homologue (IPMNs and MCMs)

PDAC or PC is composed of a firm and high sclerotic mass with poorly defined edges, which long branches of carcinoma expanded beyond the main tumor. Focusing on the light-microscopic level, it is found that PC possesses an infiltrating gland-forming neoplastic epithelium with a severe desmoplastic reaction, which intensity provokes that a minor part of mass cells formed by PC are neoplastic cells. PCs are extremely infiltrate neoplasm, in fact vascular and perineural invasion are found in most part of surgically resected cancers and metastases in tissues such as regional lymph nodes and hepatic tissue among others (260). PC has a number of histologic variants described, where are mainly included carcinomas such as colloid carcinoma, hepatoid carcinoma, medullary carcinoma or adenosquamous carcinoma among others (257), which differ in having distinct pathogenesis and better or worse prognosis (260).

In addition to PDAC, IPMN and MCN, in the exocrine pancreas there is a group of neoplastic disorders, either benign or malignant tumors or cyst, which have an occurrence around 1% each one of total neoplastic disorders of pancreas.

Solid-pseudopapillary neoplasm: Most solid-pseudopapillary neoplasms strike women in their 20s and 30s. They are characterized by having a small mass, a solid or cystic lesion or a large almost completely cystic mass, revealed by imaging, in addition to a filling of necrotic an hemorrhagic material. Structurally is observed by light

microscopy that a scarce cohesion among its cells, which form a thin layer on the blood vessels. It has been also observed the presence of diverse cells such as foam cells, clear cells, cholesterol clefts and eosinophilic hyaline globules (262). More than 90% of solid pseudopapillary neoplasms have mutations in exon 3 of the β-catenin gene (263). These mutations interfere with the degradation of the β-catenin protein, and as a result, the β-catenin protein abnormally accumulates in the neoplastic cells and is translocated to the nucleus where it increases the transcription of c-myc (myelocytomatosis viral oncogene homolog) and cyclin D1 (264), resulting in a high prevalence of solid-pseudopapillary neoplasms exhibit an altered nuclear profile of labeling with antibodies to the β- catenin protein. Furthermore, an unclear mechanism whereby the disruption of β-catenin interferes with e-cadherin provoke that the most part of solid-pseudopapillary neoplasms cannot express the extracellular domain of e-cadherin, which loss, may be the reason that explains the special dyscohesiveness of the cells happening in this neoplasm.

Pancreoblastoma: Pancreatoblastomas are neoplasm as well, but with a distinctive morphology. It has 2 common components, the first one is the existence of neoplastic cells with acinar differentiation and the second one is the presence of squamoid nests. It can be also observed other elements such as immature primitive cells as well as neoplastic cells in differentiation processes on endocrine, ductal and mesenchymal tissue. Genetically it observed a loss of copy from the short arm of chromosome 11 near the *WT-2* (Wilms tumor 2) gene locus (11p15.5) (265). Likewise molecular analyses revealed an existing parentage with hepatoblastomas and nephroblastomas, what suggests a primitive link of these neoplasms that nowadays emerge from different organs (262).

Acinar cell carcinoma: These kinds of neoplasms are characterized by the synthesis of exocrine enzymes such as trypsin, chymotrypsin or lipase by neoplastic cells (263). Acinar carcinomas of the pancreas are cellular neoplasms with minimal fibrous stroma. The cells are often pyramidal in shape, with abundant granular apical cytoplasm and the nuclei typically have single prominent nucleoli. In contrast to PDAC, most of these carcinoma do not harbor *KRAS* gene mutations, nor do they harbor mutations in the *TP53*, *p16/CDKN2A*, or *SMAD4* genes (264). Molecularly, acinar cell carcinoma possesses a loss of chromosome 11p and mutations on and β-catenin gene, suggesting thereby a greater parentage with pancreoblastoma than ductal adenocarcinomas.

Serous cystic neoplasm (SCN): SCN are benign cyst-producing neoplasms. Its cysts are composed by cuboidal cells forming a line, and which cytoplasm is clear (260). It has been reported by genetic analyses that some sporadic serous cystic neoplasms harbor somatic mutations in the *VHL* (von Hippel–Lindau syndrome) gene (266). Other neoplasms with *VHL* mutations often have clear cell morphology. Some SNC also have a prominent vasculature (267).

IX.2 TUMORS IN THE ENDOCRINE PANCREAS

Tumors in the endocrine pancreas are rare tumors currently known as pancreatic neuroendocrine tumors (PNETs). It is thought that PNETs emerge from multipotential stem cells from pancreatic ductal epithelium more frequent than from islet Langerhans. Epidemiologically PNETs account for 1-2% of all pancreatic tumors, but several studies have observed that the incidence and prevalence is increasing in recent years. PNETs increase with age and mostly occur in the fourth to sixth decades of life, although may manifest at any age. Although most PNETs are spontaneous, they can also be associated with hereditary endocrinopathies, including multiple endocrine neoplasia type 1 (MEN1), VHL, neurofibromatosis, and tuberous sclerosis (268). Like exocrine tumors of the pancreas, for its location it is required imaging techniques such as biophasic computed tomography scan or magnetic resonance, which often detect the primary tumor, as well as metastatic disease in the liver and lymph nodes. Endoscopic ultrasound is extremely helpful detecting small lesions (269).

Although WHO established a classification according to histological criteria such as the degree of differentiation, the size of the tumor, the presence or absence of angioinvasion and proliferation index, PNETs may be broadly categorized as functional or nonfunctional.

IX.2.1 Functional pancreatic neuroendocrine tumors

These kinds of tumors have an altered physiology that facilitates its recognition, moreover as it is usual that each kind of functional pancreatic neuroendocrine tumor has associated an elevated level of a specific hormone, it can be detected by commercial assays (270).

Insulinomas are the most common of the functional PNETs, are usually benign, and present classically with Whipple's triad: symptoms of hypoglycemia, documented hypoglycemia at the time of symptoms, and relief of symptoms with glucose administration. The symptoms from insulinomas may be either neuroglycopenic symptoms, such as confusion, seizure, obtundation and coma, or may be hypoglycemic-induced symptoms, such as palpitations, diaphoresis, and tachycardia (268).

Gastrinomas: The consequences of this tumor are related to gastrointestinal alterations, as it is observed gastric acid hypersecretion, peptic ulceration and diarrhea. Only half of them are malignant. Laboratory analyses revealed an elevated fasting serum gastrin and a positive secretin stimulation test (271).

Glucagonomas: It is characterized by being composed of necrolytic migratory eritrema after a skin biopsy of its inner edge, what may be helpful in order to detect, but it is usually not required because positive cases are related to elevated glucagon levels (272). It is often associated with DM, and the greater part of them are malignant, presenting metastatic processes (273), usually presented with necrolytic migratory erythema, and may also be associated with DM. Elevated glucagon levels above 1000

pg/ml may be useful for diagnostic, as well as skin biopsy (272). The most part of glucagonomas are malignant and metastatic event usually happens in these patients (273).

VIPoma: It is known as Verner-Morrison syndrome, WDHA syndrome (watery diarrhea, hypokalemia, and achlorhydria), and pancreatic cholera syndrome as well. VIPoma patients suffers several kind of diarrhea, hypokalemia because of a remarkable loss of potassium at fecal level and metabolic acidosis as bicarbonate loss are presented at fecal level as well. It can be detected by the elevated levels of serum VIP (vasoactive intestinal peptide), but due to its intermittence it is required repeated fasting VIP levels in order to assure a strong clinical suspicion (274).

Somatostatinoma syndrome: It is the least common of the mentioned well recognized functional PNETs, occurring in less than 1 in 40 million people (270). The symptoms which are observed include steatorrhea, diabetes, and cholelithiasis. In respect to its location, most of them are presented in the pancreatic head in periampullary region. For its detection, it is required measurement of somatostatin levels in serum (259).

IX.2.2 Nonfunctional pancreatic neuroendocrine tumors

These kinds of tumors comprise around two-thirds of PNETs. In contrast to functional ones, they cannot be detected by altered hormone levels in serum, but most of them produce peptide that allows its detection. Both types of PNETs secrete substances of different nature which do not cause specific syndrome, but in case of chromogranin A, a neuroendocrine secretory protein, might be useful for the detection of both PNETs. Furthermore, it could be also useful for the progression of the disease after treatment and monitoring. Anyway, it is not a universal biomarker for these kinds of tumor, but it is the most sensitive until the date (268).

Mesenchymal tumors of the pancreas: the great part of pancreatic neoplasm arise from pancreatic epithelium cells but, there is a small group of pancreatic mesenchymal tumors, while rare, can derive from the connective, lymphatic, vascular and neural tissues of the pancreas which account for 1-2% of all pancreatic tumors and are sorted regarding their histologic source (275). Subsequently it briefly mentions the main mesenchymal tumors of the pancreas.

Lymphangioma: lymphangioma has an uncertain histogenesis, some theories proposed suggesting an inherited abnormality or an embryologic or traumatic origin, whereas other authors believe lymphangioma is a true neoplastic lesion. It is typically multicystic with a varying range of size, is presented a peripheral thin capsule of fibrous tissue although calcifications are rare. Pancreatic lymphangiomas can rise within the parenchyma or it could be linked to the organ by a pedicle that can be liable to torsion (276).

Lipoma: they are uncommon in the pancreas. It is characterized by its histology, as it is composed of mature adipose cells which forming a lobules and are restricted by a slight collagen capsule, what makes differing from lipomatosis (277).

Pancreoblastoma: pancreoblastoma was previously mentioned within exocrine tumors of the pancreas, but according to what was mentioned, pancreoblastoma in rare occasion may have a mesenchymal origin.

Schwannoma: these kinds of tumor arise from an unusual site of origin, Schwann cells. This tumor provokes rare lesions because of the envelope of nervous fibers, and only few cases are considered benign (278). It comprises of two elements, an organized cellular component and a loose hypocellular component (276), known as Antoni type A area and Antoni type B area respectively.

Neurofibroma: this variant of non-functional PNETs is related to Recklinghausen disease. Structurally it is composed of fibroblast, Schwann cells and neural components. Exceptionally is located at pancreas, in fact, A regional diffusion from celiac plexus involvement in type 1 neurofibromatosis is the most common finding (276).

Lymphoma: most known as Non-Hodgkin lymphoma (NHL), has a poor pancreatic location (<1%), being the pancreas much more involved by systemic lymphoma than being primary or predominant site (279). However the 20% of cases are related to an extranodal location. It is observed a predominant structure which is uniform, hypodense and hypovascular (279).

Sarcoma: it is an infrequent pancreatic malignance, characterized by a high vascularity and early metastatic diffusion, especially to hepatic tissue (280). Although most of pancreatic malignance has a sarcomatous appearance, actually are anaplastic carcinomas, while real types of sarcomas such as primary undifferentiated sarcomas, fibrosarcomas or leiomyosarcomas have been described (276). It is thought that this tumor may emerge from the pancreatic duct as well as blood vessels in the pancreas (280).

X AGE-RELATED CHANGES OF THE PANCREAS

Pancreas as well as other tissues and organs, suffers certain changes in its structure associated to age, which make it more susceptible to age-related diseases such diabetes, pancreatitis or pancreatic cancer, and others. These alterations in the pancreas, previously mentioned, are not exclusive of aging people, as the current trend is that becoming more young people are suffering this age-related diseases. The cause of this trend seems to be related to lifestyle factors, such as diet, physical habits, smoking or alcohol consumption.

X.1 AGE-RELATED STRUCTURAL CHANGES OF THE PANCREAS

Investigations conducted in animals showed that with advancing age, the pancreas undergoes alterations, such as atrophy, fibrosis and fatty infiltration (281). Similarly, in 112 unselected autopsies of adults without clinical manifestation of pancreatic disease, an increase in lipomatosis, fibrosis and both ductal and ductal epithelial alterations were observed in old age (282). A further finding of this study was that generalized atherosclerosis was correlated with the presence of lipomatosis and fibrosis (282). Lipomatosis is a fatty replacement, in this condition, the pancreas shows near-total atrophy of acinar cells by fat, although clinical pancreatic insufficiency usually does not occur (205). These morphological alterations were accompanied by a steady decrease in the mean weight of the gland, starting at the age of 40 years, but due to lipomatosis, there is usually an increase in the weight of the gland. Lipomatous atrophy, what is also known as Shwachman syndrome, is part of a systemic syndrome with symptoms of pancreatic insufficiency, recurrent infections, neutropenia and abnormalities on skeletal muscle and skin, but pathologically it is similar to lipomatosis (205). Other changes reported in the elderly are the obtainment of a smaller size pancreas and an increase in the duct diameter (283).

At the microscopy level, the pancreatic ducts may show a dilation of peripheral ducts where it can be observed amounts augmented of thickened secretions. Likewise it is observed signs of fibrosis at periductular level and amplification of duct epithelial cells which show mucus cell hyperplasia (205, 284). All these increase with age, both in frequency and intensity, and usually appear after the age of 50 (284).

With regards to acinar cells changes, aging leads to many modifications at many levels. Firstly, at nuclear level, it is observed a reduction in size and diverse alteration of its shape. At the cytoplasm level, there is general vacuolization and lesser production of zymogen granules. In respect to acini, it is observed a generalized dilation and reduction of the acini height (205).

Several studies based on imaging, such as computed tomography (CT) (285), endoscopic retrograde cholangiopancreatography (ERCP) (286) and ultrasonography (US) (287, 288), have identified a series of structural alterations. Of particular note is a Japanese study that assessed the parenchymal perfusion of the normal pancreas using dynamic CT in correlation with the patients' demographic characteristics (289). The authors reported that the pancreas perfusion, ranging from 0.554 to 1.698 $ml \cdot min^{-1} \cdot ml^{-1}$, decreased with the patient's age. They attributed this fact to the increasing atrophy of the exocrine pancreas. Conversely, an increase in the fatty tissue with age was unlikely to be the cause of the reduced perfusion, as pancreatic parenchymal density measured before the injection of contrast material did not correlate with the perfusion measured by dynamic CT or with age.

X.2 AGE-RELATED FUNCTIONAL CHANGES OF THE PANCREAS

In animal studies, it was shown that the pancreatic function was diminished in older rats as compared with younger animals (290). In particular, in the basal condition, enzymatic and protein concentration in pancreatic tissue was not significantly different in the 2 age groups. Interestingly, after 7 days of a diet enriched with fat or starch, lipase and amylase concentration increased by about 25% in young, but not in aging rats. The authors concluded that aging induces modest changes in pancreatic digestive enzymes which are unlikely to be physiologically important, but the pancreas of aging rats did not adapt to changes in dietary intake as well as those from young rats (290). Another study performed in aged rat investigated whether aging could affect pancreatic exocrine secretion and its action mechanism. Authors concluded that pancreatic exocrine secretion was reduced with age, and that endogenous insulin secretion and/or action was involved in that phenomenon (291). The same conclusion was drawn from studies conducted in humans. In older humans, pancreatic secretion was not diminished upon initial stimulation with either secretin or cholecystokinin (292, 293) this was demonstrated by Gullo *et al*. using either a secretin-cerulein test (292) or a tubeless, a noninvasive pancreatic function test such as the pancreolauryl test (293). However upon repeated stimulation, pancreatic secretion droped significantly in older persons as compared with younger control individuals (294). Thus, it appears that in the elderly, the pancreas might be able to function well under unstressed conditions. This was confirmed in a study By Arora *et al*. (295) which showed that fat malabsorption did not occur in elderly humans up to the age of 91 years, 24-hour fecal fat excretion on a diet of 100g fat/day was found to be 2.8 g for both age groups of 19-44 and 70-91 years. However, in a Swedish study, it was found that elderly volunteers developed mild steatorrhea when the dietary fat content was increased to rather uncharacteristically high levels of 115-120 g/day (296).

With regards to carbohydrate digestion and/or absorption, a study (297) was interpreted as showing decrease carbohydrate digestive or absorptive capacity with aging. Mixed carbohydrate meals containing about 200 g of carbohydrate were fed to elderly individuals and young control subjects. Breath hydrogen test were subsequently carried out, and in the elderly group, the prevalence of positive test was about 60 %. In the control group breath hydrogen test was negative, even in subjects on the 200 g carbohydrate meal. Interpreting findings of carbohydrate maldigestion/malabsorption with as a sign of pancreatic insufficiency is complicated by the increased numbers of bacteria that can overgrow in the intestine of adults as a result of hypochlorhydria of aging (298). Hypochlorhydria happens in advanced age as a result of high prevalence of atrophic gastritis, which affects as many as 10-30 of elderly person over 60 (299). It is known that *Helicobacter pylori* infection is the main cause of atrophic gastritis. The decreased acid secretion in persons with atrophic gastritis results in increased survivability of swallowed bacteria in the stomach and small intestine, which in younger normochlorhydric persons would be destroyed by stomach acid. Thus, an abnormal result on a breath test in an elderly person might not reflect carbohydrate malabsorption,

but rather simply exposure of the carbohydrate load to the bacteria residing in the small intestine.

The demonstration that pancreatic function is well preserved with aging has the following practical consequence: encountering true pancreatic insufficiency in an elderly person should raise concerns as to whether a significant disease process is under way in the pancreas.

XI RISK FACTORS FOR PANCREATIC DISEASES

Aging itself may be considered as a risk factor for enduring the most common diseases of pancreas such as diabetes, pancreatitis, or pancreatic cancer, which are regarded as age-related diseases. Aging is not a risk factor which it could be controlled a *priori.* On the other hand, certain controllable risk factors such as physical activity, obesity, smoking, alcohol consumption and diet have been showed to be able of preventing or favoring the pancreatic diseases mentioned, and therefore if those controllable factors work in order to reduce the risk of enduring a pancreatic disease not only in the young and mid-life, but it may delay the age-related process to such diseases in aging.

XI.1 CIGARETTE SMOKING

Cigarette smoking (CS) is probably one of the most injurious habits for the public health, causing millions of death worldwide every year and increasing costs on health. It can affect to the health of the pancreas by several ways. Numerous studies have found a strong relation between CS and PC. A study analyzed data from 12 case-control studies including 6507 pancreatic cancer cases and 12890 controls. The results confirmed that CS is associated with a twofold increased risk of PC and that the risk increases with the number of cigarettes smoked and duration of smoking (300). Other similar studies obtained such results (301-306). Some of these studies also observed that smoking cessation decreases such risk, becoming up to non-smoker risk for PC elapsed 20 years. Even possibilities for surviving after a PC are decreased for smokers with respect to non-smokers (307).

CS is also related to an increase of enduring diabetes type 2 according to a systematic review and meta-analysis (308) of studies which researched for this relationship, even though mechanisms must be clarified. It was studied about nicotine effects, finding that this substance affect to insulin action and sensitiveness and therefore is connected with T2D (309). Alike, smoking cessation reduces the risk to endure diabetes type as well as the negative effects on insulin action and sensitiveness (309-312).

Pancreatitis has an association with CS, either AP or CP. Many studies confirm that CS is an independent risk factor for AP (190, 313, 314). Previously was thought that CS only enhanced the effects of alcohol consumption for AP. Although the

mechanism is not clarified enough, there is a dose-response relationship. Smoking cessation is needed for diminishing the risk for AP. Alike CS increases the risk for CP according to several studies (313, 315-317). Smoking once a patient is enduring CP is associated with morphologic and functional signs of more severe disease, observing pancreatic exocrine insufficiency, calcifications and severe morphologic changes. It was observed that such calcifications may be caused by nicotine or its metabolites, however only few studies explain possible mechanism of action. Like AP, in CP, smoking is a trigger itself but it enhances the effects produced by alcohol consumption (synergy) and likewise, it has been shown a dose-response relationship.

In addition to the known pancreatic diseases mentioned above which CS is related to, it has been observed that CS is associated with pancreatic fibrosis, specifically the intralobular pancreatic fibrosis (318), so somehow smoking accelerates the aging of the pancreas.

XI.2 ALCOHOL CONSUMPTION

Consumption of alcoholic drinks is a regular habit, which moderate consume may be compatible with a healthy life in some cases, but heavy consumption is associated with overall mortality, cardiomyopathy, hypertension, acute cerebrovascular events, gastrointestinal diseases, and cancer (319). Therefore, the pancreas is a target.

Tobacco is a well-known risk factor for PC, however the role of alcohol consumption (AC) on PC is controversial. What is in agreement is that heavy AC enhances the effect of tobacco, but some authors found a positive association between heavy AC and increased risk for PC (320, 321), even such association was found in a study which subjects consumed 3 liquor alcoholic drinks per day (322). On the other hand, it is thought that heavy AC is not individually involved in PC, but only enhancing other risk factors such as smoking (323).

AC is involved in the development of pancreatitis. AC is the second common cause for AP after gallstones (324). Although it is reversible, if AC continues, may lead to CP, which AC is the first cause for it and which effects are chronic and irreversible (321). However only 3% of heavy drinkers develop CP, so authors insinuate that AC increases the sensibility for injuring the pancreas through other factors, either genetic or environmental (324, 325). Several studies aimed how much amount of alcohol is required for developing pancreatitis. Dufour *et al.* (326) concluded that CP development is proportional to the dose and duration of alcohol consumption (minimum, 6-12 years of approximately 80 g of alcohol per day). Nevertheless the data may be different depending on the geographical situation or race (324). A therapeutic strategy is cessation of AC, it is the outset for the reversion of the injuries generated by AC such as pancreatic fibrosis (327).

With regards to the AC influence on diabetes Mellitus, authors are controversial about some aspects. On the one hand, in 2009 a review that selected meta-analyses, review articles and observational studies about this association, concluded that moderate

consumption of alcohol decreases the risk of T2D by 30%, whilst heavy drinkers have the same or slightly higher risk than abstainers (328). They attributed this beneficial effect of alcohol intake to an ethanol-mediated improvement of insulin sensitivity. A later study on Nord-Trøndelag population concluded the same (329). On the other hand, an experimental study performed on Swedish population (330) deducted that such positive effect of moderate consumption of alcohol on T2D risk was only appreciated in women, and concerning heavy drinkers, they observed an increased risk of pre-diabetes and TD2; heavy chronic consumption of alcohol deteriorates glucose tolerance and insulin resistance (331). In any case authors do not recommend become a moderate alcohol drinker to people who have a predisposition or risk of T2D, pre-diabetes or suffering T2D, especially if the patients are overweight, suffering from hypertension or hypertriglyceridemia.

XI.3 OBESITY AND OVERWEIGHT

Obesity can be measured by body mass index (BMI), that is the ratio of weight in kilograms (kg) to height in squared meters (m^2), where a BMI of 18.5 to <25 is normal, 25 to <30 is overweight and from 30 onwards is obese. Obesity can be also quantified by adiposity measurement such as waist circumference and waist to hip ratio (WHR) (5). Obesity was not considered as a disease until last few decades, when researchers associated obesity with cardiovascular diseases, T2D, pulmonary dysfunction, gallbladder disease liver disease musculoskeletal disease and increased risk of certain forms of cancer. Likewise, obesity may take part on pancreatic diseases.

A review (332) analyzed epidemiological case-control, cohort and prospective cohort studies which searched an association between obesity (as BMI or WHR) and PC. This review found a positive association between obesity/high BMI and PC risk. Particularly they observed that the risk increases 10% for a 5 kg/m^2 unit increase in BMI. They focus on the possible mechanisms for this association on hormonal and inflammatory effects of adipose tissue, increased exposure to carcinogens as a result of increased food consumption and diminished physical activity. Other study confirmed this positive association on people who are 50-71 years old (333). Another systematic review and meta-analysis of prospective studies (334) also researched for the association between BMI and pancreatic cancer risk, but in addition to this, they researched which role had abdominal fatness. The conclusion they reached was that both, regular and abdominal fat are connected with increased risk for PC, where they specify that non-linear association is observed in the overall analysis with increased risk above a BMI of 25 but most pronounced above a BMI of 35 (in nonsmokers). Besides this increased risk for PC due to obesity, obese people have a poorer prognosis and survival than non-obese people once the PC is diagnosed (332).

Develop of pancreatitis due to obesity has been also studied. The most of these studies observed the role of obesity on PC development. A study observed that obesity may act as an independent risk factor for PC, which was shown in population-based case-control study in Sweden by comparing 462 patients admitted for a first episode of

AP with 1781 control subjects (335). But if the reason of this relationship is due to total adiposity or abdominal adiposity is controversial. In a meta-analysis high BMI has been associated with an increased risk of acute pancreatitis (336), but a population-based prospective cohort study concluded that abdominal adiposity, but not total adiposity, is an independent risk factor for the development of acute pancreatitis (337). The possible mechanism which obesity could increase the risk for AC is due to a greater release of pro-inflammatory mediators such as leptin and resistin than anti-inflammatory mediator such as adiponectin by adipose tissue at obesity state. When there is an accumulation of adipose tissue at abdominal level, the release of these pro-inflammatory mediators are drained into the portal system reaching the liver which somehow alters the conversion of trypsinogen into trypsin (338). Furthermore, a review confirmed that obesity has a clinically relevant impact on the course and outcome of AP. In regards to the impact of obesity on CP development there are no many studies about, in fact only a review found a suggestion which either obesity as well as overweight are additional risk factors for development of alcohol-induced CP (339).

Association between obesity and T2D is well documented (212), in particular central obesity. Actually in a recent study was concluded that overweight and obesity confers an increased risk for diabetes at any BMI status (340), and duration of obesity is another additional risk factor for develop of T2D (341). In summary, obesity, in particular central obesity, is the most predominant risk factor for insulin resistance, which in turns, leads to T2D. As said above, the adipose tissue releases more pro-inflammatory cytokines than anti-inflammatory cytokines in obesity state, which in turns provokes the infiltration of macrophages into adipose tissue, which are attributed to exert a central role on development of insulin resistance (342).

XI.4 PHYSICAL ACTIVITY

Physical activity (PA) is widely demonstrated to be a healthy habit, contributing to cardiovascular positive effects and delay of age-related disease among others. Several studies researched for the positive effects of PA on pancreas physiology about its functionality and lower risk in pancreatic diseases such as PC and T2D.

With regards to the association between PA and PC, recent meta-analyses and reviews have approached to this relation (333, 343, 344). There was not found a strong evidence which PA is an independent risk factor for PC, but indirectly PA have a role to prevent this disease, because PA is demonstrated to prevent overweight and adiposity, and diabetes, which are well-established risk factors for PC. But there were not studies about the role of PA on patients with PC, but recently, a case study (345) aimed to determine the safety and efficacy of a 6-month supervised exercise program in a pancreatic cancer patient undergoing adjuvant treatment, concluding that PA led to improvements in a variety of patient outcomes during adjuvant therapy for pancreatic cancer, suggesting PA like a part of therapy in PC patients.

What seems to be clear is the beneficial effect of PA on diabetes disease, especially in T2D, which reviews are agree about PA reduces the risk of insulin

resistance and T2D, increases insulin action in skeletal muscle from obese and insulin resistant individual and halve the risk of progressing to T2D over a 5-year period (346). Likewise, PA is considered the single intervention with greatest efficacy to reduce the risk of virtually all chronic diseases simultaneously (346). Other authors reviewed if the risk factors for T2D may be corrected by the positive effects from regular PA, observing positive effects on different parameters, such as reduced blood pressure, better arterial response, adjustment on alterations such as dysglycaemia and dyslipidaemia, enhancement of antioxidant defenses which lead to lower oxidative damages, improving the markers of anti-inflammatory profile in respect to pro-inflammatory profile ones and amelioration of dysfunction on islet of Langerhans triggered by T2D (347). Although the guidelines for managing PA for are controversial, there is a clear consensus that regular exercise of the type and form traditionally advocated (~30–60 min of moderate intensity exercise 5–7 days per week) (346). In patients with T1D, the glycaemia is not well regulated because β-cells are impaired, so insulin cannot exert its function on glycemic levels in blood. The possible beneficial effect of regular PA on these kinds of patients have been also studied, showing that there is enough evidence which regular PA could be recommended as part of the management of patients with type 1 diabetes being as PA improved some parameters related to T1D disease, However further studies will have to search in which form and duration of PA is the most convenient for these patients (348). Nevertheless is observed that in order to avoid some risk of adverse responses to exercise in T1D patients, is required a careful screening, pre-exercise preparation, and appropriate exercise prescription (349).

XI.5 DIET

Increasing number of studies demonstrate the role of diet on health. In summary, those studies usually search for the contribution of a certain dietary pattern, food group, food product or a specific nutrient on the general health or wellness, or on a certain disease.

A well-establish studied dietary pattern is the Mediterranean diet (MD). There is a vast amount of literature that demonstrates strict relation between Mediterranean diet and health status in both general population and patients with a manifested disease. Adherence to the MD has been shown to have a protective role on cardiovascular diseases and neurodegenerative diseases such as Alzheimer and Parkinson. Furthermore, some kinds of neoplastic diseases such as colorectal cancer, breast cancer and prostate endometrial and pancreas tumors have a lower risk. Diverse studies have been also demonstrated that adherence to the MD markedly reduces the risk for T2D. As a consequence of these beneficial effects of this adherence, the MD is able to decrease the risk of mortality but also is able to determine a better and longer life with a better psychological wellbeing and a higher perceived health status (350).

Likewise, the pancreas has been under study with regards to the positive or negative influence of a specific diet on the health and functional aspects of this organ. Great part of these studies observe the influence of a modified diet regarding its

macronutrient composition (carbohydrates, proteins or fat), the quality of a macronutrient or the caloric content of the diet on the pancreas.

A high content in sugar or sucrose in diet is associated with a higher susceptibility of suffering metabolic alterations that can lead to injure pancreatic β-cells, which in turn, implies to T2D (351). Although there is no a significant direct relationship between diets high in glycemic index, glycemic load, total carbohydrates or sucrose and PC risk (352), high-sugar diet play a role concerning development of obesity (351), which in turn was previously associated with increased risk of PC, so indirectly high-sugar diet may have a role on PC. At microscopic level, a study carried out on male Sprague-dawley breeder rats with a high-sucrose diet, was observed pancreatic alterations such as hyalinization with focal inflammatory infiltrates, a greater macrophage infiltration in the pancreatic interstitium, a not-significant higher infiltration of macrophages within the islets and significantly less insulin staining in the islets (353).

Alike, a high-fat diet has been studied in animal models, observing harmful results. It has been observed an increment of insulin levels, corresponding to a pre-diabetes state (354). In addition to this, a high-fat diet or hypercaloric diet may promote a mutation concerning *Kras* genes, which ultimately promotes to neoplastic pancreatic lesions (355). Furthermore, these high-fat diets may induce to endopancreatic or exopancreatic abnormalities, increase of pro-inflammatory cytokines, fibrogenesis and may also promote to pancreatic fibrosis due to a higher oxidative stress (356).

The fatty requirements in order to achieve a balanced diet are about 25-35% of total ingest. However there are no much studies about the prevalence of a specific fat type that influence in pancreatic health or function at recommended fat-diet levels.

A feasible baseline is the fact that pancreatic cellular membranes composition is markedly influenced by lipid pattern from ingested diet. Simultaneously, this lipid pattern can modulate the secretory activity of pancreatic acinar cells which involve, at least in part, calcium signaling (357). So the studies focused on how a particular fat supplementation such as olive oil, sunflower oil or fish oil among aothers, influences to the pancreas functionality, more specifically to β-cells glucose sensitivity, either after the patient suffers diabetes mellitus or after the patient suffers any pathology that affects to pancreas, can be related to the aging process.

Olive oil is a dietary fat rich in MUFA, especially in oleic acid (18:0) and it is the predominant fat in the Mediterranean diet. It has been under study due to its healthy properties on many diseases. With Regards to the possible benefit of olive oil about pancreatic function and health, a couple studies considered. In 2004 Martínez *et al*. (358) demonstrated that a diet supplemented with virgin olive oil can modify pancreatic cell function as assessed by $[Ca^{2+}]^i$ mobilization and amylase release evoked by secretagogues in rat pancreatic acinar cells. In other study a supplementation with olive oil in diet on ovariectomized rats improved insulin stimulation after glucose ingest. Histological studies revealed a standardization of Langerhans islets size in pancreatic

tissue with respect to those rats which were not supplemented in such olive oil (359). Oleic acid from peanut was efficient in order to revert inhibitory effects of insulin production, mediated by inflammatory cytokine TNF-α (352). TNF-α is produced by adipose tissue, this is why the obesity is a risk factor to suffer diabetes mellitus (360). In addition to this, it has been observed an inverse relationship between PC and olive oil consumption in a meta-analysis (361) or by adherence to Mediterranean diet (362), which highlights the role of olive oil. Furthermore it has been observed an improvement of the management of diabetes in patients with cardiovascular diseases which were adhered to the Mediterranean diet, where olive oil has a primordial role (363, 364).

When the olive oil administered is extra virgin (EVOO), in addition to oleic acid effect, it must be considered the antioxidant effect from the organic fraction of this fat source. In fact, a study performed on metabolic tissues such as liver and β-cells from endocrine pancreas in rats, was observed that a diet rich in EVOO directly protected against oxidative stress through its antioxidant compounds as well as through indirect mechanisms that involves a higher expression and activity of certain enzymes with antioxidant activities. Such positives effects were the improvement of insulin secretion mediated by glucose, protection of lipid membrane from liver and β-cell peroxidation and higher expression of catalase and glutathione peroxidase at Langerhans islets (365).

Fish oil is a dietary fat mostly compounded by PUFA, in which the most representative ones are α-linolenic acid (ALA) (C18:3), EPA (C20:5) and DHA (C22:6). These fatty acids have been extensively studied and were found certain benefits on cardiovascular diseases, prevention of chronic diseases, mental illness and positive association with certain cancer types. It has been also studied possible effects on the pancreatic function and health (366).

A study investigated the impact of elevated tissue omega-3 (n-3) PUFA status on age-related glucose intolerance utilizing the fat-1 transgenic mouse model which can endogenously synthesize n-3 PUFA from omega-6 (n-6) PUFA, and they provided that elevated tissue n-3 PUFA status may improve age-related glucose intolerance, suggesting that beneficial effects of elevated tissue n-3 fatty acid status in the prevention and treatment of age-related chronic metabolic diseases, such as insulin resistance and type 2 diabetes (367). Another study observed that fish oil supplementation had positive effects on insulin sensibility in healthy rats only when were administered for a long-term period (368). However, according to a systematic review (369) there were not found an inverse association between fish oil consumption and decrease of PC risk.

Sunflower oil is a dietary fat rich in n-6 PUFA, mainly linoleic acid. It has been used on acute administration studies in order to prevent gastric impairments caused by injection of indomethacin. Likewise, its supplementation on diet had positive effects on plasma lipid profile (370). However, the contrary effect is also suggested, being that increased pro-inflammatory impact by a major presence of interleukine 6 (IL-6) in white adipose tissue of mice which were fed with a sunflower oil-enriched diet (371).

To elucidate these contradictory results about anti- or pro- inflammatory properties, a study assessed the impact of sunflower oil supplementation on inflammatory state and insulin resistance condition using high fat diet-induced obese mice C57BL/6 male mice after 8 weeks of experiment. It was concluded that sunflower oil supplementation has pro-inflammatory effects in macrophages and additionally it was promoted insulin-sensitive peripheral tissues and induction of insulin resistance *in vivo* and *in vitro.* In addition to this, although the lipid profile improved, the inflammatory state and insulin resistance did not improve or even worsened (372).

Nevertheless not many comparative studies of dietary fats related to the pancreatic function and health have been performed. An early approach was performed by Tinahones *et al.* (373), who carried out a comparative study of the effects of different dietary fatty acids (olive oil, sunflower oil, soybean oil, fish oil and palmitic acid (16:0) + soybean oil) on the insulin secretion during 30 days. Although they found that diet rich in sunflower oil with little supplementation of polyunsaturated fatty acids secreted greater amounts of insulin and important changes took place in the fatty acid composition of the phospholipids (like marker of fatty acid modification), there was no linear correlation between the fatty acid composition and the secretion or content of insulin. Díaz *et al.* (374) observed *in vivo*, a different pattern of secretory activity of the exocrine pancreas in rats exclusively fed by virgin olive oil or sunflower oil as fat source during 8 weeks.

However until now, there no was a comparative study of 3 of the most consumed dietary unsaturated fats (fish oil, virgin olive oil or sunflower oil) related to pancreatic function and pancreatic integrity for a long-term period and in relation to aging.

HYPOTHESIS AND OBJECTIVES

The hypothesis for the present Doctoral Thesis was that age-related damage on pancreatic tissue can be differently modulated depending on a specific dietary fat type (virgin olive oil, fish oil or sunflower oil) administered throughout life. Likewise, it is hypothesized that coenzyme Q_{10} administration could have a complementary role together with dietary fat in order to modulate age-related processes at the pancreas.

According to this hypothesis the following objectives were proposed:

1º To compare the effects of feeding rats lifelong on different dietary fat sources (virgin olive, sunflower of fish oils) on the aged pancreas.

2º To ascertain if coenzyme Q_{10} supplementation to every experimental dietary fat might exert in the rat any additional effect on the pancreas during aging to those attributed to a single dietary fat type

3º To investigate putative mechanisms behind observed effects of dietary fat and/or coenzyme Q_{10} administration on aged pancreas.

MATERIALS AND METHODS

I MATERIALS

I.1 CHEMICAL PRODUCTS

Reagents and solvents used for the analytical measurements came from Fluka (St. Louis, Missouri, USA), Merck Millipore (Hohenbrunn, Germany), Sigma-Aldrich (St. Louis, Missouri, USA), Panreac (Barcelona, Spain), Boehringer (Manheim, Germany), Roche (Basel, Switzerland), Bio-Rad (Hercules, California, USA), Cayman Chemical Company (Michigan, USA), Macherey-Nagel (Durën, Germany), and Thermo Scientific (Rockfor, Illinois, USA). Standards to fatty acids determinations, Coenzyme Q_9 and CoQ_{10} were supplied by Sigma-Aldrich and Fluka. High-performance liquid chromatography (HPLC) quality reagents and solvents were acquired in laboratories Merck and Panreac. Antibodies for histopathological analyses were adquired from Dako (Denmark), and antibodies for the same purpose were adquired from Máster Diagnóstica (Granada, Spain).

Concerning experimental dietary components, virgin olive oil came from "Venta del Llano", from Research and Training Institute for Agricultural and Fisheries of Andalusia (IFAPA); sunflower oil was bought in a local supermarket, and fish oil was acquired from Roche (Basel, Switzerland). The other components used to elaborate diets were purchased to Musal Chemical (Granada, Spain).

I.2 EQUIPMENT

- -20°C freezer Liebher (Liebher, Biberach, Denmark).
- -80°C freezer Revco VLT-1786-5-VUA (Revco, Asheville, North Carolina, USA).
- 4°C refrigerator LIEBHERR (Ochsenhausen, Alemania).
- Automatic cutter model PT 10-35 POLYTRON (Kinematical AG, Lucerne, Switzerland).
- Automatic pipettes NICHIPET EX (Nichiryo, Tokyo, Japan).
- Automatic staining equipment Leica Autostainer XL (Leica, Wetzlar, Germany).
- AutostainerPlusLink (Dako, Demark)
- Cold plate Leica EG1150C (Leica, Wetzlar, Germany).
- Computer Asus P6-P8H61E, Mini Tower, Core i3 2100 3.1 Ghz.
- Dako Automation Hematoxylin Histological Staining Reagent (Dako Denmark A/S, Denmark).
- Digital scale GRAM PRECISION (Gram Precisión, SL. Barcelona, Spain).
- DP72 camera Olympus from an Olympus BX41 microscope (x20 objective).
- Dry heat bath Dry block BD200-RE OVAN (Esper Group, Badalona, Spain).
- Electron microscope Carl Zeiss EM10C (Oberkochen, Germany).
- Electronic micropipettes EPPENDORF RESEARCH® PRO. (Eppendorf®, Hamburg, Denmark).
- Eppendorfs microcentrifuge MiniSpin® Rotor F45-12-11 (Eppendorf®, Hamburg, Denmark).
- Fully Automated Glass Coverslipper Leica CV5030 (Leica, Wetzlar, Germany).

- Gas-liquid chromatograph model HP-5890 Series II (Hewlett Packard, Palo Alto, California, USA).
- Heater MEMMERT (Memmert, Schwabach, Denmark).
- High precision electronic scale SARTORIUS BP110S (Sartorius AG, Götingen, Denmark).
- Histological bath for inclusion in paraffin Termofin (JP Selecta, Barcelona, Spain).
- HPLC system:
    - 3mm ODS Supelcosil column (Beckman Instruments, Fullerton, California, USA Sigma-Aldrich, St. Louis, Missouri, USA).
    - Analytical cell model 5011 (Thermo Scientific, Rockfor, Illinois, USA).
    - Beckman System Gold 125 pump (Beckman Instruments, Fullerton, California, USA).
    - Electrochemical detector ESA Coulochem III (Thermo Scientific, Rockfor, Illinois, USA).
    - Guard cell model 5020 (Thermo Scientific, Rockfor, Illinois, USA).
    - Water 717 plus autosampler (Milford, Massachusetts, USA).
- Ice machine SCOTSMAN AF-10 (Scotsman Ice Systems, Vernon Hills, Illinois, USA).
- Incubator (JP Selecta, Barcelona, Spain).
- Magnetic shaker with heater, AGIMATIC-E. (JP Selecta, S.A, (Barcelona, Spain).
- Microplate's reader SYNERGY HT, Multidetection Microplate Reader (BioTek Instruments, Inc. Higland Park, Vermont, USA).
- Microplate's Shaker Heidolph Titramax 100 150-1350 1/min (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany).
- Microtome blades 250 μm thick (Feather Safety Razor Co, Osaka, Japan).
- Microtome Leica RM2235 (Leica, Wetzlar, Germany).
- Optical microscope ECLIPSE E600 (Nikon Co., Tokio, Japan).
- pH-metre GLP21 (, Crison, (Barcelona, Spain).
- Platform-shaker Heidolph Polymax 1040 2-50 1/min (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany).
- Potter-Elvehjem PTFE glass tube (Sigma-Aldrich, St. Louis, Missouri, USA).
- PTM 2330 F.S column (Supelco Inc, Bellefonte, Palo Alto, California, USA).
- Refrigerated centrifuge BECKMAN model J-21 (Beckman Coulter, Inc. Fullerton, Palo Alto, California, USA).
- Scan Speed MaxiVac Alpha (Neutec Group, New York, USA).
- Teflon pestle (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany).
- Thermal Cycler of Applied Biosystems model 2720 (Life Technologies Corporation, Carlsbad, California, USA).
- UP50H sonicator (Dr Hielscher, Teltow, Germany)
- Ultracut S ultramicrotome EM UC6 (Leica, Illinois, USA).
- Vortex VWR (VWR International Eurolab S.L., Barcelona, Spain).

I.3 SOFTWARE

- Windows 7 Home Premium.
- SPSS 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA).
- Image J 1.46r, (Rasband, 2008)
- *AnaliSYS Image Processing* software (Olympus)

II METHODS

II.1 EXPERIMENTAL DESIGN: ANIMALS AND DIET

Thirty six male Wistar rats (*Rattus norvegicus*) initially weighing 80-90 grams were used for this study. The rats were randomly assigned into six experimental groups, and match-fed from weaning until 24 months of age on a semi-synthetic and isoenergetic diet according to the AIN93 criteria described by Reeves *et al*. (375, 376) but modified in relation to the dietary fat source (see below) and the addition or not of CoQ_{10} (0,7 mg/kg·day). Modified AIN-93G diet was administered to rats from weaning to 2 months of age and AIN-93M was administered for the rest of the experiment. Components of AIN-93G (caloric content 401,4 Kcal/100g) and of AIN-93M (caloric content 362,4 Kcal/100g) are shown in tables II.1, II.2 and II.3. As indicated above, AIN93 diets were modified in relation to the fat source. Original AIN93 diets are composed by soya oil. We replaced this oil by virgin olive oil (virgin olive oil fed group), sunflower oil (sunflower oil fed group) or fish oil (fish oil fed group,) to formulate the experimental diets and groups. Table II. 4 shows the fatty acid profile of experimental oils. Diets were prepared weekly and stored in darkness at 4ºC to avoid lipid peroxidation. Animals were placed in a climate-controlled room (20ºC, 12 h dark/12h light cycle) for two years in collective cages, in groups of three animals per cage, with free access to water. Diet was delivered *ad libitum* for the first two months and then at 25 grams/rat·day for the rest of the experiment (in order to avoid overweight). Food intake was indirectly monitored through the weekly body weight control and daily spillage monitoring.

Table II. 1. *AIN93 diets composition.*

COMPONENTS	AIN-93G (%)	AIN-93M (%)
Starch	38,7	46,6
Casein	20,0	14,0
Dextrose	13,2	15,5
Sucrose	10,0	10,0
Oil	8,0	4,0
Fiber (cellulose)	5,0	5,0
Mineral mixture	3,5	3,5
Vitamins mixture	1,0	1,0
L-cysteine	0,3	0,18
Methionine	-	-
Choline birtrate	0,25	0,25

Table II.2. *Vitamin mixture content.*

COMPONENTS	AIN-93G (%)	AIN-93M (%)
Nicotinic acid	0,3	0,3
Panthotenate Ca	0,16	0,16
Pirydoxine-HCl	0,07	0,07
Thiamine-HCl	0,06	0,06
Riboflavin	0,06	0,06
Folic Acid	0,02	0,02
D-Biotin	0,002	0,002
Vitamin K	0,0075	0,0075
Vitamin B-12	0,25	0,25
Vitamin A	0,08	0,08
Vitamin D	0,025	0,025
Vitamin E	1,5	1,5
Powder sucrose	97,5	97,5

Table II.3. *Mineral mixture content.*

COMPONENTS	AIN-93G (%)	AIN-93M (%)
Essentials Elements		
Calcium biphasic phosphate	-	-
Calcium carbonate anhydride, 40,04% Ca	35,7	35,7
Potassium monophasic phosphate, 22,76% P; 28,73% K	19,6	25,0
Tripotassium monohydrate citrate	7,1	2,8
Sodium chloride	7,4	7,4
Potassium sulfate	4,7	4,7
Manganese oxide	2,4	2,4
Ferric Citrate	0,606	0,606
Zinc carbonate	0,165	0,165

Magnesium carbonate	0,063	0,063
Cupric carbonate	0,03	0,03
potassium Iodate	0,001	0,001
potassium Selenate	0,001025	0,001025
Ammonium paramolybdate	0,000795	0,000795
Potential Beneficial Elements		
Sodium metasilicate	0,145	0,145
Potassium chrome sulphate	0,0275	0,0275
Lithium chloride	0,00174	0,00815
Boric acid	0,00815	0,00635
Sodium fluorouridine	0,00635	0,00318
Nickel carbonate	0,00318	0,00174
Ammonium vanadate	0,00066	0,00066
Powder sucrose	22,1	21,0

Table II.4. *Fatty-acid composition of experimental oils (g/100g).*

Fatty Acid Composition	**Virgin Olive Oil**	**Sunflower Oil**	**Fish Oil**
Myristic acid (C14:0)	0,0	0,1	7,2
Palmitic acid (C16:0)	8,3	6,4	17,1
Stearic acid (C18:0)	3,2	4,7	2,7
Lignoceric acid (C24:0)	0,0	0,1	0,3
Total SFA	12,6	11,5	30,5
Hexadecenoic acid (C16:1n9)	1,1	0,1	9,6
Oleic acid (C18:1n9)	77,7	24,2	15,1
Nervonic acid (C24:1n9)	0,0	0,0	0,9
Total MUFA	83,7	24,4	30,1
Linoleic acid (C18:2n6)	3,2	62,8	2,8
DGLA (C20:3n6)	0,1	0,9	0,1
AA (C20:4n6)	0,0	0,0	2,1
EPA (C20:5n3)	0,2	0,1	18,6
DHA (C22:6n3)	0,0	0,0	10,5
Total PUFA	3,7	64,1	39,4
n-3 PUFA	0,4	0,4	31,3
n-6 PUFA	3,3	63,7	8,2
n-6/n-3 PUFA ratio	5,5	171,0	0,3
Oleic/Linoleic ratio	24,1	0,4	5,4

AA: arachidonic acid, DGLA: dihomo-γ-linolenic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids

The animals were treated in accordance with the guidelines of the Spanish Society for Laboratory Animals and the experiment was approved by the Ethical Committee of the University of Granada (permit number 20-CEA-2004). The rats were killed by cervical dislocation followed by decapitation, at the same time of the day to avoid any

circadian fluctuation. Blood was collected in ethylenediaminetetraacetic acid (EDTA)-coated tubes and the plasma was centrifuged at 1750 *xg* for 10 min. Plasma samples were stored at -80ºC until analyses. After exsanguination, pancreatic tissue was excised and properly preserved according to the analyses to be done (see forward).

II.2 TOTAL PROTEIN EXTRACTION AND DETERMINATION IN PANCREAS TISSUE

The total protein extraction was performed with the ReadyPrep™ protein extraction kit (Bio-Rad, Hercules, California, USA). Fifty milligrams of pancreatic tissue were added to 1ml of extraction buffer, 10µl of tributylphosphine (TBP) reduction agent and 10µl of a protease inhibitor, and were homogenized with a mechanic homogenizer, formed by a Teflon pestle (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany) and glass potter-Elvehjem (Sigma-Aldrich, St. Louis, Missouri, USA). The homogenate was centrifuged at 16.000 *xg* for 30 minutes at room temperature. The supernatant was transferred to a clean tube, and the pellet was discarded. Aliquots were made and stored at -80°C until its determination.

Total protein determination was performed using the RC DC (reducing agent and detergent compatible) protein assay kit (Bio-Rad, Hercules, California, USA).

The reagents were:

-DC Reagent S

-DC Reagent A

-DC Reagent B

-Reagent I

-Reagent II

Five microliters of DC Reagent S were added to 250µl of DC Reagent A, this solution is referred to as Reagent A'. Each standard or sample assayed required 127µl of Reagent A'. A standard curve was prepared with 3 dilutions of a protein standard from 0,2mg/ml to 1,5mg/ml. 25µl of standards and 1:10 diluted protein extraction was pipetted into microfuge tubes. One hundred and twenty five µl of RD Reagent I were added into each tube, vortexed and incubated for 1 minute at room temperature. Then, 125µl of RC Reagent II was added into each tube, vortexed and spinned at 14.000 *xg* for 7 minutes. After centrifugation, supernatants were discarded and 127µl of Reagent A' were added into each tube, vortexed and incubated at room temperature for 5 minutes. The microfuge tubes were newly vortexed and 1ml of DC Reagent B were added into each tube and vortexed immediately. Microfuge tubes were incubated at room temperature for 15 minutes. After the 15 minutes incubation, 200µl of standards and samples were added into a microplate and absorbances were read at 750nm in the microplate's reader Synergy HT Bio-Tek.

II.3 DETERMINATION OF CIRCULATING FATTY ACID PROFILE

Fatty acid analysis of biological specimens by gas-liquid chromatography requires solvent extraction, purification, and derivatization procedures that are both lengthy and heavy. Lepage and Roy proposed in 1986 a technique that avoids most of the preparative steps and consists in one step methylation and transesterification of samples.

One hundred microliters of plasma sample were added with 2 ml of a methanol:benzene (4:1) mixture and 200µl of acetyl chloride. Tubes were capped and vortexed, and they were incubated for 1 hour in a 100°C bath. After this time, the tubes were removed and allowed to cool at room temperature. Next, 2 ml of a solution of 5% potassium carbonate were added to stop reaction and neutralize. After that, for 15 min at 2500 *xg* centrifugation in order to separate the phases was performed. The upper benzenic phase was removed with a Pasteur pipette and preserved with the lipid fraction in a separate tube.

Thereafter, tubes were evaporated in a speed vacuum system (Scan Speed MaxiVac Alpha, Neutec Group, New York, USA) and the lipids remain in the bottom of the tube were resuspended in 50µl of hexane for analysis. A gas-liquid chromatograph model HP-5890 Series II (Hewlett Packard, Palo Alto, California, USA) equipped with a flame-ionization detector was used to analyze the fatty acids.

Chromatography was performed using a SPTM 2330 F.S column (Supelco Inc, Bellefonte, Palo Alto, California, USA), with 60-m-long, 32 mm id and 20 mm thickness. The method has a duration of 40 minutes and the following temperature ramp: initial temperature; 160°C for 5 min; 6°C/min to 195°C; 4°C/min to 220°C; 2°C/min to 230°C; hold 12 min at 230°C; and finally 14°C/min to 160°C.

Fatty acids analyzed were: C14:0, C16:0, C16:1n9, C18, C18:1n9, C18:2n6, C20:3n6, C20:4n6, C24:0, C24:1n9, C22:6n3. Post-analytical calculations were done to determinate SFA, MUFA, PUFA, n-6 PUFA and n-3 PUFA. The results have been expressed as a percentage of total fatty acids detected in the chromatogram. Figure 1 represents a typical chromatogram showing peaks and names of the fatty acids identified.

Figure II.1. Typical chromatogram showing peaks and names of the fatty acids identified

II.4 HISTOPATHOLOGICAL ANALYSIS

The pancreatic tissue taken from each rat was fixed in 10% formalin and embedded in paraffin, and then several 4 µm sections were cut for histological examinations, Masson´s Trichrome staining, Perl´s stain and immunohistochemistry. For histological examinations, the sections were stained with haematoxylin and eosin, Masson´s Trichrome and Perls staining. Evaluation of the pathological changes was performed by two experienced pathologists who were blinded to the treatment groups. When there were discrepancies, they discussed among themselves and reached a consensus outcome. Total fields per section (the full slide) were observed and the entire available pancreas was studied in order to avoid a focal observation of the same. Acinar atrophy was scored as 0, absent; 1, cytoplasmic basophilia reduction, acinar size reduction and reduction in the content of acinar zymogen granules; 2, acinar vacuolization, early destruction of acini, inflammation and early onset of fibrosis; 3, moderate; 4, severe with metaplasia and/or acinar dilatation (transformation of acini in cystic/ductular structures coated with cuboidal or flattened cells or the formation of small glands lined by cuboidal cells in a microcystic pattern or "honeycomb"). Acinar fat infiltration (presence of adipocytes in the pancreatic parenchyma) was scored as 0, absent; 1, very low number of cells and aggregates; 2, low; 3, moderate; 4, severe. Ductular hyperplasia was scored according to its presence as 1, absent; 2, present. Acinar fibrosis was scored as 0, absent; 1, perivenular fibrosis with few septa and thin; 2, poor, thin septa with fibrous incomplete bridges between regions; 3, thin septa with extensive bridges between regions; 4, septa thickened with numerous bridges and nodular appearance. Periinsular fibrosis was scored as 0, absent; 1, focal and mild increase of peripheral collagen deposition; 2, slight increase but diffuse; 3, moderate increase; 4, severe increase. Iron deposition was scored as 0, absent; 1, splashed positive macrophages; 2, mild positive macrophage accumulation; 3, moderate accumulations; 4, numerous Perls-positive macrophage accumulation.

II.5 IMMUNOHISTOCHEMISTRY FOR INSULIN AND GLUCAGON EXPRESSION

Insulin and glucagon immunohistochemistry were used to identify β and α cells, assess their density (percentage of β and α cells per islet area) and distribution in islets. For all immunohistochemical techniques, pancreatic sections were dewaxed in xylene and rehydrated through graded alcohols to water. Antigen retrieval was performed with the PTLink module (Dako, Demark) using Dako low-pH Antigen Retrieval (AR) fluid (Dako, Demark) followed by several washes in water before being placed onto an Autostainer Plus Link (Dako, Demark) where the remainder of the immunohistochemical staining was performed using Envision ™ FLEX (Dako, Demark). Briefly, sections were first placed in washing buffer followed by blockade of endogenous peroxidase with 3% hydrogen peroxide for 5 min. Then, the primary antibody Insulin (Clonal 2D11-H5)(Master Diagnóstica© MAD-021340Q, Granada, Spain) and polyclonal Glucagon (Master Diagnóstica©MAD-021325Q) were applied for 20 min, followed by another buffer wash and visualized with 3-3’ aminobenzidine

(DAB) for 10 min. Following a water wash, sections were counterstained in haematoxylin for 7 min, washed in water, dehydrated and coverslipped.

For the image analysis, islets were defined as clusters of seven or more β cells in association with other morphologically identifiable endocrine cells. All data were obtained from immunostained sections. Islet image capture was performed using a DP72 camera Olympus from an Olympus BX41 microscope (x20 objective) and analysed using AnaliSYS Image Processing software (Olympus , Tokyo, Japan). Islets were identified and images stored. Individual islet perimeters, islet size, total islet cell number, number and area of insulin positive (β) cells, (α) cells and the percentage of islet insulin and glucagon staining were semi-automatically determined. All insulin and glucagon positive cells in tissue sections were counted, including faintly positive cells.

II.6 PROLIFERATION ASSAY: KI-67 IMMUNOSTAINING

Paraffin-embedded pancreas sections were sectioned (3 µm thickness), placed on a glass slide, deparaffinized and rehydrated through a graded alcohol series. After the antigen-retrieval procedure at pH 6 (Dako Target Retrieval Solution. Dako, Glostrup, Denmark), primary rabbit antibody Ki-67 (clone SP6. Máster Diagnóstica, Granada, Spain) was used for 10 min. Once antigen-antibody reaction was performed in an automatic immunostaining system (Autostainer Plus Link. Dako, Glostrup, Denmark), sections were blocked and treated with immunodetection solution, consisting in a horse radish peroxidase and biotinylated secondary antibody for 30 min. Diluted 1:50 liquid DAB (Dako, Glostrup, Denmark) was used as a chromogenic agent and sections were counterstained in Meyer's hematoxylin. Negative controls were carried out by the same procedure without antibody. Positive exogenous controls were pharyngeal tonsil. Results were evaluated as the percentage of positive cells per islet in a total of ten islets by an experienced pathologist.

II.7 PROTEIN, HORMONE AND METABOLITE DETERMINATIONS

II.7.1 Total insulin, glucagon and leptin

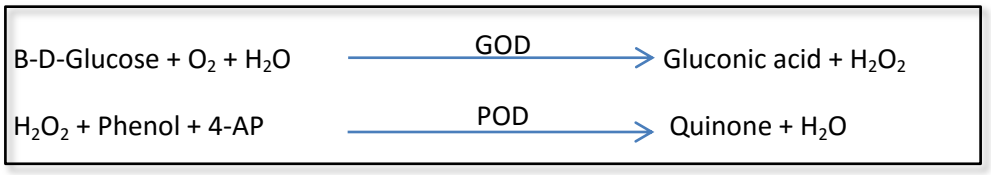
Frozen pancreatic portions (around 100 mg each) were used in order to analyze total insulin, total glucagon and total leptin. Previously, the frozen pancreatic portions were thawed and homogenized in 1 mL of alcohol:acid (50 parts of 95% ethanol:1 part of 10.2 N HCl) and then sonicated using a UP50H sonicator (Dr Hielscher, Teltow, Germany) for 3-5 pulses in an ice bath. After an overnight incubation at 4^0C, the homogenates were centrifuged at 650 *xg* for 30 minutes at 4^0C as indicated in (377). After that Total insulin was measured by radio-immuno assay (RIA) using the Coat-A-Count kit (Siemens, Los Angeles, CA). Total glucagon was measured by RIA using the Millipore kit (Billerica, MA). Leptin was determined by ELISA (LINCO, St Charles, MO). The protein content of the pancreatic homogenates was determined using the Bio-Rad assay.

II.7.2 Plasma clinical biochemistry

All the followings determinations was performed by commercial kits (Spinreact, Barcelona)

II.7.2.1 *Circulating glucose*

Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid according to reference (378). The formed hydrogen peroxide (H_2O_2) is detected by a chromogenic oxygen acceptor, phenol, 4-aminophenazone (4-AP) in the presence of peroxidase (POD). The intensity of the color formed is proportional to the glucose concentration in the sample.



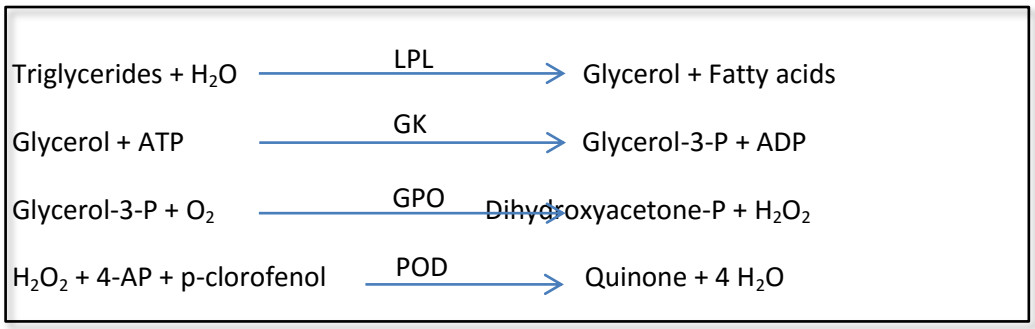
For such determinations were required 3 types of vials: the blank vial, the standard vial and the sample vials. The blank vial was only added 1ml of reagent (Phenol, GOD, POD and 4-AP). For standard vial was added 1ml of reagent and 10µl of glucose standard (100mg/dL). For each sample vial was added 1ml of reagent and 10µl of sample. All vials were mixed and incubated for 10 minutes at 37ºC. Subsequently, the absorbance (A) was measured by spectrophotometry at 505nm with a centimeter cuvette in respect to blank vial. Spectrophotometer was adjusted to zero with distilled water.

Calculation of each concentration was obtained by the following formula:

$$\frac{(A)\ sample}{(A)\ standard}\ x\ 100\ (standard\ concentration)\ =\ mg/dL\ glucose\ in\ the\ sample$$

II.7.2.2 *Circulating triglycerides*

Circulating triglycerides was determined according to reference (379) from coupled reactions of reactions of lipoprotein-lipase, glycerol-kinase, glycerol phosphate oxidase and peroxidase, giving Quinone as final product which is proportional to the amount of triglycerides



LPL: Lipoprotein-lipase ; GK: Glycerol-kinase; GPO: glicerol phosphate oxidase

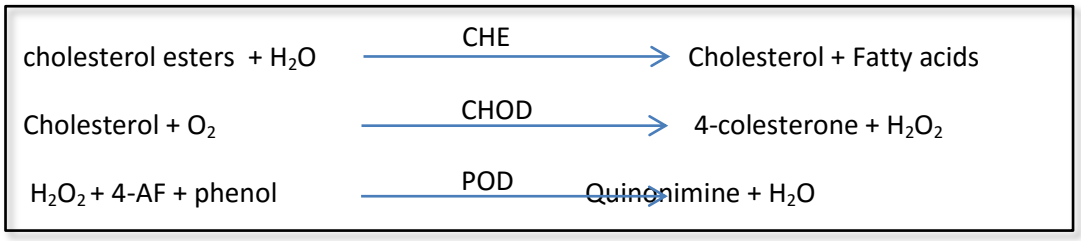
For such determination, were required 3 types of vials: the blank vial, the standard vial and the sample vials. The blank vial was only added 200µl of enzymatic mixture(LPL, GK, GPO, POD and 4-AP). For the standard vial were added 2µl of triglyceride standards (200mg/dL) and 200 µl of enzymatic mixture. For each sample vial was added 2µl of each sample and 200 µl of enzymatic mixture. All vials was shaken and incubated for 5 minutes at 37ºC. Subsequently, absorbance was measured by spectrophotometry at 505nm with a centimeter cuvette in respect to blank vial. Spectrophotometer was adjusted to zero with distilled water.

Calculation of each concentration was obtained by the following formula:

$$\frac{(A)\ sample}{(A)\ standard}\ x\ 200\ (standard\ concentration)\ =\ mg/dL\ triglycerides\ in\ the\ sample$$

II.7.2.3 *Circulating cholesterol*

Circulating cholesterol was measured by a commercial kit based on enzymatic-colorimetric reactions according to reference (379).



CHE: cholesterol esterase; CHOD: cholesterol oxidase

For such determinations were required 3 types of vials: the blank vial, the standard vial and the sample vials. For the blank vial was only added 50µl of enzymatic mixture (POD, CHE, CHOD and 4-aminoantipyrine). For the standard vial was added 2,5µl of cholesterol standards (200mg/dL) and 50 µl of enzymatic mixture. For each sample vial was added 2,5µl of sample and 50µl of enzymatic mixture. All vials were shaken and absorbance (A) was determined by spectrophotometry at 505nm after

incubation at 37ºC for 5 minutes, using cuvettes of a centimeter thick. The spectrophotometer was adjusted to Zero with distilled water.

Calculation of each concentration was obtained by the following formula:

$$\frac{(A)\ sample}{(A)\ standard}\ x\ 200\ (standard\ concentration)\ =\ mg/dL\ cholesterol\ in\ the\ sample$$

II.7.2.4 *Circulating total lipids*

Concentration of circulating total lipids was measured through a colorimetric commercial kit according to reference (380). Total lipids are treated with sulfuric acid and subsequently with phosphovainilline, giving pinkness color proportional to lipid concentration in sample. For such determinations were required a blank, a standard and the sample tube. In the sample tubes were added 2µl of each sample and 50µl of sulfuric acid, in the blank tube was added 50µl of sulfuric acid, and in the standard tube 2µl of lipid standard (750mg/dL) and 50µl of sulfuric acid were added.

Tubes were shaken and got into water-bath canner for 10 minutes at 100ºC. Later, it was taken 8µl of supernatant and was added 160µl of phosphovainilline. After vigorous shaking, they were incubated for 15 minutes at 37ºC. Absorbance (A) was measured by spectrophotometry at 520nm in respect to blank.

Calculation of each concentration was obtained by the following formula:

$$\frac{(A)\ sample}{(A)\ standard}\ x\ 750(standard\ concentration)\ =\ mg/dL\ total\ lipids\ in\ the\ sample$$

II.7.2.5 *Circulating enzymatic markers*

Circulating enzymatic markers were measured as indicated in (381).

II.7.2.5.1 Circulating phosphatase alkaline

Phosphatase alkaline (PAL) catalyzes p-nitrophenylphosphate (p-NPP) hydrolysis at pH 10,4 releasing p-nitrophenol and phosphate according the following reaction:

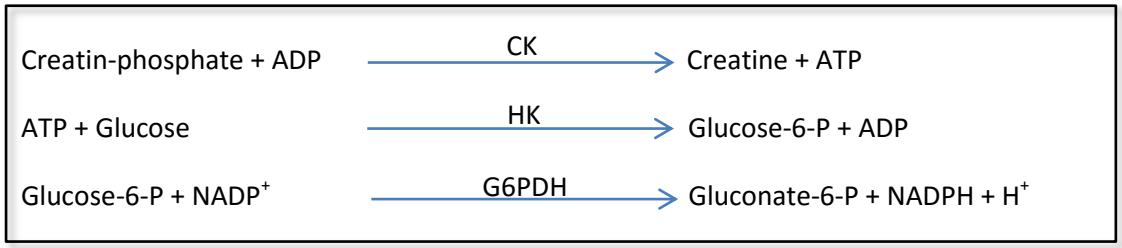
Initially it is dissolved a compressed of p-NPP on 15ml of buffer. In order to measure PAL, 75µl of working reactive and 7,5µl of sample were added into an

eppendorf. Eppendorf content was mixed and was subsequently measured in spectrophotometer at first and fourth minute after mixture. Such measurements were made in respect to air at 405nm. Calculation of each concentration was determined the mean of increment of extinction per minute (ΔE/min).

ΔE/min x 3300 = U/L

II.7.2.5.2 Circulating creatine kinase

Concentration of plasma creatine kinase (CK) was determined by a Kinetic test after activation through N-acetylcysteine.



HK: Hexoquinase; G6PDH: Glucose-6P-Dehydrogenase

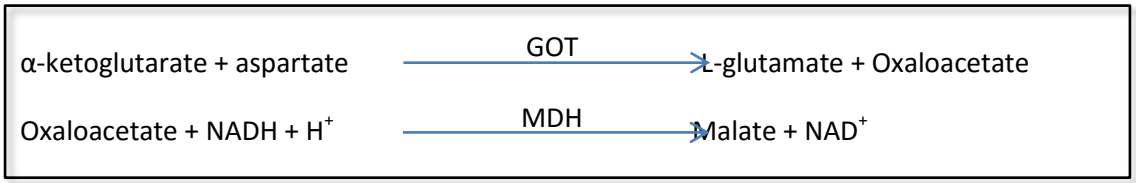
Oxidation from $NADP^+$ to NADPH is directly proportional to CK activity. For such determination was performed a semi-microtest. Firstly a compressed which contents $NADP^+$, adenosine diphosphate (ADP), adenosine monophosphate (AMP), diadenosine-5-P, hexoquinase, G6PDH, N-acetylcysteine, and creatin-phosphate is dissolved into 2,5ml of buffer. 62,5 µl from this monoreagent and 2,5µl of sample were added and mixed in an eppendorf, immediately incubated for 2 minutes and measured in spectrophotometer.

It was also measured after 5 minutes from mixture. Such measurements were made in respect to air at 340nm. In order to calculate such concentration it was determined the mean of increment of extinction per minute (ΔE/min), taking into account that such determination was performed at 25-30 ºC

ΔE/min x 4127 = U/L

II.7.2.5.3 Circulating glutamyl oxaloacetic transaminase

Concentration of plasma Circulating glutamyl oxaloacetic transaminase (GOT) was determined by a kinetic test according to the following reactions:



MDH: Malate Dehydrogenase

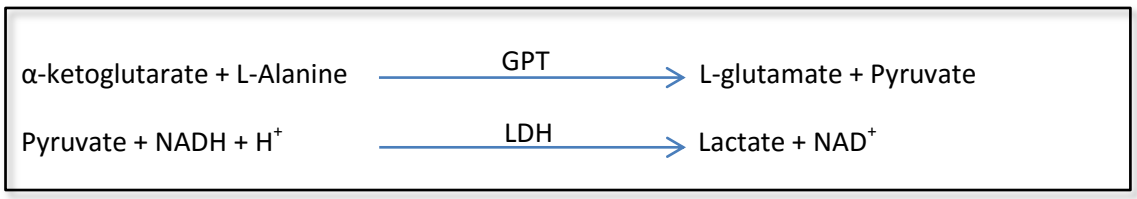
Oxidation from NADH to NAD^+ is directly proportional to GOT activity. For such determination was performed a semi-microtest. Firstly a compressed which contents NADH, lactate dehydrogenase (LDH), MDH and α-ketoglutarate is dissolved into 15ml of buffer. 75µl from this monoreagent and 7,5µl of sample were added and mixed into a eppendorf. One minute after mixture reading were taken in spectrophotometer at 1 and 3 minutes. Such measurements were made in respect to air at 340nm with a centimeter cuvette.

In order to calculate such concentration it was determined the mean of increment of extinction per minute (ΔE/min)

$$\Delta E/min \times 1750 = U/L$$

II.7.2.5.4 Circulating glutamic pyruvic transaminase (GPT)

Concentration of plasma GPT was determined by a commercial kit based on kinetic test.



Oxidation from NADH to NAD^+ is directly proportional to GPT activity. For such determination was performed a semi-microtest. Firstly a compressed which contents NADH, LDH, and α-ketoglutarate is dissolved into 15ml of buffer. 75µl from this monoreagent and 7,5µl of sample were added and mixed in a eppendorf. After mixture, reading were taken in spectrophotometer at 1 and 4 minutes. Such measurements were made in respect to air at 340nm with a centimeter cuvette at room temperature.

In order to calculate such concentration it was determined the mean of increment of extinction per minute (ΔE/min).

$$\Delta E/min \times 1750 = U/L$$

II.7.2.5.5 Circulating lactate dehydrogenase

Concentration of plasma LDH was determined by a commercial kit based on kinetic test.

Oxidation from NADH to NAD^+ in addition to a decrease of absorbance at 340nm is directly proportional to LDH activity. For such determination, firstly a compressed which contents NADH is dissolved into 3ml of buffer. 75µl from this monoreagent and 7,5µl of sample were added and mixed in a eppendorf. One minute after mixture, reading was taken in spectrophotometer at 1 and 3 minutes. Such measurements were made in respect to air at 340nm. Such measurements were made in respect to air at 340nm.

In order to calculate such concentration it was determined the mean of increment of extinction per minute (ΔE/min)

$$\Delta E/min \times 4925 = U/L$$

II.7.2.6 *Circulating Creatinine*

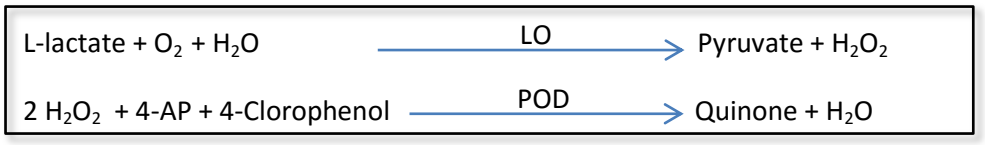
The creatinine assay is based on Jaffé direct reaction of creatinine with alkaline picrate forming a red complex which intensity is directly proportional to creatinine concentration of sample (382). For such determinations were required 3 types of vials: the blank vial, the standard vial and the sample vials. The blank vial was only added 1ml of working reagent (picric acid and sodium hydroxide). For standard vial was added 1ml of working reagent and 100µl of creatinine standard (2mg/dL). For each sample vial was added 1ml of working reagent and 100µl of sample. Every vial was mixed before starting and measurements were taken at 30 seconds (A_1) and 90 seconds (A_2) in the spectrophotometer, from which we can obtain its remainder ($\Delta A = A_2 - A_1$) . Measurements were made at 492nm with a centimeter cuvette at 37ºC. Spectrophotometer was adjusted to zero in respect to the blank vial.

In order to calculate such concentration, it was used the following equation:

$$\frac{\Delta A\ sample}{\Delta A\ standard}\ x\ 2\ (standard\ concentration)\ =\ mg/dL\ \ creatinine\ in\ the\ sample$$

II.7.2.7 *Circulating lactate*

The method consists in the oxidation of lactate by lactate oxidase (LO) to pyruvate and H_2O_2 according to reference (383). Which under the influence of POD, 4-4-AP and 4-chlorophenol form a red quinone compound.



The intensity of the color formed is proportional to the lactate concentration in the sample. For such determinations were required 3 types of vials: the blank vial, the standard vial and the sample vials. The blank vial was only added 1ml of working reagent (made of a buffer with 4-clophenol and an enzymatic component with LO, POD and 4-AP). For standard vial was added 1ml of working reagent and 10µl of lactate standard (10mg/dL). For each sample vial was added 1ml of working reagent and 10µl of sample. Every vial was mixed and subsequently incubated for 5 minutes at 37ºC. Then, the absorbance (A) was read at 505nm with a centimeter cuvette. Spectrophotometer was adjusted to zero in respect to distilled water.

In order to calculate such concentration, it was used the following equation:

$$\frac{(\mathrm{A})Sample - (A)Blank}{(\mathrm{A})Standard - (A)Blank}\ x\ 10\ (Standard\ concentration) = mg/dL\ lactate\ in\ the\ sample$$

II.7.2.8 *Homeostasis model assessment index (HOMA)*

HOMA was calculated according to (384) by the equation:

HOMA = fasting glucose (nmol/L) x fasting insuling (µUl/ml)/22.5

II.8 PROTEIN CARBONYLS ASSAY

Oxidative stress and ROS lead protein oxidation producing carbonyl groups (aldehydes and ketones) on protein side chains (especially of proline, arginine, lysine and threonine) when they are oxidized. The usage of protein carbonyl groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins.

The levels of protein carbonyl groups in pancreas were assessed using Protein Carbonyl Kit (Cayman Chemical Company, Michigan, USA), which is a convenient colorimetric assay for the measurement of oxidized proteins. Protein samples are derivatized by making use of the reaction between 2,4-dinitrophenylhydrazine (DNPH) and protein carbonyls. Formation of a Schiff base produces the corresponding hydrazone which can be analyzed spectrophotometrically at 360-385 nm.

Briefly, 100µl of pancreas protein extract were transferred to two tubes. One tube was the sample tube and the other was the control tube. After adding 400µl of DNPH to the sample tube and 400µl of 2.5 M HCl to the control tube, both of them were incubated in the dark at room temperature for 1 h, stirring them shortly every 15 minutes. Afterwards, 0.5 ml of 20% trichloroacetic acid was added to each tube and incubated in ice for 5 min. This mixture was centrifuged at 10.000 *xg* for 10 min at 4ºC, obtaining a pellet that was resuspended in 0.5 ml of 10% trichloroacetic acid and incubated in ice for 5 min and again centrifuged at 10.000 *xg* for 10 min at 4ºC. The pellet obtained was resuspended in 0.5 ml of (1:1) ethanol/ethyl acetate mixture and centrifuged at 10.000 *xg* for 10 min at 4ºC twice. Finally, the pellet obtained was resuspended in 250µl of guanidine hydrochloride and centrifuged at 10.000 *xg* for 10 min at 4ºC, obtaining a supernatant of which 220µl were transferred to a 96-well plate and absorbance read in a microplate's reader SYNERGY HT BIO-TEK at 370 nm. Total protein concentration in pancreatic samples extracts was measured using Pierce® BCA Protein Assay (Thermo scientific, Rockford, Illinois, USA) following the manufactures recommendations. The results were expressed as nmol of carbonyl proteins per mg of total proteins in the extract.

II.9 REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION

Pancreatic portions, previously preserved in RNAlater and stored at -80°C were used. Total RNA was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen , Hilden, Germany) following manufacturer's condition. The quantity and purity of the RNA were determined form the absorbance at 260/280 nm. 20ng of total RNA was reverse-transcribed into cDNA using Multiscribe enzyme (Applied Biosystems, Foster city, USA) in accordance with the manufacturer's protocol. Abi 7900 Real-Time PCR (polymerase chain reaction) system and real time PCR kit (TaqMan® Gene Expression Assays, Applied Biosystems, Foster city, USA) were employed based on the manufacturer's instruction. Probes for Bcl2, Bad and TNFα were used, as well as for 18S RNA, which was used as an internal control. PCR thermal cycling were development in three steps. Step 1 at 50°C during 2 minutes. Step 2 at 95°C during 10 minutes and a finally step with 40 cycles at 95°C during 15 seconds and 60°C during 1 minute.

II.10 STATISTICAL ANALYSES

Data are expressed as means ± standard error of the mean (SEM) of 6 animals. For quantitative parameters, prior to any statistical analysis, all variables were checked for normality and homogeneity of variance using the Kolmogorov-Smirnoff and the Levene tests, respectively. When a variable was found not to follow normality, it was log-transformed and reanalyzed. To evaluate differences in the means between groups one way analysis of variance (ANOVA) adjusted by Bonferroni correction was performed. For semiquantitative parameters (histological analysis), differences were analyzed by the Kruskal-Wallis test, and a Mann-Whitney U test was used a posteriori to evaluate mean differences between groups. For all statistical analysis, a value of $P<0.05$ was considered significant. Data were analysed using IBM® SPSS® Statistics 20 (IBM Corp. Armonk, New York, USA) statistical package.

RESULTS

In order to facilitate the designation of each group, it will be used the following abbreviated nomenclature. Animal group fed on virgin olive oil, fish oil or sunflower oil will be named as VOOF, FOF, or SOF, respectively. Their homologues supplemented with CoQ_{10} will be named as VOOFQ, FOFQ or SOFQ, respectively. Results and discussion obtained were performed separately between non-supplemented groups and supplemented groups according to the annexed papers, although in some cases differences between both groups may be mentioned.

I. DIETARY FAT EFFECTS ON PANCREAS

I.1 BODY WEIGHT EVOLUTION AND ADAPTATION TO THE DIET

After the 24 month follow-up period, non-supplemented rats did not present significant differences of weight, being FOF animals those that presented the highest values (604 ± 23.3 g), followed by VOOF rats (534±51.8 g) and finally by SOF animals (508.3±19.5 g). From the observation of food spillage, no differences concerning food intake were inferred between dietary groups.

As the differences among groups are according to dietary fat sources, which each one has a predominant fatty acid, it was required to verify a proper adaptation to the diet of the animals in order to attribute the possible differences among groups, so a circulating fatty acid profile was performed (Table I.1). As global result, it was observed that the adaptation expected was achieved, so that levels of circulating MUFAs was significant higher in VOOF than levels found in SOF as well as FOF, being oleic acid (C18:1n9) the most affluent in VOOF animals. On the other hand, levels of PUFAs were significantly higher in FOF and SOF groups in respect to VOOF group, even though SOF animals obtained significantly higher levels of linoleic (C18:2n6) and arachidonic (C20:4n6) acids, and FOF animals did it with significantly higher levels of eicosapentaenoic (C20:5n3) and docosahexaenoic (C22:6n3) acids. Altogether, the percentage of circulating MUFAs was significantly higher in VOOF rats, circulating n-6 PUFAs were significantly higher in SOF animals, and n-3 PUFAs were higher in FOF rats. Circulating saturated fatty acids levels were also measured, obtaining similar results in all groups, being the most representative fatty acids, palmitic (C16:0) and stearic (C18:0) acids, in this order.

Table I.1: Circulating fatty acid profile of VOOF, SOF and FOF rats

Fatty acid	VOOF	SOF	FOF
Myristic (C14:0)	0.7±0.1	0.6±0.0	1.1±0.1*
Palmitic (C16:0)	20.9±1.3	20.8±0.5	25.8±1.3*
Stearic (C18:0)	12.4±0.5	18.8±0.5*	9.9±0.5*
Lignoceric (C24:0)	0.8±0.1	1.6±0.4*	0.8±0.3
Saturated	37.2±1.5	38.5±1.1	40.6±1.7
Hexadecenoic (C16:1n9)	5.5±0.5	3.6±0.6*	6.7±0.3*
Oleic (C18:1n9)	26.9±5.3	10.5±5.3*	11.9±3.6*
Nervonic (C24:1n9)	0.9±0.2	1.0±0.3	1.5±0.2
Total MUFA	40.6±1.8	27.9±3.3*	31.6±2.4*
Linoleic (C18:2n6)	7.3±0.4	14.7±2.1*	2.3±0.7*
DGLA (C20:3n6)	0.3±0.1	0.4±0.1	0.2±0.0
AA (C20:4n6)	6.7±0.2	15.7±1.9*	5.8±0.4*
EPA (C20:5n3)	0.2±0.1	0.1±0.0*	8.4±1.1*
DHA (C22:6n3)	1.9±0.2	0.6±0.3*	9.0±0.6*
Total PUFA	22.2±0.7	33.6±2.5*	27.8±1.3*
n-3PUFA	2.4±0.1	1.3±0.2	18.3±1.2*
n-6PUFA	19.8±0.7	32.2±2.7*	9.4±0.4*

Notes: Results are expressed as percentage of total fatty acids. AA: arachidonic acid, DGLA: dihomo-γ-linolenic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, FOF: fish oil fed group; MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids; SOF: sunflower oil fed group; VOOF: virgin olive oil fed group. (*) $P < .05$ respect to VOOF rats.

I.2 Circulating Hormone Levels and Biochemical Parameters

Concerning circulating hormone levels (insulin and leptin), it was found significant differences on leptin levels in SOF animals, obtaining higher levels than VOOF and FOF animals, which obtained very similar results. No differences between groups were found concerning insulin values (Table I.2).

Biochemical parameters were analyzed in order to compare glycemic and lipid levels, including triglycerides, cholesterol and phospholipids. Values were not significantly different among the groups, although VOOF displayed the highest values on circulating total lipid levels. In addition to this, biochemical parameters served for measuring markers of several tissue injuries, such as GPT (marker of hepatic injury), alkaline phosphatase (marker of liver, bile ducts and bone injury), GOT (marker of muscle, heart and liver injury), CK (marker of Skeletal muscle injury), LDH (marker of hemolysis and general tissue integrity), and creatinine (marker of renal injury). Likewise, comparing VOOF with SOF and FOF independently, no significant differences were found on these parameters, even obtaining very similar values, excepting alkaline phosphatase and GTP which were significantly higher in FOF group (Table I.2). Finally homeostasis model assessment (HOMA) values did not showed significant pathological situation of insulin resistance or loss of insulin sensitivity in all groups of animals (Table I.2).

I.3 Histological examination

I.3.1 Pancreatic Parenchyma

Figure I.1 represents the histology of the pancreatic endocrine and exocrine compartment. The most important features have been quantified and represented in figure I.2.

In the endocrine pancreas, the group which displayed the less injured histology was VOOF group (reference group), without even signs of fibrosis (Figures I.1A and I.2F). On the other hand, SOF group showed grade 2 peri-insular fibrosis with macrophage infiltrates (Figure I.1B) and FOF animals displayed grade 4 peri-insular fibrosis with clear macrophage infiltrates (Figure I.1C) and even iron deposits (Figure I.2G). However, only in FOF it was found significant differences with respect to VOOF group ($P<0.05$).

Concerning exocrine pancreas, VOOF showed again the less injuried pancreas (Figure I.1J), without evident signs of acinar atrophy, fat infiltration, dilatation, fibrosis (Figures I.2A-C and E) or ductal hyperplasia (Figure I.2D). On the contrary, the other groups showed some signs of alteration. In SOF case, it was observed grade 3 acinar atrophy with vacuolization and interstitial lymphocyte infiltrates (Figure I.1K). Additional significant histological signs included ductal hyperplasia (Figure I.2D),

whereas no significant differences with respect to VOOF rats observed regarding atrophy, dilation, fat infiltration or fibrosis (Figure I.2A-C and E). In FOF case, it was observed grade 4 acinar atrophy with clear signs of cystic structure and ducts coated by plate cells (Figure I.1L). Likewise, it was found significant differences compared with VOOF group about acinar atrophy, fat infiltration (which was also significantly different to SOF), dilation, fibrosis (Figure I.2A-C and E) and ductal hyperplasia (figure I.2D).

Table I.2: Plasma circulating parameters in 24 months old rats fed on virgin olive, sunflower or fish oils.

Parameter	VOOF	SOF	FOF
Insulin (pg/mL)	190.9±47.7	330.2±98.5	246.6±69.8
Leptin (pg/mL)	17490.6±660.2	30650.9±7888.6*	14029.1±2520.7
Glucose (mM)	6.7±0.6	6.8±0.6	6.1±0.4
HOMA	1.5±0.4	2.3±0.6	1.7±0.5
Triglycerides (mM)	1.9±0.6	1.4±0.2	1.2±0.2
Cholesterol (mM)	2.5±0.4	2.2±0.3	1.8±0.3
Phospholipids (mM)	1.3±0.3	1.0±0.1	0.8±0.1
Total lipids (mg/dL)	493.1±83.2	434.1±62.3	339.7±65.6
ALP (U/mL)	63.5±8.3	57.2±6.6	104.6±9.2*
CK (U/mL)	399.2±143.6	433.1±104.8	485.4±82.5
LDH (U/mL)	438.1±67.4	460.3±43.2	384.0±51.2
GOT (U/mL)	18.5±2.1	17.7±3.6	20.2±1.3
GPT (U/mL)	5.6±0.7	6.7±1.1	9.5±1.2*
Proteins (g/dL)	6.4±0.6	5.6±0.2	5.9±0.1
Creatinine (μM)	112.7±20.1	76.4±8.9	101.9±10.5
Lactate (mM)	3.1±0.3	3.6±0.6	3.1±0.3

Notes: ALP = alkaline phosphatase; CK = creatine kinase; FOF: fish oil fed group; GOT = glutamyl oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; HOMA = homeostasis model assessment; LDH = lactate dehydrogenase; SOF: sunflower oil fed group; VOOF = virgin olive oil–fed group. (*) $P < .05$ respect to VOOF rats.

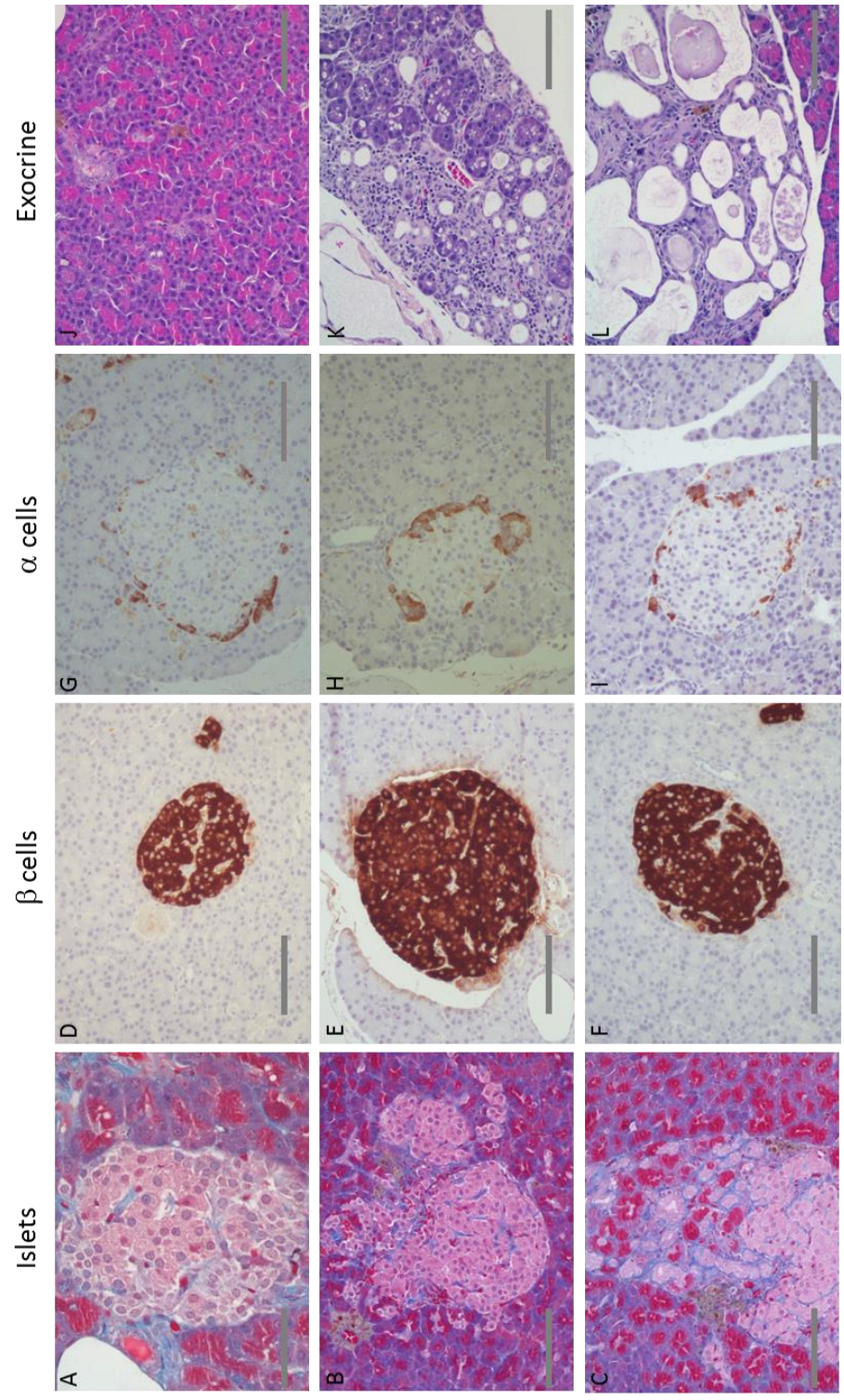


Figure I.1. Effects of feeding rats for twenty four months on different dietary fat sources (virgin olive, sunflower or fish oils) on histological changes in the pancreas. A: Tricrome Masson (TM) staining in the pancreas from virgin olive oil fed (VOOF) animals. Normal pancreatic islet. No evidence of fibrosis. B: TM staining in the pancreas from sunflower oil fed (SOF) animals. Pancreatic islet with periinsular fibrosis grade 2. Macrophages with the pigment hemosiderin are present. C: TM staining in the pancreas from fish oil fed (FOF) animals. Pancreatic islet with periinsular fibrosis grade 4. Macrophages with the pigment hemosiderin are present. D: Immunohistochemistry (IHC) of pancreas from VOOF animals with insulin antibody. E: IHC of pancreas from SOF animals with insulin antibody. F: IHC of pancreas from FOF animals with insulin antibody. G: IHC of pancreas from VOOF animals with glucagon antibody. H: IHC of pancreas from SOF animals with glucagon antibody. I: IHC of pancreas from FOF animals with glucagon antibody. J: Hematoxylin & Eosin (HE) staining in the pancreas from VOOF animals. Normal exocrine pancreas. Macrophages with the pigment hemosiderin are present. K: HE staining in the pancreas from SOF animals. Acinar vacuolation, intersticial lymphocytic infiltration. Acinar atrophy grade 3. L: HE staining in the pancreas from FOF animals. Acinar transformation with quistic or ductular structures coated with flattened cells. Acinar atrophy grade 4. Except for A (bar =50µm), bar = 100 µm.

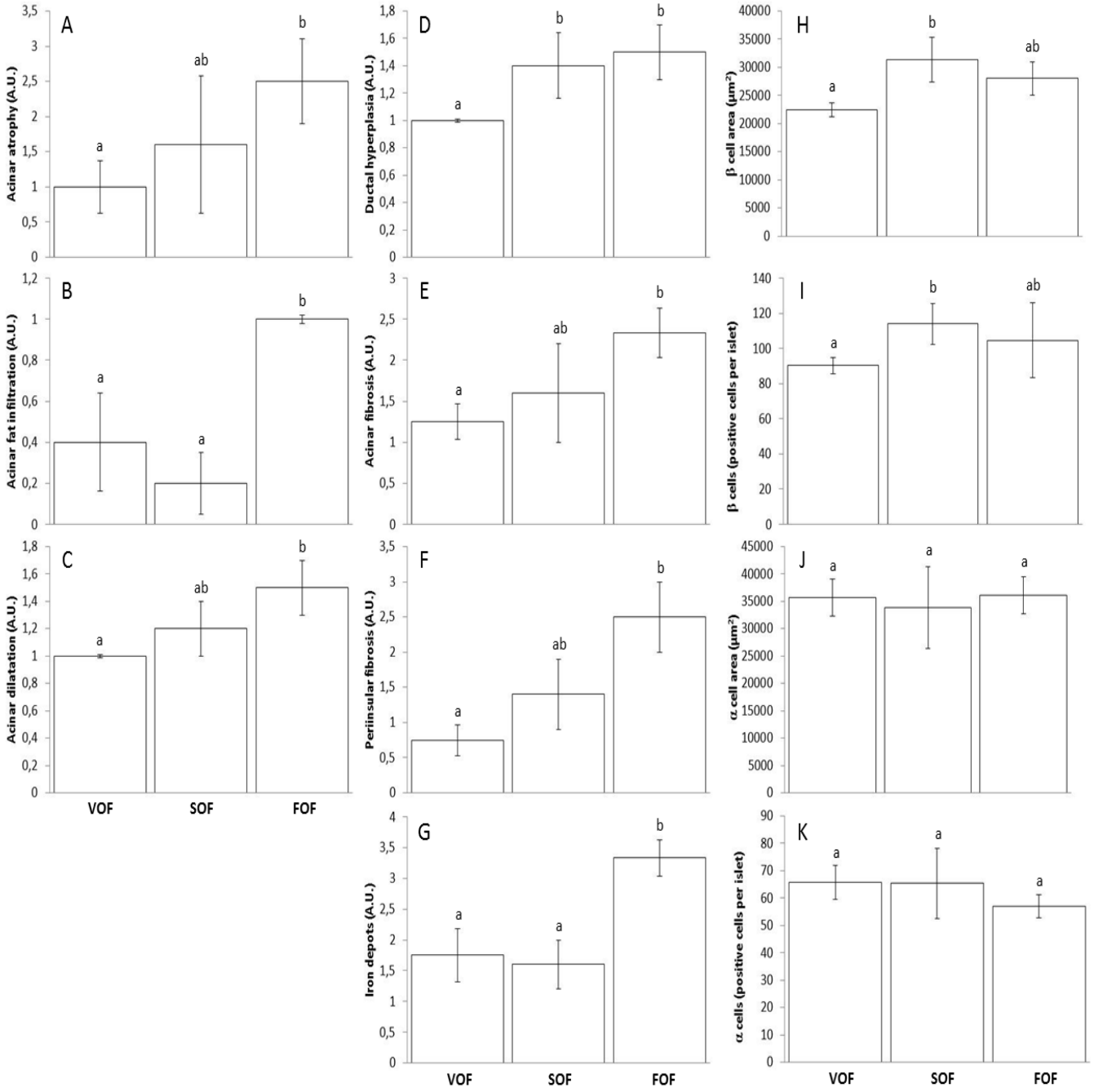


Figure I.2. Histological and immunohistochemistry (IHC) examinations in the pancreases of rats fed for 24 mo on different dietary fat sources (virgin olive, sunflower, or fish oils). (**A**–**G**) Data from histological examination. (**H**–**K**) Data from IHC analysis. Data are presented as mean ± *SEM*. Columns not sharing superscript letters are statistically different ($P < 0.05$). A.U. = arbitrary units.

I.3.2 Immunohistochemical assessment of pancreatic islets

The immunohistochemical analysis was performed in order to ascertain β and α cells distribution and density (percentage of β and α cells per islet area) by using glucagon and insulin immunohistochemistry, which thus, identify cell type (Figures I.1 and I.2). In this sense, it was observed significant higher values of insulin immunopositive area per islet in the SOF group (Figures I.1E, I.2H and I.2I) with respect to VOOF one (Figures I.1D, I.2H and I.2I). This increase may be correlated with an increase in the number of cells positive to the hormone per islet (more than an increment of delivery rate of insulin from cell). The number of insulin-positive cells in the FOF group obtained intermediated values between VOOF and SOF (Figures I.1F and I.2H and I.2I). However, concerning number of glucagon-positive cells no significant differences were found (Figures I.1G-I, I.2J and I.2K), suggesting that the islet changes seems to be β-cell specific.

I.4 PANCREATIC INSULIN AND GLUCAGON CONTENT

The histological and immunohistological studies suggested that the main alterations in the endocrine pancreas were found in the SOF group. To confirm the previous finding, a complementary analysis was performed, consisting in a measurement of insulin and glucagon pancreatic levels. As Table I.3 shows, no significant differences in insulin levels between VOOF and FOF were found, but SOF insulin levels doubled VOOF ones. Glucagon levels were similar in all groups.

Table I.3: Total insulin and glucagon contents in pancreas of 24 months old rats fed on virgin olive, sunflower or fish oils.

Parameter	VOOF	SOF	FOF
Insulin (pg/μg protein)	8028.7±2293.3	15166.6±3469.9*	9143.6±2271.8
Glucagon (pg/μg protein)	115.4±13.3	121.4±20.0	119.7±12.3

Data are presented as mean ± standard error of mean. Abbreviations: FOF: fish oil–fed group; SOF: sunflower oil–fed group; VOOF: virgin olive oil–fed group. (*) $P < .05$ respect to VOOF rats.

I.5 MECHANISTIC APPROACH TO THE OBSERVED PANCREATIC CHANGES

Proliferation, apoptotic, inflammation and oxidative stress markers were analyzed (Table I.4) in order to elucidate which feasible mechanism may be involved in the previous results in rats fed with different oils. To measure proliferation events, ki-67 test were performed, but no differences in number were found, suggesting very similar proliferation events in all groups. Expression of mitochondrial apoptotic markers was

also analyzed through Bcl2 and Bad, obtaining similar results in all groups. However, VOOF rats displayed significant lower amounts of tumor necrosis factor-α expression and protein carbonyl presence than SOF and FOF groups.

Table I.4: Ki67 proliferation assay, mRNA levels of apoptosis and inflammation related genes as well as protein oxidation in the pancreas of VOOF, SOF and FOF rats at the end of the experimental procedure (2 years).

Parameter	VOOF	SOF	FOF
Ki67 (positive cells/islet)	0.30±0.07	0.22±0.07	0.32±0.04
Bcl2 (RQ)	1.05±0.12	1.01±0.25	1.17±0.12
Bad (RQ)	1.28±0.12	1.03±0.06	1.17±0.05
TNFα (RQ)	0.69±0.17	1.34±0.32*	1.48±0.19*
Protein carbonyls (nmol/mg)	62.6±9.1	138.9±22.8*	177.5±19.5*

Notes: FOF = fish oil–fed group; mRNA = messenger RNA; RQ = relative quantity; SOF = sunflower oil–fed group; TNFα = tumor necrosis factor α; VOOF = virgin olive oil–fed group (*)$p < .05$ respect to VOOF rats.

II. DIETARY COENZYME Q EFFECTS ON PANCREAS

II.1 Body Weight Evolution and Adaptation to the Diet

Concerning supplemented groups, significant differences for body weight were observed in FOFQ group, being the group with the highest values (624± 20.0 g), the remaining supplemented animals, VOOFQ and SOFQ, led to similar body weight (496 ± 20.0 g) and (499.0 ± 6.6 g), respectively. From the observation of food spillage, no differences concerning food intake were inferred between groups. As its homologues without CoQ supplementation, the circulating fatty acid profile was assessed to verify a proper adaptation to the diet in order to attribute the possible differences among groups to them. Very similar results with respect to non-supplemented groups were obtained (Table II.1), indicating as well, a proper adaptation to the dietary fats.

II.2 Circulating Hormone Levels and Biochemical Parameters

Significantly lower concentration of insulin were found in FOFQ group compared to VOOFQ group. Higher, but not significantly different, concentration of leptin was found in FOFQ compared with SOFQ and VOOFQ groups (Table II.2).

Concerning the rest of biochemical parameters, very similar levels among groups were found with respect to glycaemia and serum lipids, including triglycerides, cholesterol and phospholipids. However, for total lipids, a lower concentration was observed in FOFQ group, and likewise HOMA index were lower in this group (Table II.2), which agree with the low insulin levels found in this group. Concerning the markers for tissue damage, FOFQ animals showed significantly higher levels of GOT with respect to SOFQ animals. Moreover, FOFQ group reported significantly higher levels of GPT with respect to SOFQ and VOOFQ groups (Table II.2).

Table II.1. Circulating fatty acid profile of VOOF, SOF and FOF rats supplemented with coenzyme Q.

Fatty acid	VOOFQ	SOFQ	FOFQ
Myristic (C14:0)	0.63±0.04	0.56±0.02	1.06±0.11*
Palmitic (C16:0)	20.13±1.13	20.90±0.92	24.40±1.44*
Stearic (C18:0)	12.63±0.49	13.47±0.28	9.77±0.17*
Lignoceric (C24:0)	0.94±0.27	1.69±0.30*	1.25±0.15
SFA	37.28±2.13	38.69±1.35	38.25±1.36
Hexadecenoic (C16:1n9)	4.64±0.55	3.48±0.18*	6.48±0.61*
Oleic (C18:1n9)	13.26±5.80	4.94±3.05	6.09±3.65
Nervonic (C24:1n9)	0.88±0.37	1.28±0.23	1.46±0.26
MUFA	38.70±4.05	28.21±2.34*	34.62±2.26
Linoleic (C18:2n6)	8.42±2.03	14.64±0.56*	2.60±0.50*
DGLA (C20:3n6)	0.28±0.09	0.36±0.05	0.19±0.05
AA (C20:4n6)	8.38±1.56	17.01±0.65*	5.78±0.51*
EPA (C20:5n3)	0.25±0.11	0.05±0.02*	9.05±0.67*
DHA (C22:6n3)	1.78±0.38	0.26±0.14*	7.95±0.54*
PUFA	24.03±2.64	33.10±1.21*	27.13±1.07
n-3PUFA	2.26±0.43	0.78±0.21*	18.06±0.86*
n-6PUFA	21.77±2.77	32.32±1.05*	9.07±0.56*

Notes: Results are expressed as percentage of total fatty acids. AA: arachidonic acid, DGLA: dihomo-γ-linolenic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids.FOFQ = fish oil–fed group plus CoQ_{10}; SOFQ = sunflower oil–fed group plus CoQ_{10}; VOOFQ = virgin olive oil–fed group plus CoQ_{10}. All supplemented with CoQ. (*) $P< 0.05$ respect to VOOFQ rats.

Table II.2. Circulating parameters in the plasma of 24 months old rats fed on different dietary fats (virgin olive, sunflower or fish oil) and supplemented with CoQ_{10}.

Parameter	VOOFQ	SOFQ	FOFQ
Insulin (pg/mL)	302.7±84.6	212.3±58.7	170.7±47.9*
Leptin (pg/mL)	14211.4±2966.2	13462.9±2275.2	21580.5±4152.9
Glucose (mM)	6.8±0.5	6.4±0.6	6.4±0.5
HOMA	2.3±0.7	1.6±0.5	1.2±0.4*
Triglycerides (mM)	1.5±0.3	1.4±0.3	1.1±0.1
Cholesterol (mM)	2.2±0.4	2.5±0.1	1.6±0.1
Phospholipids (mM)	1.1±0.1	1.0±0.1	0.8±0.1
Total lipids (mg/dL)	387.1±61.5	438.0±56.4	252.3±33.7*
ALP (U/mL)	103.3±13.0	70.3±12.7*	104.6±23.7
CK (U/mL)	407.7±117.9	443.4±101.2	379.2±85.6
LDH (U/mL)	421.1±53.9	471.6±60.7	408.4±19.1
GOT (U/mL)	15.9±1.11	6.8±1.8	20.9±2.4*
GPT (U/mL)	6.0±1.1	5.0±1.6	11.9±3.3*
Proteins (g/dL)	5.8±0.3	5.5±0.2	5.8±0.2
Creatinine (μM)	124.7±24.5	81.8±7.4*	96.5±8.0
Lactate (mM)	3.2±0.2	3.0±0.4	3.4±0.4

Data are presented as mean ± standard error of mean. Abbreviations: . FOFQ: fish oil fed group plus CoQ10; HOMA: homeostatic model assessment; CK: creatine kinase; GOT: glutamyl oxaloacetic transaminase; GPT: glutamic pyruvic transaminase; LDH: lactate dehydrogenase. SOFQ: sunflower oil–fed group plus CoQ_{10}; VOOFQ: virgin olive oil–fed group plus CoQ_{10}.
(*) P <0.05 respect to VOOQ fed rats.

II.3 HISTOLOGICAL EXAMINATION

II.3.1 Pancreatic Parenchyma

FOFQ group showed the highest number of alterations in pancreatic parenchyma. It was observed a clear acinar atrophy (Figure II.1C) accompanied by signs of inflammation (Figure II.1, F and I), and fibrosis, which was affecting the acinar (Figure II.2c) and the peri-insular compartment (Figure II.2f). Moreover, evidences of hyperplasia in the ductal region (Figure II.2i) as well as certain degree of acinar fat infiltration (Figure 1L) were detected in FOFQ group. Concerning SOFQ and VOOFQ groups, these showed a lower degree of alterations, without significant differences between them for most of the analyzed parameters (Figure II.3). VOOFQ group showed light signs of fibrosis (Figure II.2a), slight evidences of fat depots (Figure II.1j, k) and the ductal tissue and pancreatic blood vessel did not present signs of metaplasia and hyperplasia (Figure II.2 h, g). The only remarkable difference was that a slight degree of acinar atrophy was detected in VOOFQ group (Figure II.1a) whereas in SOFQ there were many samples which did not show such alteration (Figure II.1b).

II.3.2 Immunohistochemical assessment of pancreatic islets

Likewise as its non-supplemented counterparts, immunohistochemical analyses were performed in order to ascertain cell type and distribution in pancreatic islets. Thus, insulin and glucagon immunohistochemistry, identifying β (Figure II.4a) and α-cells (Figure II.4b) were evaluated. Supplemented groups did not reported significant differences concerning these parameters (Figure II.4c).

II.4 PANCREATIC INSULIN AND GLUCAGON CONTENT

The purpose of this analysis was to gain more insight into how histological changes could affect the endocrine pancreas compartment. For this reason, the main hormones produced by this compartment (insulin and glucagon) were measured. All groups obtained similar contents of both hormones, without significant differences among them (Table II.3).

Table II.3: Total insulin and glucagon contents in pancreas VOOF, SOF and FOF rats supplemented with coenzyme Q at the end of the experimental procedure (2 years).

Parameter	VOOFQ	SOFQ	FOFQ
Insulin (pg/μg protein)	18902.5±6989.5	14144±4588.5	19576±3720.3
Glucagon (pg/μg protein)	151.8±12.8	143±37.2	129.6±23.8

Data are presented as mean ± standard error of mean. Abbreviations: FOFQ: fish oil fed group supplemented on CoQ_{10}; SOFQ: sunflower oil fed group supplemented on CoQ_{10}; VOOF: virgin olive oil fed group supplemented on CoQ_{10}. (*) $P < .05$ respect to VOOFQ rats.

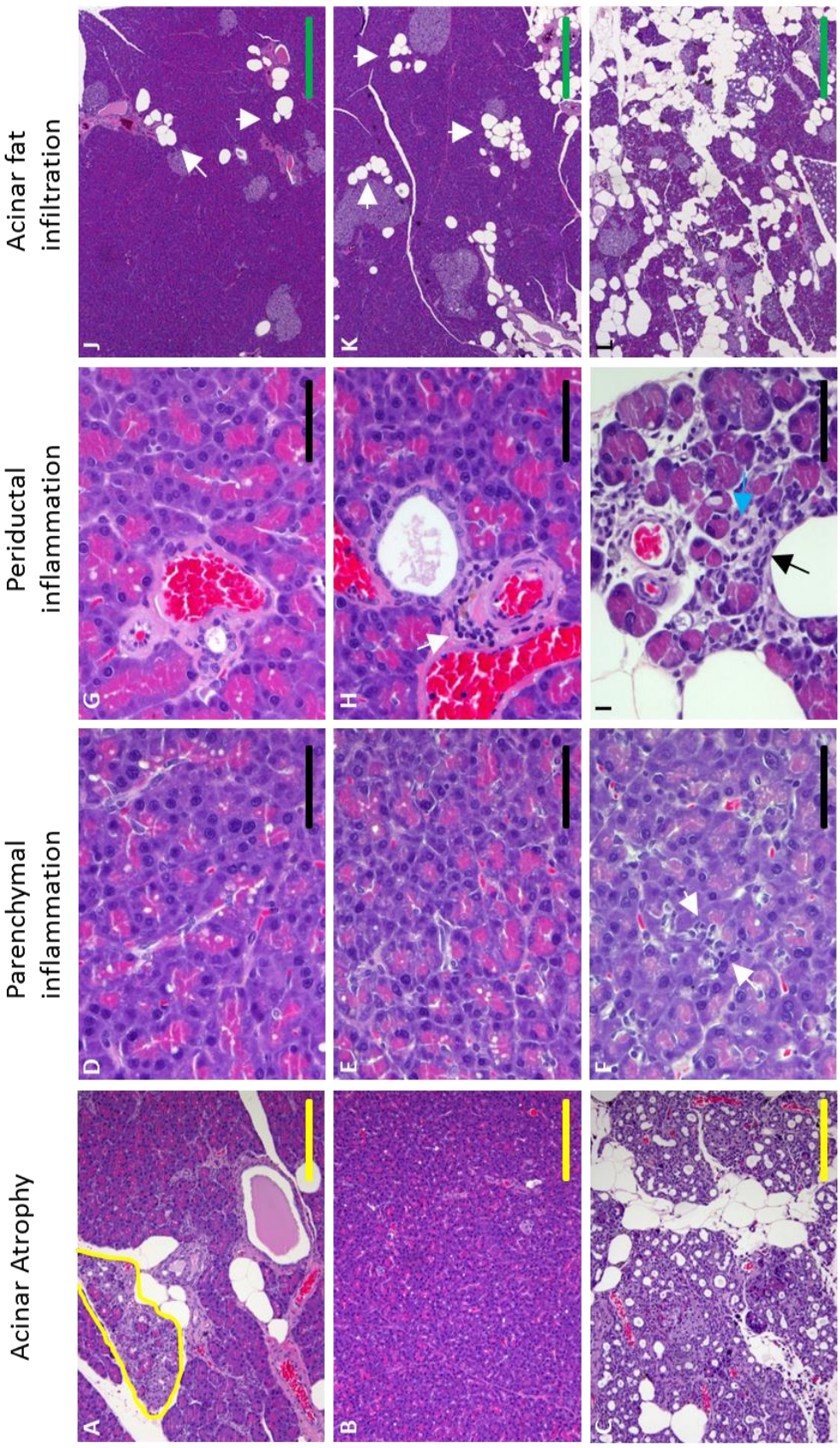


Figure II.1. Histological sections of pancreas from 24 months old rats fed on different dietary fat sources (virgin olive, sunflower or fish oils) supplemented with Coenzyme Q_{10} after hematoxilin and eosin staining for assessment of acinar atrophy, parenchymal and periductal inflammation and acinar fat infiltration, respectively. (**A**) Minimal acinar atrophy (indicated by a yellow circle); (**B**) Normal pancreatic tissues; (**C**) Severe acinar atrophy, grade 4; (**D**,**E**) Absence of inflammation; (**F**) Inflammatory cells (its presence is indicated by white arrows); (**G**) Absence of inflammation; (**H**) Minimal inflammation with some inflammatory cells (indicated by a white arrow); (**I**) More severe infiltrate (indicated by a black arrow) around the duct (indicated by a blue arrow); (**J**,**K**) Very low number of fatty cells and aggregates (grade 1–2) (indicated by white arrows); (**L**) Severe fat deposits (grade 3–4). Yellow bar = 200 µm; Black bar = 50 µm; Green bar = 500 µm. FOFQ: fish oil fed group plus CoQ_{10}; SOFQ: sunflower oil–fed group plus CoQ_{10}; VOOFQ: virgin olive oil–fed group plus CoQ_{10}.

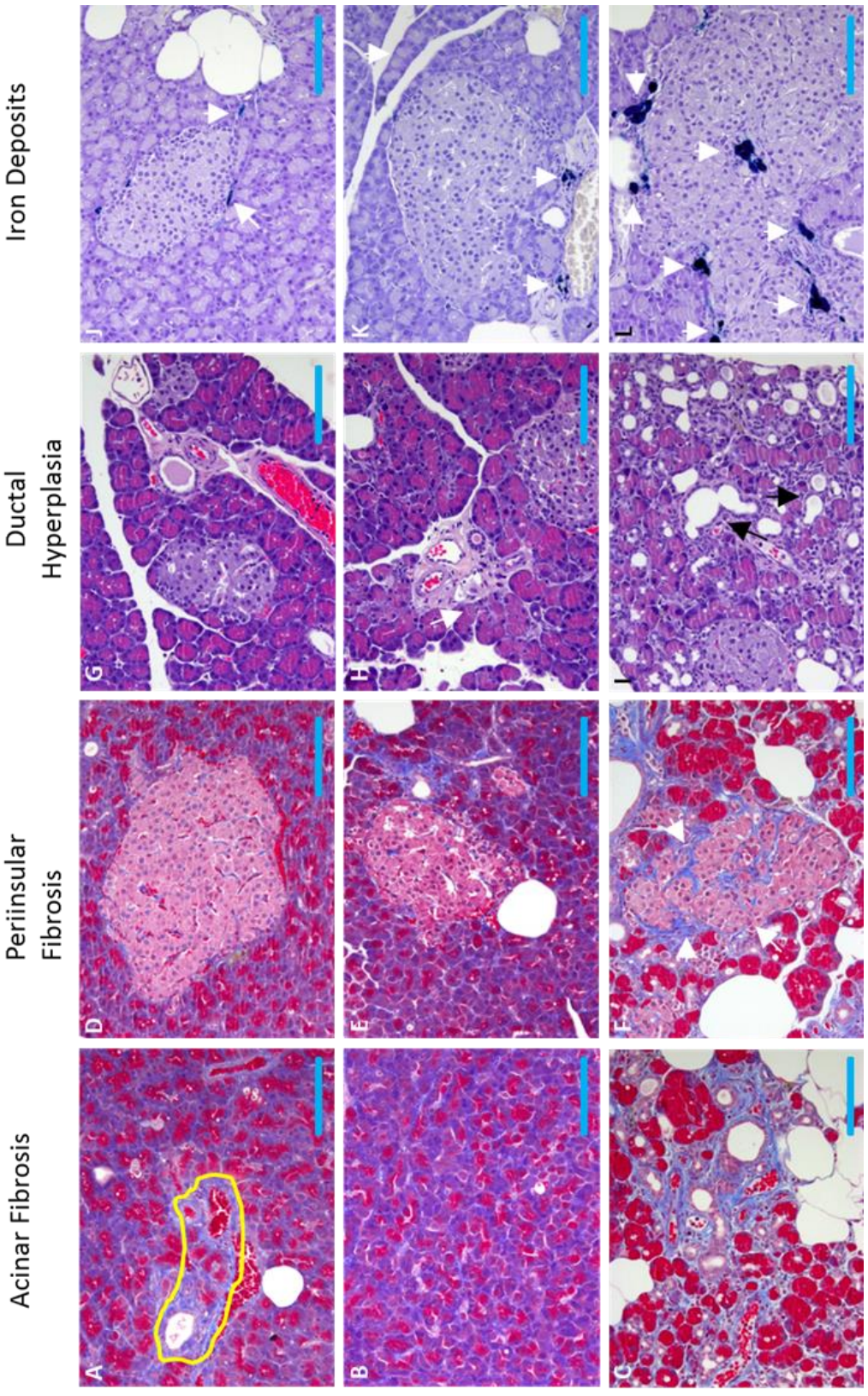


Figure II.2. Histological sections of pancreas from 24 months old rats fed on different dietary fat sources (virgin olive, sunflower or fish oils) supplemented with Coenzyme Q_{10} after Trichrome Masson (**a**–**f**), Hematoxilin and eosin (**g**–**i**) and Perls staining (**j**–**l**) for assessment of acinar and peri-insular fibrosis, ductal hyperplasia and iron deposits, respectively. (**a**) Minimal acinar fibrosis (indicated by a yellow circle); (**b**) Normal pancreatic acini; (**c**) Acinar fibrosis grade 3; (**d**,**e**) Absence of peri-insular fibrosis; (**f**) Moderate fibrosis; (**g**,**h**) Absence of ductular hyperplasia; (**i**) Ductular hyperplasia (indicated by black arrows); (**j**,**k**) Very low number of macrophages stained for hemosiderin (grade 1) with peri-insular distribution (indicated by white arrows); (**l**) High number of macrophages stained for hemosiderin (grade 3–4), (hemosiderin deposited are indicated by white arrows). Blue bar = 100 µm. FOFQ: fish oil fed group plus CoQ_{10}; SOFQ: sunflower oil–fed group plus CoQ_{10}; VOOFQ: virgin olive oil–fed group plus CoQ_{10}.

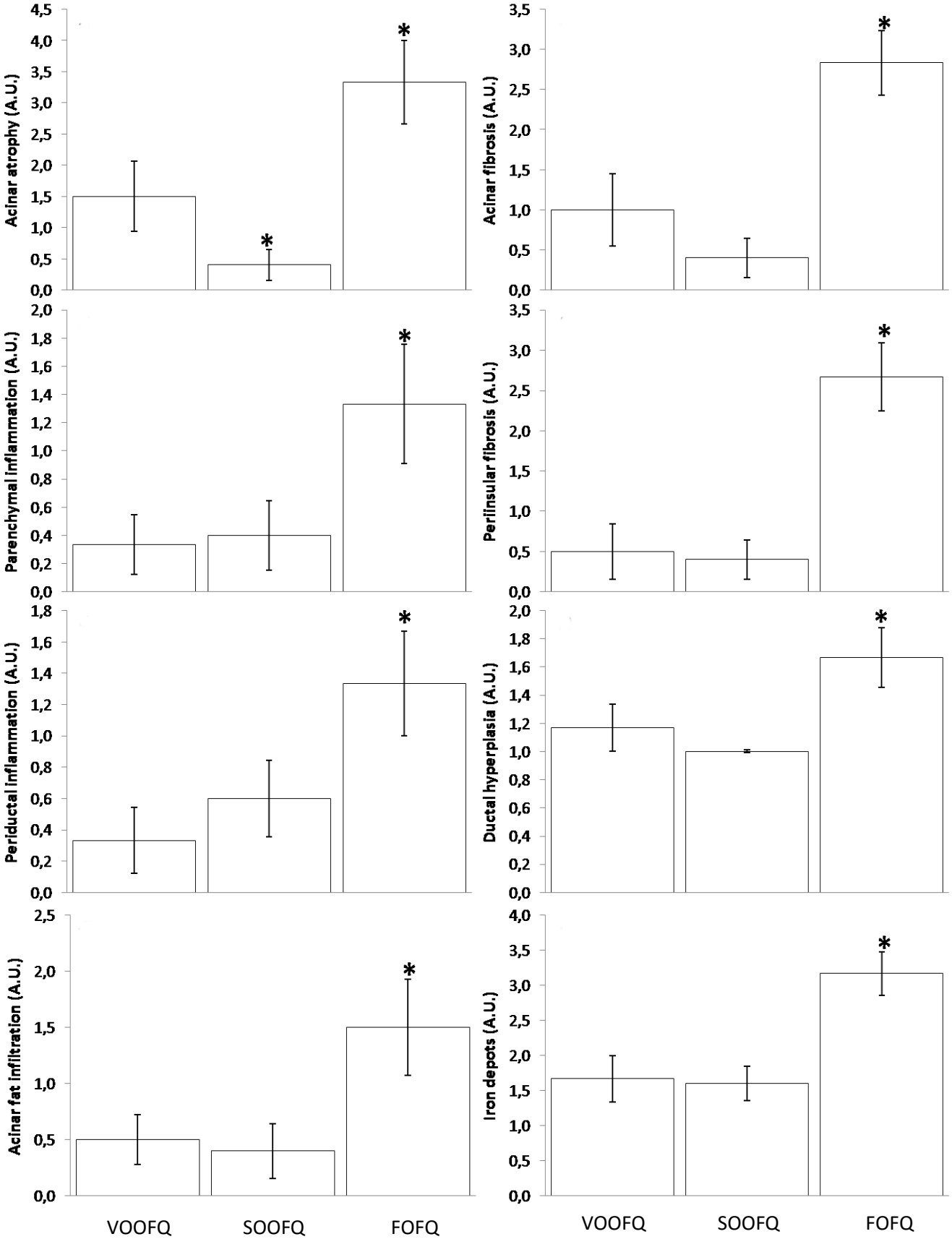


Figure II.3. Data from histological evaluations of pancreas from rats fed on different dietary fat sources (virgin olive, sunflower or fish oils) supplemented with Coenzyme Q10 . Data are presented as mean ± SEM. A.U. = arbitrary units. (*) $P < 0.05$ in respect to VOOFQ fed rats. FOFQ: fish oil fed group plus CoQ10; SOFQ: sunflower oil–fed group plus CoQ10; VOOFQ: virgin olive oil–fed group plus CoQ10.

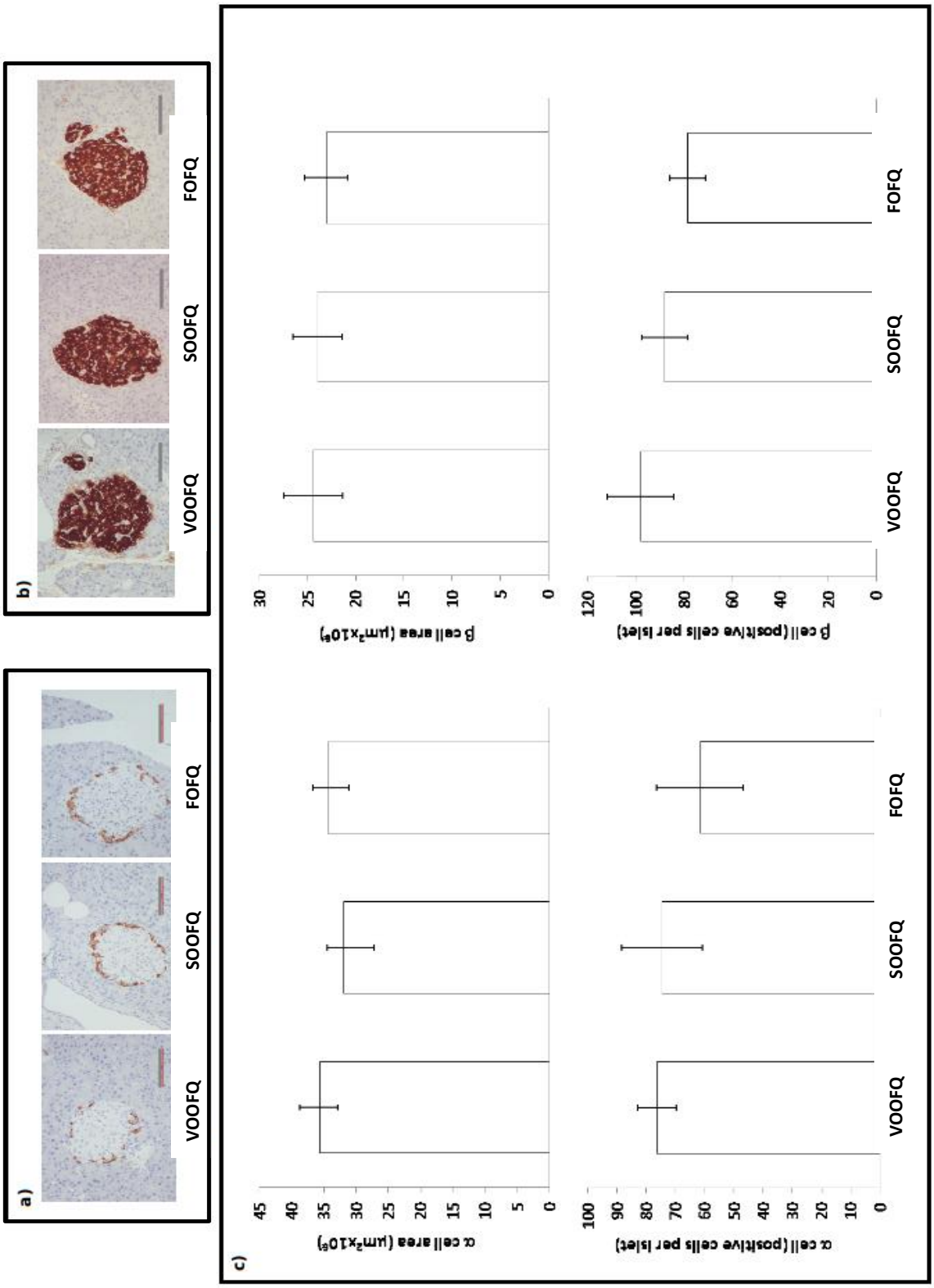


Figure II.4. Immunohistochemical assessment in the pancreas of 24 months old rats fed on different dietary fat sources (virgin olive, sunflower or fish oils) supplemented with Coenzyme Q_{10}. (a) Histological sections with pancreatic isles immunostained for glucagon; (b) Histological sections with pancreatic isles immunostained for insulin; (c) Quantitative data from histological evaluations of α- and β-cell positive density and area. Data are presented as mean ± SEM. Scare bar = 100 μm. FOFQ: fish oil fed group plus CoQ_{10}; SOFQ: sunflower oil–fed group plus CoQ_{10}; VOOFQ: virgin olive oil–fed group plus CoQ_{10}.

DISCUSSION

I. DIETARY FAT EFFECTS ON AGE-RELATED CHANGES OF PANCREAS

One of the aims of this study was to investigate the role of life-long feeding on diets varying only in the fat source. This type of dietary habits, in which diet is the same for the whole life is unlike to happens as it in the human population. However, people ha preferences for certain foods that constitute the basis of their diet. Dietary fat is one of these foods and a rat model might be very useful as a direct and easy method to observe harmful and beneficial effects derived from the exclusive intake of a particular fat type for a long period. Likewise, the rat model let us accessing to certain type of biological samples (pancreas in this case) which would be more difficult in humans, and therefore this animal model can help us to understand how diet affects organ histology in different histopathological situations. In order to avoid pathological situations such obesity or type 2 diabetes mellitus, rats were administered a balanced diet whose caloric components were controlled. In this sense, circulating parameters were measured (Table I.2). Other variables that could contribute to the observed differences, such as genetic predisposition or the rest of dietary nutrients were overruled because they should contribute equally in all the experimental groups as they were not modified. Dietary fats administered to each experimental group presented particular characteristics. Although fish oil had the highest amount of saturated fatty acids (Table I.1), this is not a key point because the circulating saturated fatty acid profile were similar in the three experimental groups after long-term consumption. Concerning unsaturated fatty acids, virgin olive oil group presented the highest amount of circulating MUFA, sunflower oil presented the highest amount of n-6 PUFA and fish oil group presented the highest amount of n-3 PUFA. This confirms the adaptation of animals to dietary fatty component, and could led to assume that found differences between groups might be attributed, at least in part, to dietary fat.

A remarkable finding of this study was the fact that the type of dietary fat affected to the exocrine and endocrine compartments of the pancreas in a different way. In this context, having VOOF islets as reference, SOF islets showed the highest number of β-cells, increased insulin content and hyperleptinemia. These signs along with other parameters are typical in alterations such as obesity, glucose intolerance, and insulin resistance, disruption of the adipoinsular axis or prediabetes (385). Nevertheless, SOF rats presented balanced levels of circulating glucose and insulin, suggesting there is no a prepathological state (386). In this sense, it cannot be given a clear explanation about the hyperleptinemia and if it was somehow correlated with the high content of insulin in islets. A possible explanation (only concerning islet tissue) may be that the high number of β-cells in SOF compared with VOOF could be interpreted as an increased replicative capacity of β-cells or a longer life span of these cells due to a reduced apoptosis. However, it has been well documented that β-cell replication decreases with age in rodents and humans (387, 388), but in compensation presents a minimal apoptosis (389). Proliferation events were very similar in all groups according to Ki-67^{+} assay on pancreases. Likewise, the expression of apoptotic markers were very similar in all

groups for Bad and Bcl2, but the expression of TNF-α apoptotic marker was lower in VOOF than SOF and FOF. All these observations together may suggest that the mitochondrial apoptotic pathway was not active in any of the of the animal groups, but in the cytoplasmic pathway, where TNF-α is most likely involved (390), it seems to be instrumental in the changes observed in SOF and FOF groups. In SOF group, it is probably that many of the positive insulin cells observed might be apoptotic, but it is required to perform functional analyses such as experiments in isolated islets so as to verify this hypothesis. Moreover SOF and FOF also obtained higher levels of oxidative stress by protein carbonyl assay compared with VOOF. So, these observations on endocrine pancreas are preliminary data, and therefore should be interpreted with caution.

Concerning the exocrine pancreas, it seems to be affected by a fish oil diet and in a lower way by a sunflower oil diet. The histopathological features typical observed in pancreatic fibrosis in the elderly (391) are very similar to what it was observed on FOF animals. In turn, consumption of olive oil at long-term suggests a possible delay in exocrine pancreatic aging. It is clear that the development of pancreatic fibrosis is a complex process where pancreatic stellate cells play an instrumental role. These cells which were initially characterized in rat and human pancreas (392, 393) are presented in the periacinar region and display droplets of lipids within the cytoplasm, and keeping a close connection with liver stellate cells which lead to hepatic fibrosis (394). The initiation of a fibrosis process requires the releasing of cytokines and growth factor from pancreatic resident cells or infiltrated inflammatory cells such as macrophages (395-397). If macrophage phagocyte damage cells release additional cytokines, it triggers to proliferation and transformation induction of stellate cells into myofibroblasts, which are responsible for producing extracellular matrix deposition and therefore fibrosis (398). Accordingly, we hypothesize that nutrients may take part in modulating the function of these cells, as it was described for glucose (399). So it is tempting to speculate that certain types of fatty acids, like those present in fish oil, might affect stellate cell function. In any case, further experiments are required to confirm this hypothesis.

In summary, according to results from the present study, the endocrine compartment of the pancreas was mostly negatively affected by a diet based on sunflower oil, meanwhile the exocrine compartment was affected in a greater extent by a fish oil diet. According to previous studies, it was observed that virgin olive oil preserved the integrity and functionality of the pancreas in the elderly, reinforcing its beneficial effects, especially in endocrine pancreas. In this sense, interventional and epidemiological studies proved that diets rich in MUFA are able to improve insulin sensitivity (400-404) and lower glycaemia and insulin requirements in type 2 diabetes patients (405). Likewise, in a cross-sectional study performed in South of Spain, an oral glucose tolerance test performed in 530 individuals in order to calculate insulin resistance using homeostasis model assessment showed a positive association between MUFA consumption and β-cell function (406). Nevertheless, it is not clear the precise

mechanisms by which virgin olive oil induced this beneficial effect on endocrine pancreas. This must be investigated in the future.. Virgin olive oil is observed to have positive effects in other tissues and organs, which indirectly may improve endocrine and exocrine pancreas (407). In this sense, virgin olive oil is rich in antioxidants, such as vitamin E or phenolic compounds, and antithrombotic agents that can improve endothelial function and reduce the risk of cardiovascular disease, insulin resistance (408-410) and possibly, pancreatitis (this study). Therefore, mechanisms responsible for virgin olive oil effects could be more complex than expected, and may be due to the synergistic effect of several compounds and not exclusively by oleic acid or MUFA.

II. DIETARY COQ EFFECTS ON AGE-RELATED CHANGES OF PANCREAS

The second objective of the present study aimed to gain more insight about the effects after the administration of a chronic low dosage of CoQ_{10} in the animals fed on the same diets as for the first objective and in the same conditions. According to that, animals from this second part of the study were fed in the same schedule as for the first part but with a supplement of CoQ_{10}.

Like in the previous study, CoQ_{10}-supplemented animals were fed on a controlled caloric intake pattern in order to avoid obesity or type 2 diabetes. Also, food spillage was indirectly monitored. FOFQ group displayed significant higher weight at sacrifice compared with the other two groups (non-supplemented animals fed on fish oil led to a higher weight too, but differences were not significant). CoQ_{10}-supplemented rats were analyzed for plasma fatty acid profile in order to ascertain adaptation to dietary fat. Results from fatty acid profile analysis lead to conclude that animals properly adapted to different dietary fat sources.

Concerning circulating hormone levels, when compared with non-supplemented rats, it was observed that SOF led to higher leptin concentration in comparison with the other two dietary fat sources. However, as for CoQ_{10}-supplemented animals are concerned, no differences were found between FOFQ and the other two groups of rats supplemented on CoQ_{10}. Somehow CoQ_{10} supplementation led to lower leptin levels associated to a SO-based diet. Leptin is produced and released primarily by adipocytes whose circulating levels are directly proportional to total body fat mass (386), but also to the percentage of body fat and BMI (411). With the present methodological approach, it is not possible to establish a clear explanation about the CoQ effect on circulating leptin levels when rats are maintaining on a sunflower oil-based diet. On the other hand, lower levels of insulin, and lower HOMA index were found in FOFQ animals in respect to VOOFQ rats. However, no clear differences were observed between animals fed on similar dietary fat in absence of CoQ supplementation for the most of the parameters analyzed. Thus, from the point of view of circulating hormones and biochemical parameters, it is very difficult establish the role of CoQ supplementation in the three studied fats, but in any case neither group of supplemented rats showed a significant pathological situation of insulin resistance or loss of insulin sensitivity (412, 413).

Histological assessment of exocrine pancreas showed that FOFQ animals presented similar alterations in pancreas parenchyma as did non-supplemented rats fed on the same dietary fat. So CoQ_{10} supplementation did not prevented alterations due to a life-long feeding non the n-3PUFA based diet. No significant differences on this tissue compartment were observed between SOFQ and VOOFQ. When compared supplemented and non-supplemented groups for these two dietary fats, better results were found for sunflower oil fed animals when they were supplemented on CoQ_{10}.

Virgin olive oil fed animals showed similar results irrespective of the supplementation with CoQ_{10}. The histological alteration degree observed in fish oil fed animals, supplemented or not, resemble to those observed in pancreatic fibrosis of the elderly (391). The higher degree of inflammation and fibrosis in pancreas found in animals fed on fish oil seems contradictory with those from animals studies where n-3 fatty acid-rich diets shows anti-inflammatory effects, although pancreas was not directly studied (414-417). Other studies have indicated an absence anti-inflammatory effect after feeding on n-3PUFA (418) or even pro-oxidant and pro-inflammatory effects for these fatty acids (419, 420). In addition to this, it has been also observed that some treatments based on these fatty acids can decrease life-span (421-424). So, the amount, the animal model, age or treatment time seems to be pivotal factors affecting n-3PUFA effects that need to be explored in more detail. Conversely, long-term consumption of diets based on virgin olive oil and sunflower oil supplemented with CoQ, would delay exocrine pancreatic aging in the present model, as these animals did not displayed a typical fibrotic pancreas of the elderly. In this sense, as previously explained above, pancreatic stellate cells are a key factor on the fibrotic process at this organ. In such condition oxidative stress is directly involved. Different antioxidant therapies have been tested demonstrating decreased pancreatic stellate cells and proinflammatory factors both *in vitro* (425, 426) and *in vivo* (395, 425-432). The findings from the present study can be associated with such facts, so that the improvement of certain aging indicators produced in SOFQ animals, might be due to the long-term addition of CoQ comparing with their corresponding non-supplemented counterparts. Nevertheless, in animals fed on fish oil and virgin olive oil, such supplementation seems to have no effect over pancreatic parenchyma. On the one hand, it could be hypothesized that the fish oil effect on the exocrine pancreas was due to a mechanism not related to oxidative stress, or at least that this was not exclusive. On the other hand, it is possible that CoQ does not act as a specific antioxidant for this particular process and/or not have enough to counteract fish oil effects. Concerning virgin olive oil, it might be so beneficial for this pancreatic compartment that CoQ supplements hardly have additional effects, but curiously the degree of acinar atrophy was lower in sunflower oil fed rats supplemented with CoQ which suggests that this combination could be even more beneficial. In any case, more studies are needed in order to understand mechanisms underlying differences among dietary fats and CoQ effects.

In regards to endocrine pancreas, it is interesting to note that CoQ-supplemented groups did not show significant differences among them. However, for the non-supplemented groups, sunflower oil fed animals led to significantly higher values, when compared to those fed on virgin olive oil, for the β-cell area, as well as a higher number of cell per islet. Age-related disorders of pancreatic endocrine function usually start with a prediabetic state of insulin resistance in which the adaptive response of pancreatic β-cells results in the presence of high levels of circulating insulin (hyperinsulinemia) in order to maintain normoglycemia. Hyperinsulinemia can be achieved by combining different mechanisms that involve expanding β-cells mass by hyperplasia and hypertrophy, enhancing insulin biosynthesis and increasing insulin

secretion in response to sustained extracellular nutrients demand (433-437). However in sunflower oil fed rats from the present study there are no signs of β-cells hypertrophy nor hyperplasia at all. This finding suggests that CoQ addition to diet prevented age-related β-cells alterations associated to long-life consumption of a sunflower oil rich diet under our experimental conditions. Leptin has been shown to have a proliferative effect on β-cells cultures under certain conditions (438-442). As previously mentioned, it is not possible to establish an explanation for CoQ effect on leptin level, but this effect on β-cells might derive from it, since this group displayed lower levels than in the non-supplemented counterparts.

Results on pancreatic contents of insulin and glucagon, in spite of observing similar values in the three groups, either insulin or glucagon, some inferences can be extracted from these results. First, FOFQ animals in which alterations at the peri-insular region level were observed, they did not yet lead to endocrine dysfunctions. Moreover, compared with non-supplemented animals, it was observed that total insulin content of supplemented rats fed on virgin olive oil and fish oil was double with respect to their non-supplemented counterparts. However, for sunflower oil-fed rats both, supplemented and non-supplemented, presented similar insulin content, although in non-supplemented rats that was associated with a higher β-cell mass. It has been hypothesized that β-cell hypertrophy could be an adaptive response to the aging situation in an attempt to maintain correct insulin levels in an organ with diminished replicative capacities (440) which would explain the observations done in non-supplemented rats. In turn, in CoQ-supplemented rats, histological measures related to β-cells were similar among groups and similar to those found in supplemented animals fed on fish or virgin olive oils. This suggests that CoQ may contribute to specifically increase the content of insulin regardless dietary fat type, but without affecting glucagon that maintained similar levels among dietary groups in both studies. HOMA values, insulin circulating levels and glycaemia were similar to those found in absence of CoQ, which suggests that there is no decrease in insulin secretion levels or demands by other tissues. Another possibility is that CoQ improves β-cells capacity to restore insulin reserves. However, because of our experimental design focused pancreas, it was not possible to elucidate it.

CONCLUSIONS

CONCLUSIONS

1º Life-long feeding on a 4% of virgin olive oil led to a good maintaining of pancreatic microstructure through aging. Both, exocrine and endocrine compartments were benefited from the continued intake of this dietary fat when compared with the other fat sources. Supplementation of virgin olive oil with coenzyme Q_{10} did not result in a subsequent improvement in the results obtained with the non-supplemented fat.

2º The intake throughout life with fish oil, rich in n-3PUFA, as dietary fat, resulted in alterations in exocrine pancreas compared to that observed with animals fed on MUFA or n-6PUFA based diets. These alterations were not prevented by supplementation of fish oil with a low dosage of coenzyme Q_{10}.

3º The use of sunflower oil as the exclusive dietary fat source throughout life led to alterations in the endocrine pancreas during aging, in relation to that observed in animals fed on virgin olive or fish oils. Sunflower oil supplementation with coenzyme Q_{10} prevented such alterations, approaching results to those found after the intake of virgin olive oil.

4º Despite that diets with a unique fat source over very long periods of time are not likely to occur in humans, animal models like the one presented here, could be very useful to examine possible effects of fat types predominant in a particular diet. In addition, these models could help to understand how diet can model organ histology in different physiopathological situations considering the difficulty to obtain certain human samples such as pancreas. Based on this approach, it was reported that different pancreatic compartments were affected according to the fat composition consumed, emphasizing the importance of dietary fatty acids in determining pancreatic structure. Moreover, chronic CoQ supplementation together with dietary fat type could play an important role in modulating aging in the pancreas, affecting different compartments of this organ in opposite ways.

CONCLUSIONES

1º La alimentación de por vida con un 4% de aceite de oliva virgen dio lugar a un adecuado mantenimiento de la microestructura del páncreas durante el envejecimiento. Tanto los compartimentos endocrino como exocrino se beneficiaron de la ingesta continuada de esta grasa de la dieta en comparación con las otras fuentes grasas. La suplementación de aceite de oliva virgen con coenzima Q_{10} no dio lugar a una posterior mejora de los resultados obtenidos con la grasa sin suplementar.

2º La ingesta durante toda la vida de aceite de pescado, rico en ácidos grasos poliinsaturados de la serie n-3, dio lugar a alteraciones en el páncreas exocrino en comparación con lo observado en los animales alimentados mayoritariamente con dietas basadas en ácidos grasos monoinsaturados o poliinsaturados de la serie n-6. Estas alteraciones no fueron impedidas por la administración de aceite de pescado suplementado con una dosis baja de coenzima Q_{10}.

3º El uso de aceite de girasol como fuente de grasa exclusiva en la dieta a lo largo de toda la vida condujo a alteraciones en el páncreas endocrino durante el envejecimiento, en relación a la observado en animales alimentados con aceites de oliva virgen o pescado. La administración de coenzima Q_{10} junto al aceite de girasol evitó tales alteraciones, acercándose entonces a los resultados obtenidos tras la ingesta de aceite de oliva virgen.

4º A pesar de que el seguimiento de una dieta basada en una única fuente de grasa durante períodos de tiempo muy largos es poco probable que ocurra en los seres humanos, los modelos animales como el que aquí se presenta, podrían ser de gran utilidad a la hora de examinar los posibles efectos de un tipo de grasa predominante en una dieta en particular. Además, estos modelos podrían ayudar a entender cómo la dieta puede modelar la histología de un órgano bajo diferentes situaciones fisiopatológicas considerando la dificultad para obtener ciertas muestras humanas, tales como ocurre con el páncreas. Sobre la base de este enfoque, se ha podido constatar que los diferentes compartimentos del páncreas se vieron afectados de acuerdo con la composición de la grasa consumida, haciendo hincapié en la importancia de los ácidos grasos de la dieta en la determinación de la estructura de este órgano. Por otra parte, la administración crónica de suplementos de CoQ_{10} a dosis bajas junto con el tipo de grasa de la dieta podría desempeñar un papel importante en la modulación de envejecimiento en el páncreas, afectando a los distintos compartimentos de este órgano de manera a veces opuesta.

REFERENCES

1.Quiles JL, Ochoa JJ, Huertas JR, Mataix J. Coenzyme Q supplementation protects from age-related DNA double-strand breaks and increases lifespan in rats fed on a PUFA-rich diet. Experimental gerontology. 2004;39(2):189-94.
2.Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Oxford university press; 1999.
3.Sohal RS, Mockett RJ, Orr WC. Mechanisms of aging: an appraisal of the oxidative stress hypothesis. Free radical biology & medicine. 2002;33(5):575-86.
4.McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). The Journal of biological chemistry. 1969;244(22):6049-55.
5.Yu BP. Cellular defenses against damage from reactive oxygen species. Physiological reviews. 1994;74(1):139-62.
6.Harman D. The biologic clock: the mitochondria? Journal of the American geriatrics society. 1972;20(4):145-7.
7.Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiological reviews. 1979;59(3):527-605.
8.Kapahi P, Boulton ME, Kirkwood TB. Positive correlation between mammalian life span and cellular resistance to stress. Free radical biology & medicine. 1999;26(5-6):495-500.
9.Miquel J, Economos AC, Fleming J, Johnson JE, Jr. Mitochondrial role in cell aging. Experimental gerontology. 1980;15(6):575-91.
10.Gutiérrez-Robledo LM. Envejecimiento. Rev Fac Med. 1998;41(5):198-206.
11.Kirkwood TB, Austad SN. Why do we age? Nature. 2000;408(6809):233-8.
12.Medvedev ZA. An attempt at a rational classification of theories of ageing. Biological reviews of the Cambridge philosophical society. 1990;65(3):375-98.
13.Finch CE, Ruvkun G. The genetics of aging. Annual review of genomics and human genetics. 2001;2:435-62.
14.Lithgow GJ, Kirkwood TB. Mechanisms and evolution of aging. Science. 1996;273(5271):80.
15.Lee CK, Klopp RG, Weindruch R, Prolla TA. Gene expression profile of aging and its retardation by caloric restriction. Science. 1999;285(5432):1390-3.
16.Kirkwood TB, Rose MR. Evolution of senescence: late survival sacrificed for reproduction. Philosophical transactions of the Royal society of London series B, biological sciences. 1991;332(1262):15-24.
17.Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature. 2000;408(6809):255-62.
18.Kirkwood TB. DNA, mutations and aging. Mutation research. 1989;219(1):1-7.
19.Catania J, Fairweather, DS. Genetic and genome factors in aging. Reviews in clinical gerontology,. 1991;1:99-111.
20.Mikhelson VM. Replicative mosaicism might explain the seeming contradictions in the telomere theory of aging. Mechanisms of ageing and development. 2001;122(13):1361-5.
21.Aviv A, Aviv H. Telomeres, hidden mosaicism, loss of heterozygosity, and complex genetic traits. Human genetics. 1998;103(1):2-4.
22.Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239-47.
23.Roth GS, Ingram DK, Lane MA. Calorie restriction in primates: will it work and how will we know? Journal of the American geriatrics society. 1999;47(7):896-903.

24.Miquel J, Economos AC. Favorable effects of the antioxidants sodium and magnesium thiazolidine carboxylate on the vitality and life span of Drosophila and mice. Experimental gerontology. 1979;14(5):279-85.
25.Furukawa T, Meydani SN, Blumberg JB. Reversal of age-associated decline in immune responsiveness by dietary glutathione supplementation in mice. Mechanisms of ageing and development. 1987;38(2):107-17.
26.Sastre J, Pallardo FV, Garcia de la Asuncion J, Vina J. Mitochondria, oxidative stress and aging. Free radical research. 2000;32(3):189-98.
27.Quiles JL, Ochoa JJ, Ramirez-Tortosa C, Battino M, Huertas JR, Martin Y, et al. Dietary fat type (virgin olive vs. sunflower oils) affects age-related changes in DNA double-strand-breaks, antioxidant capacity and blood lipids in rats. Experimental gerontology. 2004;39(8):1189-98.
28.Melov S. Therapeutics against mitochondrial oxidative stress in animal models of aging. Annals of the New York academy of sciences. 2002;959:330-40.
29.Clandinin MT, Field CJ, Hargreaves K, Morson L, Zsigmond E. Role of diet fat in subcellular structure and function. Canadian journal of physiology and pharmacology. 1985;63(5):546-56.
30.Quiles JL, Huertas JR, Manas M, Ochoa JJ, Battino M, Mataix J. Oxidative stress induced by exercise and dietary fat modulates the coenzyme Q and vitamin A balance between plasma and mitochondria. International journal for vitamin and nutrition research Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung Journal international de vitaminologie et de nutrition. 1999;69(4):243-9.
31.Mataix J, Quiles JL, Huertas JR, Battino M, Manas M. Tissue specific interactions of exercise, dietary fatty acids, and vitamin E in lipid peroxidation. Free radical biology & medicine. 1998;24(4):511-21.
32.Suzuki H, Yamazaki M, Arai S, Nagao A, Terao J. Effect of lard, palm and rapeseed oils life conservation in aged mice. Mechanisms of ageing and development. 1991;60(3):267-74.
33.Giron MD, Mataix FJ, Suarez MD. Long-term effects of dietary monounsaturated and polyunsaturated fatty acids on the lipid composition of erythrocyte membranes in dogs. Comparative biochemistry and physiology Comparative physiology. 1992;102(1):197-201.
34.Navarro MD, Periago JL, Pita ML, Hortelano P. The n-3 polyunsaturated fatty acid levels in rat tissue lipids increase in response to dietary olive oil relative to sunflower oil. Lipids. 1994;29(12):845-9.
35.Escudero A, Montilla JC, Garcia JM, Sanchez-Quevedo MC, Periago JL, Hortelano P, et al. Effect of dietary (n-9), (n-6) and (n-3) fatty acids on membrane lipid composition and morphology of rat erythrocytes. Biochimica et biophysica acta. 1998;1394(1):65-73.
36.Periago JL, Suarez MD, Pita ML. Effect of dietary olive oil, corn oil and medium-chain triglycerides on the lipid composition of rat red blood cell membranes. The Journal of nutrition. 1990;120(9):986-94.
37.Spector AA, Yorek MA. Membrane lipid composition and cellular function. Journal of lipid research. 1985;26(9):1015-35.
38.Masi I, Giani E, Galli C, Tremoli E, Sirtori CR. Diets rich in saturated, monounsaturated and polyunsaturated fatty acids differently affect plasma lipids, platelet and arterial wall eicosanoids in rabbits. Annals of nutrition & metabolism. 1986;30(1):66-72.
39.Seiquer I, Manas M, Martinez-Victoria E, Ballesta MC, Mataix FJ. Long-term influence of dietary fat (sunflower oil, olive oil, lard and fish oil) in the serum fatty acid

composition and in the different lipidic fractions, in miniature swine. International journal for vitamin and nutrition research Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung Journal international de vitaminologie et de nutrition. 1996;66(2):171-9.
40.Huertas JR, Battino M, Lenaz G, Mataix FJ. Changes in mitochondrial and microsomal rat liver coenzyme Q9 and Q10 content induced by dietary fat and endogenous lipid peroxidation. FEBS letters. 1991;287(1-2):89-92.
41.Ayre KJ, Hulbert AJ. Dietary fatty acid profile affects endurance in rats. Lipids. 1997;32(12):1265-70.
42.Gibson RA, McMurchie EJ, Charnock JS, Kneebone GM. Homeostatic control of membrane fatty acid composition in the rat after dietary lipid treatment. Lipids. 1984;19(12):942-51.
43.Soriguer FJ, Tinahones FJ, Monzon A, Pareja A, Rojo-Martinez G, Moreno F, et al. Varying incorporation of fatty acids into phospholipids from muscle, adipose and pancreatic exocrine tissues and thymocytes in adult rats fed with diets rich in different fatty acids. European journal of epidemiology. 2000;16(6):585-94.
44.Martin F. [effect of adaptation to the type of dietary fat and physical activity on the composition of microsomal and mitochondrial membranes of various organs] [doctoral thesis]. Granada: Universidad de Granada; 2002. Spanish.
45.Navarro MD, Hortelano P, Periago JL, Pita ML. Effect of dietary olive and sunflower oils on the lipid composition of the aorta and platelets and on blood eicosanoids in rats. Arteriosclerosis and thrombosis : a journal of vascular biology / American heart association. 1992;12(7):830-5.
46.Ramirez-Tortosa C, Lopez-Pedrosa JM, Suarez A, Ros E, Mataix J, Gil A. Olive oil- and fish oil-enriched diets modify plasma lipids and susceptibility of LDL to oxidative modification in free-living male patients with peripheral vascular disease: the Spanish Nutrition Study. The British journal of nutrition. 1999;82(1):31-9.
47.Aguilera CM, Ramirez-Tortosa MC, Mesa MD, Gil A. [Protective effect of monounsaturated and polyunsaturated fatty acids on the development of cardiovascular disease]. Nutricion hospitalaria. 2001;16(3):78-91.
48.Sirtori CR, Crepaldi G, Manzato E, Mancini M, Rivellese A, Paoletti R, et al. One-year treatment with ethyl esters of n-3 fatty acids in patients with hypertriglyceridemia and glucose intolerance: reduced triglyceridemia, total cholesterol and increased HDL-C without glycemic alterations. Atherosclerosis. 1998;137(2):419-27.
49.Serrano P, Yago MD, Manas M, Calpena R, Mataix J, Martinez-Victoria E. Influence of type of dietary fat (olive and sunflower oil) upon gastric acid secretion and release of gastrin, somatostatin, and peptide YY in man. Digestive diseases and sciences. 1997;42(3):626-33.
50.Naranjo JA, Martinez-Victoria E, Valverde A, Yago MD, Manas M. Effect of age on the exocrine pancreatic secretion of the preruminant milk-fed goat. Archives of physiology and biochemistry. 1997;105(2):144-50.
51.Sureda A, Arranz R, Iriondo A, Carreras E, Lahuerta JJ, Garcia-Conde J, et al. Autologous stem-cell transplantation for Hodgkin's disease: results and prognostic factors in 494 patients from the Grupo Espanol de Linfomas/Transplante Autologo de Medula Osea Spanish Cooperative Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001;19(5):1395-404.
52.Garg A. High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. The American journal of clinical nutrition. 1998;67(3 Suppl):577S-82S.
53.Quiles JL, Ochoa, J.J, Huertas, J.R., Mataix, J. [mitochondrial aspects of aging: role of dietary fat type and oxidative stress]. Endocrinología y nutrición. 2004;51(3):107-20.

54.Proceedings of a workshop on new developments on dietary fat and fiber in carcinogenesis (optimal types and amounts of fat or fiber). Preventive medicine. 1987;16(4):449-595.
55.Rose MR, Mueller LD, Long AD. Pharmacology, genomics, and the evolutionary biology of ageing. Free radical research. 2002;36(12):1293-7.
56.Quiles JL, Huertas JR, Manas M, Ochoa JJ, Battino M, Mataix J. Dietary fat type and regular exercise affect mitochondrial composition and function depending on specific tissue in the rat. Journal of bioenergetics and biomembranes. 2001;33(2):127-34.
57.Sugimura T. Nutrition and dietary carcinogens. Carcinogenesis. 2000;21(3):387-95.
58.Moral R, Escrich R, Solanas M, Vela E, Ruiz de Villa MC, Escrich E. Diets high in corn oil or extra-virgin olive oil differentially modify the gene expression profile of the mammary gland and influence experimental breast cancer susceptibility. European journal of nutrition. 2015.
59.Escrich E, Moral R, Solanas M. Olive oil, an essential component of the Mediterranean diet, and breast cancer. Public health nutrition. 2011;14(12A):2323-32.
60.Granados-Principal S, Quiles JL, Ramirez-Tortosa C, Camacho-Corencia P, Sanchez-Rovira P, Vera-Ramirez L, et al. Hydroxytyrosol inhibits growth and cell proliferation and promotes high expression of sfrp4 in rat mammary tumours. Molecular nutrition & food research. 2011;55 Suppl 1:S117-26.
61.Granados-Principal S, El-Azem N, Pamplona R, Ramirez-Tortosa C, Pulido-Moran M, Vera-Ramirez L, et al. Hydroxytyrosol ameliorates oxidative stress and mitochondrial dysfunction in doxorubicin-induced cardiotoxicity in rats with breast cancer. Biochemical pharmacology. 2014;90(1):25-33.
62.Hashim YZ, Worthington J, Allsopp P, Ternan NG, Brown EM, McCann MJ, et al. Virgin olive oil phenolics extract inhibit invasion of HT115 human colon cancer cells in vitro and in vivo. Food & function. 2014;5(7):1513-9.
63.Freeman LR, Haley-Zitlin V, Rosenberger DS, Granholm AC. Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms. Nutritional neuroscience. 2014;17(6):241-51.
64.Tchkonia T, Morbeck DE, Von Zglinicki T, Van Deursen J, Lustgarten J, Scrable H, et al. Fat tissue, aging, and cellular senescence. Aging cell. 2010;9(5):667-84.
65.Barzilai N, Gupta G. Revisiting the role of fat mass in the life extension induced by caloric restriction. The journals of gerontology Series A, Biological sciences and medical sciences. 1999;54(3):B89-96; discussion B7-8.
66.Masoro EJ. Caloric restriction and aging: controversial issues. The journals of gerontology Series A, Biological sciences and medical sciences. 2006;61(1):14-9.
67.Zamboni M, Rossi AP, Fantin F, Zamboni G, Chirumbolo S, Zoico E, et al. Adipose tissue, diet and aging. Mechanisms of ageing and development. 2014;136-137:129-37.
68.Bielski BH, Arudi RL, Sutherland MW. A study of the reactivity of HO2/O2- with unsaturated fatty acids. The Journal of biological chemistry. 1983;258(8):4759-61.
69.Pamplona R, Portero-Otin M, Ruiz C, Gredilla R, Herrero A, Barja G. Double bond content of phospholipids and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals. Mechanisms of ageing and development. 2000;112(3):169-83.
70.Pamplona R. Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. Biochimica et biophysica acta. 2008;1777(10):1249-62.
71.Pamplona R, Prat J, Cadenas S, Rojas C, Perez-Campo R, Lopez Torres M, et al. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria

from long-lived species: the pigeon and human case. Mechanisms of ageing and development. 1996;86(1):53-66.
72.Pamplona R, Portero-Otin M, Riba D, Requena JR, Thorpe SR, Lopez-Torres M, et al. Low fatty acid unsaturation: a mechanism for lowered lipoperoxidative modification of tissue proteins in mammalian species with long life spans. The journals of gerontology Series A, Biological sciences and medical sciences. 2000;55(6):B286-91.
73.Pamplona R, Barja G. Highly resistant macromolecular components and low rate of generation of endogenous damage: two key traits of longevity. Ageing research reviews. 2007;6(3):189-210.
74.Hulbert AJ. Membrane fatty acids as pacemakers of animal metabolism. Lipids. 2007;42(9):811-9.
75.Pamplona R, Barja G, Portero-Otin M. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: a homeoviscous-longevity adaptation? Annals of the New York academy of sciences. 2002;959:475-90.
76.Pamplona R, Portero-Otin M, Sanz A, Requena J, Barja G. Modification of the longevity-related degree of fatty acid unsaturation modulates oxidative damage to proteins and mitochondrial DNA in liver and brain. Experimental gerontology. 2004;39(5):725-33.
77.Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. Life and death: metabolic rate, membrane composition, and life span of animals. Physiological reviews. 2007;87(4):1175-213.
78.Lopez-Dominguez JA, Ramsey JJ, Tran D, Imai DM, Koehne A, Laing ST, et al. The Influence of Dietary Fat Source on Life Span in Calorie Restricted Mice. The journals of gerontology Series A, Biological sciences and medical sciences. 2015;70(10):1181-8.
79.Ueda Y, Wang MF, Irei AV, Sarukura N, Sakai T, Hsu TF. Effect of dietary lipids on longevity and memory in the SAMP8 mice. Journal of nutritional science and vitaminology. 2011;57(1):36-41.
80.Lee D, Hwang W, Artan M, Jeong DE, Lee SJ. Effects of nutritional components on aging. Aging cell. 2015;14(1):8-16.
81.Huhn S, Kharabian Masouleh S, Stumvoll M, Villringer A, Witte AV. Components of a Mediterranean diet and their impact on cognitive functions in aging. Frontiers in aging neuroscience. 2015;7:132.
82.Molfino A, Gioia G, Rossi Fanelli F, Muscaritoli M. The role for dietary omega-3 fatty acids supplementation in older adults. Nutrients. 2014;6(10):4058-73.
83.Denis I, Potier B, Heberden C, Vancassel S. Omega-3 polyunsaturated fatty acids and brain aging. Current opinion in clinical nutrition and metabolic care. 2015;18(2):139-46.
84.Sergeant S, McQuail JA, Riddle DR, Chilton FH, Ortmeier SB, Jessup JA, et al. Dietary fish oil modestly attenuates the effect of age on diastolic function but has no effect on memory or brain inflammation in aged rats. The journals of gerontology Series A, Biological sciences and medical sciences. 2011;66(5):521-33.
85.Ubeda N, Achon M, Varela-Moreiras G. Omega 3 fatty acids in the elderly. The British journal of nutrition. 2012;107 Suppl 2:S137-51.
86.Pitozzi V, Jacomelli M, Catelan D, Servili M, Taticchi A, Biggeri A, et al. Long-term dietary extra-virgin olive oil rich in polyphenols reverses age-related dysfunctions in motor coordination and contextual memory in mice: role of oxidative stress. Rejuvenation research. 2012;15(6):601-12.
87.Kondo K, Morino K, Nishio Y, Kondo M, Nakao K, Nakagawa F, et al. A fish-based diet intervention improves endothelial function in postmenopausal women with type 2

diabetes mellitus: a randomized crossover trial. Metabolism: clinical and experimental. 2014;63(7):930-40.
88.Haraldsdottir A, Torfadottir JE, Valdimarsdottir UA, Aspelund T, Harris TB, Launer LJ, et al. Fish and fish-liver oil consumption in adolescence and midlife and risk of CHD in older women. Public health nutrition. 2016;19(2):318-25.
89.Wilk JB, Tsai MY, Hanson NQ, Gaziano JM, Djousse L. Plasma and dietary omega-3 fatty acids, fish intake, and heart failure risk in the Physicians' Health Study. The American journal of clinical nutrition. 2012;96(4):882-8.
90.Reinders I, Murphy RA, Song X, Mitchell GF, Visser M, Cotch MF, et al. Higher Plasma Phospholipid n-3 PUFAs, but Lower n-6 PUFAs, Are Associated with Lower Pulse Wave Velocity among Older Adults. The Journal of nutrition. 2015;145(10):2317-24.
91.Djousse L, Akinkuolie AO, Wu JH, Ding EL, Gaziano JM. Fish consumption, omega-3 fatty acids and risk of heart failure: a meta-analysis. Clinical nutrition. 2012;31(6):846-53.
92.Bilato C. n-3 fatty acids and cardiovascular disease: the story is not over yet. Aging clinical and experimental research. 2013;25(4):357-63.
93.Patel JV, Tracey I, Hughes EA, Lip GY. Omega-3 polyunsaturated acids and cardiovascular disease: notable ethnic differences or unfulfilled promise? Journal of thrombosis and haemostasis : JTH. 2010;8(10):2095-104.
94.Amiot M. Olive oil and health effects: from epidemiological studies to the molecular mechanisms of phenolic fraction. OCL. 2014;21(5):D512.
95.Guasch-Ferre M, Hruby A, Salas-Salvado J, Martinez-Gonzalez MA, Sun Q, Willett WC, et al. Olive oil consumption and risk of type 2 diabetes in US women. The American journal of clinical nutrition. 2015;102(2):479-86.
96.Rozati M, Barnett J, Wu D, Handelman G, Saltzman E, Wilson T, et al. Cardio-metabolic and immunological impacts of extra virgin olive oil consumption in overweight and obese older adults: a randomized controlled trial. Nutrition & metabolism. 2015;12:28.
97.Helal O, Berrougui H, Loued S, Khalil A. Extra-virgin olive oil consumption improves the capacity of HDL to mediate cholesterol efflux and increases ABCA1 and ABCG1 expression in human macrophages. The British journal of nutrition. 2013;109(10):1844-55.
98.Loued S, Berrougui H, Componova P, Ikhlef S, Helal O, Khalil A. Extra-virgin olive oil consumption reduces the age-related decrease in HDL and paraoxonase 1 anti-inflammatory activities. The British journal of nutrition. 2013;110(7):1272-84.
99.Hohmann CD, Cramer H, Michalsen A, Kessler C, Steckhan N, Choi K, et al. Effects of high phenolic olive oil on cardiovascular risk factors: A systematic review and meta-analysis. Phytomedicine : international journal of phytotherapy and phytopharmacology. 2015;22(6):631-40.
100.Welch AA, MacGregor AJ, Minihane AM, Skinner J, Valdes AA, Spector TD, et al. Dietary fat and fatty acid profile are associated with indices of skeletal muscle mass in women aged 18-79 years. The Journal of nutrition. 2014;144(3):327-34.
101.Reinders I, Song X, Visser M, Eiriksdottir G, Gudnason V, Sigurdsson S, et al. Plasma phospholipid PUFAs are associated with greater muscle and knee extension strength but not with changes in muscle parameters in older adults. The Journal of nutrition. 2015;145(1):105-12.
102.Harris TB, Song X, Reinders I, Lang TF, Garcia ME, Siggeirsdottir K, et al. Plasma phospholipid fatty acids and fish-oil consumption in relation to osteoporotic fracture

risk in older adults: the Age, Gene/Environment Susceptibility Study. The American journal of clinical nutrition. 2015;101(5):947-55.
103.Farina EK, Kiel DP, Roubenoff R, Schaefer EJ, Cupples LA, Tucker KL. Protective effects of fish intake and interactive effects of long-chain polyunsaturated fatty acid intakes on hip bone mineral density in older adults: the Framingham Osteoporosis Study. The American journal of clinical nutrition. 2011;93(5):1142-51.
104.Bullon P, Battino M, Varela-Lopez A, Perez-Lopez P, Granados-Principal S, Ramirez-Tortosa MC, et al. Diets based on virgin olive oil or fish oil but not on sunflower oil prevent age-related alveolar bone resorption by mitochondrial-related mechanisms. PloS one. 2013;8(9):e74234.
105.Crane FL, Hatefi Y, Lester RL, Widmer C. Isolation of a quinone from beef heart mitochondria. Biochimica et biophysica acta. 1957;25(1):220-1.
106.Ernster L. Lipid peroxidation in biological membranes: mechanisms and implications. In: Yagi, K. Active oxygens, lipid peroxides and antioxidants. Boca Ratón: CRC Press; 1993. 1-38 p.
107.Lenaz G, Fato R, Degli Esposti M, Rugolo M, Parenti Castelli G. The essentiality of coenzyme Q for bioenergetics and clinical medicine. Drugs under experimental and clinical research. 1985;11(8):547-56.
108.Yamamoto Y, Yamashita S. Plasma ratio of ubiquinol and ubiquinone as a marker of oxidative stress. Molecular aspects of medicine. 1997;18 Suppl:S79-84.
109.Lopez-Lluch G, Barroso MP, Martin SF, Fernandez-Ayala DJ, Gomez-Diaz C, Villalba JM, et al. Role of plasma membrane coenzyme Q on the regulation of apoptosis. BioFactors. 1999;9(2-4):171-7.
110.Grunler J, Ericsson J, Dallner G. Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. Biochimica et biophysica acta. 1994;1212(3):259-77.
111.Olson RE, Rudney H. Biosynthesis of ubiquinone. Vitamins and hormones. 1983;40:1-43.
112.Teclebrhan H, Olsson J, Swiezewska E, Dallner G. Biosynthesis of the side chain of ubiquinone:trans-prenyltransferase in rat liver microsomes. The Journal of biological chemistry. 1993;268(31):23081-6.
113.Poulter CD, Rilling, HC. Prenyl transferases and isomerases; In: Porter, J. Biosynthesis of isoprenoide compounds. New York: John Wiley; 1981.
114.Aberg F, Appelkvist EL, Dallner G, Ernster L. Distribution and redox state of ubiquinones in rat and human tissues. Archives of biochemistry and biophysics. 1992;295(2):230-4.
115.Appelkvist EL, Kalen, A., Dallner, G. G. Biosynthesis and regulation of coenzyme Q. In: Biomedical and clinical aspects of coenzyme Q. Folkers, K., Litarru, G. P. Eds., Elsevier science publishers, 1991; 6:141-50. p. 141-50.
116.Yamamoto T, Shimizu S, Sugawara H, Momose K, Rudney H. Identification of regulatory sites in the biosynthesis of ubiquinone in the perfused rat heart. Archives of biochemistry and biophysics. 1989;269(1):86-92.
117.Fedurov VF. [Ubiquinone and sterol metabolism in the liver of rats maintained on a diet short of aromatic amino acids]. Voprosy pitaniia. 1977(6):23-5.
118.Elmberger PG, Kalen A, Brunk UT, Dallner G. Discharge of newly-synthesized dolichol and ubiquinone with lipoproteins to rat liver perfusate and to the bile. Lipids. 1989;24(11):919-30.
119.Marasco S, Salvatore, P., Franklin, L. [clinic use of coenzyme Q10]. Antioxidantes y calidad de vida [Internet].

120.Ramasarma, T. Metabolism of coenzime Q. In : Lenaz, G., ed. Coenzime Q. Chichester: John Wiley, 1985; 131-42. .
121.Turunen M, Appelkvist EL, Sindelar P, Dallner G. Blood concentration of coenzyme Q(10) increases in rats when esterified forms are administered. The Journal of nutrition. 1999;129(12):2113-8.
122.Ulrich EL, Girvin ME, Cramer WA, Markley JL. Location and mobility of ubiquinones of different chain lengths in artificial membrane vesicles. Biochemistry. 1985;24(10):2501-8.
123.Kalen A, Norling B, Appelkvist EL, Dallner G. Ubiquinone biosynthesis by the microsomal fraction from rat liver. Biochimica et biophysica acta. 1987;926(1):70-8.
124.Aberg F, Zhang Y, Teclebrhan H, Appelkvist EL, Dallner G. Increases in tissue levels of ubiquinone in association with peroxisome proliferation. Chemico-biological interactions. 1996;99(1-3):205-18.
125.Turunen M, Sindelar P, Dallner G. Induction of endogenous coenzyme Q biosynthesis by administration of peroxisomal inducers. BioFactors. 1999;9(2-4):131-9.
126.Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. Biochimica et biophysica acta. 1995;1271(1):195-204.
127.Brandt U. Proton translocation in the respiratory chain involving ubiquinone--a hypothetical semiquinone switch mechanism for complex I. BioFactors. 1999;9(2-4):95-101.
128.Yu CA, Zhang L, Deng KP, Tian H, Xia D, Kim H, et al. Structure and reaction mechanisms of multifunctional mitochondrial cytochrome bc1 complex. BioFactors. 1999;9(2-4):103-9.
129.Gille L, Nohl H. The existence of a lysosomal redox chain and the role of ubiquinone. Archives of biochemistry and biophysics. 2000;375(2):347-54.
130.Yokoyama H, Lingle DM, Crestanello JA, Kamelgard J, Kott BR, Momeni R, et al. Coenzyme Q10 protects coronary endothelial function from ischemia reperfusion injury via an antioxidant effect. Surgery. 1996;120(2):189-96.
131.Matsumoto, H. and Miyawaki F: Effect of Coenzyme Q10 pretreatment on myocardial preservation. Heart Transplantation, 1984; 3: 160-165. .
132.Kalen A, Appelkvist EL, Dallner G. Age-related changes in the lipid compositions of rat and human tissues. Lipids. 1989;24(7):579-84.
133.Aberg F, Zhang Y, Appelkvist EL, Dallner G. Effects of clofibrate, phthalates and probucol on ubiquinone levels. Chemico-biological interactions. 1994;91(1):1-14.
134.Eggens I, Elmberger PG, Low P. Polyisoprenoid, cholesterol and ubiquinone levels in human hepatocellular carcinomas. British journal of experimental pathology. 1989;70(1):83-92.
135.Guan Z, Soderberg M, Sindelar P, Prusiner SB, Kristensson K, Dallner G. Lipid composition in scrapie-infected mouse brain: prion infection increases the levels of dolichyl phosphate and ubiquinone. Journal of neurochemistry. 1996;66(1):277-85.
136.Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidants, and human disease: where are we now? The Journal of laboratory and clinical medicine. 1992;119(6):598-620.
137.Beal MF, Matthews RT. Coenzyme Q10 in the central nervous system and its potential usefulness in the treatment of neurodegenerative diseases. Molecular aspects of medicine. 1997;18 Suppl:S169-79.
138.Dallner G, Sindelar PJ. Regulation of ubiquinone metabolism. Free radical biology & medicine. 2000;29(3-4):285-94.

139.Dallner, G.: Regulatory aspects of coenzyme Q metabolism. Stockholm University: Abstracts of the second conference of the International coenzyme Q10 association. Frankfurt, Germany, 2000a; 1-3.
140.Thelin A, Schedin S, Dallner G. Half-life of ubiquinone-9 in rat tissues. FEBS letters. 1992;313(2):118-20.
141.Andersson M, Elmberger PG, Edlund C, Kristensson K, Dallner G. Rates of cholesterol, ubiquinone, dolichol and dolichyl-P biosynthesis in rat brain slices. FEBS letters. 1990;269(1):15-8.
142.Ding H, Moser CC, Robertson DE, Tokito MK, Daldal F, Dutton PL. Ubiquinone pair in the Qo site central to the primary energy conversion reactions of cytochrome bc1 complex. Biochemistry. 1995;34(49):15979-96.
143.Nohl H, Staniek K, Gille L. Imbalance of oxygen activation and energy metabolism as a consequence or mediator of aging. Experimental gerontology. 1997;32(4-5):485-500.
144.Runquist M, Parmryd I, Thelin A, Chojnacki T, Dallner G. Distribution of branch point prenyltransferases in regions of bovine brain. Journal of neurochemistry. 1995;65(5):2299-306.
145.Elmberger PG, Kalen A, Lund E, Reihner E, Eriksson M, Berglund L, et al. Effects of pravastatin and cholestyramine on products of the mevalonate pathway in familial hypercholesterolemia. Journal of lipid research. 1991;32(6):935-40.
146.Low P, Andersson M, Edlund C, Dallner G. Effects of mevinolin treatment on tissue dolichol and ubiquinone levels in the rat. Biochimica et biophysica acta. 1992;1165(1):102-9.
147.Thelin A, Peterson E, Hutson JL, McCarthy AD, Ericsson J, Dallner G. Effect of squalestatin 1 on the biosynthesis of the mevalonate pathway lipids. Biochimica et biophysica acta. 1994;1215(3):245-9.
148.Aithal HN, Joshi VC, Ramasarma T. Effect of cold exposure on the metabolism of ubiquinone in the rat. Biochimica et biophysica acta. 1968;162(1):66-72.
149.Pedersen S, Tata JR, Ernster L. Ubiquinone (coenzyme Q) and the regulation of basal metabolic rate by thryoid hormones. Biochimica et biophysica acta. 1963;69:407-9.
150.Takahashi T, Okamoto T, Mori K, Sayo H, Kishi T. Distribution of ubiquinone and ubiquinol homologues in rat tissues and subcellular fractions. Lipids. 1993;28(9):803-9.
151.Crane FL. Biochemical functions of coenzyme Q10. Journal of the American college of nutrition. 2001;20(6):591-8.
152.Villalba JM, Navas P. Plasma membrane redox system in the control of stress-induced apoptosis. Antioxidants & redox signaling. 2000;2(2):213-30.
153.Navarro F, Arroyo A, Martin SF, Bello RI, de Cabo R, Burgess JR, et al. Protective role of ubiquinone in vitamin E and selenium-deficient plasma membranes. BioFactors. 1999;9(2-4):163-70.
154.Poon WW, Do TQ, Marbois BN, Clarke CF. Sensitivity to treatment with polyunsaturated fatty acids is a general characteristic of the ubiquinone-deficient yeast coq mutants. Molecular aspects of medicine. 1997;18 Suppl:S121-7.
155.Hoppe U, Bergemann J, Diembeck W, Ennen J, Gohla S, Harris I, et al. Coenzyme Q10, a cutaneous antioxidant and energizer. BioFactors. 1999;9(2-4):371-8.
156.Arroyo A, Kagan VE, Tyurin VA, Burgess JR, de Cabo R, Navas P, et al. NADH and NADPH-dependent reduction of coenzyme Q at the plasma membrane. Antioxidants & redox signaling. 2000;2(2):251-62.

157.Villalba JM, Crane, F.L., Navas, P. Anti-oxidative role of ubiquinone in the animal plasma membrane. In: Asard, H. Plasma membrane redox system and their role in biological stress and diseases. Dordrecht: Kluwer, 1998, pp.247-266.
158.Schultz JR, Clarke , CF. Functional roles of ubiquinona. In: Cardenas, E., Packer, L. (eds): Mitochondrial oxidants and ageing. New York: M. Dekker, 1999; 95-118.
159.Rotig A, Appelkvist EL, Geromel V, Chretien D, Kadhom N, Edery P, et al. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. Lancet. 2000;356(9227):391-5.
160.Nakamura T, Ohno T, Hamamura K, Sato T. Metabolism of coenzyme Q10: biliary and urinary excretion study in guinea pigs. BioFactors. 1999;9(2-4):111-9.
161.Kagan VE, Nohl, H. and Quinn, P. J. Coenzyme Q: its role in scavenging and generation of radical membranes. In: Cadenas, E. Handbook of antioxidants. N. Y. M. Dekker, 1996; 157-201. .
162.Lass A, Kwong L, Sohal RS. Mitochondrial coenzyme Q content and aging. BioFactors. 1999;9(2-4):199-205.
163.Battino M, Bompadre S, Leone L, Villa RF, Gorini A. Coenzymes Q9 and Q10, vitamin E and peroxidation in rat synaptic and non-synaptic occipital cerebral cortex mitochondria during ageing. Biological chemistry. 2001;382(6):925-31.
164.Eriksson JG, Forsen TJ, Mortensen SA, Rohde M. The effect of coenzyme Q10 administration on metabolic control in patients with type 2 diabetes mellitus. BioFactors. 1999;9(2-4):315-8.
165.Murtaugh LC, Melton DA. Genes, signals, and lineages in pancreas development. Annual review of cell and developmental biology. 2003;19:71-89.
166.Longnecker D. Anatomy and Histology of the Pancreas. The Pancreapedia. Exocrine pancreas knowledge base. Longnecker, D. 2014.
167.Korc M. Normal function of the endocrine pancreas. Chapter 38. In: The pancreas: biology, pathobiology, and disease, second edition, edited by Go VLW, et al. Raven press Ltd., New York, pp. 751-758, 1993.
168.Hellman B. Actual distribution of the number and volume of the islets of Langerhans in different size classes in non-diabetic humans of varying ages. Nature. 1959 Nov 7;184(Suppl 19):1498–1499.
169.Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes, obesity & metabolism. 2008;10 Suppl 4:32-42.
170.Wittingen J, Frey CF. Islet concentration in the head, body, tail and uncinate process of the pancreas. Annals of surgery. 1974;179(4):412-4.
171.Hellman B. The frequency distribution of the number and volume of the islets of Langerhans in man. Acta societatis medicorum upsaliensis. 64: 432-460, 1959.
172.Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, et al. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. The Journal of clinical endocrinology and metabolism. 2003;88(5):2300-8.
173.Longnecker D, Wilson, GL,. Pancreas. In: Handbook of toxicologic pathology, edited by Haschek-Hock WM and Rousseaux CG. Academic press Inc, San Diego, pp. 253-278, 1991.
174.Williams JA. Intracellular signaling mechanisms activated by cholecystokinin-regulating synthesis and secretion of digestive enzymes in pancreatic acinar cells. Annual review of physiology. 2001;63:77-97.
175.Muallem S, Kwiatkowska K, Xu X, Yin HL. Actin filament disassembly is a sufficient final trigger for exocytosis in nonexcitable cells. The Journal of cell biology. 1995;128(4):589-98.

176.Kimmich GA. Membrane potentials and the mechanism of intestinal Na(+)-dependent sugar transport. The Journal of membrane biology. 1990;114(1):1-27.
177.Wright EM, Martin MG, Turk E. Intestinal absorption in health and disease--sugars. Best practice & research Clinical gastroenterology. 2003;17(6):943-56.
178.Hofmann AF, Borgstrom B. Hydrolysis of long-chain monoglycerides in micellar solution by pancreatic lipase. Biochimica et biophysica acta. 1963;70:317-31.
179.Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nature genetics. 1996;14(2):141-5.
180.Pubols MH, Bartelt DC, Greene LJ. Trypsin inhibitor from human pancreas and pancreatic juice. The Journal of biological chemistry. 1974;249(7):2235-42.
181.Gorry MC, Gabbaizedeh D, Furey W, Gates LK, Jr., Preston RA, Aston CE, et al. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. Gastroenterology. 1997;113(4):1063-8.
182.Omary MB, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. The Journal of clinical investigation. 2007;117(1):50-9.
183.Apte MV, Pirola RC, Wilson JS. Battle-scarred pancreas: role of alcohol and pancreatic stellate cells in pancreatic fibrosis. Journal of gastroenterology and hepatology. 2006;21 Suppl 3:S97-S101.
184.Vonlaufen A, Phillips PA, Xu Z, Goldstein D, Pirola RC, Wilson JS, et al. Pancreatic stellate cells and pancreatic cancer cells: an unholy alliance. Cancer research. 2008;68(19):7707-10.
185.Bachem MG, Zhou S, Buck K, Schneiderhan W, Siech M. Pancreatic stellate cells--role in pancreas cancer. Langenbeck's archives of surgery / Deutsche Gesellschaft fur Chirurgie. 2008;393(6):891-900.
186.Bachem MG, Zhou Z, Zhou S, Siech M. Role of stellate cells in pancreatic fibrogenesis associated with acute and chronic pancreatitis. Journal of gastroenterology and hepatology. 2006;21 Suppl 3:S92-6.
187.Pandol S, Edderkaoui M, Gukovsky I, Lugea A, Gukovskaya A. Desmoplasia of pancreatic ductal adenocarcinoma. Clinical gastroenterology and hepatology : the official clinical practice journal of the American gastroenterological association. 2009;7(11 Suppl):S44-7.
188.Nussey S, Whitehead, S. Endocrinology: An integrated approach. Oxford: BIOS scientific publishers; 2001. Chapter 2, The endocrine pancreas. Available from: http://www.ncbi.nlm.nih.gov/books/NBK30/.
189.Lankisch PG, Apte M, Banks PA. Acute pancreatitis. Lancet. 2015;386(9988):85-96.
190.Sadr-Azodi O, Andren-Sandberg A, Orsini N, Wolk A. Cigarette smoking, smoking cessation and acute pancreatitis: a prospective population-based study. Gut. 2012;61(2):262-7.
191.Lindkvist B, Appelros S, Manjer J, Berglund G, Borgstrom A. A prospective cohort study of smoking in acute pancreatitis. Pancreatology : official journal of the international association of pancreatology. 2008;8(1):63-70.
192.Tolstrup JS, Kristiansen L, Becker U, Gronbaek M. Smoking and risk of acute and chronic pancreatitis among women and men: a population-based cohort study. Archives of internal medicine. 2009;169(6):603-9.
193.Carroll JK, Herrick B, Gipson T, Lee SP. Acute pancreatitis: diagnosis, prognosis, and treatment. American family physician. 2007;75(10):1513-20.
194.Mitchell RM, Byrne MF, Baillie J. Pancreatitis. Lancet. 2003;361(9367):1447-55.

195.Lankisch P. Epidemiology of acute pancreatitis. In: Buchler MW, Uhl W, Friess H, Malfertheiner P, eds. Acute pancreatitis: novel concepts in biology and therapy. Berlin: Blackwell Science, 1999:145–53.
196.Frossard JL, Steer ML, Pastor CM. Acute pancreatitis. Lancet. 2008;371(9607):143-52.
197.Tenner S, Dubner H, Steinberg W. Predicting gallstone pancreatitis with laboratory parameters: a meta-analysis. The American journal of gastroenterology. 1994;89(10):1863-6.
198.Gorelick FS. Alcohol and zymogen activation in the pancreatic acinar cell. Pancreas. 2003;27(4):305-10.
199.Whitcomb DC. Genetic polymorphisms in alcoholic pancreatitis. Digestive diseases. 2005;23(3-4):247-54.
200.Bassi C, Falconi M, Sartori N, Bonora A, Caldiron E, Butturini G, et al. The role of surgery in the major early complications of severe acute pancreatitis. European journal of gastroenterology & hepatology. 1997;9(2):131-6.
201.Go V, DiMagno, EP., Gardner, JD. The pancreas: biology, pathobiology, and disease. 2nd ed. New York, Raven Press, 1993.
202.Elfar M, Gaber LW, Sabek O, Fischer CP, Gaber AO. The inflammatory cascade in acute pancreatitis: relevance to clinical disease. The Surgical clinics of North America. 2007;87(6):1325-40, vii.
203.Guice KS, Oldham KT, Remick DG, Kunkel SL, Ward PA. Anti-tumor necrosis factor antibody augments edema formation in caerulein-induced acute pancreatitis. The Journal of surgical research. 1991;51(6):495-9.
204.Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. American journal of surgery. 1998;175(1):76-83.
205.Wenig BM, Heffess CS. Chapter 34 - inflammatory, infectious, and other non-neoplastic disorders of the pancreas in: Goldblum, Robert D. Odzejohn R. Surgical pathology of the gastrointestinal tract, liver, biliary tract, and pancreas (Second Edition). Philadelphia: W.B. Saunders; 2009. p. 877-907.
206.Choueiri NE, Balci NC, Alkaade S, Burton FR. Advanced imaging of chronic pancreatitis. Current gastroenterology reports. 2010;12(2):114-20.
207.Layer P, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. Gastroenterology. 1994;107(5):1481-7.
208.Cohn JA, Bornstein JD, Jowell PS. Cystic fibrosis mutations and genetic predisposition to idiopathic chronic pancreatitis. The Medical clinics of North America. 2000;84(3):621-31, ix.
209.Friess H, Yamanaka Y, Buchler M, Beger HG, Do DA, Kobrin MS, et al. Increased expression of acidic and basic fibroblast growth factors in chronic pancreatitis. The American journal of pathology. 1994;144(1):117-28.
210.Di Sebastiano P, di Mola FF, Di Febbo C, Baccante G, Porreca E, Innocenti P, et al. Expression of interleukin 8 (IL-8) and substance P in human chronic pancreatitis. Gut. 2000;47(3):423-8.
211.Chey W. Neurohormonal control of the exocrine pancreas. Current opinion in gastroenterology 1997;13:375–80.
212.American diabetes association: Diagnosis and Classification of Diabetes Mellitus. Diabetes care, volume 33, supplement 1, january 2010.
213.Ahmad SI. Prologue. Diabetes. An old disease, a new insight. Advances in experimental medicine and biology. 2012;771:xxvii-xxxiii.

214.Phillips JM, Parish NM, Raine T, Bland C, Sawyer Y, De La Pena H, et al. Type 1 diabetes development requires both CD4+ and CD8+ T cells and can be reversed by non-depleting antibodies targeting both T cell populations. The review of diabetic studies : RDS. 2009;6(2):97-103.
215.van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiological reviews. 2011;91(1):79-118.
216.Nerup J, Platz P, Andersen OO, Christy M, Lyngsoe J, Poulsen JE, et al. HL-A antigens and diabetes mellitus. Lancet. 1974;2(7885):864-6.
217.Noble JA, Valdes AM, Cook M, Klitz W, Thomson G, Erlich HA. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. American journal of human genetics. 1996;59(5):1134-48.
218.Jaidane H, Sane F, Gharbi J, Aouni M, Romond MB, Hober D. Coxsackievirus B4 and type 1 diabetes pathogenesis: contribution of animal models. Diabetes/metabolism research and reviews. 2009;25(7):591-603.
219.Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. The New England journal of medicine. 2002;347(12):911-20.
220.Goldberg E, Krause I. Infection and type 1 diabetes mellitus - a two edged sword? Autoimmunity reviews. 2009;8(8):682-6.
221.Christen U, von Herrath MG. Do viral infections protect from or enhance type 1 diabetes and how can we tell the difference? Cellular & molecular immunology. 2011;8(3):193-8.
222.Knip M, Veijola R, Virtanen SM, Hyoty H, Vaarala O, Akerblom HK. Environmental triggers and determinants of type 1 diabetes. Diabetes. 2005;54 Suppl 2:S125-36.
223.Hober D, Sauter P. Pathogenesis of type 1 diabetes mellitus: interplay between enterovirus and host. Nature reviews Endocrinology. 2010;6(5):279-89.
224.Roivainen M, Ylipaasto P, Savolainen C, Galama J, Hovi T, Otonkoski T. Functional impairment and killing of human beta cells by enteroviruses: the capacity is shared by a wide range of serotypes, but the extent is a characteristic of individual virus strains. Diabetologia. 2002;45(5):693-702.
225.Hyoty H. Enterovirus infections and type 1 diabetes. Annals of medicine. 2002;34(3):138-47.
226.Haverkos HW, Battula N, Drotman DP, Rennert OM. Enteroviruses and type 1 diabetes mellitus. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2003;57(9):379-85.
227.Yin H, Berg AK, Tuvemo T, Frisk G. Enterovirus RNA is found in peripheral blood mononuclear cells in a majority of type 1 diabetic children at onset. Diabetes. 2002;51(6):1964-71.
228.Sarmiento L, Cabrera-Rode E, Lekuleni L, Cuba I, Molina G, Fonseca M, et al. Occurrence of enterovirus RNA in serum of children with newly diagnosed type 1 diabetes and islet cell autoantibody-positive subjects in a population with a low incidence of type 1 diabetes. Autoimmunity. 2007;40(7):540-5.
229.Vaarala O, Atkinson MA, Neu J. The "perfect storm" for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. Diabetes. 2008;57(10):2555-62.
230.Action to Control Cardiovascular Risk in Diabetes Study G, Gerstein HC, Miller ME, Byington RP, Goff DC, Jr., Bigger JT, et al. Effects of intensive glucose lowering in type 2 diabetes. The New England journal of medicine. 2008;358(24):2545-59.

231.Home P, Mant J, Diaz J, Turner C. Management of type 2 diabetes: summary of updated NICE guidance. British medical journal. 2008;336(7656):1306-8.
232.Barnett AH, Eff C, Leslie RD, Pyke DA. Diabetes in identical twins. A study of 200 pairs. Diabetologia. 1981;20(2):87-93.
233.Knowler WC, Saad MF, Pettitt DJ, Nelson RG, Bennett PH. Determinants of diabetes mellitus in the Pima Indians. Diabetes care. 1993;16(1):216-27.
234.Warburton DER, Nicol CW, Bredin SSD. Health benefits of physical activity: the evidence. CMAJ : Canadian medical association journal. 2006;174(6):801-9.
235.Akash MS, Rehman K, Chen S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. Journal of cellular biochemistry. 2013;114(3):525-31.
236.Kasuga M. Insulin resistance and pancreatic beta cell failure. The Journal of clinical investigation. 2006;116(7):1756-60.
237.Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature. 2001;409(6821):729-33.
238.Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, et al. Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. Cell metabolism. 2005;1(2):107-19.
239.Poitout V, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocrine reviews. 2008;29(3):351-66.
240.Cnop M, Igoillo-Esteve M, Cunha DA, Ladriere L, Eizirik DL. An update on lipotoxic endoplasmic reticulum stress in pancreatic beta-cells. Biochemical society transactions. 2008;36(Pt 5):909-15.
241.Eizirik DL, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. Endocrine reviews. 2008;29(1):42-61.
242.Lambert AJ, Brand MD. Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:ubiquinone oxidoreductase (complex I). The Journal of biological chemistry. 2004;279(38):39414-20.
243.Uchida T, Nakamura T, Hashimoto N, Matsuda T, Kotani K, Sakaue H, et al. Deletion of Cdkn1b ameliorates hyperglycemia by maintaining compensatory hyperinsulinemia in diabetic mice. Nature medicine. 2005;11(2):175-82.
244.Velho G, Froguel P. Maturity-onset diabetes of the young (MODY), MODY genes and non-insulin-dependent diabetes mellitus. Diabetes & metabolism. 1997;23 Suppl 2:34-7.
245.Muniraj T, Jamidar PA, Aslanian HR. Pancreatic cancer: a comprehensive review and update. Disease-a-month : DM. 2013;59(11):368-402.
246.Matthaei H, Dal Molin M, Maitra A. Identification and analysis of precursors to invasive pancreatic cancer. Methods in molecular biology. 2013;980:1-12.
247.Maitra A, Hruban RH. Pancreatic cancer. Annual review of pathology. 2008;3:157-88.
248.Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best practice & research clinical gastroenterology. 2006;20(2):197-209.
249.Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J, Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. American journal of epidemiology. 2001;153(7):680-7.
250.Villeneuve PJ, Johnson KC, Hanley AJ, Mao Y. Alcohol, tobacco and coffee consumption and the risk of pancreatic cancer: results from the Canadian Enhanced Surveillance System case-control project. Canadian cancer registries epidemiology research group. European journal of cancer prevention : the official journal of the European cancer prevention organisation. 2000;9(1):49-58.

251.Genkinger JM, Spiegelman D, Anderson KE, Bergkvist L, Bernstein L, van den Brandt PA, et al. Alcohol intake and pancreatic cancer risk: a pooled analysis of fourteen cohort studies. Cancer epidemiology, biomarkers & prevention : a publication of the American association for cancer research, cosponsored by the American society of preventive oncology. 2009;18(3):765-76.
252.Amundadottir LT, Thorvaldsson S, Gudbjartsson DF, Sulem P, Kristjansson K, Arnason S, et al. Cancer as a complex phenotype: pattern of cancer distribution within and beyond the nuclear family. PLoS medicine. 2004;1(3):e65.
253.Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008;321(5897):1801-6.
254.Saiki Y, Horii A. Molecular pathology of pancreatic cancer. Pathology international. 2014;64(1):10-9.
255.Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. The American journal of surgical pathology. 2001;25(5):579-86.
256.Hruban RH, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Biankin AV, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. The American journal of surgical pathology. 2004;28(8):977-87.
257.Maitra A, Fukushima N, Takaori K, Hruban RH. Precursors to invasive pancreatic cancer. Advances in anatomic pathology. 2005;12(2):81-91.
258.van Heek NT, Meeker AK, Kern SE, Yeo CJ, Lillemoe KD, Cameron JL, et al. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. The American journal of pathology. 2002;161(5):1541-7.
259.House MG, Yeo CJ, Schulick RD. Periampullary pancreatic somatostatinoma. Annals of surgical oncology. 2002;9(9):869-74.
260.Hruban RH, Pitman MB, Klimstra DS. Tumors of the pancreas. In: Atlas of tumor pathology. 4th Series. Fascicle 6. Washington, DC: Armed forces institutes of pathology; 2007.
261.Basturk O, Coban I, Adsay NV. Pancreatic cysts: pathologic classification, differential diagnosis, and clinical implications. Archives of pathology & laboratory medicine. 2009;133(3):423-38.
262.Hruban RH, Adsay NV. Molecular classification of neoplasms of the pancreas. Human pathology. 2009;40(5):612-23.
263.Shi C, Daniels JA, Hruban RH. Molecular characterization of pancreatic neoplasms. Advances in anatomic pathology. 2008;15(4):185-95.
264.Abraham SC, Klimstra DS, Wilentz RE, Yeo CJ, Conlon K, Brennan M, et al. Solid-pseudopapillary tumors of the pancreas are genetically distinct from pancreatic ductal adenocarcinomas and almost always harbor beta-catenin mutations. The American journal of pathology. 2002;160(4):1361-9.
265.Kerr NJ, Chun YH, Yun K, Heathcott RW, Reeve AE, Sullivan MJ. Pancreatoblastoma is associated with chromosome 11p loss of heterozygosity and IGF2 overexpression. Medical and pediatric oncology. 2002;39(1):52-4.
266.Mohr VH, Vortmeyer AO, Zhuang Z, Libutti SK, Walther MM, Choyke PL, et al. Histopathology and molecular genetics of multiple cysts and microcystic (serous) adenomas of the pancreas in von Hippel-Lindau patients. The American journal of pathology. 2000;157(5):1615-21.

267.Thirabanjasak D, Basturk O, Altinel D, Cheng JD, Adsay NV. Is serous cystadenoma of the pancreas a model of clear-cell-associated angiogenesis and tumorigenesis? Pancreatology : official journal of the International Association of Pancreatology. 2009;9(1-2):182-8.
268.Milan SA, Yeo CJ. Neuroendocrine tumors of the pancreas. Current opinion in oncology. 2012;24(1):46-55.
269.Dixon E, Pasieka JL. Functioning and nonfunctioning neuroendocrine tumors of the pancreas. Current opinion in oncology. 2007;19(1):30-5.
270.Kennedy EP, Brody JP, Yeo CJ. Neoplasms of the endocrine pancreas. In Mulholland MW, Lillemoe KD, Doherty GM, et al., editors. Greenfield's surgery: scientific principles and practice. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2010.
271.Wilcox CM, Seay T, Arcury JT. Zollinger-Ellison syndrome: presentation, response to therapy, and outcome. Digestive and liver disease : official journal of the Italian society of gastroenterology and the Italian association for the study of the liver. 2011;43(6):439-43.
272.Jabbour SA. Skin manifestations of hormone-secreting tumors. Dermatologic therapy. 2010;23(6):643-50.
273.Eldor R, Glaser B, Fraenkel M, Doviner V, Salmon A, Gross DJ. Glucagonoma and the glucagonoma syndrome - cumulative experience with an elusive endocrine tumour. Clinical endocrinology. 2011;74(5):593-8.
274.Adam N, Lim SS, Ananda V, Chan SP. VIPoma syndrome: challenges in management. Singapore medical journal. 2010;51(7):e129-32.
275.Kloppel G, Maillet B. Classification and staging of pancreatic nonendocrine tumors. Radiologic clinics of North America. 1989;27(1):105-19.
276.Ferrozzi F, Zuccoli G, Bova D, Calculli L. Mesenchymal tumors of the pancreas: CT findings. Journal of computer assisted tomography. 2000;24(4):622-7.
277.Katz DS, Hines J, Math KR, Nardi PM, Mindelzun RE, Lane MJ. Using CT to reveal fat-containing abnormalities of the pancreas. American journal of roentgenology. 1999;172(2):393-6.
278.Lee JY, Kim IO, Kim WS, Kim CW, Yeon KM. CT and US findings of pancreatoblastoma. Journal of computer assisted tomography. 1996;20(3):370-4.
279.Ferrozzi F, Campani R, Garlaschi G, Campodonico F. [Extranodal lymphomas: computerized tomography features and differential diagnosis]. La Radiologia medica. 1997;93(4):429-41.
280.Zalatnai A, Kovacs M, Flautner L, Sipos B, Sarkady E, Bocsi J. Pancreatic leiomyosarcoma. Case report with immunohistochemical and flow cytometric studies. Virchows Archiv : an international journal of pathology. 1998;432(5):469-72.
281.Andrew W. Senile changes in the pancreas of wistar institute rats and of man with special regard to the similarity of locule and cavity formation. The American journal of anatomy 1944;74:97-127.
282.Stamm BH. Incidence and diagnostic significance of minor pathologic changes in the adult pancreas at autopsy: a systematic study of 112 autopsies in patients without known pancreatic disease. Human pathology. 1984;15(7):677-83.
283.Schmitz-Moormann P, Otte CA, Ihm P, Schmidt G. [Comparative radiological and morphological study of the human pancreas. III. Morphometric investigation of the major pancreatic duct (author's transl)]. Zeitschrift fur gastroenterologie. 1979;17(4):256-63.

284.Schmitz-Moormann P, Hein J. [Changes of the pancreatic duct system associated with aging: their relations to parenchyma (author's transl)]. Virchows Archiv A, Pathological anatomy and histology. 1976;371(2):145-52.
285.Heuck A, Maubach PA, Reiser M, Feuerbach S, Allgayer B, Lukas P, et al. Age-related morphology of the normal pancreas on computed tomography. Gastrointestinal radiology. 1987;12(1):18-22.
286.Hastier P, Buckley MJ, Dumas R, Kuhdorf H, Staccini P, Demarquay JF, et al. A study of the effect of age on pancreatic duct morphology. Gastrointestinal endoscopy. 1998;48(1):53-7.
287.Perret RS, Sloop GD, Borne JA. Common bile duct measurements in an elderly population. Journal of ultrasound in medicine : official journal of the American institute of ultrasound in medicine. 2000;19(11):727-30; quiz 31.
288.Glaser J, Stienecker K. Pancreas and aging: a study using ultrasonography. Gerontology. 2000;46(2):93-6.
289.Tsushima Y, Kusano S. Age-dependent decline in parenchymal perfusion in the normal human pancreas: measurement by dynamic computed tomography. Pancreas. 1998;17(2):148-52.
290.Greenberg RE, Holt PR. Influence of aging upon pancreatic digestive enzymes. Digestive diseases and sciences. 1986;31(9):970-7.
291.Jiang ZE, Jiang C, Chen B, Koh CS, Yong JH, Park DH, et al. Age-associated changes in pancreatic exocrine secretion of the isolated perfused rat pancreas. Laboratory animal research. 2013;29(1):19-26.
292.Gullo L, Priori P, Daniele C, Ventrucci M, Gasbarrini G, Labo G. Exocrine pancreatic function in the elderly. Gerontology. 1983;29(6):407-11.
293.Gullo L, Ventrucci M, Naldoni P, Pezzilli R. Aging and exocrine pancreatic function. Journal of the American geriatrics society. 1986;34(11):790-2.
294.Bartos V, Groh J. The effect of repeated stimulation of the pancreas on the pancreatic secretion in young and aged men. Gerontologia clinica. 1969;11(1):56-62.
295.Arora S, Kassarjian Z, Krasinski SD, Croffey B, Kaplan MM, Russell RM. Effect of age on tests of intestinal and hepatic function in healthy humans. Gastroenterology. 1989;96(6):1560-5.
296.Werner I, Hambraeus L. The digestive capacity of elderly people; in Carsol LA (ed):Nutrition in Old age. Uppsala, Almqvist and Wicksel, 1972, pp 55-60.
297.Feibusch JM, Holt PR. Impaired absorptive capacity for carbohydrate in the aging human. Digestive diseases and sciences. 1982;27(12):1095-100.
298.Saltzman JR, Kowdley KV, Pedrosa MC, Sepe T, Golner B, Perrone G, et al. Bacterial overgrowth without clinical malabsorption in elderly hypochlorhydric subjects. Gastroenterology. 1994;106(3):615-23.
299.Hurwitz A, Brady DA, Schaal SE, Samloff IM, Dedon J, Ruhl CE. Gastric acidity in older adults. Jama. 1997;278(8):659-62.
300.Bosetti C, Lucenteforte E, Silverman DT, Petersen G, Bracci PM, Ji BT, et al. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). Annals of oncology : official journal of the European society for medical oncology / ESMO. 2012;23(7):1880-8.
301.Iodice S, Gandini S, Maisonneuve P, Lowenfels AB. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. Langenbeck's archives of surgery / Deutsche Gesellschaft fur Chirurgie. 2008;393(4):535-45.
302.Ansary-Moghaddam A, Huxley R, Barzi F, Lawes C, Ohkubo T, Fang X, et al. The effect of modifiable risk factors on pancreatic cancer mortality in populations of the Asia-Pacific region. Cancer epidemiology, biomarkers & prevention : a publication of

the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(12):2435-40.
303.Katanoda K, Marugame T, Saika K, Satoh H, Tajima K, Suzuki T, et al. Population attributable fraction of mortality associated with tobacco smoking in Japan: a pooled analysis of three large-scale cohort studies. Journal of epidemiology / Japan Epidemiological Association. 2008;18(6):251-64.
304.Lynch SM, Vrieling A, Lubin JH, Kraft P, Mendelsohn JB, Hartge P, et al. Cigarette smoking and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. American journal of epidemiology. 2009;170(4):403-13.
305.Matsuo K, Ito H, Wakai K, Nagata C, Mizoue T, Tanaka K, et al. Cigarette smoking and pancreas cancer risk: an evaluation based on a systematic review of epidemiologic evidence in the Japanese population. Japanese journal of clinical oncology. 2011;41(11):1292-302.
306.Zou L, Zhong R, Shen N, Chen W, Zhu B, Ke J, et al. Non-linear dose-response relationship between cigarette smoking and pancreatic cancer risk: evidence from a meta-analysis of 42 observational studies. European journal of cancer. 2014;50(1):193-203.
307.Pelucchi C, Galeone C, Polesel J, Manzari M, Zucchetto A, Talamini R, et al. Smoking and body mass index and survival in pancreatic cancer patients. Pancreas. 2014;43(1):47-52.
308.Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J. Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. Jama. 2007;298(22):2654-64.
309.Xie XT, Liu Q, Wu J, Wakui M. Impact of cigarette smoking in type 2 diabetes development. Acta pharmacologica Sinica. 2009;30(6):784-7.
310.Thankappan KR, Mini GK, Daivadanam M, Vijayakumar G, Sarma PS, Nichter M. Smoking cessation among diabetes patients: results of a pilot randomized controlled trial in Kerala, India. BMC public health. 2013;13:47.
311.Chang SA. Smoking and type 2 diabetes mellitus. Diabetes & metabolism journal. 2012;36(6):399-403.
312.Nagrebetsky A, Brettell R, Roberts N, Farmer A. Smoking cessation in adults with diabetes: a systematic review and meta-analysis of data from randomised controlled trials. BMJ open. 2014;4(3):e004107.
313.Alexandre M, Pandol SJ, Gorelick FS, Thrower EC. The emerging role of smoking in the development of pancreatitis. Pancreatology : official journal of the International association of pancreatology. 2011;11(5):469-74.
314.Yuhara H, Ogawa M, Kawaguchi Y, Igarashi M, Mine T. Smoking and risk for acute pancreatitis: a systematic review and meta-analysis. Pancreas. 2014;43(8):1201-7.
315.Luaces-Regueira M, Iglesias-Garcia J, Lindkvist B, Castineira-Alvarino M, Nieto-Garcia L, Larino-Noia J, et al. Smoking as a risk factor for complications in chronic pancreatitis. Pancreas. 2014;43(2):275-80.
316.Brock C, Nielsen LM, Lelic D, Drewes AM. Pathophysiology of chronic pancreatitis. World journal of gastroenterology. 2013;19(42):7231-40.
317.Andriulli A, Botteri E, Almasio PL, Vantini I, Uomo G, Maisonneuve P, et al. Smoking as a cofactor for causation of chronic pancreatitis: a meta-analysis. Pancreas. 2010;39(8):1205-10.
318.van Geenen EJ, Smits MM, Schreuder TC, van der Peet DL, Bloemena E, Mulder CJ. Smoking is related to pancreatic fibrosis in humans. The American journal of gastroenterology. 2011;106(6):1161-6; quiz 7.
319.Giacosa A, Adam-Blondon AF, Baer-Sinnott S, Barale R, Bavaresco L, Di Gaspero G, et al. Alcohol and wine in relation to cancer and other diseases. European journal of

cancer prevention : the official journal of the European cancer prevention Organisation. 2012;21(1):103-8.
320.Lucenteforte E, La Vecchia C, Silverman D, Petersen GM, Bracci PM, Ji BT, et al. Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2012;23(2):374-82.
321.Herreros-Villanueva M, Hijona E, Banales JM, Cosme A, Bujanda L. Alcohol consumption on pancreatic diseases. World journal of gastroenterology. 2013;19(5):638-47.
322.Gapstur SM, Jacobs EJ, Deka A, McCullough ML, Patel AV, Thun MJ. Association of alcohol intake with pancreatic cancer mortality in never smokers. Archives of internal medicine. 2011;171(5):444-51.
323.Duell EJ. Epidemiology and potential mechanisms of tobacco smoking and heavy alcohol consumption in pancreatic cancer. Molecular carcinogenesis. 2012;51(1):40-52.
324.Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144(6):1252-61.
325.DiMagno MJ, DiMagno EP. Chronic pancreatitis. Current opinion in gastroenterology. 2013;29(5):531-6.
326.Dufour MC, Adamson MD. The epidemiology of alcohol-induced pancreatitis. Pancreas. 2003;27(4):286-90.
327.Vonlaufen A, Phillips PA, Xu Z, Zhang X, Yang L, Pirola RC, et al. Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of LPS-induced pancreatic injury in alcohol-fed rats. Gut. 2011;60(2):238-46.
328.Pietraszek A, Gregersen S, Hermansen K. Alcohol and type 2 diabetes. A review. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2010;20(5):366-75.
329.Rasouli B, Ahlbom A, Andersson T, Grill V, Midthjell K, Olsson L, et al. Alcohol consumption is associated with reduced risk of Type 2 diabetes and autoimmune diabetes in adults: results from the Nord-Trondelag health study. Diabetic medicine : a journal of the British diabetic association. 2013;30(1):56-64.
330.Cullmann M, Hilding A, Ostenson CG. Alcohol consumption and risk of pre-diabetes and type 2 diabetes development in a Swedish population. Diabetic medicine : a journal of the British diabetic association. 2012;29(4):441-52.
331.Kim SJ, Kim DJ. Alcoholism and diabetes mellitus. Diabetes & metabolism journal. 2012;36(2):108-15.
332.Bracci PM. Obesity and pancreatic cancer: overview of epidemiologic evidence and biologic mechanisms. Molecular carcinogenesis. 2012;51(1):53-63.
333.Stolzenberg-Solomon RZ, Adams K, Leitzmann M, Schairer C, Michaud DS, Hollenbeck A, et al. Adiposity, physical activity, and pancreatic cancer in the National Institutes of Health-AARP Diet and Health Cohort. American journal of epidemiology. 2008;167(5):586-97.
334.Aune D, Greenwood DC, Chan DS, Vieira R, Vieira AR, Navarro Rosenblatt DA, et al. Body mass index, abdominal fatness and pancreatic cancer risk: a systematic review and non-linear dose-response meta-analysis of prospective studies. Annals of oncology : official journal of the European society for medical oncology / ESMO. 2012;23(4):843-52.
335.Blomgren KB, Sundstrom A, Steineck G, Wiholm BE. Obesity and treatment of diabetes with glyburide may both be risk factors for acute pancreatitis. Diabetes care. 2002;25(2):298-302.

336.Hong S, Qiwen B, Ying J, Wei A, Chaoyang T. Body mass index and the risk and prognosis of acute pancreatitis: a meta-analysis. European journal of gastroenterology & hepatology. 2011;23(12):1136-43.
337.Sadr-Azodi O, Orsini N, Andren-Sandberg A, Wolk A. Abdominal and total adiposity and the risk of acute pancreatitis: a population-based prospective cohort study. The American journal of gastroenterology. 2013;108(1):133-9.
338.Davor Štimac and Neven Franjić (2012). Obesity and Acute Pancreatitis, Acute Pancreatitis, Prof. Luis Rodrigo (Ed.), ISBN: 978-953-307-984-4, InTech, DOI: 10.5772/26839. Available from: http://www.intechopen.com/books/acute-pancreatitis/obesity-and-acute-pancreatitis.
339.Nitsche C, Simon P, Weiss FU, Fluhr G, Weber E, Gartner S, et al. Environmental risk factors for chronic pancreatitis and pancreatic cancer. Digestive diseases. 2011;29(2):235-42.
340.Twig G, Afek A, Derazne E, Tzur D, Cukierman-Yaffe T, Gerstein HC, et al. Diabetes risk among overweight and obese metabolically healthy young adults. Diabetes care. 2014;37(11):2989-95.
341.Hu Y, Bhupathiraju SN, de Koning L, Hu FB. Duration of obesity and overweight and risk of type 2 diabetes among US women. Obesity. 2014;22(10):2267-73.
342.Zeyda M, Stulnig TM. Obesity, inflammation, and insulin resistance--a mini-review. Gerontology. 2009;55(4):379-86.
343.Behrens G, Jochem C, Schmid D, Keimling M, Ricci C, Leitzmann MF. Physical activity and risk of pancreatic cancer: a systematic review and meta-analysis. European journal of epidemiology. 2015;30(4):279-98.
344.Clague J, Bernstein L. Physical activity and cancer. Current oncology reports. 2012;14(6):550-8.
345.Cormie P, Spry N, Jasas K, Johansson M, Yusoff IF, Newton RU, et al. Exercise as medicine in the management of pancreatic cancer: a case study. Medicine and science in sports and exercise. 2014;46(4):664-70.
346.Bird SR, Hawley JA. Exercise and type 2 diabetes: new prescription for an old problem. Maturitas. 2012;72(4):311-6.
347.Teixeira-Lemos E, Nunes S, Teixeira F, Reis F. Regular physical exercise training assists in preventing type 2 diabetes development: focus on its antioxidant and anti-inflammatory properties. Cardiovascular diabetology. 2011;10:12.
348.Chimen M, Kennedy A, Nirantharakumar K, Pang TT, Andrews R, Narendran P. What are the health benefits of physical activity in type 1 diabetes mellitus? A literature review. Diabetologia. 2012;55(3):542-51.
349.Burr JF, Shephard RJ, Riddell MC. Physical activity in type 1 diabetes mellitus: assessing risks for physical activity clearance and prescription. Canadian family physician Medecin de famille canadien. 2012;58(5):533-5.
350.Sofi F, Macchi C, Abbate R, Gensini GF, Casini A. Mediterranean diet and health. BioFactors. 2013;39(4):335-42.
351.Moreira PI. High-sugar diets, type 2 diabetes and Alzheimer's disease. Current opinion in clinical nutrition and metabolic care. 2013;16(4):440-5.
352.Aune D, Chan DS, Vieira AR, Navarro Rosenblatt DA, Vieira R, Greenwood DC, et al. Dietary fructose, carbohydrates, glycemic indices and pancreatic cancer risk: a systematic review and meta-analysis of cohort studies. Annals of oncology : official journal of the European society for medical oncology / ESMO. 2012;23(10):2536-46.
353.Roncal-Jimenez CA, Lanaspa MA, Rivard CJ, Nakagawa T, Sanchez-Lozada LG, Jalal D, et al. Sucrose induces fatty liver and pancreatic inflammation in male breeder

rats independent of excess energy intake. Metabolism: clinical and experimental. 2011;60(9):1259-70.
354.Honma T, Shinohara N, Ito J, Kijima R, Sugawara S, Arai T, et al. High-fat diet intake accelerates aging, increases expression of Hsd11b1, and promotes lipid accumulation in liver of SAMP10 mouse. Biogerontology. 2012;13(2):93-103.
355.Dawson DW, Hertzer K, Moro A, Donald G, Chang HH, Go VL, et al. High-fat, high-calorie diet promotes early pancreatic neoplasia in the conditional KrasG12D mouse model. Cancer prevention research. 2013;6(10):1064-73.
356.Yan MX, Ren HB, Kou Y, Meng M, Li YQ. Involvement of nuclear factor kappa B in high-fat diet-related pancreatic fibrosis in rats. Gut and liver. 2012;6(3):381-7.
357.Yago MD, Gonzalez MV, Martinez-Victoria E, Mataix J, Medrano J, Calpena R, et al. Pancreatic enzyme secretion in response to test meals differing in the quality of dietary fat (olive and sunflowerseed oils) in human subjects. The British journal of nutrition. 1997;78(1):27-39.
358.Martinez MA, Lajas AI, Yago MD, Redondo PC, Granados MP, Gonzalez A, et al. Dietary virgin olive oil enhances secretagogue-evoked calcium signaling in rat pancreatic acinar cells. Nutrition. 2004;20(6):536-41.
359.Saleh NK, Saleh HA. Olive oil improved the impairment of in vitro insulin-stimulated glucose uptake by diaphragm in ovariectomized female Wistar rats. Experimental gerontology. 2010;45(12):964-9.
360.Vassiliou EK, Gonzalez A, Garcia C, Tadros JH, Chakraborty G, Toney JH. Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNF-alpha both in vitro and in vivo systems. Lipids in health and disease. 2009;8:25.
361.Psaltopoulou T, Kosti RI, Haidopoulos D, Dimopoulos M, Panagiotakos DB. Olive oil intake is inversely related to cancer prevalence: a systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. Lipids in health and disease. 2011;10:127.
362.Bosetti C, Turati F, Dal Pont A, Ferraroni M, Polesel J, Negri E, et al. The role of Mediterranean diet on the risk of pancreatic cancer. British journal of cancer. 2013;109(5):1360-6.
363.Salas-Salvado J, Bullo M, Babio N, Martinez-Gonzalez MA, Ibarrola-Jurado N, Basora J, et al. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes care. 2011;34(1):14-9.
364.Ceriello A, Esposito K, La Sala L, Pujadas G, De Nigris V, Testa R, et al. The protective effect of the Mediterranean diet on endothelial resistance to GLP-1 in type 2 diabetes: a preliminary report. Cardiovascular diabetology. 2014;13:140.
365.Oliveras-Lopez MJ, Berna G, Carneiro EM, Lopez-Garcia de la Serrana H, Martin F, Lopez MC. An extra-virgin olive oil rich in polyphenolic compounds has antioxidant effects in OF1 mice. The Journal of nutrition. 2008;138(6):1074-8.
366.Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. Journal of the American dietetic association. 2009;109(4):668-79.
367.Romanatto T, Fiamoncini J, Wang B, Curi R, Kang JX. Elevated tissue omega-3 fatty acid status prevents age-related glucose intolerance in fat-1 transgenic mice. Biochimica et biophysica acta. 2014;1842(2):186-91.
368.Hirabara SM, Folador A, Fiamoncini J, Lambertucci RH, Rodrigues CF, Jr., Rocha MS, et al. Fish oil supplementation for two generations increases insulin sensitivity in rats. The Journal of nutritional biochemistry. 2013;24(6):1136-45.

369.Qin B, Xun P, He K. Fish or long-chain (n-3) PUFA intake is not associated with pancreatic cancer risk in a meta-analysis and systematic review. The Journal of nutrition. 2012;142(6):1067-73.
370.Odabasoglu F, Halici Z, Cakir A, Halici M, Aygun H, Suleyman H, et al. Beneficial effects of vegetable oils (corn, olive and sunflower oils) and alpha-tocopherol on anti-inflammatory and gastrointestinal profiles of indomethacin in rats. European journal of pharmacology. 2008;591(1-3):300-6.
371.Laugerette F, Vors C, Peretti N, Michalski MC. Complex links between dietary lipids, endogenous endotoxins and metabolic inflammation. Biochimie. 2011;93(1):39-45.
372.Masi LN, Martins AR, Rosa Neto JC, do Amaral CL, Crisma AR, Vinolo MA, et al. Sunflower oil supplementation has proinflammatory effects and does not reverse insulin resistance in obesity induced by high-fat diet in C57BL/6 mice. Journal of biomedicine & biotechnology. 2012;2012:945131.
373.Tinahones FJ, Pareja A, Soriguer FJ, Gomez-Zumaquero JM, Cardona F, Rojo-Martinez G. Dietary fatty acids modify insulin secretion of rat pancreatic islet cells in vitro. Journal of endocrinological investigation. 2002;25(5):436-41.
374.Diaz RJ, Yago MD, Martinez-Victoria E, Naranjo JA, Martinez MA, Manas M. Comparison of the effects of dietary sunflower oil and virgin olive oil on rat exocrine pancreatic secretion in vivo. Lipids. 2003;38(11):1119-26.
375.Reeves PG, Nielsen FH, Fahey GC, Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of nutrition. 1993;123(11):1939-51.
376.Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. The Journal of nutrition. 1997;127(5 Suppl):838S-41S.
377.Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. Diabetes. 2004;53(7):1714-20.
378.Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of clinical pathology. 1969;22(2):158-61.
379.Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clinical chemistry. 1973;19(5):476-82.
380.Naito HK, David JA. Laboratory considerations: determination of cholesterol, triglyceride, phospholipid, and other lipids in blood and tissues. Laboratory and research methods in biology and medicine. 1984;10:1-76.
381.Young D, Friedman, RB. Effects of Disease on Clinical Laboratory Tests. Washington, DC: AACC Press; 2001
382.Bowers LD, Wong ET. Kinetic serum creatinine assays. II. A critical evaluation and review. Clinical chemistry. 1980;26(5):555-61.
383.Weisshaar D, Gossrau E, Faderl B. [Normal ranges of alpha-HBDH, LDH, AP, and LAP as measured with substrate-optimated test charges]. Die Medizinische Welt. 1975;26(9):387-92.
384.Wallace TM, Matthews DR. The assessment of insulin resistance in man. Diabetic medicine : a journal of the British diabetic association. 2002;19(7):527-34.
385.Jones HB, Nugent D, Jenkins R. Variation in characteristics of islets of Langerhans in insulin-resistant, diabetic and non-diabetic-rat strains. International journal of experimental pathology. 2010;91(3):288-301.

386.Sattar N, Wannamethee SG, Forouhi NG. Novel biochemical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities? Diabetologia. 2008;51(6):926-40.
387.Finegood DT, Scaglia L, Bonner-Weir S. Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. Diabetes. 1995;44(3):249-56.
388.Meier JJ, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. Diabetes. 2008;57(6):1584-94.
389.Teta M, Long SY, Wartschow LM, Rankin MM, Kushner JA. Very slow turnover of beta-cells in aged adult mice. Diabetes. 2005;54(9):2557-67.
390.Chu WM. Tumor necrosis factor. Cancer letters. 2013;328(2):222-5.
391.Kloppel G, Detlefsen S, Feyerabend B. Fibrosis of the pancreas: the initial tissue damage and the resulting pattern. Virchows Archiv : an international journal of pathology. 2004;445(1):1-8.
392.Apte MV, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, et al. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. Gut. 1998;43(1):128-33.
393.Bachem MG, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, et al. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. Gastroenterology. 1998;115(2):421-32.
394.Bedossa P, Paradis V. Liver extracellular matrix in health and disease. The Journal of pathology. 2003;200(4):504-15.
395.Andoh A, Takaya H, Saotome T, Shimada M, Hata K, Araki Y, et al. Cytokine regulation of chemokine (IL-8, MCP-1, and RANTES) gene expression in human pancreatic periacinar myofibroblasts. Gastroenterology. 2000;119(1):211-9.
396.Luttenberger T, Schmid-Kotsas A, Menke A, Siech M, Beger H, Adler G, et al. Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: implications in pathogenesis of pancreas fibrosis. Laboratory investigation; a journal of technical methods and pathology. 2000;80(1):47-55.
397.Saurer L, Reber P, Schaffner T, Buchler MW, Buri C, Kappeler A, et al. Differential expression of chemokines in normal pancreas and in chronic pancreatitis. Gastroenterology. 2000;118(2):356-67.
398.Shek FW, Benyon RC, Walker FM, McCrudden PR, Pender SL, Williams EJ, et al. Expression of transforming growth factor-beta 1 by pancreatic stellate cells and its implications for matrix secretion and turnover in chronic pancreatitis. The American journal of pathology. 2002;160(5):1787-98.
399.Czako L, Hegyi P, Rakonczay Z, Jr., Wittmann T, Otsuki M. Interactions between the endocrine and exocrine pancreas and their clinical relevance. Pancreatology : official journal of the international association of pancreatology. 2009;9(4):351-9.
400.Maron DJ, Fair JM, Haskell WL. Saturated fat intake and insulin resistance in men with coronary artery disease. The Stanford Coronary Risk Intervention Project Investigators and Staff. Circulation. 1991;84(5):2020-7.
401.Soriguer F, Esteva I, Rojo-Martinez G, Ruiz de Adana MS, Dobarganes MC, Garcia-Almeida JM, et al. Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study). European journal of endocrinology / European federation of endocrine societies. 2004;150(1):33-9.
402.Mayer EJ, Newman B, Quesenberry CP, Jr., Selby JV. Usual dietary fat intake and insulin concentrations in healthy women twins. Diabetes care. 1993;16(11):1459-69.

403.Ryan M, McInerney D, Owens D. Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. Monthly journal of the association of physicians. 2000;93(2):85-91.
404.Marshall JA, Bessesen DH, Hamman RF. High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: the San Luis Valley Diabetes Study. Diabetologia. 1997;40(4):430-8.
405.Garg A, Bonanome A, Grundy SM, Zhang ZJ, Unger RH. Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. The New England journal of medicine. 1988;319(13):829-34.
406.Rojo-Martinez G, Esteva I, Ruiz de Adana MS, Garcia-Almeida JM, Tinahones F, Cardona F, et al. Dietary fatty acids and insulin secretion: a population-based study. European journal of clinical nutrition. 2006;60(10):1195-200.
407.Umezawa M, Higuchi K, Mori M, Matushita T, Hosokawa M. Effect of dietary unsaturated fatty acids on senile amyloidosis in senescence-accelerated mice. The journals of gerontology Series A, Biological sciences and medical sciences. 2009;64(6):646-52.
408.Serra-Majem L, de la Cruz JN, Ribas L, Salleras L. Mediterranean diet and health: is all the secret in olive oil? Pathophysiology of haemostasis and thrombosis. 2003;33(5-6):461-5.
409.Madigan C, Ryan M, Owens D, Collins P, Tomkin GH. Dietary unsaturated fatty acids in type 2 diabetes: higher levels of postprandial lipoprotein on a linoleic acid-rich sunflower oil diet compared with an oleic acid-rich olive oil diet. Diabetes care. 2000;23(10):1472-7.
410.Perona JS, Vogler O, Sanchez-Dominguez JM, Montero E, Escriba PV, Ruiz-Gutierrez V. Consumption of virgin olive oil influences membrane lipid composition and regulates intracellular signaling in elderly adults with type 2 diabetes mellitus. The journals of gerontology Series A, Biological sciences and medical sciences. 2007;62(3):256-63.
411.Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. The New England journal of medicine. 1996;334(5):292-5.
412.Zethelius B, Lithell H, Hales CN, Berne C. Insulin sensitivity, proinsulin and insulin as predictors of coronary heart disease. A population-based 10-year, follow-up study in 70-year old men using the euglycaemic insulin clamp. Diabetologia. 2005;48(5):862-7.
413.Flier JS. Leptin expression and action: new experimental paradigms. Proceedings of the national academy of sciences of the United States of America. 1997;94(9):4242-5.
414.Bargut TC, Mandarim-de-Lacerda CA, Aguila MB. A high-fish-oil diet prevents adiposity and modulates white adipose tissue inflammation pathways in mice. The Journal of nutritional biochemistry. 2015;26(9):960-9.
415.Kalupahana NS, Claycombe K, Newman SJ, Stewart T, Siriwardhana N, Matthan N, et al. Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. The Journal of nutrition. 2010;140(11):1915-22.
416.Kalupahana NS, Claycombe KJ, Moustaid-Moussa N. (n-3) Fatty acids alleviate adipose tissue inflammation and insulin resistance: mechanistic insights. Advances in nutrition. 2011;2(4):304-16.

417.Cai A, Hutchison E, Hudson J. Metabolic enrichment of omega-3 polyunsaturated fatty acids does not reduce the onset of idiopathic knee osteoarthritis in mice. Osteoarthritis Research Society. 2014;22(9):1301-9.
418.Boulis TS, Rochelson B, Novick O, Xue X, Chatterjee PK, Gupta M, et al. Omega-3 polyunsaturated fatty acids enhance cytokine production and oxidative stress in a mouse model of preterm labor. Journal of perinatal medicine. 2014;42(6):693-8.
419.Lionetti L, Mollica MP, Sica R, Donizzetti I, Gifuni G, Pignalosa A, et al. Differential effects of high-fish oil and high-lard diets on cells and cytokines involved in the inflammatory process in rat insulin-sensitive tissues. International journal of molecular sciences. 2014;15(2):3040-63.
420.Chen CC, Ho CY, Chaung HC, Tain YL, Hsieh CS, Kuo FY, et al. Fish omega-3 fatty acids induce liver fibrosis in the treatment of bile duct-ligated rats. Digestive diseases and sciences. 2013;58(2):440-7.
421.Church MW, Jen KL, Anumba JI, Jackson DA, Adams BR, Hotra JW. Excess omega-3 fatty acid consumption by mothers during pregnancy and lactation caused shorter life span and abnormal ABRs in old adult offspring. Neurotoxicology and teratology. 2010;32(2):171-81.
422.Liu Y, Zhang D, Wu Y, Ji B. Docosahexaenoic acid aggravates photooxidative damage in retinal pigment epithelial cells via lipid peroxidation. Journal of photochemistry and photobiology B, Biology. 2014;140:85-93.
423.Spindler SR, Mote PL, Flegal JM. Dietary supplementation with Lovaza and krill oil shortens the life span of long-lived F1 mice. Age. 2014;36(3):9659.
424.Valencak TG, Ruf T. N-3 polyunsaturated fatty acids impair lifespan but have no role for metabolism. Aging cell. 2007;6(1):15-25.
425.Kikuta K, Masamune A, Satoh M, Suzuki N, Satoh K, Shimosegawa T. Hydrogen peroxide activates activator protein-1 and mitogen-activated protein kinases in pancreatic stellate cells. Molecular and cellular biochemistry. 2006;291(1-2):11-20.
426.Masamune A, Kikuta K, Satoh M, Suzuki N, Shimosegawa T. Green tea polyphenol epigallocatechin-3-gallate blocks PDGF-induced proliferation and migration of rat pancreatic stellate cells. World journal of gastroenterology. 2005;11(22):3368-74.
427.Tanioka H, Mizushima T, Shirahige A, Matsushita K, Ochi K, Ichimura M, et al. Xanthine oxidase-derived free radicals directly activate rat pancreatic stellate cells. Journal of gastroenterology and hepatology. 2006;21(3):537-44.
428.Kikuta K, Masamune A, Satoh M, Suzuki N, Shimosegawa T. 4-hydroxy-2, 3-nonenal activates activator protein-1 and mitogen-activated protein kinases in rat pancreatic stellate cells. World journal of gastroenterology. 2004;10(16):2344-51.
429.Masamune A, Watanabe T, Kikuta K, Satoh K, Shimosegawa T. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. American journal of physiology Gastrointestinal and liver physiology. 2008;294(1):G99-G108.
430.Asaumi H, Watanabe S, Taguchi M, Tashiro M, Otsuki M. Externally applied pressure activates pancreatic stellate cells through the generation of intracellular reactive oxygen species. American journal of physiology gastrointestinal and liver physiology. 2007;293(5):G972-8.
431.Masamune A, Satoh M, Kikuta K, Suzuki N, Satoh K, Shimosegawa T. Ellagic acid blocks activation of pancreatic stellate cells. Biochemical pharmacology. 2005;70(6):869-78.
432.Zhou CH, Lin L, Zhu XY, Wen T, Hu DM, Dong Y, et al. Protective effects of edaravone on experimental chronic pancreatitis induced by dibutyltin dichloride in rats. Pancreatology : official journal of the international association of pancreatology. 2013;13(2):125-32.

433.Jiang F, Liao Z, Hu LH, Du YQ, Man XH, Gu JJ, et al. Comparison of antioxidative and antifibrotic effects of alpha-tocopherol with those of tocotrienol-rich fraction in a rat model of chronic pancreatitis. Pancreas. 2011;40(7):1091-6.
434.Nolan CJ, Prentki M. The islet beta-cell: fuel responsive and vulnerable. Trends in endocrinology and metabolism. 2008;19(8):285-91.
435.Steil GM, Trivedi N, Jonas JC, Hasenkamp WM, Sharma A, Bonner-Weir S, et al. Adaptation of beta-cell mass to substrate oversupply: enhanced function with normal gene expression. American journal of physiology Endocrinology and metabolism. 2001;280(5):E788-96.
436.Gonzalez AM, Garcia T, Samper E, Rickmann M, Vaquero EC, Molero X. Assessment of the protective effects of oral tocotrienols in arginine chronic-like pancreatitis. American journal of physiology gastrointestinal and liver physiology. 2011;301(5):G846-55.
437.Liu YQ, Jetton TL, Leahy JL. beta-Cell adaptation to insulin resistance. Increased pyruvate carboxylase and malate-pyruvate shuttle activity in islets of nondiabetic Zucker fatty rats. The Journal of biological chemistry. 2002;277(42):39163-8.
438.Jetton TL, Lausier J, LaRock K, Trotman WE, Larmie B, Habibovic A, et al. Mechanisms of compensatory beta-cell growth in insulin-resistant rats: roles of Akt kinase. Diabetes. 2005;54(8):2294-304.
439.Islam MS, Morton NM, Hansson A, Emilsson V. Rat insulinoma-derived pancreatic beta-cells express a functional leptin receptor that mediates a proliferative response. Biochemical and biophysical research communications. 1997;238(3):851-5.
440.Tanabe K, Okuya S, Tanizawa Y, Matsutani A, Oka Y. Leptin induces proliferation of pancreatic beta cell line MIN6 through activation of mitogen-activated protein kinase. Biochemical and biophysical research communications. 1997;241(3):765-8.
441.Islam MS, Sjoholm A, Emilsson V. Fetal pancreatic islets express functional leptin receptors and leptin stimulates proliferation of fetal islet cells. International journal of obesity and related metabolic disorders : journal of the international association for the study of obesity. 2000;24(10):1246-53.
442.Khalaileh A, Gonen-Gross T, Magenheim J, Nir T, Porat S, Salpeter S, et al. Determinants of pancreatic beta-cell regeneration. Diabetes, obesity & metabolism. 2008;10 Suppl 4:128-35.

APPENDIX 1: CONTENT ORIGINALITY REPORT

In order to verify the thesis originality, it was subjected to a detection analysis by "Turnitin Ephorus" software version 2.3.5 (Turnitin LLC, Utrech, Netherlands, 2015). Such analysis was performed separately by sections, indicating number of matching words to the total, matching percentage per section, and the sources from matching. Matching words from own texts were not considered. Table 1 Shows the summary of the results.

Table 1. Content percentage of the present PhD thesis that matches that of other published documents.

Section	***Total Words***	***Matching words***	***Percentage per section***	***Source***	***Matching words of source***
Introduction	27397	86	0,31%	I	26
				II	19
				III	16
				IV	13
				V	12
Hypothesis and Objectives	237	0	0%	————	————
Materials and Methods	5863	527	8,99%	VI	401
				VII	40
				VIII	35
				IX	34
				X	17
Results	2342	11	0,47%	XI	11
Discussion	2665	0	0%	————	————
Conclusions	597	0	0%	————	————

Sources:

I: Body mass index, abdominal fatness and pancreatic cancer risk: a systematic review and non-linear dose–response meta-analysis of prospective studies. Annals of Oncology 23: 843–852, 2012.

II: Molecular classification of neoplasms of the pancreas. Human Pathology (2009) 40, 612–623.

III: Mesenchymal Tumors of the Pancreas: CT Findings. Journal of Computer Assisted Tomography 24(4), July/August 2000, pp 622-627.

IV: Pathogenesis of type 1 diabetes mellitus: interplay between enterovirus and host. Nature Reviews Endocrinology 6, 279-289 (May 2010).

V: https://en.wikipedia.org/w/index.php?title=SMARCD2&oldid=679040448

VI:https://www1.ephorus.com/reportjs/rest/restricted/documents/download/document?docGuid=a9414b7e-567c-4577-b6a5-2f976dab45b6

VII: Variation in characteristics of islets of Langerhans in insulin-resistant, diabetic and non-diabetic-rat strains. Int. J. Exp. Path. (2010), 91, 288–301

VIII:http://www.tdx.cat/bitstream/handle/10803/11052/GarciaAlonso2de2.pdf?sequence=2

IX: Effect of quercetin and desferrioxamine on 6-hydroxydopamine (6-OHDA) induced neurotoxicity in striatum of rats. The Journal of Toxicological Sciences Vol. 38 (2013) No. 1 February p. 25-33

X: Hydroxytyrosol inhibits growth and cell proliferation and promotes high expression of sfrp4 in rat mammary tumours. Mol. Nutr. Food Res. 2011, 55, S117–S126

XI: Antioxidized LDL Antibodies Are Associated With Different Metabolic Pathways in Patients With Atherosclerotic Plaque and Type 2 Diabetes. Diabetes Care 36:1006–1011, 2013

APPENDIX 2: *CURRICULUM VITAE*

Adrián González Alonso

University of Granada
Department of Physiology
Nutrition and Food Technology Institute "José Mataix Verdú"
Nutritional Physiology and Biochemistry of Oxidative Stress Research Group
Biomedical research center
Avenida del conocimiento S/N 18100 Armilla, Granada, Spain. Tel: 958241000 ext. 20316
Email: agonzalezalonso@ugr.es

UNIVERSITY EDUCATION

- PhD student in Human Nutrition. Thesis title: *Functional and Structural Aspects of Aged Pancreas Related To The Nature of Dietary Fat Supplemented or Not With Coenzyme Q_{10}*. Directors: José Luis Quiles Morales, María del Carmen Ramírez Tortosa, Cesar Luis Ramírez Tortosa. Department of Physiology, University of Granada, Spain. **2011-2016**
- Master Degree in Human Nutrition. Final Project (translated title): *Behavior of Endocrine Pancreas During Aging in Response To Dietary Fat Type (Fish, Sunflower And Olive Oil)*. Director: José Luis Quiles Morales. Department of Physiology, University of Granada, Spain. **2010.**
- Graduate in Food Science and technology. University of Granada, Spain. **2009.**

SCIENTIFIC RESEARCH EXPERIENCE

Grants from Public Authorities

- International Mobility Grant for PhD students. Ministry of Education, Government of Spain. Department of Food and Nutritional Sciences; School of Chemistry, Food & Pharmacy; University of Reading. **February 2014 - April 2014**.
- Predoctoral Fellowship FPU. Ministry of Education, Government of Spain, Nutritional Physiology and Biochemistry of Oxidative Stress Research Group, Department of Physiology, University of Granada. **December 2010 - December 2014**.
- Grant for Research Staff Trainning. UGR-Empresa General Foundation. Project translated title: *Study of Mitochondrial Dysfunction During Sarcopenia Development Associated to Aging in Skeletal Muscle With or Without Nutritional Intervention*. Main researchers: José Luis Quiles Morales and María del Carmen Ramírez Tortosa. Funding by Abbott Laboratories S.A., Nutritional

Physiology and Biochemistry of Oxidative Stress Research Group, Nutrition and Food Technology Institute "José Mataix", University of Granada. **November 2009 - September 2010**.

Participation in Research Projects

- *Oxidative Stress Markers in Diabetes Nephropathy.* Main researchers: Ramírez-Tortosa, M.C. and Quiles, J.L., funding by Abbott Laboratories, S.A. Duration: 1 year.
- *Targeting mitochondrial function for Preventing Adverse Developmental Programming of Obesity-Related Disorders Later in Life. The Role of Maternal Nutrition*. Main researchers: Ramírez-Tortosa, M.C. and Quiles, J.L., Funding by Abbott Laboratories, S.A. Duration: 1 year.
- *Effect of the Compounds NST0021 and NST0037 on Atherosclerosis Development in an Experimental Rabbit Model (translated title)*. Main researchers: Ramírez-Tortosa, M.C. and Quiles, J.L., funding by Neuron Biopharma S.A., duration: 1 year.
- *Performing of Clinical Testing on Klocker Essential Implants, Surfaces Assessing and Analyzing, Setting Procedures of Immediate Charge and Many Research Protocols as Monitoring Committee Determines* (translated title). Main researchers: Ramírez-Tortosa, M.C. and Quiles, J.L., funding by "Fundación de Investigación de la Universidad de Sevilla"., Duration: 2 Years.

TEACHING EXPERIENCE

Universitary

2013-2014

- Practicum in Human Physiology, Sciences of Physical Activity and Sport Degree, University of Granada. (10 hours).
- Practicum in Human and Cellular Physiology II, Pharmacy Degree, University of Granada. (35.5 hours).
- Human and Cellular Physiology II, Pharmacy Degree, University of Granada. (14hours).

2012-2013

- Practicum in Human and Cellular Physiology I, Pharmacy Degree, University of Granada. (8 hours)

- Practicum in Molecular Physiology of Animals, Biochemistry Degree, University of Granada (19 hours).
- Practicum in Human and Cellular Physiology II, Pharmacy Degree, University of Granada. (19.5 hours).
- Human and Cellular Physiology I, Pharmacy Degree, University of Granada. (7 hours).
- Human Physiology, Nutrition and Dietetics Degree, University of Granada (6.5 hours).

Other

- Tutor Work IVT, Laboratory Training Cycle. Clinical Diagnosis of Higher Degree in The Health Professional Field, I.E.S Aynadamar, Granada, **2012-2013 (**300 hours).

RESEARCH AND TEACHING TRAINING

- Distance Education and Training in Animal Research and Protection for Technical Caregiver Staff. Category A, University of Granada. **November-2009.**
- Specialization itinerary I: *Virtual Teaching*. Virtual Learning Center, University of Granada, **October 2013**.
- Seminar: *Update of Oxygen Free Radicals and Antioxidant Enzymes*. Department of Biochemistry and Molecular Biology, Vicerectorate for Quality Assurance, University of Granada. **April-May 2012**. (10 hours).
- Participation as Student in the Course: "*Training for Novel Professorate on New Teaching Methodologies of Degree in Pharmacy, Nutrition and Food Science and Technology*". Vicerectorate for Quality Assurance, University of Granada. **September-2012**. (32 hours).
- Participation as Student in the Course: "*Application and Diffusion of Teaching Innovation Performed by the Department of Physiology Within the Guide of EEES*". Vicerectorate for Quality Assurance, University of Granada. **November 2012-February 2013**. (40 hours).

SCIENTIFIC PRODUCTION

Articles in Indexed Journals

- *Sunflower Oil but Not Fish Oil Resembles Positive Effects of Virgin Olive Oil on Aged Pancreas after Life-Long Coenzyme Q Addition.* **González-Alonso A**, Ramírez-Tortosa CL, Varela-López A, Roche E, Arribas MI, Ramírez-Tortosa MC, Giampieri F, Ochoa JJ, Quiles JL. Int J Mol Sci. **2015** Sep.
- *Doxorubicin-Induced Oxidative Stress in Rats Is Efficiently Counteracted by Dietary Anthocyanin Differently Enriched Strawberry (Fragaria × ananassa Duch.).* Diamanti J, Mezzetti B, Giampieri F, Alvarez-Suarez JM, Quiles JL, **Gonzalez-Alonso A**, Ramirez-Tortosa MD, Granados-Principal S, Gonzáles-Paramás AM, Santos-Buelga C, Battino M. J Agric Food Chem. **2014** Mar 11.
- *Diets Based on virgin Olive Oil or Fish Oil but not on Sunflower Oil Prevent Age-Related Alveolar Bone Resorption by Mitochondrial-Related Mechanisms.* Bullon P, Battino M, Varela-Lopez A, Perez-Lopez P, Granados-PrincIFal S, Ramirez-Tortosa MC, Ochoa JJ, Cordero MD, **Gonzalez-Alonso A**, Ramirez-Tortosa CL, Rubini C, Zizzi A, Quiles JL. J Gerontol A Biol Sci Med Sci **2014** August.
- *Comparative Analysis of Pancreatic Changes in Aged Rats Fed Life Long With Sunflower, Fish, or Olive Oils.* Roche E, Ramírez-Tortosa CL, Arribas MI, Ochoa JJ, Sirvent-Belando JE, Battino M, Ramírez-Tortosa MC, **González-Alonso A**, Pérez-López MP, Quiles JL. J Gerontol A Biol Sci Med Sci. **2013**
- *Autophagy in Periodontitis Patients and Gingival Fibroblasts: Unraveling the Link Between Chronic Diseases and Inflammation.* Bullon P, Cordero MD, Quiles JL, Ramirez-Tortosa MC, **Gonzalez-Alonso A**, Alfonsi A, Garcia-Marin R, de Miguel M and Battino M. BMC Medicine **2012** Oct.

Articles in Non-Indexed Journal

- *Experimental Evidence on the Role of Different Types Unsaturated Fats in the Diet on Ageing* [translated title]. **González-Alonso A**, Pérez-López P, Varela-López A, Ramírez-Tortosa MC, Battino M, Quiles JL. Rev Esp Geriatr Gerontol. **2015**.

Book Chapters

- *Relationship between Cardiovascular Risk Factors and Periodontal Disease: Current Knowledge,* Sergio Granados-Principal , Jose L Quiles , Nuri El-Azem , Patricia Perez-Lopez , **Adrián Gonzalez** , M Carmen Ramirez-Tortosa., in: :

Cardiovascular Risk Factors. Editor: Armen Yuri Gasparyan. Editorial: Intech. Pages: 193-216. ISBN: 978-953-51-0240-3**. 2012**.

- *Olive Oil, Mitochondrial Oxidative Stress and Aging*, Pérez-López P, Ramirez-Tortosa MC, El-Azem N, **González A**, Granados-Principal S, Battino M, Quiles JL., in: Olive Oil, Mitocondrial Oxidative Stress and Aging. Editor: Savalas CA and Nicolau SN. Editorial: Nova Publishers. ISBN: 978-1-62100-774-6. **2012.**

Oral Communications in Congresses, Conferences, Meetings and Workshops

- *Development of an experimental rabbit model that allows studying the periodontitis relationship with cardiovascular and liver diseases (translated title).* Varela-López, A.; Román-Malo, L.; Ramírez-Tortosa, M.C.; Pérez-López, P.; Pulido-Morán, M.; **González-Alonso, A**.; Cordero, M.D.; Battino, M.; Ramírez-Tortosa, C.L.; Bullón, P. and Quiles, J.L., 48th Annual Meeting of Spanish Society of Periodontology and Osseointegration (SEPA), Valladolid, May 22 – 25, **2014**.

Abstracts in Congresses, Conferences, Meetings and Workshops

- *Gene expression profiling of aging in liver of rats feed life-long on a N-6 polyunsaturated diet with or without coenzyme Q_{10} supplementation*. P. Pérez-López, MC. Ramírez-Tortosa, JJ. Ochoa, S. Granados-Principal, **A. González**, CL. Ramírez-Tortosa, M. Battino, JL.Quiles. The sixth conference of the international coenzyme Q_{10} association, Brussels, May 27-30, **2010**.

Panels in congresses, conferences, meetings and workshops

- *Effects of Squalene, Hydroxytyrosol and Tyrosol on The Expression of CD36 Receptor in Macrophages.* S. Granados-Principal, J.L Quiles, C. Ramírez-Tortosa, N. El azem-De haro, P. Pérez-lópez, **A. González**, L. Vera-Ramirez, P.Camacho-Corencia, M.C. Ramírez-Tortosa., 2º International Mediterranean Meeting. Nutrition and Metabolism, Granada, June 16-19, **2010**.
- *Hydroxityrosol Restrains the Growth of Experimental Breast Tumours in Rats.* S. Granados-Principal, J.L Quiles, P. Pérez-lópez, N. El azem-De haro, **A. González**, P.Camacho-Corencia, L. Vera-Ramirez, C. Ramírez-Tortosa, P. Sánchez-Rovira, M.C. Ramírez-Tortosa., 2º International Mediterranean Meeting. Nutrition and metabolism, Granada, June 16-19, **2010**.
- *Expression Changes in Apotosis-Related Genes in Old vs. Young Rats Fed Life-Long on Virgin Olive, Sunflower or Fish Oils.* P. Pérez-lópez, M.C. Ramírez-Tortosa, J.J. Ochoa, S. Granados-Principal, N. El azem-De haro, **A. González**,

M. López-Frías, CL Ramírez-Tortosa, M. Battino, J.L Quiles., 2º International Mediterranean Meeting. Nutrition and Metabolism, Granada, June 16-19, **2010**.

- *Gene Expression Profiling of Aging in Liver of Rats Feed Life-Long on a n-6 Polyunsaturated Diet With or Without Coenzyme Q10 Supplementation*. P. Pérez-lópez, M.C. Ramírez-Tortosa, J.J. Ochoa, S. Granados-Principal**, A. González**, CL Ramírez-Tortosa, M. Battino, J.L Quiles., 2º International Mediterranean Meeting. Nutrition and Metabolism, Granada, June 16-19, **2010**.

LANGUAGE SKILLS

- English: speaking (B1) writing (B1) reading (B1)

APPENDIX 3: RELATED PUBLICATIONS

Journals of Gerontology: BIOLOGICAL SCIENCES
Cite journal as: J Gerontol A Biol Sci Med Sci 2014 August;69(8):934–944
doi:10.1093/gerona/glt157

Advance Access publication October 17, 2013

Comparative Analysis of Pancreatic Changes in Aged Rats Fed Life Long With Sunflower, Fish, or Olive Oils

Enrique Roche,[1] César L. Ramírez-Tortosa,[2] María I. Arribas,[1] Julio J. Ochoa,[3,4] José E. Sirvent-Belando,[5] Maurizio Battino,[6] M. Carmen Ramírez-Tortosa,[3,7] Adrián González-Alonso,[3,4] M. Patricia Pérez-López,[3,4] and José L. Quiles[3,4]

[1]Bioengineering Institute, University Miguel Hernandez, Elche (Alicante), Spain.
[2]Department of Pathology, Complejo Hospitalario de Jaén, Spain.
[3]Institute of Nutrition and Food Technology "José Mataix Verdú" and
[4]Department of Physiology, University of Granada, Spain.
[5]Department of Analytical Chemistry, Nutrition and Bromatology, University of Alicante, Spain.
[6]Dipartimento di Scienze Biomediche e Sanità Pubblica, Università Politecnica delle Marche, Ancona, Italy.
[7]Department of Biochemistry and Molecular Biology II, University of Granada, Spain.

Address correspondence to José L. Quiles, PhD, Departamento de Fisiología. Instituto de Nutrición y Tecnología de los Alimentos "José Mataix Verdú", Laboratorio 120, Parque Tecnológico de Ciencias de la Salud, Avda del Conocimiento sn, Armilla (Granada) 18100, Spain. Email: jlquiles@ugr.es

An adequate pancreatic structure is necessary for optimal organ function. Structural changes are critical in the development of age-related pancreatic disorders. We aimed to study the effect of oil consumption on pancreas histology in order to find aging-related signs. To this end, three groups of rats were fed an isocaloric diet for 2 years, where virgin olive, sunflower, or fish oil was included. Pancreatic samples for microscopy and blood samples were collected at the moment of sacrifice. As a result, the sunflower oil–fed rats presented higher β-cell numbers and twice the insulin content than virgin olive oil–fed animals. In addition, rats fed with fish oil developed acinar fibrosis and macrophage infiltrates in peri-insular regions, compared with counterparts fed with virgin olive oil. Inflammation signs were less prominent in the sunflower group. The obtained data emphasize the importance of dietary fatty acids in determining pancreatic structure.

Key Words: β-Cell—Endocrine pancreas—Exocrine pancreas—Pancreatic aging—Pancreatic fibrosis of the elderly.

Received January 24, 2013; Accepted August 12, 2013

Decision Editor: Rafael de Cabo, PhD

THE pancreas is vital for nutrient digestion and consumption. This dual function can be performed due to the presence of two compartments: the exocrine and the endocrine pancreas. The exocrine portion is the most abundant (98% of pancreatic mass) and is formed by interstitial mesenchymal, ductal, and acinar cells, the latter producing enzymes responsible for food digestion in the duodenum. The endocrine compartment is formed by cell aggregates (also called islets of Langerhans) disseminated throughout the exocrine structure and by different cell types specialized in the biosynthesis and secretion of specific hormones: insulin-producing β cells (around 60% of islet mass), glucagon-producing α cells (30% of islet mass), somatostatin-producing δ cells, pancreatic polypeptide–producing cells, and finally, ghrelin-synthesizing E cells (only present during embryogenesis) (1).

An adequate cell mass is necessary for optimal organ function, and changes in pancreatic cell mass are critical in the development of pancreatic disorders (2). In this context, throughout life the persistent bad habits in diet and lifestyle can provoke the developments of certain pathologies that disturb pancreatic cell mass and accelerate organ's aging process (3). In this context, alcohol abuse or cigarette smoking can disturb the exocrine pancreatic compartment by producing pancreatitis (4). This pathological entity is characterized by a pancreatic insufficiency (decrease in digestive enzyme production) due to the destruction of functional exocrine pancreatic tissue that results in nutrient malabsorption in the intestine (5). Pathologic anatomy reveals the presence of tissue necrosis or apoptosis, together with inflammation, ductal obstruction, and fibrosis. The latter results in extracellular matrix deposition in affected areas of the organ, disturbing lobular structure and causing duct deformation (6). Damage is associated with the transformation of resident pancreatic stellate cells into myofibroblasts, which produce extracellular matrix rich in collagen types I and III, and fibronectin (6–9), matrix metalloproteinases, and inhibitors (10). The fibrosis patterns are instrumental in the clinic to identify the different types of human chronic pancreatitis, which include alcoholic, hereditary, autoimmune, idiopathic, tropical, and other rare types (6). At the same time, the rare types include pancreatic fibrosis of the elderly adults, which varies from person to person and affects 50% of people older than 60 years of age (6). This

condition is characterized by the narrowing of the lumen in medium-sized ducts, most likely due to the hyperplasia of ductal epithelium. Therefore, fibrosis and inflammation mainly occur in the intra-acinar regions closest to hyperplastic ducts (6).

On the other hand, type 2 diabetes is one of the most prevalent pancreatic diseases worldwide (180 million people affected in 2010) that disturbs the endocrine pancreatic compartment and in particular the β cells (11). This pathology is frequent in adult and elderly and is closely related to an excessive and unbalanced intake of calorinergic nutrients such as fat and sugars (12). This pathology presents a prediabetic state of insulin resistance in which the adaptive response of pancreatic β cells results in the presence of high levels of circulating insulin (hyperinsulinemia) in order to maintain normoglycemia. Hyperinsulinemia can be achieved by combining different mechanisms that imply expanding β-cell mass by hyperplasia and hypertrophy, enhancing insulin biosynthesis and increasing insulin secretion in response to sustained extracellular nutrient demands (11,13–15). Type 2 diabetes appears when adaptation of the β-cell mass fails to compensate the increased insulin demand, resulting in β-cell apoptosis (11). Therefore, insulin resistance and hyperinsulinemia are components of age-related metabolic disorders such as, glucose intolerance, dyslipemias, obesity, cardiovascular disease, and metabolic syndrome (16–20) that present a high risk of evolving towards type 2 diabetes.

In addition, it is obvious that due to the close proximity, pathological states affecting one of the pancreatic compartments will inevitably affect the other (20). Impairment of the endocrine function, that is, diabetes, can affect the function of the exocrine gland. In this sense, atrophy of the exocrine pancreas may be due to the lack of insulin, and hyperglycemia can cause activation of stellate cells that eventually contribute to developing pancreatic fibrosis. On the other hand, severe impairment of the exocrine tissue (acute or chronic pancreatitis) affects the endocrine compartment, increasing the risk of developing glucose intolerance and diabetes (20–22).

Therefore, the identification of factors that are capable of reverting or avoiding these conditions is instrumental to design prevention strategies against the different pancreatic pathologies that occur during aging. The abovementioned examples clearly support the key role of diet in this particular context. We have previously shown that specific unsaturated dietary fat sources may help to attenuate certain deleterious aspects of aging (23–27). However, despite overall benefits exerted by unsaturated dietary fats on health, particular consideration should be done depending on each type of fat (28–30). In this report, we compared three groups of rats fed during 2 years with an isocaloric diet but with different fat composition due to the inclusion of different food oils: virgin olive, sunflower, and fish oils. At the end of the experiment, no pathological symptom was detected, including obesity; however, histologically we observed that the different oils affected different pancreas compartments. Specifically, sunflower oil–fed (SOF) rats possessed increased β-cell numbers, whereas those fed with fish oil presented signs of pancreatic fibrosis of the elderly.

Methods

Animals

Eighteen male Wistar rats (*Ratus norvegicus*) weighing 80–90 g initially were used for this study. The rats were randomly assigned into three experimental groups and matchfed from weaning until 24 months of age on a semisynthetic and isoenergetic diet according to the AIN-93 criteria (31) but modified in relation to the dietary fat source. Modified AIN-93G diet was administered to rats from weaning to 2 months of age, and AIN-93M was administered for the rest of the experiment. Components of AIN-93G diet in weight percentage were 38.7% starch, 20% casein, 13.2% dextrose, 10% sucrose, 8% oil, 5% cellulose (fiber), 3.5% mineral mix, 1% vitamin mix, 0.3% L-cysteine, and 0.25% choline bitartrate. Therefore, the caloric content for AIN-93G diet was 401.4 kcal/100 g. AIN-93M diet presented a similar weight composition than AIN-93G except for the following: 46.6% starch, 14% casein, 15.5% dextrose, 4% oil, and 0.18% L-cysteine. The corresponding caloric content for AIN-93GM diet was 362.4 kcal/100 g. As indicated above, AIN-93 diets were modified in relation to the fat source. Original AIN-93 diets are composed by soya oil. We replaced this oil by virgin olive oil (virgin olive oil–fed group [VOOF]), sunflower oil (SOF), or fish oil (fish oil–fed group [FOF]) to formulate the experimental diets and groups. Table 1 shows a summary of the fatty acid profile of experimental oils (total fatty acid profile of dietary fat sources is presented as Supplementary Material). Diets were prepared weekly and stored in darkness at 4°C to avoid lipid peroxidation. Animals were placed in a climate-controlled room (20°C, 12-h dark/12-h light cycle) for 2 years in collective cages, in groups of three animals per cage, with free access to water. Diet was delivered ad libitum for the first 2 months and then at 25 g/rat/d for the rest of the experiment (in order to avoid overweight). Food intake was indirectly monitored through the weekly body weight control and daily spillage monitoring. The food completely disappeared every day. Amount calculations were done in order to avoid overfeeding and also any significant caloric restriction. Because animals were allocated in groups, maybe some animals on some days ate some more diet than other animals. However, the systematic supervision of weight gain every week allowed us to discard the possibility of overfeeding or restricted animals, as can be shown in body weight calculations (see the Results section).

Table 1. Relevant Fatty Acid Composition of Virgin Olive, Sunflower, and Fish Oils Administered to Each Group of Animals

Fatty Acid	Virgin Olive	Sunflower	Fish
Myristic (C14:0)	0.0	0.1	7.2
Palmitic (C16:0)	8.3	6.4	17.1
Stearic (C18:0)	3.2	4.7	2.7
Lignoceric (C24:0)	0.0	0.1	0.3
Total saturated	12.6	11.5	30.5
Hexadecenoic (C16:1n9)	1.1	0.1	9.6
Oleic (C18:1n9)	77.7	24.2	15.1
Nervonic (C24:1n9)	0.0	0.0	0.9
Total monounsaturated	83.7	24.4	30.1
Linoleic (C18:2n6)	3.2	62.8	2.8
DGLA* (C20:3n6)	0.1	0.9	0.1
Arachidonic (C29:4n6)	0.0	0.0	2.1
EPA† (C20:5n3)	0.2	0.1	18.6
DHA‡ (C22:6n3)	0.0	0.0	10.5
Total polyunsaturated	3.7	64.1	39.4
Polyunsaturated (n-3)	0.4	0.4	31.3
Polyunsaturated (n-6)	3.3	63.7	8.2
Polyunsaturated (n-6/n-3)	5.5	171.0	0.3
Oleic/linoleic	24.1	0.4	5.4

Note: Results are expressed as percentage of total fat (total fatty acids are presented as Supplementary material).
*Dihomo-γ-linolenic.
†Eicosapentaenoic.
‡Docosahexaenoic.

The animals were treated in accordance with the guidelines of the Spanish Society for Laboratory Animals, and the experiment was approved by the Ethical Committee of the University of Granada. The rats were killed by cervical dislocation followed by decapitation at the same time of the day to avoid any circadian fluctuation.

Blood was collected in ethylenediaminetetraacetic acid-coated tubes, and the plasma was centrifuged at 1,750*g* for 10 minutes. Plasma samples were stored at −80°C until analysis. After exsanguination, pancreatic tissue was excised and properly preserved according to the analyses to be done (see below).

Histological Examination

The pancreatic tissue taken from each rat was fixed in 10% formalin and embedded in paraffin, and then several 4-μm sections were cut for histological examinations, Masson's trichrome staining, Perl's stain, and immunohistochemistry. For histological examinations, the sections were stained with hematoxylin and eosin, Masson's trichrome, and Perl's staining. Evaluation of the pathological changes was performed by two experienced pathologists who were blinded to the treatment groups. When there were discrepancies, they discussed among themselves and reached a consensus outcome. Total fields per section (the full slide) were observed, and the entire available pancreas was studied in order to avoid a focal observation of the same. Acinar atrophy was scored as 0, absent; 1, cytoplasmic basophilia reduction, acinar size reduction, and reduction in the content of acinar zymogen granules; 2, acinar vacuolization, early destruction of acini, inflammation, and early onset of fibrosis; 3, moderate; 4, severe with metaplasia and acinar dilatation (transformation of acini in cystic/ductular structures coated with cuboidal or flattened cells or the formation of small glands lined by cuboidal cells in a microcystic pattern or "honeycomb"). Acinar fat infiltration (presence of adipocytes in the pancreatic parenchyma) was scored as 0, absent; 1, very low number of cells and aggregates; 2, low; 3, moderate; 4, severe. Ductular hyperplasia was scored according to its presence as 1, absent; 2, present. Acinar fibrosis was scored as 0, absent; 1, perivenular fibrosis with few septa and thin; 2, poor, thin septa with fibrous incomplete bridges between regions; 3, thin septa with extensive bridges between regions; 4, septa thickened with numerous bridges and nodular appearance. Peri-insular fibrosis was scored as 0, absent; 1, focal and mild increase of peripheral collagen deposition; 2, slight increase but diffuse; 3, moderate increase; 4, severe increase. Iron deposition was scored as 0, absent; 1, splashed positive macrophages; 2, mild positive macrophage accumulation; 3, moderate accumulations; 4, numerous Perl's-positive macrophage accumulation.

Immunohistochemistry for Insulin and Glucagon Expression

Insulin and glucagon immunohistochemistry were used to identify β and α cells and assess their density (percentage of β and α cells per islet area) and distribution in islets. For all immunohistochemical techniques, pancreatic sections were dewaxed in xylene and rehydrated through graded alcohols to water. Antigen retrieval was performed with the PTLink module (Dako, Glostrup, Denmark) using Dako low-pH Antigen Retrieval fluid (Dako) followed by several washes in water before being placed onto an Autostainer Plus Link (Dako, Demark) where the remainder of the immunohistochemical staining was performed using Envision FLEX (DAKO). Briefly, sections were first placed in washing buffer followed by blockade of endogenous peroxidase with 3% hydrogen peroxide for 5 minutes. Then, the primary antibody Insulin (Clonal 2D11-H5, Máster Diagnóstica MAD-021340Q; Máster Diagnóstica, Granada, Spain) and polyclonal Glucagon (Máster Diagnóstica MAD-021325Q) were applied for 20 minutes, followed by another buffer wash, and visualized with 3,3'-diaminobenzidine for 10 minutes. Following a water wash, sections were counterstained in hematoxylin for 7 minutes, washed in water, dehydrated, and coverslipped.

For the image analysis, islets were defined as clusters of seven or more β cells in association with other morphologically identifiable endocrine cells. All data were obtained from immunostained sections. Islet image capture was performed using a DP72 camera Olympus from an Olympus BX41 microscope (×20 objective) and analyzed using AnaliSYS Image Processing software (Olympus). Islets

were identified and images stored. Individual islet perimeters, islet size, total islet cell number, number and area of insulin-positive (β) cells, (α) cells, and the percentage of islet insulin and glucagon staining were semiautomatically determined. All insulin- and glucagon-positive cells in tissue sections were counted, including faintly positive cells.

Determination of Circulating Fatty Acid Profile

The circulating fatty acid profile was determined by gas–liquid chromatography as described in reference (32).

Protein, Hormone, and Metabolite Determinations

Frozen pancreatic portions (around 100 mg each) were thawed and homogenized in 1 mL of alcohol:acid (50 parts of 95% ethanol:1 part of 10.2 N HCl) and then sonicated using a UP50H sonicator (Dr. Hielscher, Teltow, Germany) for 3–5 pulses in an ice bath. Homogenates were processed as indicated in (33). Total insulin was measured by radioimmunoassay using the Coat-A-Count kit (Siemens, Los Angeles, CA). Total glucagon was measured by radioimmunoassay using the Millipore kit (Millipore, Billerica, MA). Leptin was determined by enzyme-linked immunosorbent assay (LINCO, St. Charles, MO). The protein content of the pancreatic homogenates was determined using the Bio-Rad assay.

Circulating glucose was determined using the glucose oxidase method coupled to the peroxidase reaction (34). Circulating triglycerides were determined from coupled reactions of lipoprotein-lipase, glycerol-kinase, glycerol phosphate oxidase, and peroxidase, giving a color end-adduct as previously reported (35). Circulating cholesterol and total lipids were determined as indicated in references (35,36), respectively. Circulating markers alkaline phosphatase, creatine kinase (CK), glutamyl oxaloacetic transaminase, glutamic pyruvic transaminase (GPT)/alanine aminotransferase, and lactate dehydrogenase were measured as indicated in reference (37). Briefly, alkaline phosphatase was measured by following the reaction with p-nitrophenilphosfate, CK was measured by the linking reaction of CK with the transformation of glucose plus adenosine triphosphate into glucose-6-P, and finally glucose-6-P plus $NADP^+$ to gluconate-6-P plus NADPH and H^+, with NADPH concentration directly related to CK activity. Glutamyl oxaloacetic transaminase activity was measured by the linked reaction with oxaloacetate to malate by the malate dehydrogenase. GPT was measured by linking pyruvate production by GPT with reaction of pyruvate with lactate dehydrogenase to lactate. Finally, lactate dehydrogenase activity was assayed by the measurement of NAD^+ production during lactate formation. Creatinine was determined by Jaffé direct reaction of creatinine with alkaline picrate forming a red complex (38). Lactate was determined by lactate oxidase/peroxidase coupled colorimetric reaction (39). Homeostasis model assessment index was calculated according to reference (40) by the equation homeostasis model assessment = fasting glucose (mmol/L) × fasting insulin (μUI/mL)/22.5.

Proliferation Assay: Ki-67 Immunostaining

Paraffin-embedded pancreas sections were sectioned (3-μm thickness), placed on a glass slide, deparaffinized, and rehydrated through a graded alcohol series. After the antigen-retrieval procedure at pH 6 (Dako Target Retrieval Solution; Dako), primary rabbit antibody Ki-67 (clone SP6; Máster Diagnóstica) was used for 10 minutes. Once antigen–antibody reaction was performed in an automatic immunostaining system (Autostainer Plus Link; Dako), sections were blocked and treated with immunodetection solution consisted of horseradish peroxidase and biotinylated secondary antibody for 30 minutes. Diluted 1:50 liquid 3,3'-diaminobenzidine (Dako) was used as a chromogenic agent, and sections were counterstained in Meyer's hematoxylin. Negative controls were carried out by the same procedure without antibody. Positive exogenous controls were pharyngeal tonsil. An experienced pathologist evaluated the results as the percentage of positive cells per islet in a total of 10 islets.

Reverse Transcription–Polymerase Chain Reaction

Pancreatic portions, previously preserved in RNA and later stored at −80°C, were used. Total RNA was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen) following manufacturer's condition. The quantity and purity of the RNA were determined form the absorbance at 260/280 nm. Total RNA of 20 ng was reverse transcribed into complementary DNA using Multiscribe enzyme (Applied Biosystems) in accordance with the manufacturer's protocol. Abi 7900 Real-Time polymerase chain reaction system and real-time polymerase chain reaction kit (TaqMan Gene Expression Assays; Applied Biosystems) were employed based on the manufacturer's instruction. Probes for Bcl2, Bad, and tumor necrosis factor-α were used, as well as for 18S RNA, which was used as an internal control. Polymerase chain reaction thermal cycling was followed in three steps: step 1 at 50°C during 2 minutes; step 2 at 95°C during 10 minutes; and the final step, step 3, with 40 cycles at 95°C during 15 seconds and 60°C during 1 minute.

Protein Carbonyl Assay

The levels of protein carbonyl groups in pancreas were assessed using Protein Carbonyl Kit (Cayman Chemical Company, Ann Arbor, MI). Briefly, 100 μl of pancreatic protein extract were transferred to two tubes. One tube was the sample tube and the other was the control tube. After adding 400 μl of 2,4-dinitrophenylhydrazine to the sample tube and 400 μl of 2.5 M HCl to the control tube, both were incubated in the dark at room temperature for 1 hour, stirring the tubes shortly every 15 minutes. Afterwards, 0.5 mL of 20%

trichloroacetic acid was added to each tube and incubated in ice for 5 minutes. This mixture was centrifuged at 10,000*g* for 10 minutes at +4°C, obtaining a pellet that was resuspended in 0.5 mL of 10% trichloroacetic acid and incubated in ice for 5 minutes and again centrifuged at 10,000*g* for 10 minutes at +4°C. The pellet obtained was resuspended in 0.5 mL of (1:1) ethanol/ethyl acetate mixture and centrifuged at 10,000*g* for 10 minutes at +4°C twice. Finally, the pellet obtained was resuspended in 250 µl of guanidine hydrochloride and centrifuged at 10,000*g* for 10 minutes at +4°C, obtaining a supernatant of which 220 µl were transferred to a 96-well plate and absorbance read (SYNERGY HT, Multi-Detection Microplate Reader; BioTek Instruments, Inc., Winooski, VT) at 370 nm. Total protein concentration in pancreas sample extracts was measured using Pierce bicinchoninic acid Protein Assay (Thermo Scientific, Rockford, IL). The results were expressed as nmol of carbonyl proteins per mg of total proteins in the extract.

Statistics

Data are expressed as means ± *SEM* of six animals. For quantitative parameters, prior to any statistical analysis, all variables were checked for normality and homogeneous variance using the Kolmogorov–Smirnoff and the Levene tests, respectively. When a variable was found not to follow normality, it was log-transformed and reanalyzed. To evaluate differences in the means between groups (virgin olive versus sunflower and virgin olive versus fish), one-way analysis of variance adjusted by Bonferroni correction was performed. For semiquantitative parameters (histological analysis), differences were analyzed by the Kruskal–Wallis test, and a Mann–Whitney *U*-test was used a posteriori to evaluate mean differences between groups. For all statistical analysis, a value of $p < .05$ was considered significant. Data were analyzed using IBM SPSS Statistics 20 (IBM corp., Armonk, NY) statistical package.

Results

Body Weight Evolution

During the 24-month follow-up, FOF animals presented the highest values (604.0 ± 23.3 g), followed by VOOF rats (534.5 ± 51.8 g), and finally by SOF animals (508.3 ± 19.5 g). However, weight differences were not significant. From the observation of body weight evolution and food spillage, no differences concerning food intake were inferred between groups or in relation to age.

Histological Pancreas Analyses

Figure 1 depicts the histology of the pancreatic endocrine and exocrine compartments from the different groups of animals. The most important features have been quantified, and the results are presented in Figure 2.

In the endocrine compartment, VOOF rats presented normal pancreatic islet histology with no evident signs of fibrosis (Figures 1A and 2F). However, SOF animals presented grade 2 peri-insular fibrosis with macrophage infiltrates (Figure 1B), although the net differences with respect to VOOF animals were not significant (Figure 2F). The situation seemed to be clearer in FOF animals that presented grade 4 peri-insular fibrosis with clear macrophage infiltrates (Figure 1C), being significant with respect to VOOF rats (Figure 2F) and with iron deposits (Figure 2G).

In the immunohistochemical analysis (insulin and glucagon), the insulin immunopositive area per islet was significantly higher in the SOF group (Figures 1E and 2H) compared with the VOOF (Figures 1D and 2H) animals. This increase seemed to correlate with an increase in the number of cells positive to the hormone per islet (Figure 2I). The number of insulin-positive cells in the FOF group was in the intermediate range between VOOF and SOF (Figures 1F, 2H and I). On the other hand, the number of glucagon-positive cells was similar in all three groups (Figures 1G–I, 2J and K), indicating that the islet changes seemed to be β-cell specific.

Regarding the exocrine pancreas compartment, VOOF rats presented a normal histology (Figure 1J) with no evident signs of acinar atrophy, fat infiltration, dilatation, fibrosis (Figure 2A–C and E), or ductal hyperplasia (Figure 2D). Conversely, the SOF group presented grade 3 acinar atrophy with vacuolization and interstitial lymphocyte infiltrates (Figure 1K). Additional significant histological signs included ductal hyperplasia (Figure 2D), whereas no significant differences with respect to VOOF rats were observed in the exocrine acinar tissue regarding atrophy, fat infiltration, dilation or fibrosis (Figure 2A–C and E). Finally, histological analysis of the exocrine pancreas in FOF animals resulted in grade 4 acinar atrophy with clear signs of cystic structures and ducts coated by plate cells (Figure 1L). Compared with VOOF animals, the FOF group had significant signs of acinar atrophy, fat infiltration (which was also significantly different to SOF), dilatation, fibrosis (Figure 2A–C and E), and ductal hyperplasia (Figure 2D).

Pancreatic Insulin and Glucagon Contents

Histological data indicated that the differences observed in the pancreas of SOF rats seemed to affect mainly the endocrine compartment; whereas the exocrine pancreas seemed to be more affected in FOF rats. To confirm this point, insulin and glucagon content were measured. As a result, there were no significant differences in FOF and VOOF groups (Table 2). However, the SOF group contained twice the amount of insulin detected in the other groups, whereas glucagon levels remain unchanged (Table 2).

Circulating Parameters

Due to the experimental design, we only have the data obtained at the moment of sacrifice after one overnight

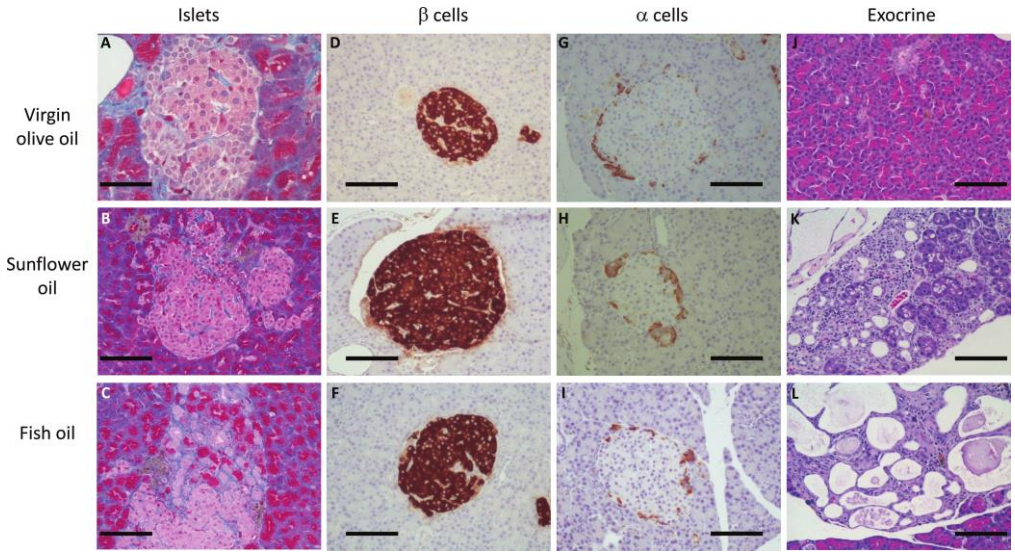


Figure 1. Effects of feeding rats for 24 mo on different dietary fat sources (virgin olive, sunflower, or fish oils) on histological changes in the pancreas. (**A**) Trichrome Masson (TM) staining in the pancreas from virgin olive oil–fed (VOOF) animals. Normal pancreatic islet. No evidence of fibrosis. (**B**) TM staining in the pancreas from sunflower oil–fed (SOF) animals. Pancreatic islet with peri-insular fibrosis grade 2. Macrophages stained with hemosiderin are present. (**C**) TM staining in the pancreas from fish oil–fed (FOF) animals. Pancreatic islet with peri-insular fibrosis grade 4. Macrophages stained with hemosiderin are present. (**D**–**F**) Insulin staining in VOOF, SOF, and FOF animals, respectively. (**G**–**I**): Glucagon staining in VOOF, SOF, and FOF animals, respectively. (**J**) Hematoxylin and eosin (HE) staining in the pancreas from VOOF animals. Normal exocrine pancreas. Macrophages stained with hemosiderin are present. (**K**) HE staining in the pancreas from SOF animals. Acinar vacuolation, interstitial lymphocytic infiltration. Acinar atrophy grade 3. (**L**) HE staining in the pancreas from FOF animals. Acinar transformation with quistic or ductular structures coated with flattened cells. Acinar atrophy grade 4. Except for A (bar = 50 μm), bar = 100 μm.

fasting. No significant differences were observed in the levels of circulating insulin in VOOF, SOF, and FOF animals at the moment of sacrifice (Table 3). However, circulating leptin levels significantly differed in the SOF rats compared with VOOF animals, whereas leptin levels in VOOF and FOF rats were very similar (Table 3).

In addition, at the moment of sacrifice all animals presented similar glycemic and lipidic levels, including triglycerides, cholesterol, and phospholipids, although VOOF rats displayed higher, but not significant, levels of circulating total lipids (Table 3). Comparing SOF and VOOF rats, no significant changes were detected in GPT (marker of hepatic injury), alkaline phosphatase (marker of liver, bile ducts, and bone injury), glutamyl oxaloacetic transaminase (marker of muscle, heart, and liver injury), CK (marker of skeletal muscle injury), and lactate dehydrogenase (marker of hemolysis and in general for tissue integrity; Table 3). Similar results were observed when comparing FOF and VOOF groups, with the exception of alkaline phosphatase and GPT which were significantly increased in FOF (Table 3). Similar creatinine levels in all groups (Table 3) indicated a correct kidney function. Finally, the calculated homeostasis model assessment values did not show a significant pathological situation of insulin resistance or loss of insulin sensitivity in all groups of animals (Table 3).

Because diet fat composition was the only differen-tial parameter in all groups of animals, we wanted to gain more insight by analyzing the main circulating fatty acids (Table 4). All groups of animals expressed similar levels of circulating saturated fatty acids, being palmitic acid (C16:0) the most abundant (which was significantly increased in FOF with respect to VOOF) and stearic acid (C18:0) the second-most abundant (which was signifi-cantly increased in SOF with respect to VOOF). There were also differences in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The circulating levels of MUFAs in VOOF animals were sig-nificantly higher than the levels found in the SOF and FOF rats, being oleic acid (C18:1n9) the most abundant in VOOF animals. On the other hand, circulating PUFAs were significantly higher in SOF and FOF animals com-pared with VOOF rats. In the case of SOF animals, this was due to the significantly higher levels of linoleic (C18:2n6) and arachidonic (C20:4n6) acids. For FOF animals, this was due to the significantly higher levels of eicosapentae-noic acid (C20:5n3) and docosahexaenoic acid (C22:6n3). Altogether, the percentage of circulating MUFAs was significantly higher in VOOF rats, circulating n-6 PUFAs were significantly higher in SOF animals, and n-3 PUFAs were higher in FOF rats.

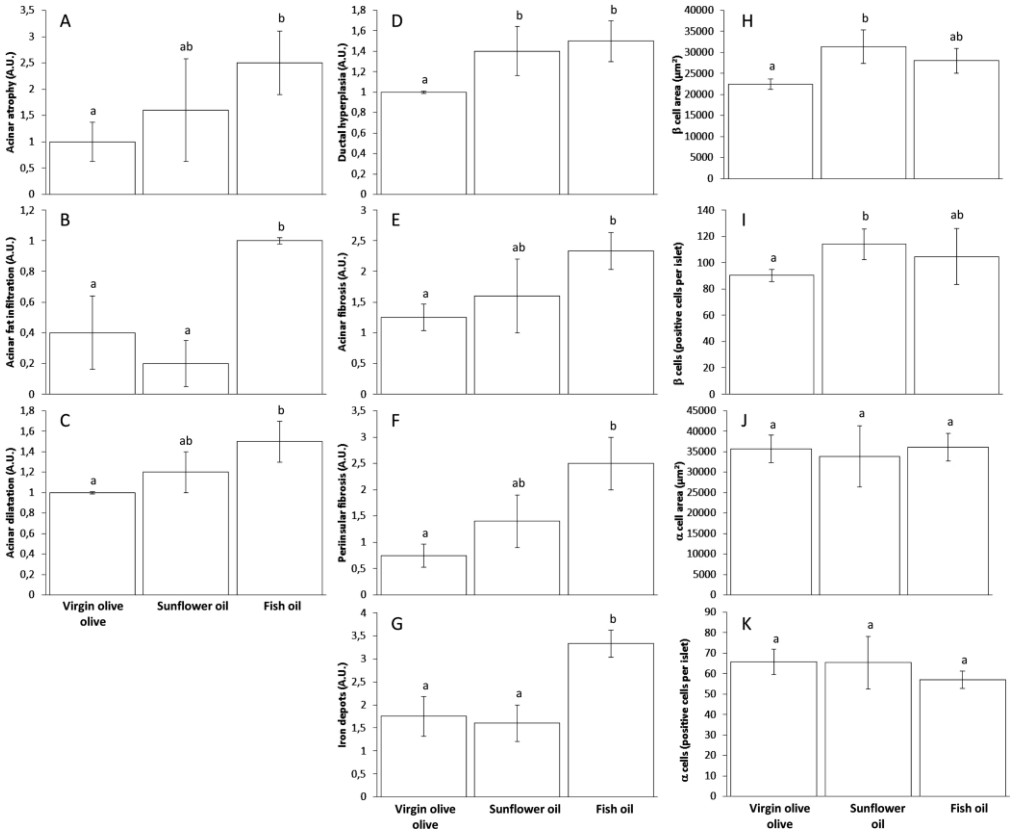


Figure 2. Histological and immunohistochemistry (IHC) examinations in the pancreases of rats fed for 24 mo on different dietary fat sources (virgin olive, sunflower, or fish oils). (**A**–**G**) Data from histological examination. (**H**–**K**) Data from IHC analysis. Data are presented as mean ± *SEM*. Columns not sharing superscript letters are statistically different ($p < .05$). A.U. = arbitrary units.

Table 2. Total Insulin and Glucagon Contents in Pancreata VOOF, SOF, and FOF Rats at the End of the Experimental Procedure (2 y)

Parameter	VOOF	SOF	FOF
Insulin (pg/μg protein)	8,028.7 ± 2,293.3	15,166.6 ± 3,469.9*	9,143.6 ± 2,271.8
Glucagon (pg/μg protein)	115.4 ± 13.3	121.4 ± 20.0	119.7 ± 12.3

Notes: FOF = fish oil–fed group; SOF = sunflower oil–fed group; VOOF = virgin olive oil–fed group.
*$p < .05$ respect to VOOF rats.

Experimental Approach to Explain the Observed Pancreatic Changes

In order to elucidate the possible mechanisms involved in the previously mentioned pancreatic changes in rats fed with different oils, several proliferation and apoptotic markers were analyzed (Table 5). As a result, no significant differences were observed in the number of Ki-67$^+$ cells, indicating that pancreatic proliferation events were very similar in all groups of animals. A similar observation was detected in the expression of mitochondrial apoptotic markers Bcl2 and Bad. However, VOOF rats presented significant lower amounts of tumor necrosis factor-α expression and protein carbonyl presence than SOF and FOF animals.

Discussion

Diets with a similar fat composition ingested over very long periods of time are not likely to occur. However, this observational study uses the rat model to mimic a nutritional situation that occurs in humans, where the consumption

Table 3. Circulating Parameters of VOOF, SOF, and FOF Rats at the End of the Experimental Procedure (2 y)

Parameter	VOOF	SOF	FOF
Insulin (pg/mL)	190.9 ± 47.7	330.2 ± 98.5	246.6 ± 69.8
Leptin (pg/mL)	17,490.6 ± 660.2	30,650.9 ± 7,888.6*	14,029.1 ± 2,520.7
Glucose (mM)	6.7 ± 0.6	6.8 ± 0.6	6.1 ± 0.4
HOMA	1.5 ± 0.4	2.3 ± 0.6	1.7 ± 0.5
Triglicerides (mM)	1.9 ± 0.6	1.4 ± 0.2	1.2 ± 0.2
Cholesterol (mM)	2.5 ± 0.4	2.2 ± 0.3	1.8 ± 0.3
Phospholipids (mM)	1.3 ± 0.3	1.0 ± 0.1	0.8 ± 0.1
Total lipids (mg/dL)	493.1 ± 83.2	434.1 ± 62.3	339.7 ± 65.6
ALP (U/mL)	63.5 ± 8.3	57.2 ± 6.6	104.6 ± 9.2*
CK (U/mL)	399.2 ± 143.6	433.1 ± 104.8	485.4 ± 82.5
LDH (U/mL)	438.1 ± 67.4	460.3 ± 43.2	384.0 ± 51.2
GOT (U/mL)	18.5 ± 2.1	17.7 ± 3.6	20.2 ± 1.3
GPT (U/mL)	5.6 ± 0.7	6.7 ± 1.1	9.5 ± 1.2*
Proteins (g/dL)	6.4 ± 0.6	5.6 ± 0.2	5.9 ± 0.1
Creatinine (μM)	112.7 ± 20.1	76.4 ± 8.9	101.9 ± 10.5
Lactate (mM)	3.1 ± 0.3	3.6 ± 0.6	3.1 ± 0.3

Notes: ALP = alkaline phosphatase; CK = creatine kinase; GOT = glutamyl oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; HOMA = homeostasis model assessment; LDH = lactate dehydrogenase; VOOF = virgin olive oil–fed group.
*$p < .05$ respect to VOOF rats.

Table 4. Circulating Fatty Acid Profile of VOOF, SOF, and FOF Rats

Fatty Acid	VOOF	SOF	FOF
Myristic (C14:0)	0.7 ± 0.1	0.6 ± 0.0	1.1 ± 0.1*
Palmitic (C16:0)	20.9 ± 1.3	20.8 ± 0.5	25.8 ± 1.3*
Stearic (C18:0)	12.4 ± 0.5	18.8 ± 0.5*	9.9 ± 0.5*
Lignoceric (C24:0)	0.8 ± 0.1	1.6 ± 0.4*	0.8 ± 0.3
Saturated	37.2 ± 1.5	38.5 ± 1.1	40.6 ± 1.7
Hexadecenoic (C16:1n9)	5.5 ± 0.5	3.6 ± 0.6*	6.7 ± 0.3*
Oleic (C18:1n9)	26.9 ± 5.3	10.5 ± 5.3*	11.9 ± 3.6*
Nervonic (C24:1n9)	0.9 ± 0.2	1.0 ± 0.3	1.5 ± 0.2
Monounsaturated	40.6 ± 1.8	27.9 ± 3.3*	31.6 ± 2.4*
Linoleic (C18:2n6)	7.3 ± 0.4	14.7 ± 2.1*	2.3 ± 0.7*
DGLA (C20:3n6)	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.0
Arachidonic (C20:4n6)	6.7 ± 0.2	15.7 ± 1.9*	5.8 ± 0.4*
EPA (C20:5n3)	0.2 ± 0.1	0.1 ± 0.0*	8.4 ± 1.1*
DHA (C22:6n3)	1.9 ± 0.2	0.6 ± 0.3*	9.0 ± 0.6*
Polyunsaturated	22.2 ± 0.7	33.6 ± 2.5*	27.8 ± 1.3*
Polyunsaturated (n-3)	2.4 ± 0.1	1.3 ± 0.2	18.3 ± 1.2*
Polyunsaturated (n-6)	19.8 ± 0.7	32.2 ± 2.7*	9.4 ± 0.4*

Notes: Results are expressed as percentage of total fat. DGLA = dihomo-γ-linolenic; DHA = docosahexaenoic; EPA = eicosapentaenoic; FOF = fish oil–fed group; SOF = sunflower oil–fed group; VOOF = virgin olive oil–fed group.
(*) $p < .05$ respect to VOOF rats.

of a certain type of fat predominates in diet. In this context, the animal model could serve as a direct and easy method to observe harmful and beneficial effects caused by the presence of particular fats in diet. In addition, owing to the difficult accessibility of certain human samples (ie, pancreas), the development of animal models can help to understand how diet can model organ histology in different physiopathological situations. Here, we report that different pancreatic compartments were affected according to the fat composition consumed by the animals over long periods of time. This attempt was performed in the context of a balanced diet in order to eliminate other variables generated from a pathological situation, such as obesity or type 2 diabetes. This is reflected in the circulating parameters of each experimental group and shown in Table 3. In addition, the other variables that could contribute to the observed differences, such as genetic predisposition or the intake of other nutrients, are overruled because they seem to contribute equally in all experimental groups.

In this context, the oils provided to each experimental group presented particular characteristics. Although fish oil contains the highest amounts of saturated fatty acids (Table 1), this is not a key point because the circulating sat-urated fatty acid profiles reached in the three experimental groups after long-term consumption were similar (Table 4). Therefore, we can assume that the distinctive feature of the different oils concerns other types of fatty acids. In this context, olive oil presents the highest amount of MUFAs,

Table 5. Ki-67 Proliferation Assay, mRNA Levels of Apoptosis and Inflammation-Related Genes as Well as Protein Oxidation in the Pancreas of VOOF, SOF, and FOF Rats at the End of the Experimental Procedure (2 y)

Parameter	VOOF	SOF	FOF
Ki67 (positive cells/islet)	0.30 ± 0.07	0.22 ± 0.07	0.32 ± 0.04
Bcl2 (RQ)	1.05 ± 0.12	1.01 ± 0.25	1.17 ± 0.12
Bad (RQ)	1.28 ± 0.12	1.03 ± 0.06	1.17 ± 0.05
TNFα (RQ)	0.69 ± 0.17	1.34 ± 0.32*	1.48 ± 0.19*
Protein carbonyls (nmol/mg)	62.6 ± 9.1	138.9 ± 22.8*	177.5 ± 19.5*

Notes: FOF = fish oil–fed group; mRNA = messenger RNA; RQ = relative quantity; SOF = sunflower oil–fed group; TNFα = tumor necrosis factor α; VOOF = virgin olive oil–fed group

*$p < .05$ respect to VOOF rats.

whereas the other two food oils possess the highest PUFA contents, specifically n-6 in sunflower and n-3 in fish. This profile is maintained thereafter in circulation in VOOF, SOF, and FOF animals, although with less marked differences compared with the original oil composition.

The important finding of this study is that the type of fat can affect the exocrine and endocrine compartments of the pancreas differently. In this context and taking islets of VOOF rats as reference, SOF islets contained a higher number of β cells, reflected by increased insulin contents, and hyperleptinemia. These signs, along with other parameters, have been described in obesity, glucose intolerance, insulin resistance, disruption of the adipoinsular axis or prediabetes (41). However, SOF rats were normoglycemic and normoinsulinemic, indicating the absence of a prepathological state (42). In this context, we do not have a clear explanation for the high levels of circulating leptin and if there is a correlation with the high insulin contents found in the islets of this group. In this context, and regarding only islet tissue, the high β-cell number found in SOF rat islets with respect to VOOF could be interpreted as an increased replicative capacity of β cells or a longer life span of these cells due to a reduced apoptosis. However, it has been well documented that β-cell replication decreases with age in rodents and humans (43,44), but in compensation presents a minimal apoptosis (45).

Considering these observations, the results shown in Table 5, although preliminary, seem to indicate increased apoptosis, inflammation, and oxidative stress in SOF and FOF animals. First, and according to the number of $Ki67^+$ cells, it seems that proliferation events were very similar in the pancreases of all groups. The same occurred for the expression levels of the apoptotic markers Bcl2 and Bad but not for tumor necrosis factor-α, which presented lower expression levels in VOOF animals compared with SOF and FOF rats. Altogether, these results might suggest that the mitochondrial apoptotic pathway was not active in any of the animal groups and that the cytoplasmic pathway, where tumor necrosis factor-α is most likely participating (46), seems to be instrumental in the changes observed in SOF and FOF rats. In SOF rats, it is very likely that many of $insulin^+$ cells observed could be apoptotic, but we need to perform functional analysis, such as insulin secretion experiments in isolated islets, in order to verify this hypothesis. In addition, an increased oxidative stress in SOF and FOF animals, corroborated by protein carbonyl determinations, can be observed. In conclusion, these are preliminary data that have to be interpreted with caution.

On the other hand, the presence of fish oil in animal diet seems to mainly affect the exocrine pancreas compartment, as observed by the intermediate alteration detected in the SOF group. The histological pancreatic alterations observed in FOF animals resemble those observed in pancreatic fibrosis of the elderly (6). This indicates at the same time that long-term virgin olive consumption should delay exocrine pancreatic aging. It is clear that the development of pancreatic fibrosis is a complex process in which stellate cells play an instrumental role. These cells, which initially were characterized in human and rat pancreas (47,48), are generally distributed in the periacinar region, presenting lipid droplets within the cytoplasm. These cells closely resemble liver stellate cells, explaining why hepatic fibrosis presents many similarities with pancreatic fibrosis (49). The initial damage causes the release of cytokines and growth factors from pancreatic resident cells or infiltrated inflammatory cells, mainly macrophages (50–52). Macrophage phagocyte damage cells, releasing additional cytokines that induce the proliferation and transformation of stellate cells into myofibroblasts that produce extracellular matrix deposition and therefore fibrosis (53). We hypothesize that nutrients can modulate the function of these cells, as it has been described for glucose (20). In this context, it is tempting to speculate that certain types of fatty acids, like those present in fish oil, could affect stellate cell function. In any case, additional experiments are necessary to confirm this hypothesis.

Finally, the data of this study reinforce in some way the already published favorable effects of olive oil in diet, although the major part of published studies have been focused on endocrine pancreas function. In this context, intervention and epidemiological studies clearly state that MUFA-rich diets tend to improve insulin sensitivity (54–58) and lower glycemia and insulin requirements in type 2 diabetic subjects (59). In a cross-sectional study carried out in the South of Spain, an oral glucose tolerance test was performed in 538 individuals in order to calculate insulin resistance using homeostasis model assessment. The study

showed a positive association between MUFA consumption and β-cell function (60). However, the exact mechanisms by which olive oil induces this effect remain to be explored.

It is clear that olive oil has a beneficial effect in other tissues or systems that indirectly can improve endocrine and exocrine pancreas function (61). In this context, olive oil is rich in antioxidants (vitamin E and phenolic compounds) and antithrombotic agents that can improve endothelial function and reduce the risk of cardiovascular disease, insulin resistance (62–64), and possibly, pancreatitis (this study). Therefore, the mechanisms for this effect could be more complex than expected, and may be due to the synergistic effect of several components and not to the sole effect of oleic acid or MUFAs.

In conclusion, although preliminary, this study emphasizes from a histological point of view that the composition of fat could play an important role in pancreatic aging, affecting different compartments of this organ. In addition, it strengthens the beneficial effect of olive oil consumption in maintaining pancreatic microstructure.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

Funding

M.I.A. is recipient of a fellowship of VALi+d program from Generalitat Valenciana (APOSTD/2012/021). E.R. is recipient of a grant from Instituto de Salud Carlos III-FEDER (PS09/01093) and PROMETEO/2012/007 from Generalitat Valenciana. E.R. is a member of the "Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición" CIBERobn (CB12/03/30038). M.P.P.-L. is recipient of a fellowship of Formación de Personal Investigador program from the Spanish Ministry of Education. A.G.-A. is recipient of a fellowship of Formación de Personal Universitario program from the Spanish Ministry of Education. This study was supported by I+D grants from the Spanish Ministry of Education and Science (AGL2008-01057) and the Autonomous Government of Andalusia (AGR832) to J.L.Q.

References

1. Murtaugh LC, Melton DA. Genes, signals, and lineages in pancreas development. *Annu Rev Cell Dev Biol*. 2003;19:71–89.
2. Ouyang D, Dhall D, Yu R. Pathologic pancreatic endocrine cell hyperplasia. *World J Gastroenterol*. 2011;17:137–143.
3. Halter JB. Diabetes mellitus in an aging population: the challenge ahead. *J Gerontol A Biol Sci Med Sci*. 2012;67:1297–1299.
4. Braganza JM, Lee SH, McCloy RF, McMahon MJ. Chronic pancreatitis. *Lancet*. 2011;377:1184–1197.
5. Keller J, Layer P. Human pancreatic exocrine response to nutrients in health and disease. *Gut*. 2005;54(suppl 6):vi1–28.
6. Klöppel G, Detlefsen S, Feyerabend B. Fibrosis of the pancreas: the initial tissue damage and the resulting pattern. *Virchows Arch*. 2004;445:1–8.
7. Kuroda J, Suda K, Hosokawa Y. Periacinar collagenization in patients with chronic alcoholism. *Pathol Int*. 1998;48:857–868.
8. Apte MV, Haber PS, Darby SJ, et al. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. *Gut*. 1999;44:534–541.
9. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol*. 2003;200:500–503.
10. Patel M, Fine DR. Fibrogenesis in the pancreas after acinar cell injury. *Scand J Surg*. 2005;94:108–111.
11. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest*. 2006;116:1802–1812.
12. Nolan CJ, Prentki M. The islet beta-cell: fuel responsive and vulnerable. *Trends Endocrinol Metab*. 2008;19:285–291.
13. Steil GM, Trivedi N, Jonas JC, et al. Adaptation of beta-cell mass to substrate oversupply: enhanced function with normal gene expression. *Am J Physiol Endocrinol Metab*. 2001;280:E788–E796.
14. Liu YQ, Jetton TL, Leahy JL. beta-Cell adaptation to insulin resistance. Increased pyruvate carboxylase and malate-pyruvate shuttle activity in islets of nondiabetic Zucker fatty rats. *J Biol Chem*. 2002;277:39163–39168.
15. Jetton TL, Lausier J, LaRock K, et al. Mechanisms of compensatory beta-cell growth in insulin-resistant rats: roles of Akt kinase. *Diabetes*. 2005;54:2294–2304.
16. Tierney AC, Roche HM. The potential role of olive oil-derived MUFA in insulin sensitivity. *Mol Nutr Food Res*. 2007;51:1235–1248.
17. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1595–1607.
18. Bonora E, Kiechl S, Willeit J, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. *Diabetes*. 1998;47:1643–1649.
19. Zethelius B, Lithell H, Hales CN, Berne C. Insulin sensitivity, proinsulin and insulin as predictors of coronary heart disease. A population-based 10-year, follow-up study in 70-year old men using the euglycaemic insulin clamp. *Diabetologia*. 2005; 48:862–867.
20. Czakó L, Hegyi P, Rakonczay Z Jr, Wittmann T, Otsuki M. Interactions between the endocrine and exocrine pancreas and their clinical relevance. *Pancreatology*. 2009;9:351–359.
21. Wu D, Xu Y, Zeng Y, Wang X. Endocrine pancreatic function changes after acute pancreatitis. *Pancreas*. 2011;40:1006–1011.
22. Sasikala M, Talukdar R, Pavan kumar P, et al. β-Cell dysfunction in chronic pancreatitis. *Dig Dis Sci*. 2012;57:1764–1772.
23. Ochoa JJ, Quiles JL, Ibáñez S, et al. Aging-related oxidative stress depends on dietary lipid source in rat postmitotic tissues. *J Bioenerg Biomembr*. 2003;35:267–275.
24. Quiles JL, Martínez E, Ibáñez S, et al. Ageing-related tissue-specific alterations in mitochondrial composition and function are modulated by dietary fat type in the rat. *J Bioenerg Biomembr*. 2002;34:517–524.
25. Quiles JL, Ochoa JJ, Ramirez-Tortosa C, et al. Dietary fat type (virgin olive vs. sunflower oils) affects age-related changes in DNA double-strand-breaks, antioxidant capacity and blood lipids in rats. *Exp Gerontol*. 2004;39:1189–1198.
26. Quiles JL, Ochoa JJ, Ramirez-Tortosa MC, Huertas JR, Mataix J. Age-related mitochondrial DNA deletion in rat liver depends on dietary fat unsaturation. *J Gerontol A Biol Sci Med Sci*. 2006;61:107–114.
27. Quiles JL, Pamplona R, Ramirez-Tortosa MC, et al. Coenzyme Q addition to an n-6 PUFA-rich diet resembles benefits on age-related mitochondrial DNA deletion and oxidative stress of a MUFA-rich diet in rat heart. *Mech Ageing Dev*. 2010;131:38–47.
28. Quiles JL, Huertas JR, Mañas M, Ochoa JJ, Battino M, Mataix J. Oxidative stress induced by exercise and dietary fat modulates the coenzyme Q and vitamin A balance between plasma and mitochondria. *Int J Vitam Nutr Res*. 1999;69:243–249.
29. Battino M, Quiles JL, Huertas JR, et al. Feeding fried oil changes antioxidant and fatty acid pattern of rat and affects rat liver mitochondrial respiratory chain components. *J Bioenerg Biomembr*. 2002;34:127–134.
30. Quiles JL, Huertas JR, Battino M, et al. The intake of fried virgin olive or sunflower oils differentially induces oxidative stress in rat liver microsomes. *Br J Nutr*. 2002;88:57–65.
31. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr*. 1997;127(suppl 5):838S–841S.
32. Ochoa JJ, Pamplona R, Ramirez-Tortosa MC, et al. Age-related changes in brain mitochondrial DNA deletion and oxidative stress are differentially modulated by dietary fat type and coenzyme Q10. *Free Radic Biol Med*. 2011;50:1053–1064.

33. Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. *Diabetes*. 2004;53:1714–1720.
34. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol*. 1969;22:158–161.
35. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem*. 1973;19:476–482.
36. Naito HK, David JA. Laboratory considerations: determination of cholesterol, triglyceride, phospholipid, and other lipids in blood and tissues. *Lab Res Methods Biol Med*. 1984;10:1–76.
37. Young DS, Friedman RB. *Effects of Disease on Clinical Laboratory Tests*. Washington, DC: AACC Press; 2001.
38. Bowers LD, Wong ET. Kinetic serum creatinine assays. II. A critical evaluation and review. *Clin Chem*. 1980;26:555–561.
39. Weisshaar D, Gossrau E, Faderl B. [Normal ranges of alpha-HBDH, LDH, AP, and LAP as measured with substrate-optimated test charges]. *Med Welt*. 1975;26:387–392.
40. Wallace TM, Matthews DR. The assessment of insulin resistance in man. *Diabet Med*. 2002;19:527–534.
41. Jones HB, Nugent D, Jenkins R. Variation in characteristics of islets of Langerhans in insulin-resistant, diabetic and non-diabetic-rat strains. *Int J Exp Pathol*. 2010;91:288–301.
42. Sattar N, Wannamethee SG, Forouhi NG. Novel biochemical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities? *Diabetologia*. 2008;51:926–940.
43. Finegood DT, Scaglia L, Bonner-Weir S. Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. *Diabetes*. 1995;44:249–256.
44. Meier JJ, Butler AE, Saisho Y, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes*. 2008;57:1584–1594.
45. Teta M, Long SY, Wartschow LM, Rankin MM, Kushner JA. Very slow turnover of beta-cells in aged adult mice. *Diabetes*. 2005;54:2557–2567.
46. Chu WM. Tumor necrosis factor. *Cancer Lett*. 2013;328:222–225.
47. Apte MV, Haber PS, Applegate TL, et al. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. *Gut*. 1998;43:128–133.
48. Bachem MG, Schneider E, Gross H, et al. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology*. 1998;115:421–432.
49. Bedossa P, Paradis V. Liver extracellular matrix in health and disease. *J Pathol*. 2003;200:504–515.
50. Andoh A, Takaya H, Saotome T, et al. Cytokine regulation of chemokine (IL-8, MCP-1, and RANTES) gene expression in human pancreatic periacinar myofibroblasts. *Gastroenterology*. 2000;119:211–219.
51. Luttenberger T, Schmid-Kotsas A, Menke A, et al. Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: implications in pathogenesis of pancreas fibrosis. *Lab Invest*. 2000;80:47–55.
52. Saurer L, Reber P, Schaffner T, et al. Differential expression of chemokines in normal pancreas and in chronic pancreatitis. *Gastroenterology*. 2000;118:356–367.
53. Shek FW, Benyon RC, Walker FM, et al. Expression of transforming growth factor-beta 1 by pancreatic stellate cells and its implications for matrix secretion and turnover in chronic pancreatitis. *Am J Pathol*. 2002;160:1787–1798.
54. Maron DJ, Fair JM, Haskell WL. Saturated fat intake and insulin resistance in men with coronary artery disease. The Stanford Coronary Risk Intervention Project Investigators and Staff. *Circulation*. 1991;84:2020–2027.
55. Mayer EJ, Newman B, Quesenberry CP Jr, Selby JV. Usual dietary fat intake and insulin concentrations in healthy women twins. *Diabetes Care*. 1993;16:1459–1469.
56. Marshall JA, Bessesen DH, Hamman RF. High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: the San Luis Valley Diabetes Study. *Diabetologia*. 1997;40:430–438.
57. Ryan M, McInerney D, Owens D, Collins P, Johnson A, Tomkin GH. Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *QJM*. 2000;93:85–91.
58. Soriguer F, Esteva I, Rojo-Martinez G, et al. Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study). *Eur J Endocrinol*. 2004;150:33–39.
59. Garg A, Bonanome A, Grundy SM, Zhang ZJ, Unger RH. Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1988;319:829–834.
60. Rojo-Martínez G, Esteva I, Ruiz de Adana MS, et al. Dietary fatty acids and insulin secretion: a population-based study. *Eur J Clin Nutr*. 2006;60:1195–1200.
61. Umezawa M, Higuchi K, Mori M, Matushita T, Hosokawa M. Effect of dietary unsaturated fatty acids on senile amyloidosis in senescence-accelerated mice. *J Gerontol A Biol Sci Med Sci*. 2009;64:646–652.
62. Serra-Majem L, de la Cruz JN, Ribas L, Salleras L. Mediterranean diet and health: is all the secret in olive oil? *Pathophysiol Haemost Thromb*. 2003;33:461–465.
63. Madigan C, Ryan M, Owens D, Collins P, Tomkin GH. Dietary unsaturated fatty acids in type 2 diabetes: higher levels of postprandial lipoprotein on a linoleic acid-rich sunflower oil diet compared with an oleic acid-rich olive oil diet. *Diabetes Care*. 2000;23:1472–1477.
64. Perona JS, Vögler O, Sánchez-Domínguez JM, Montero E, Escribá PV, Ruiz-Gutierrez V. Consumption of virgin olive oil influences membrane lipid composition and regulates intracellular signaling in elderly adults with type 2 diabetes mellitus. *J Gerontol A Biol Sci Med Sci*. 2007;62:256–263.

Int. J. Mol. Sci. **2015**, *16*, 23425-23445; doi:10.3390/ijms161023425

OPEN ACCESS
International Journal of
Molecular Sciences
ISSN 1422-0067
www.mdpi.com/journal/ijms

Article

Sunflower Oil but Not Fish Oil Resembles Positive Effects of Virgin Olive Oil on Aged Pancreas after Life-Long Coenzyme Q Addition

Adrián González-Alonso [1], César L. Ramírez-Tortosa [2], Alfonso Varela-López [1], Enrique Roche [3], María I. Arribas [3], M. Carmen Ramírez-Tortosa [4], Francesca Giampieri [5], Julio J. Ochoa [1] and José L. Quiles [1,*]

[1] Department of Physiology, Institute of Nutrition and Food Technology "Jose Mataix", University of Granada, Biomedical Research Center, lab. 250, Avda. Conocimiento s/n, 18100 Armilla, Granada, Spain; E-Mails: kulturadrian@hotmail.com (A.G.-A.); alvarela@ugr.es (A.V.-L.); jjoh@ugr.es (J.J.O.)

[2] Department of Pathology, Complejo Hospitalario de Jaén, 23007 Jaén, Spain; E-Mail: cesarl.ramirez.sspa@juntadeandalucia.es

[3] Bioengineering Institute, University Miguel Hernandez and CIBEROBN (CB 12/03/30038, Fisiopatología de la Obesidad y la Nutrición, Instituto de Salud Carlos III), 03202 Elche, Spain; E-Mails: eroche@umh.es (E.R.); miarribas@umh.es (M.I.A.)

[4] Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "Jose Mataix", University of Granada, 18071 Granada, Spain; E-Mail: mramirez@ugr.es

[5] Dip. Scienze Cliniche Specialistiche ed Odontostomatologiche, Università Politecnica delle Marche, 60121 Ancona, Italy; E-Mail: f.giampieri@univpm.it

***** Author to whom correspondence should be addressed; E-Mail: jlquiles@ugr.es; Tel.: +34-958-241000 (ext. 20316).

Academic Editor: Maurizio Battino

Received: 28 July 2015 / Accepted: 22 September 2015 / Published: 29 September 2015

Abstract: An adequate pancreatic structure is necessary for optimal organ function. Structural changes are critical in the development of age-related pancreatic disorders. In this context, it has been reported that different pancreatic compartments from rats were affected according to the fat composition consumed. Since there is a close relationship between mitochondria, oxidative stress and aging, an experimental approach has been developed to gain more insight into this process in the pancreas. A low dosage of coenzyme Q was administered life-long in rats in order to try to prevent pancreatic aging-related

alterations associated to some dietary fat sources. According to that, three groups of rats were fed normocaloric diets containing Coenzyme Q (CoQ) for two years, where virgin olive, sunflower, or fish oil was included as unique fat source. Pancreatic samples for microscopy and blood samples were collected at the moment of euthanasia. The main finding is that CoQ supplementation gives different results according to fat used in diet. When sunflower oil was the main fat in the diet, CoQ supplementation seems to improve endocrine pancreas structure and in particular β-cell mass resembling positive effects of virgin olive oil. Conversely, CoQ intake does not seem to improve the structural alterations of exocrine compartment previously observed in fish oil fed rats. Therefore CoQ may improve pancreatic alterations associated to the chronic intake of some dietary fat sources.

Keywords: β-cell; endocrine pancreas; exocrine pancreas; inflammation; pancreatic aging; pancreatic fibrosis of the elderly; fat; dietary lipids

1. Introduction

The pancreas is a key organ in nutrient digestion as well as in nutrient utilization. This dual function can be performed due to the presence of two compartments: the exocrine and the endocrine pancreas. The exocrine portion is the most abundant (98% of pancreatic mass) and is formed by interstitial mesenchymal, ductal and acinar cells, these last producing enzymes responsible for food digestion in the duodenum. The endocrine compartment is formed by cell aggregates (also called islets of Langerhans) disseminated through the exocrine structure and formed by different cell types specialized in the biosynthesis and secretion of specific hormones: insulin by β-cells (around 60% of islet mass), glucagon by α-cells (30% of islet mass), somatostatin by δ-cells, pancreatic polypeptide by γ-cells and ghrelin by ε-cells, these last only appearing during embryogenesis [1,2]. During the life cycle, persistent bad habits in diet and life style can predispose to the appearance of certain pathologies that can disturb independently both pancreatic compartments and accelerate the organ aging process. In addition, it is obvious that due to the close proximity, pathological states affecting one of the pancreatic compartments will inevitably affect the other [3].

In this context, dietary habits have shown to exert effects on both compartments. On one hand, exocrine pancreatic compartment have been shown to be disturbed by alcohol abuse [4] that lead to destruction of functional exocrine pancreatic tissue resulting in nutrient malabsorption in the intestine [5]. On the other hand, endocrine pancreatic compartments, particularly the β-cells, are disturbed in type 2 diabetes mellitus [6], which is closely related to an excessive and unbalanced intake of high calorie nutrients such as fat and sugars [7]. Concerning fat in the diet, depending of the nature of this nutrient, different effects have been reported both on the endocrine as well as on the exocrine function. In this sense, different intervention and epidemiological studies clearly state that monounsaturated fat-rich diets improve insulin sensitivity [8–12] and lead to lower glycaemia and insulin requirements in type 2 diabetic subjects [13,14]. On the other hand, as far as exocrine pancreas compartment is concerned, extensive work has been carried out mainly comparing virgin olive oil against sunflower oil from the point of view of exocrine pancreatic secretion [15]. Authors demonstrated that exocrine pancreatic

secretory activity in response to food was greater in dogs fed on a diet rich in sunflower oil than in animals given the same diet with virgin olive oil [16,17]. These authors, working on rats found that chronic intake of diets differing only in the type of fat (olive oil or sunflower oil) influences the fatty acid profile of pancreatic cell membranes; with rats fed the olive oil diet showing higher levels of oleic acid, whereas those fed sunflower oil had increased linoleic acid and PUFA n-6 contents. In these animals, exocrine pancreatic secretion in anesthetized animals was also affected [18]. Regarding dietary fat type in the aged pancreas, we have previously reported the effects of life-long feeding rats with virgin olive, sunflower or fish oil on the endocrine function and histology of the pancreas [19]. The main finding was related to the beneficial effect of monounsaturated fat-rich diets in maintaining pancreatic microstructure and endocrine function. Thus, animals fed on sunflower oil showed higher β-cell numbers and insulin content compared with those fed on virgin olive oil. In addition, animals fed on fish oil developed acinar fibrosis and presented macrophage infiltrates in peri-insular regions, also compared with animals fed on virgin olive oil.

Coenzyme Q (CoQ) is the general term that refers to a class of benzoquinones widely distributed in the phospholipidic bilayers of cellular and organelle membranes of living organisms [20–22]. CoQ is composed of a redox active quinoid moiety linked to an isoprenoid side chain (2,3-dimethoxy-5 methyl-6-multiprenyl-1-4-benzoquinone) comprising six to ten units, depending on the species [21,23]. In humans, the predominant form of CoQ comprises 10 isoprenoid repeats in the side chain [24] and it is also referred as ubiquinone, meanwhile in rats the predominant form contains nine isoprenoid subunits [25]. CoQ is synthesized by the mevalonate pathway and presents a varying distribution among body tissues [22,26]. CoQ plays an instrumental role in mitochondrial energy production by transferring electrons from respiratory complexes I and II to complex III of the electron transport chain with simultaneous proton translocation to the intermembrane space, contributing to the generation of the transmembrane proton gradient that drives the synthesis of ATP from APD phosphorylation [27]. Ubiquinol is the two-electron reduction product of CoQ [28], functioning as an antioxidant in mitochondria and lipidic membranes [29] and representing the 80%–90% of the CoQ pool in many fluids and tissues including plasma, liver, pancreas and intestine [30,31]. However, the partially reduced form of CoQ is the primary source of superoxide radical in the mitochondrial respiratory chain [32]. In this context, the oxidized form of CoQ predominates in brain and lung [33]. Several reports have implicated direct or indirectly CoQ deficiency in the development of pathologies and physiological states associated with an impaired mitochondrial bioenergetic function such as aging and age-associated diseases [26,34–38]. Concerning aging, the most extended hypothesis is that mitochondrial respiration tend to decline during aging as well as mitochondrial CoQ levels, resulting in a decrease of ATP biosynthesis and an increase in superoxide generation with associated damage in biological macromolecules such as membrane lipids, proteins and DNA [39,40]. In this context, results from several studies indicate that CoQ intake results in an amelioration of age-related disorders [41–44]. In the same way, it has been previously reported how CoQ feeding may counteract some alterations associated to age in different tissues in the rat, like the liver [45], brain [46], heart [47,48], skeletal muscle [49], and periodontal tissue [50] after feeding pro-oxidant fats like sunflower oil (including an impaired function and an increased oxidative stress at the mitochondria); or even expanding lifespan [51].

According to the above-mentioned variations in pancreas structure and function after lifelong feeding on different dietary fats, and the potentially beneficial effects on these aspects by CoQ supplementation,

the present study was designed with the aim to compare three groups of rats maintained for two years with isocaloric diets containing as the only dietary fat virgin olive (VOO), sunflower (SO) or fish oils (FO), all of them supplemented with CoQ.

2. Results and Discussion

2.1. Body Weight Evolution and Adaptation to the Diet

After the 24 month follow-up period, rats fed on FO + CoQ displayed significantly higher body weight (624 ± 20 g) than those receiving VOO + CoQ (496 ± 20 g) and SO + CoQ (499.0 ± 6.6 g). However, from the observation of food spillage, no differences concerning food intake were inferred between dietary groups or in relation to age.

As it has been shown, dietary fat sources as those used in this study are rich in different groups of fatty acids [18,52]. Because dietary fat composition was the main differential parameter in all groups of animals, we needed to verify a proper adaptation to the diet of the animals in order to attribute the possible differences among groups to them. For this reason, circulating fatty acids profile was analyzed. Results showed that for C18:1n-9 (oleic acid, the most representative fatty acid found in virgin olive oil), the highest percentage was found for VOO fed animals (13.3% ± 2.8%), that was significantly higher than that found in SO (4.9% ± 1.1%) and FO (6.1% ± 1.7%) animals. Concerning C18:2n-6 (linolenic acid, the most representative fatty acid found in sunflower oil), SO fed animals showed significantly higher percentage (14.6% ± 0.6%) than VOO (8.4% ± 1.9%) and FO (2.6% ± 0.5%) groups. Finally, for C22:6n-3 (docosahexaenoic acid, mostly present in marine species, FO group led to the significantly highest percentage (8.1% ± 0.5%), compared with VOO (1.8% ± 0.4%) and SO groups (0.3% ± 0.1%). Something similar was found for the other typical marker for fish oil, namely, eicosapentaenoic acid (EPA); the FO group reported the highest percentage (9.9% ± 0.7%), compared with VOO (0.2% ± 0.1%) and SO groups (not detected). This lipid profile resembles that present in the original composition of oils used in the diets and it would indicate a proper adaptation of the rats to the different dietary fats as previous studies [18,53,54].

2.2. Circulating Hormone Levels and Biochemical Parameters

A major pancreatic alteration associated to aging is type 2 diabetes melllitus that appears when adaptation of the β-cell mass fails to compensate the increased insulin demand, resulting in β-cell apoptosis [6]. Increased insulin circulating levels and hyperleptinemia along with other parameters have been described in obesity, glucose intolerance, insulin resistance, disruption of the adipoinsular axis or prediabetes [55]. According to that, insulin resistance and hyperinsulinemia are components of the age-related metabolic disorders such as glucose intolerance, dyslipidemia, obesity, cardiovascular disease, and metabolic syndrome [3,56–59] that present a high risk of evolving towards type 2 diabetes [19]. To evaluate the health of the animals concerning these potential risks, both, circulating levels of insulin and leptin, as well as biochemical parameters related to their actions were measured.

Regarding hormone levels, no significant differences were observed in the amounts of circulating leptin found between CoQ supplemented rats fed on VOO and SO or FO, meanwhile supplemented FO-fed animals presented lower circulating insulin values compared with VOO fed rats (Table 1). When

the levels of these hormones were studied in animals fed on similar dietary fats without CoQ supplementation [19], it was noted that significantly higher values were found only for leptin in animals fed on SO. Eicosapentaenoic acid (EPA), one of the n-3 polyunsaturated fatty acids, has been shown to stimulate leptin mRNA expression and secretion in 3T3-L1 cells [60]. According to that, it is possible that CoQ increased leptin levels only in animals fed on FO and SO, although no additive effects might be accounted for those fed on FO.

In addition, at the moment of euthanasia, all groups of CoQ supplemented animals displayed very similar glycemia and serum lipids, including triglycerides, cholesterol and phospholipids, but not total lipids, which were lower in FO + CoQ rats than in VOO + CoQ animals (Table 1). Likewise, the calculated HOMA (homeostasis model assessment) index showed significantly lower values in FO + CoQ fed rats (Table 1), which is in accordance with the lower insulin levels. However no clear differences were observed between animal fed on similar dietary fats in absence of CoQ supplementation for the last seven parameters [19]. Thus, from the point of view of circulating hormones and biochemical parameters, it is difficult to establish the role of CoQ supplementation in the three studied dietary fats; in any case, neither group of rats showed a significant pathological situation of insulin resistance or loss of insulin sensitivity [61]. On the other hand, CoQ supplementation seemed to reduce leptin levels associated to a SO-based diet. Leptin is produced and released primarily by adipocytes whose circulating levels are directly proportional to total fat mass [62], but also to the percentage of body fat and BMI [63,64]. Nevertheless, with the present methodological approach, it is not possible to establish a clear explanation for CoQ effects on circulating leptin levels when rats are maintained on a SO-based diet.

Table 1. Circulating parameters of rats fed on CoQ supplemented diet based on virgin olive oil (VOO), sunflower oil (SO) and fish oil (FO) at the end of the experimental procedure.

Parameter	VOO	SO	FO
Insulin (pg/mL)	302.7 ± 84.6	212.3 ± 58.7	170.7 ± 47.9 *
Leptin (pg/mL)	14,211.4 ± 2966.2	13,462.9 ± 2275.2	21,580.5 ± 4152.9
Glucose (mM)	6.8 ± 0.5	6.4 ± 0.6	6.4 ± 0.5
HOMA	2.3 ± 0.7	1.6 ± 0.5	1.2 ± 0.4 *
Triglycerides (mM)	1.5 ± 0.3	1.4 ± 0.3	1.1 ±0.1
Cholesterol (mM)	2.2 ± 0.4	2.5 ± 0.1	1.6 ± 0.1
Phospholipids (mM)	1.1 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
Total lipids (mg/dL)	387.1 ± 61.5	438.0 ± 56.4	252.3 ± 33.7 *

Data are presented as mean ± standard error of mean. Abbreviations: HOMA: Homeostatic model assessment; Symbols: * p <0.05 respect to VOO fed rats.

2.3. Histological Study

2.3.1. Pancreatic Parenchyma

Histological assessment of pancreas revealed a clear acinar atrophy (Figure 1C) in rats fed on FO-based diet supplemented with CoQ, accompanied by signs of inflammation (Figure 1F,I) and fibrosis, which was affecting the acinar (Figure 2c) but also peri-insular compartment (Figure 2f). In addition, it was also noted an evident degree of hyperplasia in the ductal region (Figure 2i) as well as certain degree of acinar fat infiltration (Figure 1L). In turn, the other two experimental groups generally

showed lower alteration degree and no significant differences existed between them for most of the features analyzed. (Figure 3). In this sense, light signs of fibrosis in animals fed on VOO (Figure 2a) have been observed. Additionally, acini scarcely display fat depots (Figure 1J,K) and the ductal tissue and pancreatic blood vessels did not present signs of metaplasia or hyperplasia (Figure 2g,h). The only difference was that a slight degree of acinar atrophy was noted in rats fed with VOO and CoQ (Figure 1A) whereas in those fed with SO and CoQ, there were many samples in which it was absent (Figure 1B). Previously, in rats fed on FO and VOO-based diets, but without CoQ, similar alterations were found [19]. However, SO fed rats did not show significant differences in respect to any of the other group for many of the analyzed features, even they showed a similar degree of ductal hyperplasia that those fed on FO. Consequently, pancreatic alteration degree in this group was intermediate between the other two. Histological alterations observed in FO fed animals, supplemented or not, resemble those observed in pancreatic fibrosis of the elderly [65]. The higher degree of inflammation and fibrosis in pancreas found in animals fed on FO seems contradictory with those from animal studies where n-3 fatty acid-rich diets shows anti-inflammatory effects, although pancreas was not directly studied [66–69]. However, other studies have also indicated an absence of such effects [70] or even pro-oxidant and pro-inflammatory effects for these types of fatty acids [71,72]; in addition, it has also been observed that some treatment based on them can decrease lifespan [73–76]. So, the amount, the animal model, age or treatment time seem to be important factors affecting n-3 fatty acid effects that need to be explored in more detail.

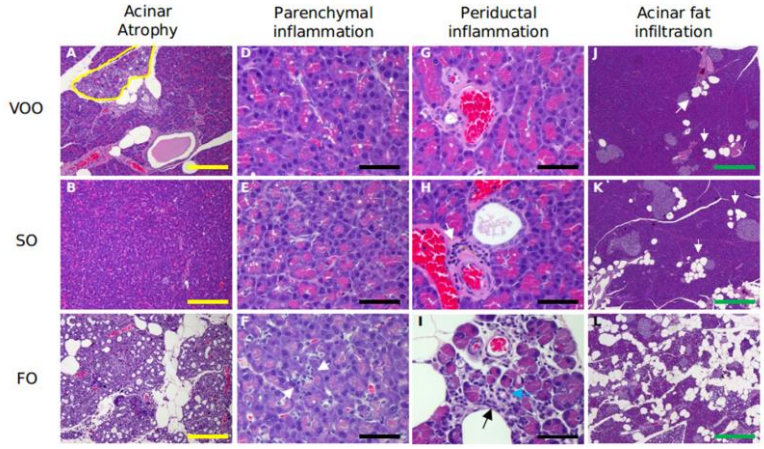


Figure 1. Histological sections of pancreas from rats fed on different dietary fat sources supplemented with Coenzyme Q: virgin olive (VOO), sunflower (SO) and fish oil (FO) after hematoxilin and eosin staining for assessment of acinar atrophy, parenchymal and periductal inflammation and acinar fat infiltration, respectively. (**A**) Minimal acinar atrophy (indicated by a yellow circle); (**B**) Normal pancreatic tissues; (**C**) Severe acinar atrophy, grade 4; (**D**,**E**) Absence of inflammation; (**F**) Inflammatory cells (its presence is indicated by white arrows); (**G**) Absence of inflammation; (**H**) Minimal inflammation with some inflammatory cells (indicated by a white arrow); (**I**) More severe infiltrate (indicated by a black arrow) around the duct (indicated by a blue arrow); (**J**,**K**) Very low number of fatty cells and aggregates (grade 1–2) (indicated by white arrows); (**L**) Severe fat deposits (grade 3–4). Yellow bar = 200 μm; Black bar = 50 μm; Green bar = 500 μm.

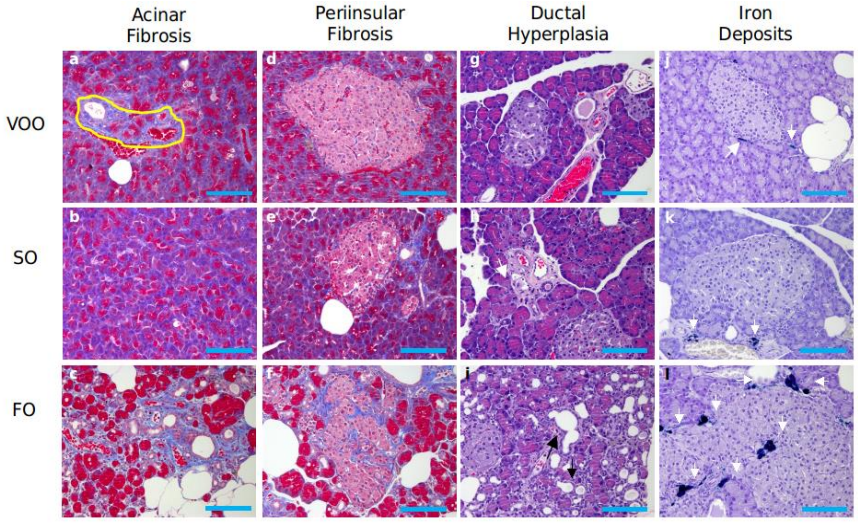


Figure 2. Histological sections of pancreas from rats fed on different dietary fat sources supplemented with coenzyme Q: virgin olive (VOO), sunflower (SO) and fish oil (FO) after Trichrome Masson (**a**–**f**), Hematoxilin and eosin (**g**–**i**) and Perls staining (**j**–**l**) for assessment of acinar and peri-insular fibrosis, ductal hyperplasia and iron deposits, respectively. (**a**) Minimal acinar fibrosis (indicated by a yellow circle); (**b**) Normal pancreatic acini; (**c**) Acinar fibrosis grade 3; (**d**,**e**) Absence of peri-insular fibrosis; (**f**) Moderate fibrosis; (**g**,**h**) Absence of ductular hyperplasia; (**i**) Ductular hyperplasia (indicated by black arrows); (**j**,**k**) Very low number of macrophages stained for hemosiderin (grade 1) with peri-insular distribution (indicated by white arrows); (**l**) High number of macrophages stained for hemosiderin (grade 3–4), (hemosiderin deposited are indicated by white arrows). Blue bar = 100 μm.

Conversely, long-term consumption of diets only based on VOO and SO but supplemented with CoQ, would delay exocrine pancreatic aging in the present model. Pancreatic fibrosis is a complex process in which pancreatic stellate cells (PSCs) play a pivotal role [77,78]. In the normal pancreas, PSCs present a quiescent stage featured by vitamin A-containing lipid droplets, but they can transform into myofibroblast-like cells expressing α-smooth muscle actin [79,80], which are responsible for extracellular matrix deposition and therefore fibrosis [79]. This occurs in response to cytokines and growth factors released by infiltrated inflammatory cells (mainly macrophages) and pancreatic resident cells against cell or tissue damage that stimulate PSCs growth and production of extracellular matrix proteins [81–88]. In turn, activated PSCs are able to produce many inflammatory mediators when they are stimulated by interleukin (IL)-1β and Tumor Necrosis Factor-α (TNF-α), which contributes to accelerating the process of inflammation [89]. In this sense, there is considerable evidence indicating that oxidative stress is implicated in PSC activation [65,66,90–94].

Different antioxidants therapies have been tested demonstrating decreased PSCs and proinflammatory factors both, *in vitro* [90,94] and *in vivo* [87–95]. Likewise, histological findings from the present study suggest that long-term addition of a low-dosage of CoQ to a diet with SO as unique dietary fat results in improvement of certain aging indicators in the exocrine compartment of pancreas

compared with their corresponding non-supplemented counterparts [19]. Nevertheless, in animals fed on FO and VOO, CoQ supplementation seems to have no effect over pancreatic parenchyma. On the one hand, it could be hypothesized that the FO effect on the exocrine compartment of pancreas was due to a mechanism not related to oxidative stress, or at least that this was not exclusive. On the other hand, it is possible that CoQ does not act as a specific antioxidant for this particular process and/or not have enough to counteract FO effects. Regarding VOO, it might be so beneficial for this pancreatic compartment that CoQ supplements hardly have additional effects, but curiously the degree of acinar atrophy was lower in SO fed rat supplemented with CoQ which suggests that this combination could be even more beneficial. In any case, more studies are needed in order to understand mechanisms underlying differences among dietary fats and CoQ effects.

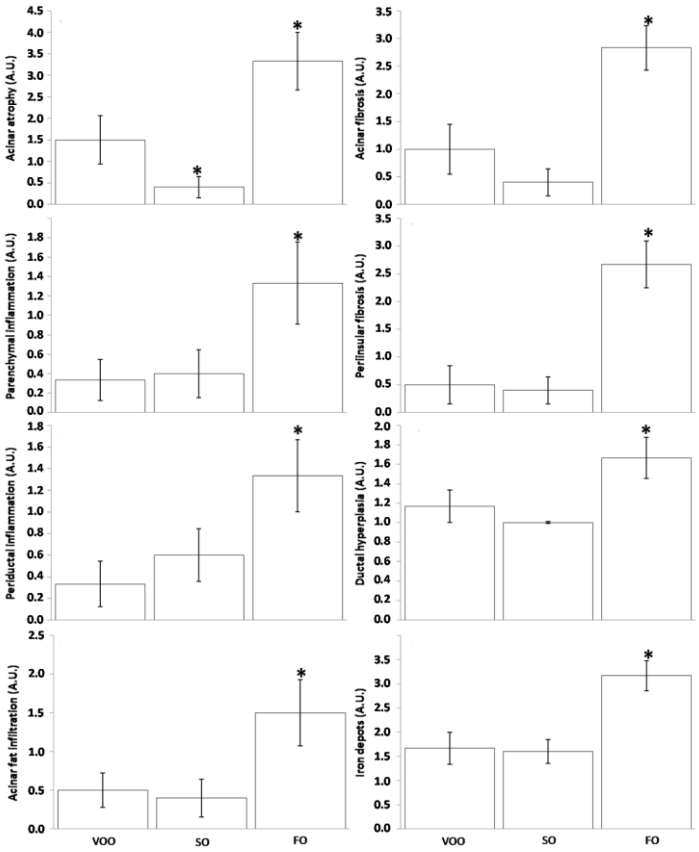


Figure 3. Data from histological evaluations of pancreas from rats fed on different dietary fat sources supplemented with Coenzyme Q: virgin olive (VOO), sunflower (SO) and fish oil (FO). Data are presented as mean ± SEM. A.U. = arbitrary units. * $p < 0.05$ in respect to VOO fed rats.

2.3.2. Immunohistochemical Assessment of Pancreatic Islets

Concerning pancreatic islets, cell type distribution in islets and their density were assessed by using insulin and glucagon immunohistochemistry to identify β- (Figure 4b) and α-cells (Figure 4a),

respectively. In relation to these, no significant differences were found among dietary groups for any of the studied parameters (Figure 4c). This absence of differences is interesting since in the previous study with non CoQ supplemented diets based on similar fats, rats fed on SO presented higher values than those fed on VOO for the β-cell area, as well as a higher number of cells per islet [19]. Age-related disorders of pancreatic endocrine function usually start with a prediabetic state of insulin resistance in which the adaptive response of pancreatic β-cells results in the presence of high levels of circulating insulin (hyperinsulinemia) in order to maintain normoglycemia. Hyperinsulinemia can be achieved by combining different mechanisms that imply expanding β-cell mass by hyperplasia and hypertrophy, enhancing insulin biosynthesis and increasing insulin secretion in response to sustained extracellular nutrient demands [6,96–99]. However, in SO fed rats from the present study there are no signs of β-cell hypertrophy, nor hyperplasia at all. This finding suggests that CoQ addition to diet prevented age-related β-cell alterations associated to long-life consumption of a SO-rich diet under our experimental conditions. Leptin has been shown to have a proliferative effect on β-cell cultures under certain conditions [100–104]. As mentioned before, it is not possible to establish an explanation for CoQ effect on leptin levels, but this effect on β-cells might derive from it, since this group displayed lower levels than in the non-supplemented counterparts [19].

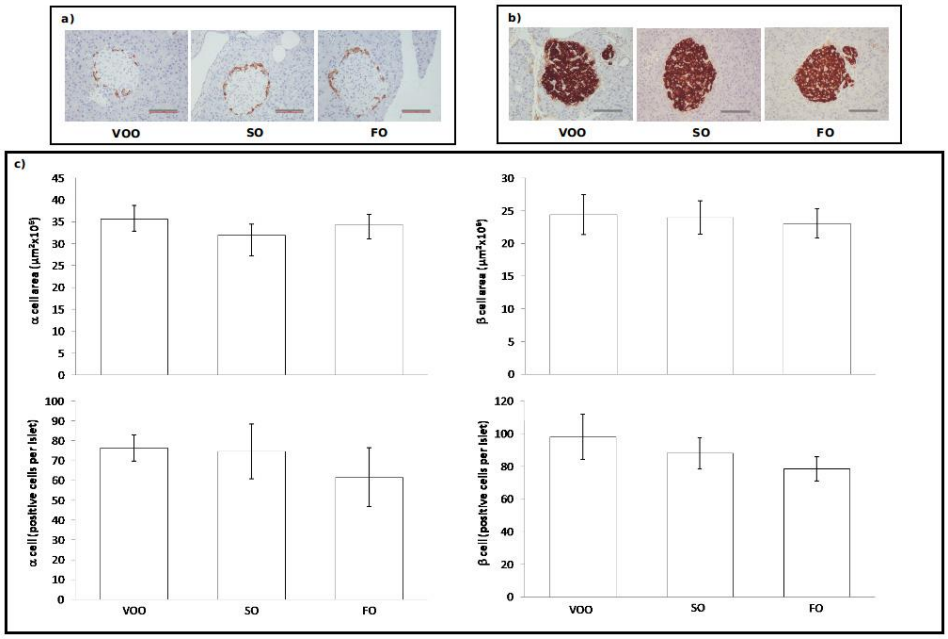


Figure 4. Immunohistochemical assessment in the pancreas of rats fed on different dietary fat sources supplemented with Coenzyme Q: virgin olive oil (VOO), sunflower oil (SO) and fish oil (FO). (**a**) Histological sections with pancreatic isles immunostained for glucagon from rats fed on VOO, SO and FO; (**b**) Histological sections with pancreatic isles immunostained for insulin from rats fed on VOO, SO and FO; (**c**) Quantitative data from histological evaluations of α- and β-cell positive density and area. Data are presented as mean ± SEM. Scare bar = 100 μm.

2.4. Pancreatic Contents of Insulin and Glucagon

In order to gain more insight into about how histological changes could affect the endocrine pancreas compartment, main hormones produced by this micro-organ, *i.e.*, insulin and glucagon, were measured. The three groups of animals presented similar pancreatic content for both insulin and glucagon (Table 2). In particular, FO fed animals supplemented with CoQ, that started to develop certain islet affectation, did not show lower hormone content. This suggests that the alterations observed at the level of the peri-insular region have not lead yet to endocrine dysfunction. In addition, and compared to previous determinations in non-supplemented animals from previous studies [19], it was observed that total insulin content in rats fed on VOO and FO supplemented with CoQ was double that in the non-supplemented counterparts. Meanwhile, supplemented and non-supplemented SO fed rats presented similar insulin contents. However in the second case, they were associated with a higher β-cell mass [19]. It has been hypothesized that β-cell hypertrophy could be an adaptive response to the aging situation in an attempt to maintain correct insulin levels in an organ with diminished replicative capacities [103] which would explain observations from the previous study [19]. In turn, here, histological measures related to β-cells were similar among groups and similar to those found in supplemented animals fed FO or VOO [19]. This suggest that CoQ may contribute to specifically increase the content of insulin regardless dietary fat type, but without affecting glucagon that maintained similar levels among dietary groups in both studies. HOMA values, insulin circulating levels and glycaemia were similar to those found in absence of CoQ [19], which suggests that there is no decrease in insulin secretion levels or demands by other tissues. Another possibility is that CoQ improves β-cells capacity to restore insulin reserves. However, because of our experimental design focused on pancreas, it was not possible to elucidate it.

Table 2. Total insulin and glucagon pancreatic contents in rats fed on CoQ supplemented diet based on virgin olive oil (VOO), sunflower oil (SO) and fish oil (FO) at the end of the experimental procedure.

Parameter	VOO	SO	FO
Insulin (pg/μg protein)	18,902.5 ± 6989.5	14,144 ± 4588.5	19,576 ± 3720.3
Glucagon (pg/μg protein)	151.8 ± 12.8	143 ± 37.2	129.6 ± 23.8

3. Experimental Section

3.1. Animals and Diet

Eighteen male Wistar *rats* (Rattus *norvegicus*) initially weighing 80–90 g were housed during 2 years and fed with the semisynthetic and isocaloric AIN-93G (the first two months) and AIN-93M diets [105]. Components of AIN-93G diet in weight percentage were: 38.7% starch, 20% caseine, 13.2% dextrose, 10% sucrose, 8% oil, 5% cellulose (fibre), 3.5% mineral mix, 1% vitamin mix, 0.3% L-cysteine and 0.25% choline bitartrate. This corresponded to a caloric distribution of 43.3% polysaccharides, 11.1% disaccharides, 14.6% monosaccharides, 22.2% proteins and 8.8% lipids. AIN-93M diet presents similar weight composition than AIN-93G except for: 46.6% starch, 14% caseine, 15.5% dextrose, 4% oil and 0.18% L-cysteine. The corresponding

caloric composition for AIN-93GM diet was: 51.8% polysaccharides, 11.1% disaccharides, 16.7% monosaccharides, 25.8% proteins and 4.4% lipids.

Three groups of 6 animals each were fed with the described diets presenting different lipid composition: VOO, SO and FO. FO diet was prepared by using pure fish oil ROPUFA 30 (DSM, Kaiseraugst, Switzerland). Sunflower oil was acquired from a local supermarket. Extra virgin olive oil was a kind gift from the agricultural research center "Venta del Llano" from Mengibar, Jaén, Spain. Additionally all diets were supplemented with 50 mg/kg/day of CoQ. CoQ dosage has been chosen according with human intake recommendation for this molecule. Previous studies from our laboratory support the use of this dosage in an aging model in the rat [47,50,52,106]. Animals were placed in a climate-controlled room (20 °C, 12 h dark/12 h light cycle) for two years in collective cages, in groups of three animals per cage, with free access to water. Diet was delivered *ad libitum* for the first two months and then at 25 g/rat/day for the rest of the experiment (in order to avoid overweight). Food intake was indirectly monitored through the weekly body weight control and daily spillage monitoring.

The animals were treated in accordance with the guidelines of the Spanish Society for Laboratory. Animals and the experiment was approved by the Ethical Committee of the University of Granada. The rats were killed by cervical dislocation followed by decapitation, at the same time of the day to avoid any circadian fluctuation. At the end of the experiment, rats underwent overnight fasts and were euthanasized next day. Different tissues and samples were quickly removed for different studies. In our particular case, pancreas was quickly dissected and excised in 3 portions. One portion was snap frozen in liquid nitrogen for protein, insulin and glucagon determinations. The other portions were fixed in formaldehyde for immuno-histological analyses. Blood was collected in EDTA-coated tubes and the plasma was centrifuged at 1750× *g* for 10 min. Plasma samples were stored at −80 °C until analyzed.

3.2. Histological Assessments

Pancreatic tissue taken from each rat was fixed in 10% formalin and embedded in paraffin to cut several 4 μm sections. For histological examinations, the sections were stained with haematoxylin and eosin, Masson's Trichrome and Perls staining. Evaluation of the pathological changes was performed by two experienced pathologists who were blinded to the treatment groups. When there were discrepancies, they discussed among themselves and reached a consensus outcome. Total fields per section (the full slide) were observed and the entire available pancreas was studied in order to avoid a focal observation of the same. Acinar atrophy was scored as 0, absent; 1, cytoplasmic basophilia reduction, acinar size reduction and reduction in the content of acinar zymogen granules; 2, acinar vacuolization, early destruction of acini, inflammation and early onset of fibrosis; 3, moderate; 4, severe with metaplasia and/or acinar dilatation (transformation of acini in cystic/ductular structures coated with cuboidal or flattened cells or the formation of small glands lined by cuboidal cells in a microcystic pattern or "honeycomb"). Parenchymal and periductal inflammation: number of groups of inflammatory cells into parenchyma or around of ducts in total fields per section. Acinar fat infiltration (presence of adipocytes in the pancreatic parenchyma) was scored as 0, absent; 1, very low number of cells and aggregates; 2, low; 3, moderate; 4, severe. Ductal hyperplasia was scored according to its presence as 1, absent; 2, present. Acinar fibrosis was scored as 0, absent; 1, perivenular fibrosis with few septa and thin; 2, poor, thin septa with fibrous incomplete bridges between regions; 3, thin septa with extensive

bridges between regions; 4, septa thickened with numerous bridges and nodular appearance. Peri-insular fibrosis was scored as 0, absent; 1, focal and mild increase of peripheral collagen deposition; 2, slight increase but diffuse; 3, moderate increase; 4, severe increase. Iron deposition was scored as 0, absent; 1, splashed positive macrophages; 2, mild positive macrophage accumulation; 3, moderate accumulations; 4, numerous Perls-positive macrophage accumulation.

3.3. Immunohistochemistry for Insulin and Glucagon Expression

β and α cells distribution in islets and their density (percentage of β and α cells per islet area) were assesed by using insulin and glucagon immunohistochemistry to indentify cell type. For all immunohistochemical techniques, pancreatic sections were dewaxed in xylene and rehydrated through graded alcohols to water. Antigen retrieval was performed with the PTLink module (Dako, Demark) using Dako low-pH Antigen Retrieval (AR) fluid (Dako, Demark) followed by several washes in water before being placed onto an AutostainerPlusLink (Dako, Demark) where the remainder of the immunohistochemical staining was performed using Envision™ FLEX (Dako, Demark). Briefly, sections were first placed in washing buffer followed by blockade of endogenous peroxidase with 3% hydrogen peroxide for 5 min. Then, the primary antibody Insulin (Clonal 2D11-H5) (Master Diagnóstica© MAD-021340Q) and polyclonal Glucagon (Master Diagnóstica© MAD-021325Q) were applied for 20 min, followed by another buffer wash and visualized with DAB for 10 min. Following a water wash, sections were counterstained in haematoxylin for 7 min, washed in water, dehydrated and coverslipped. For the image analysis, islets were defined as clusters of seven or more β cells in association with other morphologically identifiable endocrine cells. All data were obtained from immunostained sections. Islet image capture was performed using a DP72 camera Olympus (Tokyo, Japan) from an Olympus BX41 microscope (×20 objective) and analysed using *AnaliSYS Image Processing* software (Olympus). Islets were identified and images stored. Individual islet perimeters, islet size, total islet cell number, number and area of insulin positive (β) cells, (α) cells and the percentage of islet insulin and glucagon staining were semi-automatically determined. All insulin and glucagon positive cells in tissue sections were counted, including faintly positive cells.

3.4. Protein, Hormone and Metabolite Determinations

Frozen pancreas portions (around 100 mg each) were thawed and homogenized in 1 mL of alcohol:acid (50 parts of 95% ethanol:1 part of 10.2 N HCl) and then sonicated in a UP50H sonicator (Dr Hielscher, Teltow, Germany) for 3–5 pulses in ice bath. Homogenates were processed as was indicated by Duttaroy *et al.* [107]. Total insulin was measured by radio-immuno assay (RIA) by using the Coat-A-Count kit (Siemens, Los Angeles, CA, USA). Total glucagon was measured by RIA by using the Milipore kit (Billerica, MA, USA). Leptin was determined by ELISA (LINCO, St. Charles, MO, USA). The protein content of the pancreatic homogenates was determined by Bio-Rad assay.

Circulating glucose was determined by the glucose oxidase method coupled to the peroxidase reaction [108]. Circulating triglycerides were determined from coupled reactions of lipoprotein-lipase, glycerol-kinase, glycerol phosphate oxidase and peroxidase giving a colour end-adduct according to previously reported [109]. Circulating cholesterol and total lipids were determined as was indicated by

Naito and David [110] and Bucolo and David [109] respectively. HOMA index was calculated according to Wallace and Matthews [111].

3.5. Statistics

Data are expressed as means ± standard error of the mean (SEM) of 6 animals. For quantitative parameters, prior to any statistical analysis, all variables were checked for normality and homogeneous variance using the Kolmogorov-Smirnoff and the Levene tests, respectively. When a variable was found not to follow normality, it was log-transformed and reanalyzed. To evaluate differences in the means between groups (virgin olive *vs.* sunflower and virgin olive *vs.* fish) one way ANOVA adjusted by Bonferroni correction was performed. For semiquantitative parameters (histological analysis), differences were analysed by the Kruskal-Wallis test, and a Mann-Whitney U test was used *a posteriori* to evaluate mean differences between groups. For all statistical analysis, a value of $p < 0.05$ was considered significant. Data were analysed using IBM® SPSS® Statistics 20 (IBM corp. Armonk, NY, USA) statistical package.

4. Conclusions

Despite that diets with a unique fat source over very long periods of time are not likely to occur in humans, animal models like the one presented here, could be very useful to examine possible effects of fat types predominant in a particular diet. In addition, these models could help to understand how diet can model organ histology in different physiopathological situations considering the difficulty to obtain certain human samples such as pancreas. Based on this approach, it was reported that different pancreatic compartments were affected according to the fat composition consumed, emphasizing the importance of dietary fatty acids in determining pancreatic structure. SO-rich diets mainly led to endocrine alterations with higher β-cell numbers and twice the insulin content, whereas diets rich in FO are associated to signs of pancreatic fibrosis of the elderly in rats [19]. Since there is a close relationship between mitochondria, oxidative stress and aging, here, we wanted to gain more insight into this process in pancreas by administering chronic low-dosages of CoQ. The main finding was that CoQ supplementation led to different results according to fat used in diet. It seems that in the context of endocrine pancreas structure and in particular β-cell, SO as the main fat in the diet, when it is supplemented on CoQ, resembles features associated to VOO, observing a reduction of cell mass but maintaining pancreatic insulin contents. However, results found in animals fed on FO could be interpreted in several ways. A first way might be that oxidative stress is not very important in relation to exocrine pancreas aging in the context of FO-rich diets. A second option might be that CoQ does not act as an antioxidant in this context. Nevertheless, these and other options will be investigated in future work. Therefore, CoQ may improve pancreas function when some deleterious dietary fats are being used as the basis of the diet. Thus, the effect of CoQ has to be assessed for each particular organ-compartment, taking into account the fat component of diet. The present study emphasizes from a histological point of view that chronic CoQ supplementation together with dietary fat type could play an important role in modulating aging in the pancreas, affecting different compartments of this organ in opposite ways.

Acknowledgments

This work was funded by grants to José L. Quiles from the Spanish Ministry of Education and Science (AGL2008-01057) and the Autonomous Government of Andalucia (AGR832). And to ER from CIBEROBN 12/03/30038 (Fisiopatologia de la Obesidad y la Nutrición, Instituto de Salud Carlos III) and from PROMETEO 2012/007 (Generalitat Valenciana) and from the Spanish Ministry of Health (Red de Envejecimiento y Fragilidad RD06/0013/0012, PI04/0355, and PI05/2214). Adrian González-Alonso and Alfonso Varela-López are recipients of a fellowship of FPU program from the Spanish Ministry of Education. MI Arribas was recipient of a fellowship VALi+d APOSTD/2012/021 from Generalitat Valenciana.

Author Contributions

Adrián González-Alonso and Alfonso Varela-López performed some of the determinations, contributed to the discussion of results and to the writing of the manuscript. César L. Ramírez-Tortosa performed pathological analysis of the study, discussed results and contributed to manuscript writing. María I. Arribas performed hormone determinations; Enrique Roche analyzed data and contributed to manuscript writing. M. Carmen Ramírez-Tortosa, Julio J. Ochoa and Francesca Giampieri contributed to experiment design and to the discussion of the results. José L. Quiles designed the experiment, performed some of the experiments, discussed results and contributed to the writing of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Kim, S.K.; MacDonald, R.J. Signaling and transcriptional control of pancreatic organogenesis. *Curr. Opin. Genet. Dev.* **2002**, *12*, 540–547.
2. Murtaugh, L.C.; Melton, D.A. Genes, signals, and lineages in pancreas development. *Ann. Rev. Cell Dev. Biol.* **2003**, *19*, 71–89.
3. Czako, L.; Hegyi, P.; Rakonczay, Z., Jr.; Wittmann, T.; Otsuki, M. Interactions between the endocrine and exocrine pancreas and their clinical relevance. *Pancreatology* **2009**, *9*, 351–359.
4. Braganza, J.M.; Lee, S.H.; McCloy, R.F.; McMahon, M.J. Chronic pancreatitis. *Lancet* **2011**, *377*, 1184–1197.
5. Keller, J.; Layer, P. Human pancreatic exocrine response to nutrients in health and disease. *Gut* **2005**, *54*, 1–28.
6. Prentki, M.; Nolan, C.J. Islet β cell failure in type 2 diabetes. *J. Clin. Investig.* **2006**, *116*, 1802–1812.
7. Nolan, C.J.; Prentki, M. The islet β-cell: Fuel responsive and vulnerable. *Trends Endocrinol. Metab.* **2008**, *19*, 285–291.
8. Maron, D.J.; Fair, J.M.; Haskell, W.L. Saturated fat intake and insulin resistance in men with coronary artery disease. The Stanford Coronary Risk Intervention Project Investigators and Staff. *Circulation* **1991**, *84*, 2020–2027.

9. Mayer, E.J.; Newman, B.; Quesenberry, C.P.; Selby, J.V. Usual dietary fat intake and insulin concentrations in healthy women twins. *Diabetes Care* **1993**, *16*, 1459–1469.
10. Marshall, J.A.; Bessesen, D.H.; Hamman, R.F. High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: The san luis valley diabetes study. *Diabetologia* **1997**, *40*, 430–438.
11. Ryan, M.; McInerney, D.; Owens, D.; Collins, P.; Johnson, A.; Tomkin, G.H. Diabetes and the Mediterranean diet: A beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *QJM* **2000**, *93*, 85–91.
12. Soriguer, F.; Esteva, I.; Rojo-Martinez, G.; de Adana, M.R.; Dobarganes, M.C.; Garcia-Almeida, J.M.; Tinahones, F.; Beltran, M.; Gonzalez-Romero, S.; Olveira, G.; *et al.* Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study). *Eur. J. Endocrinol.* **2004**, *150*, 33–39.
13. Garg, A.; Bonanome, A.; Grundy, S.M.; Zhang, Z.-J.; Unger, R.H. Comparison of a high-carbohydrate diet with a high-monounsaturated-fat diet in patients with non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **1988**, *319*, 829–834.
14. Rojo-Martínez, G.; Esteva, I.; Ruiz de Adana, M.S.; García-Almeida, J.M.; Tinahones, F.; Cardona, F.; Morcillo, S.; García-Escobar, E.; García-Fuentes, E.; Soriguer, F. Dietary fatty acids and insulin secretion: A population-based study. *Eur. J. Clin. Nutr.* **2006**, *60*, 1195–1200.
15. Mañas, M.; Yago, M.D.; Martínez-Victoria, E.; Quiles, J.L.; Ramírez-Tortosa, M.C.; Yaqoob, P. Others olive oil and regulation of gastrointestinal function. In *Olive Oil and Health*; Quiles, J.L., Ramírez-Tortosa, M.C., Yaqoob, P., Eds.; CABI Publishing: London, UK, 2006; pp. 284–308.
16. Ballesta, M.C.; Mañas, M.; Mataix, F.J.; Martínez-Victoria, E.; Seiquer, I. Long-term adaptation of pancreatic response by dogs to dietary fats of different degrees of saturation: Olive and sunflower oil. *Br. J. Nutr.* **1990**, *64*, 487–496.
17. Yago, M.D.; Martinez-Victoria, E.; Huertas, J.R.; Mañas, M. Effects of the amount and type of dietary fat on exocrine pancreatic secretion in dogs after different periods of adaptation. *Arch. Physiol. Biochem.* **1997**, *105*, 78–85.
18. Díaz, R.J.; Yago, M.D.; Martínez-Victoria, E.; Naranjo, J.A.; Martínez, M.A.; Mañas, M. Comparison of the effects of dietary sunflower oil and virgin olive oil on rat exocrine pancreatic secretion *in vivo*. *Lipids* **2003**, *38*, 1119–1126.
19. Roche, E.; Ramírez-Tortosa, C.L.; Arribas, M.I.; Ochoa, J.J.; Sirvent-Belando, J.E.; Battino, M.; Ramírez-Tortosa, M.C.; González-Alonso, A.; Pérez-López, M.P.; Quiles, J.L. Comparative analysis of pancreatic changes in aged rats fed life long with sunflower, fish, or olive oils. *J. Gerontol. A Biol. Sci. Med. Sci.* **2014**, *69*, 934–944.
20. Battino, M.; Ferri, E.; Gorini, A.; Villa, R.F.; Huertas, J.F.R.; Fiorella, P.; Genova, M.L.; Lenaz, G.; Marchetti, M. natural distribution and occurrence of coenzyme Q homologues. *Mol. Membr. Biol.* **1990**, *9*, 179–190.
21. Nohl, H.; Gille, L.; Kozlov, A.V. Antioxidant-derived prooxidant formation from ubiquinol. *Free Radic. Biol. Med.* **1998**, *25*, 666–675.
22. Budavari, S. *The Merck Index*, 12th ed.; Merck & Co: Hunterdon County, NJ, USA, 1996; p. 1606.
23. Ibrahim, W.H.; Bhagavan, H.N.; Chopra, R.K.; Chow, C.K. Dietary coenzyme Q10 and vitamin E alter the status of these compounds in rat tissues and mitochondria. *J. Nutr.* **2000**, *130*, 2343–2348.

24. Lenaz, G. The mitochondrial production of reactive oxygen species: Mechanisms and implications in human pathology. *IUBMB Life* **2001**, *52*, 159–164.
25. Ramasarma, T. Natural occurrence and distribution of coenzyme Q. *Coenzyme Q* **1985**, 67–81.
26. Grossi, G.; Bargossi, A.M.; Fiorella, P.L.; Piazzi, S.; Battino, M.; Bianchi, G.P. Improved high-performance liquid chromatographic method for the determination of coenzyme Q10 in plasma. *J. Chromatogr. A* **1992**, *593*, 217–226.
27. Lass, A.; Kwong, L.; Sohal, R.S. Mitochondrial coenzyme Q content and aging. *Biofactors* **1999**, *9*, 199–205.
28. Ernster, L.; Dallner, G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim. Biophys. Acta* **1995**, *1271*, 195–204.
29. Pepping, J. Coenzyme Q10. *Am. J. Health Syst. Pharm.* **1999**, *56*, 519–521.
30. Forsmark-Andrée, P.; Lee, C.-P.; Dallner, G.; Ernster, L. Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochondrial particles. *Free Radic. Biol. Med.* **1997**, *22*, 391–400.
31. Aberg, F.; Appelkvist, E.L.; Dallner, G.; Ernster, L. Distribution and redox state of ubiquinones in rat and human tissues. *Arch. Biochem. Biophys.* **1992**, *295*, 230–234.
32. Okamoto, T.; Fukunaga, Y.; Ida, Y.; Kishi, T. Determination of reduced and total ubiquinones in biological materials by liquid chromatography with electrochemical detection. *J. Chromatogr. B* **1988**, *430*, 11–19.
33. Turrens, J.F.; Alexandre, A.; Lehninger, A.L. Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. *Arch. Biochem. Biophys.* **1985**, *237*, 408–414.
34. Kitano, M.; Watanabe, D.; Oda, S.; Kubo, H.; Kishida, H.; Fujii, K.; Kitahara, M.; Hosoe, K. Subchronic oral toxicity of ubiquinol in rats and dogs. *Int. J. Toxicol.* **2008**, *27*, 189–215.
35. Battino, M.; Gorini, A.; Villa, R.F.; Genova, M.L.; Bovina, C.; Sassi, S.; Littarru, G.P.; Lenaz, G. Coenzyme Q content in synaptic and non-synaptic mitochondria from different brain regions in the ageing rat. *Mech. Ageing Dev.* **1995**, *78*, 173–187.
36. Onur, S.; Niklowitz, P.; Fischer, A.; Metges, C.C.; Grune, T.; Menke, T.; Rimbach, G.; Döring, F. A comparative study into alterations of coenzyme Q redox status in ageing pigs, mice, and worms. *Biofactors* **2014**, *40*, 346–354.
37. Pignatti, C.; Cocchi, M.; Weiss, H. Coenzyme Q10 levels in rat heart of different age. *Biochem. Exp. Biol.* **1980**, *16*, 39–42.
38. Kalén, A.; Appelkvist, E.L.; Dallner, G. Age-related changes in the lipid compositions of rat and human tissues. *Lipids* **1989**, *24*, 579–584.
39. Söderberg, M.; Edlund, C.; Kristensson, K.; Dallner, G. Lipid compositions of different regions of the human brain during aging. *J. Neurochem.* **1990**, *54*, 415–423.
40. Petrosillo, G.; Matera, M.; Casanova, G.; Ruggiero, F.M.; Paradies, G. Mitochondrial dysfunction in rat brain with aging: Involvement of complex I, reactive oxygen species and cardiolipin. *Neurochem. Int.* **2008**, *53*, 126–131.
41. Kwong, L.K.; Sohal, R.S. Age-related changes in activities of mitochondrial electron transport complexes in various tissues of the mouse. *Arch. Biochem. Biophys.* **2000**, *373*, 16–22.
42. Marcoff, L.; Thompson, P.D. The role of coenzyme Q10 in statin-associated myopathy. *J. Am. Coll. Cardiol.* **2007**, *49*, 2231–2237.

43. Rosenfeldt, F.; Hilton, D.; Pepe, S.; Krum, H. Systematic review of effect of coenzyme Q10 in physical exercise, hypertension and heart failure. *Biofactors* **2003**, *18*, 91–100.
44. Rosenfeldt, F.L.; Haas, S.J.; Krum, H.; Hadj, A.; Ng, K.; Leong, J.-Y.; Watts, G.F. Coenzyme Q10 in the treatment of hypertension: A meta-analysis of the clinical trials. *J. Hum. Hypertens.* **2007**, *21*, 297–306.
45. Shults, C.W. Therapeutic role of coenzyme Q_{10} in Parkinson's disease. *Pharmacol. Ther.* **2005**, *107*, 120–130.
46. Bello, R.I.; Gómez-Díaz, C.; Burón, M.I.; Alcaín, F.J.; Navas, P.; Villalba, J.M. Enhanced anti-oxidant protection of liver membranes in long-lived rats fed on a coenzyme Q10-supplemented diet. *Exp. Gerontol.* **2005**, *40*, 694–706.
47. Ochoa, J.J.; Pamplona, R.; Ramirez-Tortosa, M.C.; Granados-Principal, S.; Perez-Lopez, P.; Naudí, A.; Portero-Otin, M.; López-Frías, M.; Battino, M.; Quiles, J.L. Age-related changes in brain mitochondrial DNA deletion and oxidative stress are differentially modulated by dietary fat type and coenzyme Q10 *Free Radic. Biol. Med.* **2011**, *50*, 1053–1064.
48. Huertas, J.R.; Martinez-Velasco, E.; Ibáñez, S.; López-Frias, M.; Ochoa, J.J.; Quiles, J.; Parenti Castelli, G.; Mataix, J.; Lenaz, G. Virgin olive oil and coenzyme Q10 protect heart mitochondria from peroxidative damage during aging. *Biofactors* **1999**, *9*, 337–343.
49. Quiles, J.L.; Pamplona, R.; Ramirez-Tortosa, M.C.; Naudí, A.; Portero-Otin, M.; Araujo-Nepomuceno, E.; López-Frías, M.; Battino, M.; Ochoa, J.J. Coenzyme Q addition to an n-6 PUFA-rich diet resembles benefits on age-related mitochondrial DNA deletion and oxidative stress of a MUFA-rich diet in rat heart. *Mech. Ageing Dev.* **2010**, *131*, 38–47.
50. Ochoa, J.J.; Quiles, J.L.; López-Frías, M.; Huertas, J.R.; Mataix, J. Effect of lifelong coenzyme Q10 supplementation on age-related oxidative stress and mitochondrial function in liver and skeletal muscle of rats fed on a polyunsaturated fatty acid (PUFA)-rich diet. *J. Gerontol. A Biol. Sci. Med. Sci.* **2007**, *62*, 1211–1218.
51. Varela-Lopez, A.; Bullon, P.; Battino, M.; Ramirez-Tortosa, M.C.; Ochoa, J.J.; Cordero, M.D.; Ramirez-Tortosa, C.L.; Rubini, C.; Zizzi, A.; Quiles, J.L. Coenzyme Q protects against age-related alveolar bone loss associated to n-6 PUFA rich-diets by modulating mitochondrial mechanisms. *J. Gerontol. A Biol. Sci. Med. Sci.* **2015**, in press.
52. Quiles, J.L.; Ochoa, J.J.; Huertas, J.R.; Mataix, J. Coenzyme Q supplementation protects from age-related DNA double-strand breaks and increases lifespan in rats fed on a PUFA-rich diet. *Exp. Gerontol.* **2004**, *39*, 189–194.
53. Bullon, P.; Battino, M.; Varela-Lopez, A.; Perez-Lopez, P.; Granados-Principal, S.; Ramirez-Tortosa, M.C.; Ochoa, J.J.; Cordero, M.D.; Gonzalez-Alonso, A.; Ramirez-Tortosa, C.L.; *et al.* Diets based on virgin olive oil or fish oil but not on sunflower oil prevent age-related alveolar bone resorption by mitochondrial-related mechanisms. *PLoS ONE* **2013**, *8*, e74234.
54. Ochoa, J.J.; Quiles, J.L.; Ibáñez, S.; Martínez, E.; López-Frías, M.; Huertas, J.R.; Mataix, J. Aging-related oxidative stress depends on dietary lipid source in rat postmitotic tissues. *J. Bioenerg. Biomembr.* **2003**, *35*, 267–275.
55. Aguilera, C.M.; Mesa, M.D.; Ramirez-Tortosa, M.C.; Nestares, M.T.; Ros, E.; Gil, A. Sunflower oil does not protect against LDL oxidation as virgin olive oil does in patients with peripheral vascular disease. *Clin. Nutr.* **2004**, *23*, 673–681.

56. Jones, H.B.; Nugent, D.; Jenkins, R. Variation in characteristics of islets of Langerhans in insulin-resistant, diabetic and non-diabetic-rat strains. *Int. J. Exp. Pathol.* **2010**, *91*, 288–301.
57. Tierney, A.C.; Roche, H.M. The potential role of olive oil-derived MUFA in insulin sensitivity. *Mol. Nutr. Food Res.* **2007**, *51*, 1235–1248.
58. Reaven, G.M. Role of Insulin Resistance in Human Disease. *Diabetes* **1988**, *37*, 1595–1607.
59. Bonora, E.; Kiechl, S.; Willeit, J.; Oberhollenzer, F.; Egger, G.; Targher, G.; Alberiche, M.; Bonadonna, R.C.; Muggeo, M. Prevalence of insulin resistance in metabolic disorders: The bruneck study. *Diabetes* **1998**, *47*, 1643–1649.
60. Pérez-Matute, P.; Marti, A.; Martínez, J.A.; Fernández-Otero, M.P.; Stanhope, K.L.; Havel, P.J.; Moreno-Aliaga, M.J. Eicosapentaenoic fatty acid increases leptin secretion from primary cultured rat adipocytes: Role of glucose metabolism. *Am. J. Physiol.* **2005**, *288*, R1682–R1688.
61. Zethelius, B.; Lithell, H.; Hales, C.N.; Berne, C. Insulin sensitivity, proinsulin and insulin as predictors of coronary heart disease. A population-based 10-year, follow-up study in 70-year old men using the euglycaemic insulin clamp. *Diabetologia* **2005**, *48*, 862–867.
62. Sattar, N.; Wannamethee, S.G.; Forouhi, N.G. Novel biochemical risk factors for type 2 diabetes: Pathogenic insights or prediction possibilities? *Diabetologia* **2008**, *51*, 926–940.
63. Considine, R.V.; Sinha, M.K.; Heiman, M.L.; Kriauciunas, A.; Stephens, T.W.; Nyce, M.R.; Ohannesian, J.P.; Marco, C.C.; McKee, L.J.; Bauer, T.L.; Caro, J.F. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med.* **1996**, *334*, 292–295.
64. Flier, J.S. Leptin expression and action: New experimental paradigms. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4242–4245.
65. Klöppel, G.; Detlefsen, S.; Feyerabend, B. Fibrosis of the pancreas: The initial tissue damage and the resulting pattern. *Virchows Arch.* **2004**, *445*, 1–8.
66. Bargut, T.C.L.; Mandarim-de-Lacerda, C.A.; Aguila, M.B. A high-fish-oil diet prevents adiposity and modulates white adipose tissue inflammation pathways in mice. *J. Nutr. Biochem.* **2015**, *26*, 960–969.
67. Kalupahana, N.S.; Claycombe, K.; Newman, S.J.; Stewart, T.; Siriwardhana, N.; Matthan, N.; Lichtenstein, A.H.; Moustaid-Moussa, N. Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. *J. Nutr.* **2010**, *140*, 1915–1922.
68. Kalupahana, N.S.; Claycombe, K.J.; Moustaid-Moussa, N. (n-3) Fatty acids alleviate adipose tissue inflammation and insulin resistance: Mechanistic insights. *Adv. Nutr.* **2011**, *2*, 304–316.
69. Cai, A.; Hutchison, E.; Hudson, J.; Kawashima, Y.; Komori, N.; Singh, A.; Brush, R.S.; Anderson, R.E.; Sonntag, W.E.; Matsumoto, H.; *et al.* Metabolic enrichment of omega-3 polyunsaturated fatty acids does not reduce the onset of idiopathic knee osteoarthritis in mice. *Osteoarthr. Cartil.* **2014**, *22*, 1301–1309.
70. Boulis, T.S.; Rochelson, B.; Novick, O.; Xue, X.; Chatterjee, P.K.; Gupta, M.; Solanki, M.H.; Akerman, M.; Metz, C.N. Omega-3 polyunsaturated fatty acids enhance cytokine production and oxidative stress in a mouse model of preterm labor. *J. Perinat. Med.* **2014**, *42*, 693–698.
71. Lionetti, L.; Mollica, M.P.; Sica, R.; Donizzetti, I.; Gifuni, G.; Pignalosa, A.; Cavaliere, G.; Putti, R. Differential effects of high-fish oil and high-lard diets on cells and cytokines involved in the inflammatory process in rat insulin-sensitive tissues. *Int. J. Mol. Sci.* **2014**, *15*, 3040–3063.

72. Chen, C.-C.; Ho, C.-Y.; Chaung, H.-C.; Tain, Y.-L.; Hsieh, C.-S.; Kuo, F.-Y.; Yang, C.-Y.; Huang, L.-T. Fish omega-3 fatty acids induce liver fibrosis in the treatment of bile duct-ligated rats. *Dig. Dis. Sci.* **2013**, *58*, 440–447.
73. Church, M.W.; Jen, K.-L.C.; Anumba, J.I.; Jackson, D.A.; Adams, B.R.; Hotra, J.W. Excess omega-3 fatty acid consumption by mothers during pregnancy and lactation caused shorter life span and abnormal ABRs in old adult offspring. *Neurotoxicol. Teratol.* **2010**, *32*, 171–181.
74. Liu, Y.; Zhang, D.; Wu, Y.; Ji, B. Docosahexaenoic acid aggravates photooxidative damage in retinal pigment epithelial cells via lipid peroxidation. *J. Photochem. Photobiol. B Biol.* **2014**, *140*, 85–93.
75. Spindler, S.R.; Mote, P.L.; Flegal, J.M. Dietary supplementation with Lovaza and krill oil shortens the life span of long-lived F1 mice. *Age* **2014**, *36*, 9659, doi:10.1007/s11357-014-9659-7.
76. Valencak, T.G.; Ruf, T. n-3 polyunsaturated fatty acids impair lifespan but have no role for metabolism. *Aging Cell* **2007**, *6*, 15–25.
77. Apte, M.V.; Haber, P.S.; Applegate, T.L.; Norton, I.D.; McCaughan, G.W.; Korsten, M.A.; Pirola, R.C.; Wilson, J.S. Periacinar stellate shaped cells in rat pancreas: Identification, isolation, and culture. *Gut* **1998**, *43*, 128–133.
78. Bachem, M.G.; Schneider, E.; Gross, H.; Weidenbach, H.; Schmid, R.M.; Menke, A.; Siech, M.; Beger, H.; Grünert, A.; Adler, G. Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* **1998**, *115*, 421–432.
79. Shek, F.W.-T.; Benyon, R.C.; Walker, F.M.; McCrudden, P.R.; Pender, S.L.F.; Williams, E.J.; Johnson, P.A.; Johnson, C.D.; Bateman, A.C.; Fine, D.R.; *et al.* Expression of transforming growth factor-β1 by pancreatic stellate cells and its implications for matrix secretion and turnover in chronic pancreatitis. *Am. J. Pathol.* **2002**, *160*, 1787–1798.
80. Apte, M.V.; Haber, P.S.; Darby, S.J.; Rodgers, S.C.; McCaughan, G.W.; Korsten, M.A.; Pirola, R.C.; Wilson, J.S. Pancreatic stellate cells are activated by proinflammatory cytokines: Implications for pancreatic fibrogenesis. *Gut* **1999**, *44*, 534–541.
81. Haber, P.S.; Keogh, G.W.; Apte, M.V.; Moran, C.S.; Stewart, N.L.; Crawford, D.H.G.; Pirola, R.C.; McCaughan, G.W.; Ramm, G.A.; Wilson, J.S. Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis. *Am. J. Pathol.* **1999**, *155*, 1087–1095.
82. Luttenberger, T.; Schmid-Kotsas, A.; Menke, A.; Siech, M.; Beger, H.; Adler, G.; Grünert, A.; Bachem, M.G. Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: Implications in pathogenesis of pancreas fibrosis. *Lab. Investig. J. Tech. Methods Pathol.* **2000**, *80*, 47–55.
83. Mews, P.; Phillips, P.; Fahmy, R.; Korsten, M.; Pirola, R.; Wilson, J.; Apte, M. Pancreatic stellate cells respond to inflammatory cytokines: Potential role in chronic pancreatitis. *Gut* **2002**, *50*, 535–541.
84. Schneider, E.; Schmid-Kotsas, A.; Zhao, J.; Weidenbach, H.; Schmid, R.M.; Menke, A.; Adler, G.; Waltenberger, J.; Grünert, A.; Bachem, M.G. Identification of mediators stimulating proliferation and matrix synthesis of rat pancreatic stellate cells. *Am. J. Physiol.* **2001**, *281*, C532–C543.
85. Shimizu, K. Mechanisms of pancreatic fibrosis and applications to the treatment of chronic pancreatitis. *J. Gastroenterol.* **2008**, *43*, 823–832.

86. Saurer, L.; Reber, P.; Schaffner, T.; Büchler, M.W.; Buri, C.; Kappeler, A.; Walz, A.; Friess, H.; Mueller, C. Differential expression of chemokines in normal pancreas and in chronic pancreatitis. *Gastroenterology* **2000**, *118*, 356–367.
87. Andoh, A.; Takaya, H.; Saotome, T.; Shimada, M.; Hata, K.; Araki, Y.; Nakamura, F.; Shintani, Y.; Fujiyama, Y.; Bamba, T. Cytokine regulation of chemokine (IL-8, MCP-1, and RANTES) gene expression in human pancreatic periacinar myofibroblasts. *Gastroenterology* **2000**, *119*, 211–219.
88. Tanioka, H.; Mizushima, T.; Shirahige, A.; Matsushita, K.; Ochi, K.; Ichimura, M.; Matsumura, N.; Shinji, T.; Tanimoto, M.; Koide, N. Xanthine oxidase-derived free radicals directly activate rat pancreatic stellate cells. *J. Gastroenterol. Hepatol.* **2006**, *21*, 537–544.
89. Kikuta, K.; Masamune, A.; Satoh, M.; Suzuki, N.; Shimosegawa, T. 4-hydroxy-2,3-nonenal activates activator protein-1 and mitogen-activated protein kinases in rat pancreatic stellate cells. *World J. Gastroenterol.* **2004**, *10*, 2344–2351.
90. Kikuta, K.; Masamune, A.; Satoh, M.; Suzuki, N.; Satoh, K.; Shimosegawa, T. Hydrogen peroxide activates activator protein-1 and mitogen-activated protein kinases in pancreatic stellate cells. *Mol. Cell. Biochem.* **2006**, *291*, 11–20.
91. Asaumi, H.; Watanabe, S.; Taguchi, M.; Tashiro, M.; Otsuki, M. Externally applied pressure activates pancreatic stellate cells through the generation of intracellular reactive oxygen species. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2007**, *293*, G972–G978.
92. Masamune, A.; Watanabe, T.; Kikuta, K.; Satoh, K.; Shimosegawa, T. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *294*, G99–G108.
93. Masamune, A.; Satoh, M.; Kikuta, K.; Suzuki, N.; Satoh, K.; Shimosegawa, T. Ellagic acid blocks activation of pancreatic stellate cells. *Biochem. Pharmacol.* **2005**, *70*, 869–878.
94. Masamune, A.; Kikuta, K.; Satoh, M.; Suzuki, N.; Shimosegawa, T. Green tea polyphenol epigallocatechin-3-gallate blocks PDGF-induced proliferation and migration of rat pancreatic stellate cells. *World J. Gastroenterol.* **2005**, *11*, 3368–3374.
95. Zhou, C.-H.; Li, L.; Zhu, X.-Y.; Tang, W.; Hu, D.-M.; Dong, Y.; Li, L.-Y.; Wang, S.-F. Protective effects of edaravone on experimental chronic pancreatitis induced by dibutyltin dichloride in rats. *Pancreatology* **2013**, *13*, 125–132.
96. Jiang, F.; Liao, Z.; Hu, L.-H.; Du, Y.-Q.; Man, X.-H.; Gu, J.-J.; Gao, J.; Gong, Y.-F.; Li, Z.-S. Comparison of antioxidative and antifibrotic effects of α-tocopherol with those of tocotrienol-rich fraction in a rat model of chronic pancreatitis. *Pancreas* **2011**, *40*, 1091–1096.
97. González, A.M.; Garcia, T.; Samper, E.; Rickmann, M.; Vaquero, E.C.; Molero, X. Assessment of the protective effects of oral tocotrienols in arginine chronic-like pancreatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2011**, *301*, G846–G855.
98. Steil, G.M.; Trivedi, N.; Jonas, J.-C.; Hasenkamp, W.M.; Sharma, A.; Bonner-Weir, S.; Weir, G.C. Adaptation of β-cell mass to substrate oversupply: Enhanced function with normal gene expression. *Am. J. Physiol.* **2001**, *280*, E788–E796.
99. Liu, Y.Q.; Jetton, T.L.; Leahy, J.L. β-Cell Adaptation to insulin resistance increased pyruvate carboxylase and malate-pyruvate shuttle activity in islets of non-diabetic Zucker fatty rats. *J. Biol. Chem.* **2002**, *277*, 39163–39168.

100. Jetton, T.L.; Lausier, J.; LaRock, K.; Trotman, W.E.; Larmie, B.; Habibovic, A.; Peshavaria, M.; Leahy, J.L. Mechanisms of compensatory β-cell growth in insulin-resistant rats: Roles of Akt kinase. *Diabetes* **2005**, *54*, 2294–2304.
101. Islam, M.S.; Morton, N.M.; Hansson, A.; Emilsson, V. Rat insulinoma-derived pancreatic β-cells express a functional leptin receptor that mediates a proliferative response. *Biochem. Biophys. Res. Commun.* **1997**, *238*, 851–855.
102. Islam, M.S.; Sjöholm, A.; Emilsson, V. Fetal pancreatic islets express functional leptin receptors and leptin stimulates proliferation of fetal islet cells. *Int. J. Obes.* **2000**, *24*, 1246–1253.
103. Tanabe, K.; Okuya, S.; Tanizawa, Y.; Matsutani, A.; Oka, Y. Leptin induces proliferation of pancreatic β cell line MIN6 through activation of mitogen-activated protein kinase. *Biochem. Biophys. Res. Commun.* **1997**, *241*, 765–768.
104. Khalaileh, A.; Gonen-Gross, T.; Magenheim, J.; Nir, T.; Porat, S.; Salpeter, S.; Stolovich-Rain, M.; Swisa, A.; Weinberg, N.; Dor, Y. Determinants of pancreatic β-cell regeneration. *Diabetes Obes. Metab.* **2008**, *10*, 128–135.
105. Reeves, P.G. Components of the AIN-93 diets as improvements in the AIN-76A diet. *J. Nutr*. **1997**, *127*, 838S–841S.
106. Ochoa, J.J.; Quiles, J.L.; Huertas, J.R.; Mataix, J. Coenzyme Q10 protects from aging-related oxidative stress and improves mitochondrial function in heart of rats fed a polyunsaturated fatty acid (PUFA)-rich diet. *J. Gerontol. A Biol. Sci. Med. Sci.* **2005**, *60*, 970–975.
107. Duttaroy, A.; Zimliki, C.L.; Gautam, D.; Cui, Y.; Mears, D.; Wess, J. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. *Diabetes* **2004**, *53*, 1714–1720.
108. Trinder, P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J. Clin. Pathol.* **1969**, *22*, 158–161.
109. Bucolo, G.; David, H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **1973**, *19*, 476–482.
110. Naito, H.K.; David, J.A. Laboratory considerations: Determination of cholesterol, triglyceride, phospholipid, and other lipids in blood and tissues. *Lab. Res. Methods Biol. Med.* **1984**, *10*, 1–76.
111. Wallace, T.M.; Matthews, D.R. The assessment of insulin resistance in man. *Diabet. Med.* **2002**, *19*, 527–534.

G Model
REGG-664; No. of Pages 4

Rev Esp Geriatr Gerontol. 2015;xxx(xx):xxx–xxx

Revista Española de Geriatría y Gerontología

www.elsevier.es/regg

REVISIÓN

Evidencias experimentales con respecto al papel de distintos tipos de grasas insaturadas de la dieta en el envejecimiento

Adrian González-Alonso[a], Patricia Pérez-López[a], Alfonso Varela-López[a], M. Carmen Ramírez-Tortosa[b], Maurizio Battino[c] y José L. Quiles[a,*]

[a] Instituto de Nutrición y Tecnología de los Alimentos José Mataix Verdú, Departamento de Fisiología, Universidad de Granada, Granada, España
[b] Instituto de Nutrición y Tecnología de los Alimentos José Mataix Verdú, Departamento de Bioquímica y Biología Molecular II, Universidad de Granada, Granada, España
[c] Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche, Università Politecnica delle Marche, Ancona, Italia

información del artículo

Historia del artículo:
Recibido el 11 de septiembre de 2014
Aceptado el 13 de mayo de 2015
On-line el xxx

Palabras clave:
Aceite de oliva virgen
Aceite de girasol
Aceite de pescado
Longevidad
Mitocondria

resumen

La nutrición ha sido relacionada ampliamente con el proceso fisiológico del envejecimiento. Varios nutrientes, como ciertos tipos de grasa de la dieta y diversos antioxidantes, han demostrado poseer efectos positivos en enfermedades relacionadas con la edad. El tipo de grasa de la dieta afecta a la estructura y función mitocondrial, así como a su susceptibilidad al estrés oxidativo, todos, factores implicados en el envejecimiento. La presente revisión trata de resumir los estudios realizados por nuestro grupo de investigación en los últimos 10 años empleando aceite de oliva virgen, aceite de girasol o aceite de pescado como fuente de grasa insaturada de la dieta en relación con un modelo de envejecimiento en rata.

Experimental evidence on the role of different types unsaturated fats in the diet on ageing

Keywords:
Virgin olive oil
Sunflower oil
Fish oil
Longevity
Mitochondria

abstract

Nutrition has been largely related to the physiological ageing process. Several nutrients, such as certain types of dietary fat and various antioxidants have been shown to have positive effects on age-related diseases. The type of dietary fat affects mitochondrial structure and function, as well as its susceptibility to oxidative stress, all factors involved in ageing. The present review aims to summarise the studies conducted by our research group in the past 10 years, using virgin olive oil, sunflower oil, or fish oil as a source of unsaturated fat diet relative to a rat model of ageing.

Introducción

En los últimos anos, numerosas evidencias científicas han mostrado que ciertos factores nutricionales podrían jugar un papel importante en la etiología de diversas enfermedades crónicas, deterioro cognitivo, así como diversos procesos con base inflamatoria y cancerígena, proponiendo la existencia de un vínculo entre patrones dietéticos y longevidad. Varios nutrientes, como ciertos tipos de grasa de la dieta y diversos antioxidantes, han demostrado poseer efectos positivos en enfermedades relacionadas con la edad. Asimismo, el control de la ingesta ha sido propuesto como una herramienta efectiva para modular el envejecimiento.

Además de la producción de especies reactivas del oxígeno (ERO), hay otra característica fundamental en animales de vida larga que relaciona el envejecimiento con el estrés oxidativo, en concreto, el grado de insaturación de los ácidos grasos de las membranas de los tejidos celulares, adaptado para protegerse frente a las ERO[1]. De este modo, algunos estudios han puesto de manifiesto

* Autor para correspondencia.
Correo electrónico: jlquiles@ugr.es (J.L. Quiles).

http://dx.doi.org/10.1016/j.regg.2015.05.003
0211-139X/

Cómo citar este artículo: González-Alonso A, et al. Evidencias experimentales con respecto al papel de distintos tipos de grasas insaturadas de la dieta en el envejecimiento. Rev Esp Geriatr Gerontol. 2015. http://dx.doi.org/10.1016/j.regg.2015.05.003

G Model
REGG-664; No. of Pages 4

que el grado de insaturación de los ácidos grasos en membranas biológicas de diferentes tejidos en mamífero se correlaciona negativamente con su longevidad[2,3].

Las membranas biológicas son estructuras dinámicas que generalmente constan de bicapas de moléculas anfipáticas que se mantienen unidas mediante enlaces no covalentes[4]. El lípido de membrana predominante es el fosfolípido, cuyas cadenas acilo son saturadas, monoinsaturadas o poliinsaturadas por cadenas hidrocarbonadas con una longitud media de 18 átomos de carbono en células eucarióticas de especies vertebradas, donde la distribución relativa entre ácidos grasos saturados e insaturados sigue una proporción cercana a 40:60, respectivamente. La susceptibilidad de las membranas biológicas a sufrir alteraciones oxidativas se relaciona con la reactividad química de los ácidos grasos que la componen[3]. Los ácidos grasos insaturados son las moléculas más sensibles al daño de las ERO debido a la alta presencia de electrones inestables cerca de sus dobles enlaces, y también debido a que su sensibilidad a la peroxidación lipídica es tanto mayor cuanto más moléculas con dobles enlaces tienen[5,6].

Por otra parte, los productos derivados de la oxidación lipídica pueden producir modificaciones covalentes de proteínas. En este sentido, los niveles más bajos de aductos de proteína N < épsilon > -(malondialdehído)-lisina y S-(carboximetil)-lisina se han hallado en mitocondrias y tejidos de mamíferos y aves de larga vida que muestran un bajo grado de insaturación de ácidos grasos[7]. Estudios recientes indican que la reducción experimental en el grado de insaturación de los ácidos grasos de la dieta induce a un menor daño oxidativo y alteraciones en ADN mitocondrial (ADNmt) de hígado[8], corazón[9] y cerebro[10].

La alta concentración de ácidos grasos insaturados en los fosfolípidos de las membranas celulares no solo les hace más susceptibles a las reacciones de oxidación, sino que también les hace partícipes en las reacciones de radicales libres en cadena. De ese modo, un bajo grado de insaturación en los ácidos grasos de las membranas biológicas podría disminuir su sensibilidad a la peroxidación lipídica, la cual, a su vez, puede proteger a otras moléculas del daño derivado de la lipooxidación[11].

Según lo mencionado anteriormente, el tipo de grasa de la dieta afecta a la estructura y función mitocondrial, así como a su susceptibilidad al estrés oxidativo. En este sentido, si pudiéramos construir membranas biológicas «personalizadas» según el tipo de grasa de la dieta, tal vez se podría modificar de forma positiva la manera en la cual los órganos envejecen. Esta hipótesis de trabajo representa un nuevo enfoque en el estudio del envejecimiento desde el punto de vista de la nutrición, y podría tener implicaciones importantes para el estudio del fenómeno del envejecimiento. La presente revisión trata de resumir los estudios realizados por nuestro grupo de investigación en los últimos 10 años empleando aceite de oliva virgen, aceite de girasol o aceite de pescado como fuente de grasa insaturada de la dieta en relación con un modelo de envejecimiento en rata.

Deleciones en el ADN mitocondrial, estrés oxidativo y alteraciones ultraestructurales durante el envejecimiento en función de la fuente grasa

El papel de la grasa de la dieta durante el envejecimiento se ha analizado en nuestro laboratorio a nivel mitocondrial desde el enfoque del estrés oxidativo, las alteraciones ultraestructurales y la frecuencia de aparición de deleciones en el ADNmt en diversos tejidos en rata, tanto mitóticos (hígado) como posmitóticos (corazón y cerebro).

La dieta puede tener influencia sobre el grado de envejecimiento del hígado y, en consecuencia, sobre la salud del mismo[12,13]. La respuesta podría estar en la teoría de envejecimiento mitocondrial (el envejecimiento es consecuencia de la acumulación del daño en el ADNmt). Para estudiar esta posibilidad, alimentamos ratas macho Wistar durante toda su vida con aceite de oliva virgen (rico en ácido oleico, monoinsaturado) o de girasol (rico en ácido linoleico, poliinsaturado). A los 6 y 24 meses se analizaron las mitocondrias de hígado en relación con la frecuencia de aparición de deleción común en ADNmt, ERO, antioxidantes y alteraciones ultraestructurales[8]. Se observó un aumento relacionado con el envejecimiento en la cantidad relativa de ERO en ambos grupos dietéticos, siendo mayor en los animales alimentados con aceite de girasol. El envejecimiento condujo a mayores actividades de superóxido dismutasa, catalasa y glutatión peroxidasa, así como a mayores concentraciones de a-tocoferol y coenzima Q. Por otro lado, las mitocondrias de animales viejos alimentados con aceite de girasol exhibieron un menor número de crestas y una circularidad superior, factores ambos que han sido relacionados con una menor funcionalidad del orgánulo[8]. Del mismo modo, la frecuencia de aparición de deleción común en el ADNmt de los animales viejos fue superior en las ratas alimentadas con aceite de girasol. En este estudio se puso de manifiesto, por tanto, una relación entre la producción de ERO y la aparición de alteraciones ultraestructurales y a nivel de ADN en mitocondria de hígado de animales viejos. Pero lo más interesante fue poder comprobar cómo estos aspectos, que podrían ser definitorios de la aparición del fenotipo de envejecimiento, podían ser modulados a través de la dieta mediante la elección de una fuente grasa más o menos insaturada, lo cual da pie a la posibilidad de modular el envejecimiento a través de la dieta.

El tejido miocárdico durante el envejecimiento se caracteriza por la acumulación de tejido conectivo, un mayor volumen de miocitos y un aumento en los procesos necróticos y apoptóticos[14]. Además, el corazón es un tejido posmitótico con un gran metabolismo aeróbico gracias a su abundancia de mitocondrias. En un estudio similar al descrito anteriormente, pero centrado en el corazón, se estudiaron animales jóvenes y viejos (6 y 24 meses de edad) alimentados con aceite de oliva virgen o de girasol (este último con o sin suplementación de coenzima Q10) con el objetivo de estudiar las deleciones mitocondriales relacionadas con el envejecimiento y la dieta[9]. Se obtuvieron resultados parecidos a los conseguidos en tejido hepático con respecto a parámetros ultraestructurales[8]. También se estudió la frecuencia de una deleción concreta en ADNmt correspondiente al complejo i de la cadena respiratoria mitocondrial, ya que es uno de los complejos más afectados por el envejecimiento[15]. La frecuencia de aparición de esta deleción fue menor durante el envejecimiento en los animales alimentados durante toda su vida con aceite de oliva virgen extra o los alimentados con aceite de girasol suplementado en coenzima Q10, demostrando así que el aumento de la frecuencia de las deleciones en el ADNmt debidas a la edad pueden ser atenuadas interviniendo dietéticamente también desde el punto de vista cardiaco[9].

Diversos estudios han puesto de manifiesto una disminución de la actividad enzimática de ciertos complejos de la cadena de transporte electrónico mitocondrial debida a la edad en el cerebro[16], aunque no se sabe con certeza si dichas alteraciones se traducen necesariamente en una disminución respiratoria o, incluso, en una disminución en la producción de ATP[17]. Al igual que en el hígado y el corazón, nuestro grupo ha estudiado los cambios en la frecuencia de deleción común del ADNmt de tejido cerebral debidos a la edad, con intervención dietética al variar la fuente grasa de la dieta[10]. Con respecto a la peroxidación lipídica, se demostró que los niveles de peróxidos lipídicos eran más bajos en los grupos alimentados con aceite de oliva virgen y en los animales alimentados con aceite de girasol y suplementados con coenzima Q10, así como la frecuencia de aparición de deleciones en ADNmt[10].

Cómo citar este artículo: González-Alonso A, et al. Evidencias experimentales con respecto al papel de distintos tipos de grasas insaturadas de la dieta en el envejecimiento. Rev Esp Geriatr Gerontol. 2015. http://dx.doi.org/10.1016/j.regg.2015.05.003

G Model
REGG-664; No. of Pages 4

Tipo de grasa de la dieta y pérdida de hueso alveolar debida a la edad

La periodontitis es una enfermedad inflamatoria crónica caracterizada por una destrucción progresiva de los tejidos que soportan los dientes. Se ha relacionado con los desórdenes debidos a la edad mediante la rotura de los tejidos que sostienen al diente, principalmente la pérdida del hueso alveolar. Por otro lado, evidencias crecientes vinculan la periodontitis a enfermedades sistémicas como la aterosclerosis, habiéndose puesto atención en el síndrome metabólico y el estrés oxidativo como lazos potenciales que explican esta relación[18,19]. Tradicionalmente, muchas de las investigaciones sobre dieta y enfermedades periodontales se focalizan en unos pocos nutrientes con roles bien establecidos en la formación y manutención de los componentes estructurales de los tejidos orales como el colágeno (vitamina C) y el hueso (calcio), la integridad del tejido epitelial (vitamina A) o en la formación de placa que alberga los patógenos periodontales (hidratos de carbono). De forma más reciente, las investigaciones se han ampliado para incluir nutrientes que parecen atenuar los procesos inflamatorios o que poseen propiedades antiinflamatorias, como son los ácidos grasos poliinsaturados (AGPI) n3. Sin embargo, no se ha estudiado el posible papel de otros ácidos grasos con conocidas propiedades beneficiosas para otros aspectos de la salud, como son los ácidos grasos monoinsaturados (AGMI)[20]. Con base en esto, Bullon et al. recientemente han reproducido un modelo de periodontitis asociada al envejecimiento, dándole un enfoque completamente fisiológico a las condiciones periodontales para evaluar el impacto del tipo de grasa de la dieta sobre el tejido[21]. Así, los animales fueron alimentados durante toda su vida con dietas basadas en AGMI (procedentes de aceite de oliva virgen extra), AGPI n6 (procedentes de aceite de girasol) o AGPI n3 (procedentes de aceite de pescado). Estudiaron la altura del hueso alveolar a 2 niveles, en concreto a nivel mesial y a nivel distal, en el primer molar y segundo premolar. También se analizó la histología del tejido gingival en la mucosa molar gingival. Los resultados mostraron diferencias con respecto a la pérdida de hueso alveolar durante el envejecimiento en función de la grasa ingerida a través de la dieta. En concreto, la mayor pérdida de masa ósea, junto con un alto grado de fibrosis y un moderado grado de inflamación se observaron en los animales alimentados con aceite de girasol, seguidos de aquellos alimentados con aceite de pescado. Por otro lado, se observaron niveles circulantes de citocinas inflamatorias superiores en todos los grupos viejos, pero sin diferencias significativas en función del tratamiento dietético, lo cual fue corroborado cuando se estudió la expresión génica de estas moléculas a nivel de ARNm de tejido gingival. El hecho de no existir diferencias en los niveles de citocinas podría deberse a que la inflamación periodontal no fue promovida en exceso mediante tratamientos más intensivos, sino que la periodontitis se generó simplemente a través del envejecimiento fisiológico y con una dieta optimizada en todos los casos. Con el objeto de intentar vincular mecanismos a los hallazgos observados a nivel de pérdida de hueso alveolar, y ante la ausencia de inflamación, se procedió al análisis de los niveles de ARNm y de niveles circulantes de 2 de los marcadores más importantes asociados con la resorción ósea, en concreto, el ligando de receptor activador para el factor nuclear kB (RANKL) y la osteoprotegerina (OPG). La cantidad relativa de RANKL fue la más elevada en animales alimentados con aceite de oliva virgen a los 6 meses de edad, mientras que la cantidad relativa de OPG fue la menor para los animales alimentados con aceite de pescado. Las diferencias de niveles plasmáticos de RANKL fueron asociadas únicamente a la edad, mostrando los animales de 6 meses niveles más altos que los de 24 meses de edad. Sin embargo, se mostraron diferencias en los niveles plasmáticos de OPG entre animales jóvenes y viejos, siendo los animales alimentados con aceite de oliva virgen los que presentaron los niveles más bajos. Estos resultados son de gran interés, ya que se ha establecido previamente que la periodontitis cursa con niveles elevados de RANKL junto con bajas concentraciones de OPG, lo que en su conjunto provoca un aumento de la ratio RANKL/OPG, marcador que podría ser usado para realizar un seguimiento más real de la enfermedad[22]. Parece, por tanto, que la pérdida de hueso alveolar debida a la edad podría estar condicionada por la grasa de la dieta. Según lo observado en este estudio, un exceso de AGPI n6 a lo largo de la vida podría provocar un bloqueo de la capacidad celular de adaptación al envejecimiento. Los análisis de expresión genética evidenciaron que los AGMI o los AGPI n3 permitían a la mitocondria mantener un recambio adecuado mediante la inducción de biogénesis o autofagia. Dichos ácidos grasos serían también capaces de inducir a los sistemas antioxidantes pertinentes a contrarrestar el estrés oxidativo debido a la edad y no inhibir la cadena de transporte electrónico mitocondrial. Un tratamiento dietético adecuado podría reducir, por tanto, el exceso de pérdida de hueso alveolar, característico de las enfermedades periodontales[21].

Comportamiento del páncreas durante el envejecimiento en función del tipo de grasa ingerido

El páncreas es un órgano vital para la digestión y consumo de los nutrientes de la dieta. Una adecuada estructura pancreática es requisito para su funcionamiento óptimo[23]. Ciertas enfermedades asociadas con la dieta alteran la masa celular pancreática, desembocando en inflamación, obstrucción ductal, fibrosis e, incluso, necrosis, acelerando el proceso de envejecimiento de los órganos[24]. La identificación de factores capaces de revertir o evitar estas condiciones patológicas es un instrumento útil para diseñar estrategias de prevención frente a los distintos desórdenes pancreáticos que ocurren durante el envejecimiento. En línea con el estudio de diferentes fuentes grasas insaturadas en relación con diversos aspectos de la salud durante el envejecimiento, Roche et al.[25] compararon 3 grupos de ratas alimentadas durante 2 años con dietas isocalóricas de diferente composición para estudiar su implicación en el envejecimiento del páncreas.

Las ratas se dividieron en 3 grupos experimentales alimentados con dietas isoenergéticas de acuerdo con el criterio AIN93, pero modificando su fuente grasa, usando aceite de oliva virgen, de girasol o de pescado, respectivamente. Al final del experimento no se detectaron síntomas patológicos como la obesidad, no obstante, el hallazgo importante de este estudio fue que el tipo de grasa afectó al compartimento endocrino y exocrino del páncreas de modo diferente. Tomando islotes de ratas alimentadas con aceite de oliva como referencia, los islotes de las ratas alimentadas con aceite de girasol contuvieron un número mayor de células b, reflejado por un aumento en el contenido de insulina e hiperleptinemia. Estos signos, junto con otros parámetros, han sido descritos en obesidad, intolerancia a la glucosa, resistencia a la insulina, desorganización del axis adipoinsular o prediabetes[26]. En los análisis inmunohistoquímicos (insulina y glucagón), las áreas inmunopositivas por islote fueron significativamente mayores en el grupo de aceite de girasol en comparación con el grupo de aceite de oliva virgen. Este incremento parecía correlacionarse con un aumento en el número de células positivas a la hormona en el islote. Por otra parte, el número de células positivas para glucagón fue similar en los 3 grupos, evidenciando que los cambios de los islotes parecían ser exclusivos de las células b. Ha sido bien documentado que la replicación de las células b disminuye con la edad en roedores y humanos[27], pero, en compensación, se presenta una apoptosis mínima[28]. Por otro lado, la presencia de aceite de pescado en la dieta parece afectar principalmente al compartimento exocrino del páncreas. Las alteraciones histológicas pancreáticas observadas en los animales alimentados con aceite de pescado se parecen a aquellas observadas en la fibrosis pancreática de la vejez[29]. El contenido de insulina y glucagón

Cómo citar este artículo: González-Alonso A, et al. Evidencias experimentales con respecto al papel de distintos tipos de grasas insaturadas de la dieta en el envejecimiento. Rev Esp Geriatr Gerontol. 2015. http://dx.doi.org/10.1016/j.regg.2015.05.003

G Model
REGG-664; No. of Pages 4

medido confirmó que el grupo de aceite de girasol contenía 2 veces la cantidad de insulina detectada en los otros grupos, mientras que los niveles de glucagón permanecieron intactos.

Es bien conocido que la grasa de la dieta puede modificar la composición de los ácidos grasos circulantes[11]. Como sería de esperar, los porcentajes de AGMI circulantes fueron significativamente superiores en ratas alimentadas con aceite de oliva virgen; los niveles más altos de AGPI n6 se lograron en las ratas alimentadas con aceite de girasol, y los niveles más altos de AGPI n3 fueron superiores en aquellas ratas alimentadas con aceite de pescado.

Con el propósito de aclarar los mecanismos asociados a los cambios antes descritos, se analizaron diversos marcadores de proliferación y apoptosis. Así, en relación con el número de células positivas para el marcador de proliferación Ki-67 no se observaron diferencias entre los distintos grupos experimentales. Lo mismo ocurrió para la expresión de los marcadores apoptóticos Bcl-2 y Bad, pero no para el factor de necrosis tumoral a, el cual presentaba una menor expresión en sus niveles para el aceite de oliva virgen comparado con los grupos de rata alimentadas con aceite de pescado y girasol. En conjunto, estos resultados podrían demostrar que la ruta mitocondrial apoptótica no se activó en ninguno de los grupos animales, y que en caso de producirse apoptosis, fuese a través de la ruta citoplasmática, donde se implica de forma mayoritaria el factor de necrosis tumoral a[30].

Lo más importante del estudio de Roche et al. es el hecho de que el tipo de grasa puede afectar a los compartimentos exocrinos y endocrinos del páncreas de manera diferente. Así, las fuentes grasas poliinsaturadas de tipo n6 condicionan fundamentalmente un sobreesfuerzo a nivel endocrino, lo que podría predisponer a un entorno prediabético, lo cual, en caso de tener lugar un episodio patológico adicional, podría acelerar el paso hacia la diabetes como consecuencia de la ingesta de ese tipo de grasa. Por su parte, los AGPI de tipo n3 alteran sobre todo el componente exocrino. De este modo, la fuente grasa de elección desde el punto de vista de un envejecimiento óptimo del páncreas sería el aceite de oliva virgen como fuente de AGMI, lo cual refuerza hallazgos previos anteriores[31].

Financiación

Los resultados expuestos en esta revisión han sido financiados con diferentes proyectos de investigación nacionales (ALI95-1036-C05-04, 1FD97-0457-C02-01, AGL2008-01057) y regionales (AGR832).

Conflicto de intereses

Adrian González-Alonso y Alfonso Varela-López son contratados FPU del Ministerio de Educación, Cultura y Deporte.

Agradecimientos

Los autores agradecen a la Universidad de Granada y a la Junta de Andalucía el soporte que proporcionan al Grupo de Investigación.

Bibliografía

1. Naudí A, Jové M, Ayala V, Portero-Otín M, Barja G, Pamplona R. Membrane lipid unsaturation as physiological adaptation to animal longevity. Front Physiol. 2013;4:372, http://dx.doi.org/10.3389/fphys.2013.00372.
2. Pamplona R, Portero-Otín M, Ruiz C, Gredilla R, Herrero A, Barja G. Double bond content of phospholipids and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals. Mech Ageing Dev. 2000;112:169–83.
3. Pamplona R. Membrane phospholipids, lipoxidative damage and molecular integrity: A causal role in aging and longevity. Biochim Biophys Acta. 2008;1777:1249–62.
4. Vance DE, Vance JE. Biochemistry of lipids, lipoproteins and membranes. Amsterdam: Elsevier Science BV; 1996. p. 1–553.
5. Holman RT. Autoxidation of fats and related substances. En: Holman RT, Lundberg WO, Malkin T, editores. Progress in chemistry of fats and other lipids. London: Pergamon Press; 1954. p. 51–98.
6. Bielski BH, Arudi RL, Sutherland MW. A study of the reactivity of HO_2/O_2^- with unsaturated fatty acids. J Biol Chem. 1983;258:4759–61.
7. Pamplona R, Barja G, Portero-Otín M. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: A homeoviscous-longevity adaptation? Annu N Y Acad Sci. 2002;959:475–90.
8. Quiles JL, Ochoa JJ, Ramírez-Tortosa MC, Huertas JR, Mataix J. Age-related mitocondrial DNA deletion in rat liver depends on dietary fat unsaturation. J Gerontol A Biol Sci Med Sci. 2006;61:107–14.
9. Quiles JL, Pamplona R, Ramírez-Tortosa MC, Naudí A, Portero-Otin M, Araujo-Nepomuceno E, et al. Coenzyme Q addition to an n-6 PUFA-rich diet resembles benefits on age-related mitochondrial DNA deletion and oxidative stress of a MUFA-rich diet in rat heart. Mech Ageing Dev. 2010;131:38–47.
10. Ochoa JJ, Pamplona R, Ramírez-Tortosa MC, Granados-Principal S, Pérez-López P, Naudí A, et al. Age-related changes in brain mitochondrial DNA deletion and oxidative stress are differentially modulated by dietary fat type and coenzyme Q_{10}. Free Radic Biol Med. 2011;50:1053–64.
11. Mataix J, Quiles JL, Huertas JR, Battino M, Manas M. Tissue specific interactions of exercise, dietary fatty acids, and vitamin E in lipid peroxidation. Free Radic Biol Med. 1998;24:511–21.
12. Anantharaju A, Feller A, Chedid A. Aging liver. A review. Gerontology. 2002;48:343–53.
13. Quiles JL, Martínez E, Ibánez S, Ochoa JJ, Martín Y, López-Frías M, et al. Ageing-related tissue-specific alterations in mitochondrial composition and function are modulated by dietary fat type in the rat. J Bioenerg Biomembr. 2002;34:517–24.
14. Kajstura J, Cheng W, Sarangarajan R, Li P, Li B, Nitahara JA, et al. Necrotic and apoptotic myocyte cell death in the aging heart of Fischer 344 rats. Am J Physiol. 1996;271:1215–28.
15. Lenaz G, Bovina C, D'Aurelio M, Fato R, Formiggini G, Genova ML, et al. Role of mitochondria in oxidative stress and aging. Ann N Y Acad Sci. 2002;959:199–213.
16. Gilmer LK, Ansari MA, Roberts KN, Scheff SW. Age-related changes in mitochondrial respiration and oxidative damage in the cerebral cortex of the Fischer 344 rat. Mech Ageing Dev. 2010;131:133–43.
17. Van Remmen H, Richardson A. Oxidative damage to mitochondria and aging. Exp Gerontol. 2001;36:957–68.
18. Bullon P, Morillo JM, Ramírez-Tortosa MC, Quiles JL, Newman HN, Battino M. Metabolic syndrome and periodontitis: Is oxidative stress a common link? J Dent Res. 2009;88:503–18.
19. Bullon P, Cordero MD, Quiles JL, Morillo JM, Ramírez-Tortosa MC, Battino M. Mitochondrial dysfunction promoted by Porphyromonas gingivalis lipopolysaccharide as a possible link between cardiovascular disease and periodontitis. Free Radic Biol Med. 2011;50:1336–43.
20. Kaye EK. n-3 fatty acid intake and periodontal disease. J Am Diet Assoc. 2010;110:1650–2.
21. Bullon P, Battino M, Varela-Lopez A, Perez-Lopez P, Granados-Principal S, Ramirez-Tortosa MC, et al. Diets based on virgin olive oil or fish oil but not on sunflower oil prevent age-related alveolar bone resorption by mitochondrial-related mechanisms. PLoS One. 2013;8:e74234.
22. Jules J, Ashley JW, Feng S. Selective targeting of RANK signaling pathways as new therapeutic strategies for osteoporosis. Expert Opin Ther Targets. 2010;14:923–34.
23. Ouyang D, Dhall D, Yu R. Pathologic pancreatic endocrine cell hyperplasia. World J Gastroenterol. 2011;17:137–43.
24. Halter JB. Diabetes mellitus in an aging population: The challenge ahead. J Gerontol A Biol Sci Med Sci. 2012;67:1297–9.
25. Roche E, Ramírez-Tortosa CL, Arribas MI, Ochoa JJ, Sirvent-Belando JE, Battino M, et al. Comparative analysis of pancreatic changes in aged rats fed life long with sunflower, fish, or olive oils. J Gerontol A Biol Sci Med Sci. 2014;69:934–17944.
26. Sattar N, Wannamethee SG, Forouhi NG. Novel biochemical risk factors for type 2 diabetes: Pathogenic insights or prediction possibilities? Diabetologia. 2008;51:926–40.
27. Meier JJ, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. Diabetes. 2008;57:1584–94.
28. Teta M, Long SY, Wartschow LM, Rankin MM, Kushner JA. Very slow turnover of beta-cells in aged adult mice. Diabetes. 2005;54:2557–67.
29. Klöppel G, Detlefsen S, Feyerabend B. Fibrosis of the pancreas: The initial tissue damage and the resulting pattern. Virchows Arch. 2004;445:1–8.
30. Chu WM. Tumor necrosis factor. Cancer Lett. 2013;328:222–5.
31. Rojo-Martínez G, Esteva I, Ruiz de Adana MS, García-Almeida JM, Tinahones F, Cardona F, et al. Dietary fatty acids and insulin secretion: A population-based study. Eur J Clin Nutr. 2006;60:1195–200.

Cómo citar este artículo: González-Alonso A, et al. Evidencias experimentales con respecto al papel de distintos tipos de grasas insaturadas de la dieta en el envejecimiento. Rev Esp Geriatr Gerontol. 2015. http://dx.doi.org/10.1016/j.regg.2015.05.003