Tesis Doctoral

Regulation of thyroid function by lifestyle factors and its implication on energy homeostasis and cardiometabolic health in young euthyroid adults

Regulación de la función tiroidea por factores del estilo de vida y su implicación en la homeostasis energética y salud cardiometabólica en adultos jóvenes eutiroideos

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Que la Tesis Doctoral titulada **"Regulación de la función tiroidea por factores del estilo de vida y su implicación en la homeostasis energética y salud cardiometabólica en adultos jóvenes eutiroideos"** que presenta Dña. Mª Elisa Merchán Ramírez al superior juicio del Tribunal que designe la Universidad de Granada, ha sido realizada bajo mi dirección durante los años 2016-2021, siendo expresión de la capacidad técnica e interpretativa de su autora en condiciones tan aventajadas que le hacen merecedora al Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

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Garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando 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.

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La doctoranda Dña. Mª Elisa Merchán Ramírez ha realizado la presente Tesis Doctoral siendo beneficiario de un contrato laboral de personal técnico o de gestión de I+D+I (Referencia: (PEJ2018-003843-A), enmarcado en el Sistema Nacional de Garantía Juvenil y financiado por parte del Fondo Social Europeo y de la Iniciativa de Empleo Juvenil (IEJ). Resolución publicada el 02/09/2019 por el Vicerrectorado de Investigación y Transferencia de la Universidad de Granada. Subvención concedida por Resolución de 10 de mayo de 2019 de la Secretaría de Estado de investigación, Desarrollo e Innovación del Ministerio de Economía y competitividad, en el marco del Plan estatal de Investigación Científica y Técnica y de Innovación 2017-2020.

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"Denme un punto de apoyo, y moveré el mundo"
-Arquímedes-

A mi familia. A los que disfruto, y a los que extraño.

Especialmente, a mis padres y hermanos.
Vuestro amor incondicional
es mi mejor punto
de apoyo.

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List of abbreviations

^{18}F-FDG: ^{18}F-fluorodeoxyglucose
ALP: Alkaline phosphatase
APOA1: Apolipoprotein A-1
APOB: Apolipoprotein B
BAT: Brown adipose tissue
BMI: Body mass index
BP: Blood pressure
CI: Cold induced
CIT: Cold induced thermogenesis
CRM: Cardiometabolic
CT: Computed tomography
DASH: Dietary Approaches to Stop Hypertension
DII: Dietary inflammatory index
DQI: Diet quality index
DXA: Dual Energy X-ray Absorptiometry
FFQ: Food frequency questionnaire
FM: Fat mass
FMI: Fat mass index
FT3: Free Tri-iodothyronine.
FT4: Free Thyroxine
GGT: Gamma-glutamyltransferase
HDL-C: High density lipoprotein
HOMA-IR: Homeostatic model assessment index of insulin resistance
HPT: Hypothalamus-pituitary-thyroid
LDL-C: Low density lipoprotein
LM: Lean mass
LMI: Lean mass index
MeD-P: A priori Mediterranean dietary pattern
MeD-S: Mediterranean diet score
MeD-DQI: Dietary quality index for a Mediterranean diet
MIT: Meal induced thermogenesis
NAT: Neck adipose tissue
PA: Physical activity
PET: Positron emission tomography
PTFQI: Parametric Thyroid Feedback Quantile-based Index
REE: Resting energy expenditure
RMR: Resting metabolic rate
STT: Shivering threshold test
SUV: Standardized uptake value
TC: Total cholesterol
TG: Triglycerides
Tg: Thyroglobulin
TGB: Thyroxine-binding-globulin
THs: Thyroid hormones
TRH: Thyroid releasing hormone
TSH: Thyroid-stimulating hormone
TTR: Transthyretin
UCP1: Uncoupling protein 1
VAS: Visual analogue scales
VAT: Visceral adipose tissue
VO_2max: Maximum oxygen consumption
WC: Waist circumference
WP: Warm period

ABSTRACT

Thyroid function plays an important role on body temperature, weight and energy metabolism regulation. Even in the absence of thyroid pathology, slight differences in thyroid hormones (THs, i.e. triiodothyronine, T3, and thyroxine, T4) levels have implications for cardiometabolic health.

Several studies have investigated the relationship of thyroid function with lifestyle factors, brown adipose tissue (BAT), obesity and cardiometabolic risk. However, inconsistent results have been found in euthyroid subjects, and the link between thyroid function and lifestyle factors and cardiometabolic health is still uncertain.

Recent evidence suggests that nutrient availability, physical activity and sleep disturbances are influential factors that modulate the hypothalamus-pituitary-thyroid axis activity, influencing the THs production, and hence body weight and metabolic homeostasis.

The aims of this Doctoral Thesis are to analyse, in young euthyroid adults, the relationship between habitual nutrient intake, dietary patterns, physical activity levels and sleep habits and thyroid function in young euthyroid adults (Study 1); the association of thyroid function with body composition and cardiometabolic risk (Study 2) and the effect of a cold exposure on thyroid function and the association between thyroid function and BAT volume and function (Study 3).

The results of the present Doctoral Thesis show that lifestyle factors such as dietary energy intake, is directly associated with thyroid function, which is inversely related to adherence to the Mediterranean diet and physical activity levels in young euthyroid adults. These results also show that free T3 was positively associated with central adiposity and cardiometabolic risk factors, such as insulin resistance, fatty liver index and blood pressure, even in young euthyroid adults. Lastly, despite the serum thyroid-stimulating hormone and free T4 levels decrease in response to a personalized mild-cold exposure, thyroid function is not associated with BAT volume or function in young euthyroid adults.

Collectively, the results of this Doctoral Thesis increase our knowledge about the lifestyle determinants of thyroid function in young euthyroid adults, and the relationship of thyroid function with cardiometabolic health and BAT metabolism. Dietary habits and physical activity appear to be associated with thyroid function in healthy individuals. On the other hand, even in euthyroid individuals, thyroid function seems to be linked to central adiposity and cardiometabolic risk, although no association seems to exist between thyroid and BAT metabolism in young euthyroid adults.

RESUMEN

La función tiroidea juega un papel importante en la regulación de la temperatura corporal, el peso y el metabolismo energético. Incluso en ausencia de patología tiroidea, las ligeras diferencias en los niveles de hormonas tiroideas (HTs, es decir, triyodotironina, T3 y tiroxina, T4) tienen implicaciones para la salud cardiometabólica.

Varios estudios han investigado la relación de la función tiroidea con factores de estilo de vida, tejido adiposo pardo (TAP), obesidad y riesgo cardiometabólico. Sin embargo, los resultados encontrados en sujetos eutiroideos son inconsistentes, y el vínculo entre la función tiroidea y factores de estilo de vida y salud cardiometabólica, aún se mantiene incierto.

La reciente evidencia sugiere que la disponibilidad de nutrientes, la actividad física y las alteraciones del sueño son factores influyentes que modulan la actividad del eje hipotálamico-pituitario-tiroideo, influyendo en la producción de HTs y, por tanto, en el peso corporal y la homeostasis metabólica.

Los objetivos de esta Tesis Doctoral son analizar, en adultos jóvenes eutiroideos, la relación entre la ingesta habitual de nutrientes, los patrones dietéticos, los niveles de actividad física y los hábitos de sueño y la función tiroidea en adultos jóvenes eutiroideos (Estudio 1); la asociación entre la función tiroidea y la composición corporal y el riesgo cardiometabólico (Estudio 2); y el efecto de una exposición al frío sobre la función tiroidea, y la asociación entre la función tiroidea y el volumen y función del TAP (Estudio 3).

Los resultados de la presente Tesis Doctoral muestran que factores del estilo de vida, como la ingesta energética de la dieta, están asociados directamente con la función tiroidea, la cual está inversamente relacionada con la adherencia a la dieta mediterránea y los niveles de actividad física en adultos jóvenes eutiroideos. Estos resultados también muestran que la T3 libre se asoció positivamente con la adiposidad central y factores de riesgo cardiometabólicos, como la resistencia a la insulina, el índice de hígado graso y la presión arterial, incluso en adultos jóvenes eutiroideos. Por último, a pesar de que la hormona estimulante del tiroides en suero, y los niveles de T4 libre disminuyen en respuesta a una exposición personalizada leve al frío, la función tiroidea no se asocia con el volumen o función de TAP en adultos jóvenes eutiroideos.

En conjunto, los resultados de esta Tesis Doctoral aumentan nuestro conocimiento de los determinantes del estilo de vida sobre función tiroidea en adultos jóvenes eutiroideos, y la relación de la función tiroidea con la salud cardiometabólica y el metabolismo del TAP. Los hábitos alimentarios y la actividad física parecen estar asociados con la función tiroidea en individuos sanos. Por otro lado, incluso en individuos eutiroideos, la función tiroidea parece estar relacionada con la adiposidad central y el riesgo cardiometabólico, aunque no parece existir asociación entre la función tiroidea y el metabolismo de TAP en adultos jóvenes eutiroideos.

GENERAL INTRODUCTION

GENERAL INTRODUCCTION

ENERGY HOMEOSTASIS AND CARDIOMETABOLIC HEALTH

Nowadays, diabetes, hypertension and/or dyslipidemia, are responsible for approximately one-third of deaths worldwide, and this number is expected to continue increasing (1). The prevalence of these disorders is being encouraged by the growing epidemic of obesity (2). Accordingly, obesity is associated with several metabolic abnormalities and have been related with the increase of cardiovascular disease (CVD), morbidity and mortality (2).

Obesity is a non-communicable and chronic disease, defined as an excessive or abnormal fat accumulation, which has reached epidemic proportions globally (3). The obese population has been tripled on the last 40 years. In 2016, 1.9 billion adults over 18 years old were overweight, of which, 650 million were obese (3). Worryingly, these increasing trends are also noted for children and adolescents. In 2016, around 340 million of children and adolescents were overweight or obese (4).

The World Health Organization (WHO) established the body mass index (BMI) as measure to assess total body fat, which is calculated by dividing the body weight in kilograms (kg) by the square of the height in metres (m) (3). BMI is well correlated with body fat percentage in young and middle-aged obese population (3,5). Obesity is diagnosed in subjects whose BMI is higher than 30 kg/m^2 (3,5).

An accurate balance between energy intake and energy expenditure is necessary to maintain body weight within a healthy range (6). However, the obesogenic environment of the industrialized societies is certainly promoting overnutrition, which together with the raise in sedentary habits leads to a deregulation of energy balance and consequently, fat accumulation. Excessive fat accumulation in organs not specialized on lipid storage, such as the liver or skeletal muscle, produce metabolic disturbances and disorders, such as type II diabetes, insulin resistance, CVD, hepatitis or even cancer (6,7).

Therefore, disturbances in energy homeostasis produces importantly alterations in the metabolism, promoting an increase of obesity and cardiometabolic disease (6). Consequently, energy homeostasis is an essential determinant of cardiometabolic health (6). Thyroid function plays an important role on body

weight and energy metabolism regulation (8), and therefore its modulation might affect to the energy homeostasis and hence the risk of developing cardiometabolic diseases.

THYROID FUNCTION AND METABOLISM

Thyroid function is regulated by the hypothalamic-pituitary-thyroid (HPT) axis to orchestrate processes such as cell differentiation, and growth, development and function of several tissues (8,9). Thyrotropin (TRH), which is synthesized and secreted from the hypothalamus, stimulate the thyrotropin releasing hormone (TSH) secretion by the pituitary gland (8). The increase concentration of TSH in turn signals in the thyroid gland, inducing the production of thyroid hormones (THs, i.e. triiodothyronine, T3, and thyroxine, T4) which are the ultimate effectors of the axis. The HPT-axis responds to an endocrine negative feedback loop, due to the circulating levels of T3 and T4 negatively modulate the hypothalamic TRH and TSH synthesis and secretion (8,9).

T4 is the main secretory product of the thyroid gland in all vertebrates. However, it is recognized as a minimally active molecule. Consequently, most of T4 is converted, through deiodination, to T3, which is the active form of THs. The process consists in removing one iodine residue from the T4 molecule to produce T3, reaction catalysed by the type 1 (D1) and type 2 iodothyronine (D2) deiodinases (10). Over 80% of the T3 production in healthy adults is produced by peripheral deiodination of T4 by D2. Therefore, only over ~20% of T3 serum levels is secreted by the thyroid gland through D1 action (11).

In humans, after being secreted by the thyroid gland, most of THs (99.5-99.9 %, approximately), circulate through the bloodstream bound to specific serum proteins such as thyroxine-binding-globulin (TBG), transthyretin (TTR) and albumin (10%) (10). These THs bound to serum carriers do not penetrate cells, being biologically inactive and functioning as storage reservoirs for THs in circulation. Relevantly, less than 1% of all serum THs circulate freely (free T3 (FT3) and free T4 (FT4)) and is available for uptake by the different tissues (11).

Nevertheless, serum THs levels do not reflect tissue THs concentrations. Tissue THs levels are determined by a several factors, such as the local, intracellular activity of the different metabolic pathways of THs (11). Therefore, circulating THs levels can be metabolized by different pathways playing an essential role in determining

tissue THs levels and action (11). Contrary, tissue THs metabolism may influence the serum THs levels (11).

Finally, it is important to note that thyroid dysfunction is one of the most important endocrine disorders, representing over the 30-40% of the patients seen in an endocrine consultation (12). According to the meta-analysis of Garmendia-Madariaga et al. (13), over the 11% of Europeans suffer thyroid dysfunction. Nevertheless, even in euthyroid subjects, the thyroid function might be involved in the pathogenesis of several cardiometabolic diseases.

Therefore, due to the determinant role of THs on metabolic pathways for optimal growth, development and metabolism regulation in humans, it is important to understand the external factors involved in the modulation of thyroid function even in young euthyroid adults.

LIFESTYLE FACTORS AND THYROID FUNCTION

In recent years, several studies have presented evidence suggesting that lifestyle factors, such as diet, physical activity and sleep affect the thyroid hormone production, and hence body weight and metabolic homeostasis (8,14,15).

Nutrient availability is one of the most influential environmental factors modulating the HPT axis activity (16). During caloric restriction and prolonged starvation, the hormonal milieu is dominated by low levels of insulin and THs (17). This in turn reduces whole-body energy expenditure to preserve energy stores by modulating behaviour and metabolism (18). Despite this mechanism evolved to prevent excessive weight loss and increase survival, it is regarded as an important barrier for obesity treatment (17,18). Notably, despite many of the counterregulatory hormonal changes induced by weight loss have been shown to persist unaltered for a long time (19), thyroid activity is restored promptly upon refeeding (20).

On the other hand, an increase in serum FT3 might contribute to the increase in energy expenditure observed in response to overnutrition (18,20,21). Importantly, beyond food availability, different types of diet seem to modulate THs concentrations (22,23). For instance, Zupo et al. (24) showed that the adherence to the Mediterranean diet was independently associated to reduced thyroid function in euthyroid individuals. Moreover, micronutrients such as iodine, selenium, zinc, iron, copper, and vitamin A are important regulators of THs synthesis and metabolism (25). Indeed, associations between circulating levels of THs and

micronutrient intake have been reported in humans (26–28). However, the heterogeneity in previous studies results prevent drawing firm conclusions (25). Therefore, analysing the association of dietary patterns and habitual nutrient intake with thyroid function is needed to elucidate the role of dietary habits in the thyroid metabolism in euthyroid individuals.

It is widely known that exercise affects the activity of many endocrine glands, including the thyroid, modulating THs secretion (29). Abnormal THs circulating levels have a major impact on exercise tolerance and might result in decreased ability to perform vigorous activities. Additionally, physical activity itself could have direct effects on thyroid function because as it produces alterations in the HPT axis, either due to acute or long-lasting effects (30). Most of the studies have been conducted in trained subjects and/or patients with thyroid disease (31), thus, it is of great interest to study the relationship between habitual physical activity levels and thyroid function in sedentary and euthyroid subjects.

Sleep disturbances are an important public health problem that frequently affect millions of people worldwide (32). Thyroid disorders are often related to sleep problems, since sleep deprivation causes alterations in the HPT axis activity (33,34). In humans, acute sleep deprivation is associated with an increase of TSH, FT4, and FT3 circulating concentration (35,36), whereas sleep seem to acutely inhibit TSH secretion overnight (37). It is widely known that hyperthyroid subjects are usually short sleepers, and hypothyroid subjects are often long sleepers, which suggest an inhibitory effect of THs on sleep (38). Therefore, the thyroid function seems to have an important role in sleep regulation. However, the association between sleep quality and TSH/THs serum levels in euthyroid adults is still controversial and requires further research.

ROLE OF THYROID FUNCTION ON BODY COMPOSITION AND CARDIOMETABOLIC HEALTH

Thyroid function plays an important role on body weight and energy metabolism regulation (8). It is well recognized that THs status is closely related to body weight and energy expenditure (8,39,40). Moreover, several studies supported that thyroid dysfunction (clinical or subclinical) is related to metabolic disturbances (13). Hyperthyroidism (elevated THs levels), stimulate a hypermetabolic condition defined as lower cholesterol levels, higher resting energy expenditure (REE), weight loss, increased lipolysis and gluconeogenesis (8). Conversely,

hypothyroidism (decreased THs levels) is associated with hypometabolism state described as higher cholesterol levels, lower REE, weight gain, reduced lipolysis and gluconeogenesis (8). Due to the important role of thyroid function in energy homeostasis and cardiovascular health, several studies have investigated its relationship with obesity and cardiometabolic risk factors in euthyroid subjects (8,41,42). Both BMI and waist circumference (WC) are positively associated with serum FT3 and TSH levels in euthyroid subjects (43–45). However, the relationship between obesity and FT4 levels in euthyroid subjects is still controversial (43,45). On the other hand, previous studies have shown that moderately low (within the normal range) FT4 and TSH levels are associated with increased glucose levels, insulin resistance, higher atherogenic lipid levels, and low-grade chronic inflammation in euthyroid subjects (46,47). However, the associations between insulin resistance and serum FT4 or TSH are still controversial (43,48,49). Moreover, some studies have shown a positive association between serum FT3 levels and insulin resistance (43,48,49).

More recently, a thyroid hormone resistance index (the Parametric Thyroid Feedback Quantile based Index, PTFQI, based on the circulating concentrations of T4 and TSH) (50), has been related to the prevalence of obesity, metabolic syndrome, type 2 diabetes mellitus, and the incidence of diabetes-related deaths in the U.S.A general population, even in euthyroid subjects. Despite the advances achieved in this field, the relationship of thyroid function with obesity and cardiometabolic risk factors in young euthyroid adults requires further investigation.

Despite BMI is the most used method to diagnose overweight and obesity, this method is not completely accurate due to the lack of fat mass (FM) and lean mass (LM) information. FM and LM have different physiological role on metabolic health (51), and different FM/LM proportion at the same BMI are differentially related to insulin resistance and cardiometabolic risk factors (51). Particularly, although the association between THs levels and body weight has been largely analysed, the association between THs levels and body composition (i.e., FM and LM) is still unknown. Moreover, not only fat accumulation, but fat partitioning is an important risk factor for the development of cardiometabolic diseases. Visceral adipose tissue (VAT) causes insulin resistance and low-grade systemic inflammation and precedes pathological states such as the metabolic syndrome

and type 2 diabetes mellitus (52). Crucially, despite VAT mass is generally associated with total FM, it can largely vary between individuals with similar BMI and total FM (53). Therefore, it is of great relevance to determine whether thyroid function is associated with VAT mass in euthyroid adults. Recently, other ectopic fat depots, such as neck adipose tissue (NAT) mass, have also been suggested to be a relevant indicator of cardiometabolic risk and pro-inflammatory profile (54,55). Since the thyroid gland function might be specially affected by this ectopic fat accumulation, to further understand the association between thyroid function and NAT mass is of clinical interest. Despite thyroid function has been shown to be associated with classic cardiometabolic risk factors in euthyroid adults (48), whether it is also true in young euthyroid adults (18-25 years) remains to be determined.

THYROID FUNCTION, THERMOGENESIS AND BROWN ADIPOSE TISSUE

The relationship between thyroid function, energy homeostasis and cardiometabolic health, might be partially explained, by the role of brown adipose tissue (BAT) in the energy metabolism regulation.

BAT plays an important role in the energy homeostasis of many mammalian species, acting as a key thermogenic tissue. This thermogenic activity is made possible by the activity of the uncoupling protein 1 (UCP1) in the inner mitochondrial membrane of brown adipocytes and is mainly regulated by the sympathetic nervous system (56). BAT activation has been suggested as a means of therapeutically regulating energy balance and energy homeostasis (56,57).

The THs play an important role in energy homeostasis and body temperature regulation. Both in vitro and animal studies have shown that THs contribute to BAT recruitment and differentiation (58,59). Indeed, previous results suggest that the expression of UCP1 is partially due to the presence of T3 (58). D2, which is present in brown adipocytes, accelerates the intracellular formation of T3 via the de-iodination of T4 (60), which leads to the saturation of the nuclear T3 receptors, rendering possible the expression of UCP1 upon cold stimulus (58).

In murine models, TSH binds to TSH receptors in the thyroid gland, increasing the secretion of THs (61). Interestingly, TSH-receptors are also expressed in other tissues, including BAT (59). It is, therefore, biologically plausible that cold-induced increases in the secretion of TSH and THs in humans contribute to BAT recruitment.

However, few studies have addressed this issue in euthyroid subjects, and those undertaken have returned contradictory results. Some reported stable levels of TSH and THs after acute cold exposure (62,63), others indicate a reduction in FT3 and no change in FT4 or TSH after prolonged cold stimulation (64,65), and others still have reported a reduction in TSH and THs in response to cold (66,67). Such controversy might be due, at least in part, to the heterogeneity of the cold exposure protocols followed. Analysing the effect of a personalized cold exposure designed to elicit non-shivering thermogenesis, and to activate BAT, might help clarifying the effect of cold exposure on the thyroid function in euthyroid subjects.

Whether circulating levels of THs are related to BAT volume or activity in humans is unclear (59). It has been suggested that BAT ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake may be associated with the circulating levels of THs in euthyroid and hypothyroid subjects (59), yet studies actually performed with hyperthyroid patients have returned contradictory results (68,69). Such inconsistent results might be explained by the important influence of the BAT assessment method on BAT volume and ^{18}F-FDG uptake estimates (70). Analyses of the association between BAT volume/^{18}F-FDG uptake, strictly following current standards, and thyroid function are needed to clarify the role of THs in the recruitment of BAT in euthyroid humans.

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AIMS

AIMS

Overall aim

The overall aim of this Doctoral Thesis is to identify lifestyle factors related to the inter-individual differences in thyroid function in young euthyroid adults, and to explore the relationship between thyroid function and cardiometabolic health in this population.

Specific aims

Chapter 1: To examine the association between lifestyle factors and thyroid function in young euthyroid adults.

Chapter 2: To investigate the association of thyroid function with body composition and cardiometabolic risk factors in young euthyroid adults.

Chapter 3: To analyse the effect of a personalized cold exposure on the circulating levels of TSH, FT4 and FT3 and analyse the association between thermoneutral and cold-induced levels of circulating TSH, FT4 and FT3 levels with BAT volume, ^{18}F-FDG uptake and mean radiodensity assessed after a 2 h cold exposure in young healthy euthyroid adults.

MATERIALS AND METHODS

METHODOLOGICAL OVERVIEW OF THE PRESENT DOCTORAL THESIS

The present Doctoral Thesis is based on three observational studies, all of them framed in the baseline data of ACTIBATE project. In this Doctoral Thesis, different analyses were performed to study the association between lifestyle factors and thyroid function (study 1), the associations between body composition and cardiometabolic risk factors with thyroid function (study 2), to study the effect of an acute cold exposure on thyroid function, and the association between thyroid function and BAT metabolism (study 3).

Table 1 show the characteristics of the studies included in the present doctoral thesis, characteristics of participant, independent and dependent variables and statistical approach used in every study.

Table 1. Methodological overview of all studies included in the Doctoral Thesis

Study	Design	Participants	Independent variables	Dependent variables	Statistical approach
Study 1	Cross-sectional	105 young euthyroid adults 71 W, 34 M Age= 22±2	Self-reported energy intake, Dietary energy density, Fat, Protein, Carbohydrates, Fibre, Ethanol, SFA, MUFA, PUFA, Cholesterol, MeD-P, MeD-S, MeD-DQI, DASH, DQI, DII. Sedentary time, LPA, MPA, VPA, MVPA, overall PA. Night onset, wake up, sleep, time, sleep efficiency, sleep quality, WASO.	Serum TSH, FT3, FT4 and Tg levels.	Simple and multiple linear regression
Study 2	Cross-sectional	106 young euthyroid adults 72 W, 34 M Age= 22±2	Serum TSH, FT3 and FT4 levels, and PTFQI.	BMI, lean mass, fat mass, VAT mass, WC, Total NAT mass Glucose, insulin, HOMA-IR, total-Cholesterol, HDL-C, LDL-C, Triglycerides, LDL-C/HDL-C ratio, TG/HDL-C ratio, TC/HDL-C ratio, APOA1, APOB, C-reactive-protein, Homocysteine, leptin, adiponectin, GGT, ALP, CMR Score, fatty liver index, systolic BP, diastolic BP, Mean BP, VO2max	Square root transformation to normalize Simple and multiple linear regressions
Study 3	Longitudinal	106 young euthyroid adults 72 W, 34 M Age= 22±2	Cold exposure	TSH, THs and PTFQI thermoneutral and cold-induced levels	Repeated measures analyses of variance Analysis of covariance
	Cross-sectional	106 young euthyroid adults 72 W, 34 M Age= 22±2	Serum TSH, FT3 and FT4 levels, and PTFQI. TSH, THs and PTFQI cold-induced levels.	BAT volume BAT SUVmean BAT SUVpeak BAT radiodensity CIT	Simple and multiple linear regressions

Abbreviations: W, Women; M, Men; BMI, Body mass index; LMI, Lean mass index, FMI, Fat mass index; ^{18}F-FDG, ^{18}F-Fluorodeoxyglucose; BAT, Brown adipose tissue; SUV, Standardized uptake value; TSH, Thyroid-Stimulating Hormone; FT4, free thyroxine; FT3, free triiodothyronine; PTFQI, Parametric Thyroid Feedback Quantile based Index; Tg, Thyroglobulin; BMI, Body mass index; LMI, Lean mass index, FMI, Fat mass index; VAT, visceral adipose tissue; NAT, Neck adipose tissue; HOMA-IR, homeostatic model assessment-Insulin resistence; HDL-C, High density lipoprotein choesterol; LDL-C, low density lipoprotein cholesterol; TG, Triglycerides; TC, total cholesterol; APOA1, Apolipoprotein A-1; APOB, Apolipoprotein B; GGT, gamma-glutamyltransferase; ALP, *Alkaline phosphatase;* CRM Score, Cardiometabolic risk score; BP, blood pressure; VO_2max, maximum oxygen consumption; WC, Waist Cincumference; MeD-P, a priori Mediterranean diet pattern; MeD-S, Mediterranean diet score; MeD-DQI, dietary quality index for a Mediterranean diet; DASH, Dietary Approaches to Stop Hypertension; DQI, diet quality index; DII, dietary inflammatory index; LPA, Light physical activity; MPA, Moderate physical activity; VPA, Vigorous physical activity; MVPA, Moderate-Vigorous physical activity; PA, Physical activity; WASO, wake after sleep onset.

THE ACTIVATING BROWN ADIPOSE TISSUE THROUGH EXERCISE (ACTIBATE) STUDY: DESIGN AND METHODOLOGY

Study design

The ACTIBATE study is a randomized controlled trial (ClinicalTrials.gov ID: NCT02365129). The Human Research Ethics Committee of both University of Granada (nº 924) and *Servicio Andaluz de Salud* (Centro de Granada, CEI-Granada) approved the study protocols and design and informed consent procedure, according to ethical guidelines of the Declaration of Helsinki (2013 revision). Participants were randomly allocated to a usual care (control), moderate-intensity exercise or vigorous-intensity exercise groups. Follow-up evaluations were carried out after 6 months of exercise intervention. All baseline examinations were performed at the Instituto Mixto Deporte y Salud (iMUDS) at the University of Granada and the Hospital Universitario Virgen de las Nieves, Granada, Spain, and by the same research personnel. Baseline evaluations were accomplished between October and November 2015 (n≈60 participants) and 2016 (n≈90 participants).

Recruitment of participant

The study was divulged on local media, social networks, and posters at the Faculties of the University of Granada. Information sessions at the different faculties were also offered. People interested contacted the research group through Facebook, Twitter or e-mail. Thereafter, they visited the research center to receive an exhaustive explanation about the study characteristics and procedures.

Subsequently, potential interested participants received a short online survey to get information about eligibility criteria: age, height, present and past (previous 3 months) weight, physical activity, medication use, current medical history, smoking, alcohol habits, and residence. Individuals who accepted to participate in the study were invited to a second information meeting. In the second visit, participants obtained a detailed written information about the study and eligible participants understood and signed the informed consent.

Participants and selection criteria

The inclusion and exclusion selection criteria of participants are showed in Table 2.

Table 2. Inclusion and exclusion criteria of ACTIBATE project (1).

Inclusion criteria	Exclusion criteria
Age: 18–25 years.	History of cardiovascular disease.
BMI: 18.5–35 kg/m^2.	Diabetes or hypertension.
Not engaged in regular physical activity >20 min on >3 days/week.	Pregnancy, or planning to get pregnant during the study period.
Not participating in a weight loss programme.	Medication for hypertension, hyperlipidemia, hyperuricemia or other illness.
Stable weight over the last 3 months (body weight changes <3 kg).	Beta blockers or benzodiazepines use.
Normal electrocardiogram.	Smoking.
Participants must be capable and willing to provide consent, understand exclusion criteria and accept the randomized group assignment.	Frequent exposure to cold temperatures.
	Taking medication for thyroid disease.
	Other significant medical conditions that are life-threatening or that can interfere with or be aggravated by exercise.
	Unwillingness to either complete the study requirements or to be randomized into control or training group.
	A first-degree relative with history of cancer.

Outcomes measures in ACTIBATE study

The main outcome measure in ACTIBATE study was BAT volume and ^{18}F-FDG. Secondarily, other variables were included, such as REE, meal-induced thermogenesis (MIT), cold-induced thermogenesis (CIT), body temperature regulation and shivering threshold, body composition and cardiometabolic disease risk factors, cardiorespiratory fitness and muscular strength, dietary habits, physical activity, sleep habits, health-related quality of life and other psychosocial variables, ad libitum energy intake and appetite-related sensations, and demographic characteristics. Biopsies from abdominal subcutaneous white adipose tissue (WAT) and skeletal muscle were also collected.

Baseline assessments of ACTIBATE study

In the first visit, participants were subjected to a thorough medical examination. Then, baseline assessments were performed in 8 different days, whose order was altered according to convenience.

- **Day 1:** After medical examination, muscle strength tests was conducted. Different online questionnaires about health and life quality were sent to the participants to fill it out at home.
- **Day 2:** First, heart rate variability was measured during 15 min (Polar RS800X, Polar Electro Öy, Kempele, Finland). Then, REE was measured by indirect calorimetry. Later, a standardized breakfast was administered to participants (50% REE, T-Diet, Vegenat®: 1.6 Kcal/ml; 47% CHO, 30% FAT; 15% PRO, 3% FIBRE) and energy expenditure was measured for other 3h and 30 minutes (2). Appetite-related sensations, thermal comfort and thermal sensation were collected through visual analogue scales (VAS), and skin temperature was also monitored during the test. Afterwards, Dual Energy X-ray Absorptiometry (DXA) scan (HOLOGIC, Discovery Wi) was performed to determinate the body composition. Finally, an ad libitum test meal was offered to participants 4 h and 15 minutes after the breakfast.
- **Day 3:** Fasting blood samples and muscle and adipose tissue biopsies were collected in the morning after an overnight fasting period.
- **Day 4:** Maximum graded exercise test was conducted under medical supervision. During the test, blood pressure, respiratory gases interchange, and electrocardiogram were collected.
- **Day 5:** Submaximal walking effort test was performed, in a fasting condition, in order to assess the Maximal fat oxidation, through a treadmill (H/P/cosmos pulsar, H/P/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany). Respiratory gas exchange was also obtained during the test.
- **Day 6:** The participants´ shivering threshold test (SST) was measured. For 30 minutes, participant rested in a warm room (22-23ºC), and then, they were entered into a mild cold room (19.5-20ºC) and were dressed in a water perfused vest (Polar Products Inc., Ohio, USA) set at ≈17ºC. After, the water temperature was slightly decreased until shivering occurred, or the test finished. The water temperature at which shivering occurred was

considered the shivering threshold. Moreover, participants filled their thermal sensation and thermal comfort (VAS).

- **Day 7:** Positron emission tomography-Computerized tomography (PET/CT) scans were conducted to determine the BAT mass and activity, and blood samples were collected at thermoneutral and after cold exposure (1h and 2 h), to explore changes on biomarkers in response to cold, at the Hospital Universitario Virgen de las Nieves (Granada, Spain).
- **Day 8:** Finally, 48-72 hours later than the PET-CT, CIT and CI-NUTox were assessed. Resting metabolic rate (RMR) was measure to the participants in a warm room (22-23ºC). Then, the participants entered into a cold room (19.7±0.4ºC), and they were dressed in the water perfused cooling vest, whose temperature was 4ºC above the individual's shivering threshold temperature (Polar Products Inc., Ohio, USA). Later, indirect calorimetry was performed for 1 hour.

In different days, participants filled several web-based questionnaires at home, which were based on collected data about dietary habits, quality of life, sleep habits, etc. In addition, physical activity and sleep related variables were measured by means of accelerometry (during 1 week), and dietary intake was assessed by three 24 h recalls and food frequency questionary (FFQ).

The flow-chart of the final participants included in the studies of this doctoral thesis is shown in **Figure 1**.

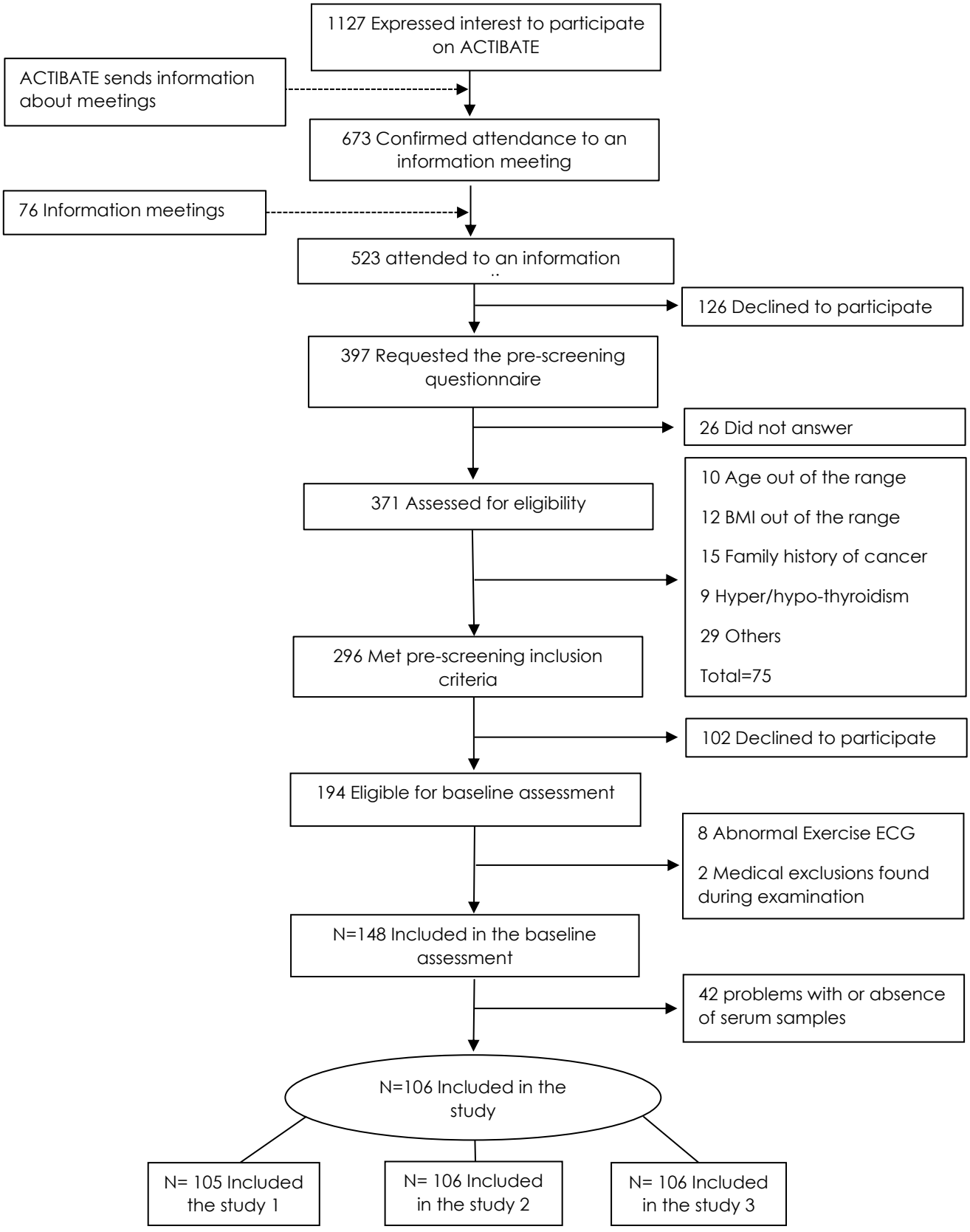

Figure 1. Flow-chart of the studies included in the present doctoral thesis. Abbreviations: BMI, Body mass index; ECG, Electrocardiogram.

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RESULTS AND DISCUSSION

CHAPTER 1:

Association between lifestyle factors and thyroid function in young euthyroid adults

ABSTRACT

Purpose: Several lifestyle factors are known to influence thyroid function. However, this relationship remains unclear in young euthyroid adults. The present work examines the associations of dietary habits, sedentarism, physical activity levels and sleep habits, with circulating thyroid-stimulating hormone (TSH) and thyroid hormones (THs) concentrations in young euthyroid adults.

Methods: A total of 105 young euthyroid adults (71 women; 22±2 years old) participated in this cross-sectional study. Serum TSH and THs concentrations were determined in fasting conditions (>6 h), using standard biochemical methods. Dietary habits were measured by a food frequency questionnaire and three non-consecutive 24 h recalls (analysed with the EvalFINUT® software), and different dietary intake and patterns were then estimated. The time spent in sedentary behaviour and different-intensities of physical activity, and sleep habits were objectively measured using a wrist-worn accelerometer that subjects wore for 7 consecutive days.

Results: Self-reported energy and carbohydrate intake were positively associated with TSH concentration, whereas fat intake was negatively associated with TSH concentration (all $P \leq 0.022$). Self-reported energy intake was also positively associated with free triiodothyronine (FT3) ($P < 0.032$). Further, adherence to the Mediterranean dietary pattern was negatively related to TSH and free thyroxine (FT4) (all $P \leq 0.020$). Time spent on vigorous-intensity and overall physical activity were negatively associated with FT4 ($P \leq 0.041$). In contrast, no associations were found between sleep parameters and TSH or THs concentrations.

Conclusions: Lifestyle factors such as dietary intake and physical activity levels, but not sleep habits, seems to be related to thyroid function in young euthyroid adults.

BACKGROUND

The thyroid gland and THs are among the main regulators of energy balance (1,2), highly influencing the basal metabolic rate (3). Importantly, thyroid dysfunction is closely related to body weight (4) and cardiometabolic alterations (2), even in euthyroid subjects. Recent evidence suggest that lifestyle might influence the THs production, and hence energy homeostasis, affecting to body composition and cardiometabolic health (3,5,6).

HPT axis activity is modulated by environmental factors, such as nutrient intake (7). During caloric restriction, thyroid function decrease, and conversely, it is restoring after refeeding (8–10). Moreover, different types of diet, such as Mediterranean diet, seem to modulate the thyroid function, even in euthyroid individuals (11–13). It is also important to note that several micronutrients are essential regulators of THs metabolism in humans (14–17). However, the heterogeneity in previous studies results prevent drawing firm conclusions (14).

On the other hand, exercise affects the HPT axis functionality, and therefore, the thyroid gland activity, modulating THs secretion (18,19). Abnormal serum THs levels also affect to the exercise tolerance, might alter the ability to perform vigorous activities. HPT axis activity is also altered by sleep deprivation, affecting to thyroid function (20,21), which translates into an increase of TSH and THs levels (22,23), and the inhibition of the TSH levels during sleep time (24). Therefore, sleep regulation seems to be closely related to thyroid function in humans.

The present work examines the associations between dietary habits, sedentarism, physical activity levels and sleep habits, and circulating TSH and THs concentrations in young euthyroid adults.

MATERIALS AND METHODS

Study design and study subjects

The study subjects were 105 young adults (34 men, 71 women) aged 22 ± 2 years (Table 3). All were enrolled in the ACTIBATE study (25), an exercise-based randomized controlled trial (ClinicalTrials.gov ID: NCT02365129). All participants were young (18-25 years old), and euthyroid adults (TSH, FT4, and FT3 levels within the normal range: 0.34-5.6 µUI/ml, 0.38-1.5 ng/dL and 2.5-4.94 pg/ml, respectively). The inclusion criteria of the study were: i) being sedentary (less than 20 minutes on less than 3 days/week of physical activity), ii) not smoking or taking any medication, iii) having had a stable body weight (changes <3 kg in the last 3 months), iv) not having acute/ chronic diseases, and v) not being pregnant. The study protocol and written informed consent were performed in accordance with the Declaration of Helsinki (revised in 2013). The study was accepted by the Ethics Committee of Human Research of the University of Granada (nº 924) and the *Servicio Andaluz de Salud* (Centro de Granada, CEI-Granada). All measurements were performed in Granada (Spain) during the months of October, November, and December of 2015, and 2016.

Table 3. Characteristics of the study participants.

	All (n=105)		Men (n=34)		Women (n=71)		P
Age	22.1	(2.1)	22.2	(2.1)	22.0	(2.1)	0.590
BMI	24.9	(4.6)	26.9	(5.5)	24.1	(3.9)	0.002
FM%	36.2	(7.2)	30.8	(7.3)	38.7	(5.8)	<0.001
Thyroid function							
TSH (µUI/ml)	1.7	(0.8)	2.0	(0.9)	1.6	(0.6)	0.004
FT4 (ng/dl)	0.9	(0.1)	1.0	(0.1)	0.9	(0.1)	0.081
FT3 (pg/ml)	3.4	(0.4)	3.5	(0.3)	3.3	(0.4)	0.029
Tg (ng/mL)	10.3	(8.2)	9.0	(6.0)	11.0	(9.1)	0.264
Self-reported dietary intake							
Energy intake (kcal/day)	1848.8	(417.8)	1991.8	(469.9)	1783.9	(377.7)	0.015
Dietary energy density (kcal/	1.5	(0.3)	1.5	(0.3)	1.5	(0.3)	0.642
Fat (g/day)	82.4	(24.0)	88.5	(28.7)	79.7	(21.1)	0.074
Fat (%EI)	40.2	(6.7)	39.8	(7.7)	40.2	(6.0)	0.789
Protein (g/day)	74.1	(18.1)	85.1	(21.4)	69.0	(13.9)	<0.001
Protein (%EI)	16.6	(3.2)	17.6	(3.3)	16.1	(3.0)	0.020
Carbohydrates (g/day)	197.7	(60.8)	207.2	(72.0)	193.4	(55.0)	0.275
Carbohydrates (%EI)	42.7	(7.4)	41.9	(7.9)	43.3	(6.9)	0.351
Fibre (g/day)	16.2	(7.0)	15.3	(4.4)	16.6	(7.9)	0.369
Ethanol (g/day)	1.3	(2.9)	1.8	(3.8)	1.1	(2.5)	0.278
SFA (g/day)	26.2	(9.8)	28.9	(11.8)	25.0	(8.6)	0.059
SFA (%EI)	12.6	(3.0)	12.8	(3.1)	12.5	(2.9)	0.645
MUFA (g/day)	37.3	(11.6)	40.4	(13.4)	35.9	(10.5)	0.061
MUFA (%EI)	18.3	(4.4)	18.3	(4.6)	18.2	(4.3)	0.891
PUFA (g/day)	12.6	(5.5)	13.5	(6.4)	12.2	(5.0)	0.237
PUFA (%EI)	6.1	(2.0)	6.1	(2.2)	6.1	(1.9)	0.986
Cholesterol (mg/day)	262.6	(108.5)	283.6	(121.9)	253.1	(101.4)	0.176
Adherence to dietary patterns							
MeD-P	24.0	(5.1)	23.0	(4.3)	24.4	(5.4)	0.182
MeD-S	4.2	(1.5)	4.0	(1.7)	4.3	(1.4)	0.363
MeD-DQI	7.0	(2.3)	7.5	(2.2)	6.7	(2.3)	0.092
DASH	24.9	(4.9)	22.2	(4.9)	26.1	(4.5)	<0.001
DQI	9.2	(2.0)	9.2	(2.2)	9.2	(2.0)	0.934
DII	-0.1	(1.5)	-0.1	(1.4)	-0.1	(1.6)	0.794
Physical Activity							
Sedentary time (min/day)	934.0	(49.4)	951.1	(53.7)	926.8	(46.1)	0.021
LPA (min/day)	22.4	(8.6)	21.5	(11.9)	22.8	(6.8)	0.474
MPA (min/day)	57.7	(21.3)	54.3	(21.2)	59.1	(21.3)	0.297
VPA (min/day)	1.5	(2.4)	1.5	(2.1)	1.5	(2.6)	0.973
MVPA (min/day)	59.2	(22.3)	55.8	(22.0)	60.6	(22.4)	0.317
Overall PA	14.9	(4.7)	14.4	(5.0)	15.2	(4.6)	0.450
Sleep habits							
Night onset (hh:mm)	01:06	(01:00)	01:18	(01:06)	01:06	(01:00)	0.367
Wake up (hh:mm)	08:48	(01:00)	09:00	(01:12)	08:48	(00:54)	0.313
Sleep time (min/day)	385.1	(45.6)	368.3	(44.1)	392.2	(44.7)	0.014
Sleep efficiency	0.9	(0.0)	0.8	(0.1)	0.9	(0.0)	0.017
Sleep quality	-5.7	(2.5)	-5.8	(2.4)	-5.6	(2.5)	0.705
WASO (min/day)	58.5	(25.9)	67.8	(36.2)	54.6	(19.2)	0.016

Data are presented as mean (standard deviation). P value is from an independent samples t-test comparing men vs women. Abbreviations: BMI, Body mass index; FM, Fat mass; TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; MeD-P, a priori Mediterranean diet pattern; MeD-S, Mediterranean diet score; MeD-DQI, dietary quality index for a Mediterranean diet; DASH, Dietary Approaches to Stop Hypertension; DQI, diet quality index; DII, dietary inflammatory index; LPA, Light physical activity; MPA, Moderate physical activity; VPA, Vigorous physical activity; MVPA, Moderate-Vigorous physical activity; PA, Physical activity; WASO, wake after sleep onset.

Thyroid function

A blood draw was taken between 8 am and 6.30 pm for determining thyroid function (no associations were found between the time of the day when the samples were collected and the TSH, FT4, FT3 and thyroglobulin (Tg) concentrations), after fasting for >6 hours and after avoiding moderate (24 previous hours) and vigorous (48 previous hours) physical activity. Blood samples were centrifuged and stored at 4 °C until analyses. To determinate the TSH, FT4, FT3, and Tg circulating levels, a Beckman Coulter DXI (33820, 33880, A13422, and 33860, respectively) chemiluminescent immunoassay system was used.

Body composition

Fat mass was determined by DXA scan (Discovery Wi, Hologic, Inc., Bedford, MA, USA) and data were analysed using the Hologic APEX 4.0.2. (Hologic, Inc., Bedford, MA, USA) software. Then, fat mass (%) was calculated. Weight and height were measured by a SECA scale and stadiometer respectively (model 799, Electronic Column Scale, Hamburg, Germany), and BMI was calculated (kg/m^2).

Dietary assessment

Dietary intake was recorded using three non-consecutive 24h dietary recalls (two working days, one non-working day), in a face-to-face interview performed by qualified and trained research dietitians. In order to improve the accuracy of food quantification, a photograph book of portion sizes was used during the interviews (26). Data were entered independently by two research dieticians in the EvalFINUT® software (https://www.finut.org/evalfinut/), which employs the USDA (United States Department of Agriculture) and BEDCA (Base de Datos Española de Composición de Alimentos) databases, and energy and daily macro/micronutrient intake were estimated. The mean of the two datasets obtained by the two independent researchers was used when the coefficient of variance (CV) was less than 5%. When the CV was greater than 5%, a third dietician analysed the data and the mean for the two datasets with the highest percentage of agreement was used. Dietary energy density was calculated by dividing the energy of foods and beverages (excluding water) by the total weight of foods and beverages consumed (expressed as kcal/g) (27). The 24h recalls were carried out within a three-week period and subjects were not informed when there were going to be performed.

In addition to the self-reported energy intake estimated with the 24 h dietary recalls, energy intake was also assessed by an *ad libitum* test meal. Subjects received a plate of refined wheat pasta with minced pork, fried tomato sauce, and virgin olive oil (45.5% carbohydrates, 38.5% fat, 16% proteins; 3% fiber, dietary energy density = 1.54 kcal/g). Four hours and fifteen minutes after consuming a standardized liquid breakfast (energy content equivalent to 50% of the measured resting metabolic rate, T-Diet Energy neutral flavour, Vegenat S. A., Badajoz, Spain), the subjects were moved into a quiet room without distractions where they found their plate (1500 g for men and 1000 g for women) and a glass of water (450 mL). Then, they were left alone and were instructed to eat as much or as little as necessary for feeling pleasantly satiated (28). Food intake was measured as the difference between the plate weight before and after the meal (in grams), and then energy intake was calculated by multiplying the grams of food consumed by their energy content.

A validated food frequency questionnaire (FFQ) of 100-food items was used to record the food frequency consumption (29). The subjects were asked to report their habitual intake frequency of each food item during the last three months. To improve measurement accuracy, a lay description of portion size was detailed for each item (e.g., cups, teaspoons, etc.). Then, each item was converted into standardized portions (weight).

Dietary patterns

A priori Mediterranean Dietary Pattern (MeD-P). The MeD-P is based on six protective dietary items (olive oil, fibre, fruit, vegetable, fish, and ethanol), and two unprotective dietary items (meat and derived products and the combination of white bread, non-whole rice, and pasta) (30). The distribution of consumption was calculated into quintiles for each item. For each protective dietary item, a score of 1 to 5 was awarded to the subjects whose intake falling into quintile in ascending order. On the contrary, for the harmful dietary items, a score of 1 to 5 was given when subject's diet fell into quintile in descending order. The total score was calculated by summing the scores of all items (30). A higher MeD-P score means greater adherence to the Mediterranean Diet.

Mediterranean Diet Score (MeD-S). The MeD-S is focused on six beneficial dietary items (fruits and nuts, vegetables, legumes, cereals, fish, and the monounsaturated/saturated fatty acid ratio), and three unfavourable dietary

items (dairy products, meat and ethanol consumption) (31). For beneficial dietary items, a value of 0 was assigned to subjects below the median (which was calculated applying the sex-specific medians), and a value of 1 was assigned to those above it. On the contrary, for the unfavourable dietary items, a value of value of 0 was assigned to subjects above the median, and a value of 1 to those below it. To subjects whose ethanol consumption was within the sex-specific range (10-50 g/day for men, and 5-25 g/day for women), one point was assigned. Conversely, the value of 0 was assigned to subjects with ethanol consumption outside of this range. The sum of the total items scores was calculated to obtain the overall score (31). A higher MeD-S score means greater adherence to the Mediterranean diet.

Diet Quality Index for a Mediterranean Diet (MeD-DQI). The MeD-DQI contemplates seven dietary items: vegetables and fruits, cereals, fish, olive oil, meat, cholesterol, and saturated fatty acids (32). The tertiles according to the consumption of each item were calculated. Scores of 0 to 2 were assigned, from the highest to the lowest intake tertile, for olive oil, fish, cereals, fruits, and vegetables. Inversely scores of 0 to 2 were assigned to meat consumption (from the lowest to the highest intake tertile). A score of 0 to 2 was assigned for cholesterol and saturated fatty acid intake (lower score for a lower consumption), according to the recommended intake guidelines (32). The sum of all items scores was calculated to obtain the overall score (32). The lower MeD-DQI score, the higher Mediterranean diet quality.

Dietary Approaches to Stop Hypertension (DASH). The DASH score takes into account eight dietary items: vegetables, fruits, legumes, low-fat dairy products, whole grains, sweetened beverages, processed and red meats, and sodium. The quintiles according to the intake of each item were calculated for each subject. To the subjects whose intake of fruits, vegetables, legumes, dairy products, and whole grains were in the first quintile, a score of 1 was assigned. On the contrary, a score of 5 was assigned to the subjects whose intake was included in the fifth quintile. For sodium, sweetened beverages, and red and processed meats, a score of 1 was awarded to the subjects whose intake was into the fifth quintile, whereas a score of 5 was awarded to those with the intake in the first quintile. The total DASH score was obtained by summing the scores of each item. A higher DASH score means better adherence to the DASH guidelines (33).

Diet Quality Index (DQI). The DQI is determined from the intake of eight dietary items: total fat, cholesterol, saturated fatty acids, fruit and vegetables, complex carbohydrates, protein, and calcium and sodium (34). Subjects were classified with a score of 0, 1, or 2 for each range of the dietary items according to the recommended guidelines of Diet and Health (34). The overall DQI score was obtained by the sum of all items. A higher DQI score represents a lower diet quality.

Dietary Inflammatory Index (DII). The DII score is based on the intake of 28 food components/nutrients, including: energy, carbohydrate, protein, fibre, alcohol, fat, cholesterol, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, β-carotene, vitamin C, vitamin D, vitamin E, niacin, thiamine, riboflavin, vitamin B6, vitamin B12, iron, folate magnesium, zinc, selenium, onion, garlic, pepper, and tea. To the subjects' DII scores calculation, the global mean intake (acquired from Shivappa et al. (35)) was obtained from recorded made to the subjects, and was divided by the standard deviation of this mean intake. Then, this value was converted to a centred percentile score. The percentile score for every food item was multiplied by its respective inflammatory effect score (35). The total DII score of each subject was acquired by summing the new values obtained. A higher DII score means a more inflammatory diet.

Sedentarism behaviour, physical activity and sleep habits

Sedentary time, physical activity and sleep time were objectively assessed for 7 consecutive days (24 h/d) by a wrist-worn accelerometer (ActiGraph GT3X+, Pensacola, FL, USA) (25). The participants were instructed to wear the accelerometer on the non-dominant wrist, only removing it when doing water activities. Participants were also instructed to note their daily wake-up time, bedtime, and the time that at which took off and on the accelerometers in a diary. The accelerometers were programmed to archive raw accelerations using an epoch length of 5 s at 100 Hz of sampling frequency (36). Once the record was over, raw accelerations were downloaded and transformed to ".csv" format using ActiLife software, version 6.13.3. (ActiGraph, Pensacola, FL, USA). Afterwards, the GGIR package (version 1.6-0; https://cran.r-project.org/web/packages/GGIR/) in R (version 3.1.2, https://www.cran.r-project.org/) was used.

The GGIR package to obtain the physical activity outcomes included (1) a signal auto-calibration of the data according to the local gravity (37); (2) an

identification of non-wear time; (3) calculation of the Euclidean Norm Minus One (ENMO); (4) detection and evaluation of sustained abnormally high values accelerations; (5) imputation of identification non-wear time and abnormal high values accelerations; (6) detection of waking and sleeping time with an automatized algorithm obtained by the participants' daily reports (37); and (7) evaluation of the time invested in sedentary behaviour and in different physical activity (PA) intensities [light (LPA), moderate (MPA), vigorous (VPA), and moderate-vigorous (MVPA) PA], calculated by age-specific thresholds for ENMO (38,39).

The mean ENMO (mG) during waking time was used to obtain a general indicator of physical activity level. The GGIR package to obtain the sleep outcomes included (1) a signal auto-calibration of the data according to the local gravity (36); (2) an identification of non-wear time; (3) evaluation of sustained abnormally high values accelerations; (4) calculation of the Euclidean Norm Minus One (ENMO); and (5) identification of waking and sleeping time by an automatized algorithm (37). Actigraphy data were used to obtain sleep efficiency (percentage of bedtime determined as sleep time), and total sleep time (total time spent in bed excluding sleep latency) (40). Moreover, the Pittsburgh Sleep Quality Index (PSQI), a validated self-reported questionnaire, was performed to determinate sleep quality (41). The total score was reversed so that lower values indicated poorer sleep quality. Finally, we classify as good sleepers the participants that obtained an overall PSQI score ≥ −5, and bad sleepers were considered those with overall score of ≤−6 (42).

Statistical analyses

Data are reported as mean and standard deviation. We conducted simple linear regressions to study the association of TSH, FT4, FT3, and Tg serum concentrations with dietary outcomes, sedentary time, physical activity, and sleep-related variables. Moreover, we also tested these associations using multiple linear regressions adjusting for fat free mass (only for the energy intake analyses), energy intake, sex and BMI. The *sex* x *TSH*/*THs*/*Tg* interaction effect on dietary intake, physical activity levels and sleep outcomes was also analysed, and exploratory analyses were performed for men and women separately when significant interaction effects were detected. The Statistical Package for Social Sciences (SPSS, v. 22.0, IBM SPSS Statistics, IBM Corporation) was used to perform the

analyses. The GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used to build the graphical plots. Statistical significance was set at P<0.05.

RESULTS

Association between dietary outcomes and thyroid function

Self-reported energy intake was positively associated with serum TSH (β<0.001, R^2=0.102, P=0.022) and FT3 (β <0.001; R^2=0.137; P=0.004) levels (Table 4), but no associations were found between energy intake on the *ad libitum* meal test and TSH or THs (Figure 2). On the other hand, carbohydrate intake was positively associated with TSH levels (β=0.005, R^2=0.129, P=0.007), whereas fat (β=-0.014, R^2=0.137, P=0.004), saturated fatty acid (SFA) (β=-0.036, R^2=0.145; P=0.003) and monounsaturated fatty acid (MUFAs) (β=-0.022, R^2=0.132, P=0.006) intake was negatively associated with TSH levels. These results persisted after adjusting for energy intake, sex and BMI (all P≤0.039; Table 4). Further, positive associations were observed between different fatty acids intake and FT3 [SFA (β<0.001; R^2=0.097; P=0.032), MUFAs (β <0.001; R^2=0.105; P=0.002), polyunsaturated fatty acid (PUFAs) (β <0.001; R^2=0.098; P=0.004) and total cholesterol intake (β <0.001; R^2=0.096; P=0.004)]. However, these results disappeared after adjusting for sex and BMI (all P≥0.297, Table 4). No associations were found between self-reported energy intake or macronutrient intake and FT4 or Tg (P≥0.170; Table 4). We detected a *Sex x FT3* interaction effect on self-reported energy, energy density, protein and alcohol intake (P< 0.029). Associations between self-reported energy and alcohol intake and FT3 were found in women (β <0.001; R^2=0.164; P=0.001 and β= -0.038; R^2=0.213; P=0.035) but not in men (P>0.291). No other *Sex x TSH/THs/Tg* interaction effect on macronutrient intake were observed.

Table 4. Associations between self-reported energy and macronutrients intake with thyroid function.

	TSH (µUI/ml)			FT4 (ng/dl)			FT3 (pg/ml)			Tg (ng/mL)		
	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P
Energy (kcal/day)												
*Model 1**	<0.001	0.102	**0.022**	-3.623	0.008	0.991	<0.001	0.137	**0.004**	-0.002	0.015	0.377
*Model 2**	<0.001	0.115	**0.039**	-9.428	0.035	0.763	<0.001	0.138	**0.004**	-0.002	0.018	0.438
*Model 3**	<0.001	0.103	**0.021**	-2.276	0.009	0.943	<0.001	0.149	**0.002**	-0.002	0.015	0.406
Dietary energy density (kcal/g)												
Model 1	0.346	0.083	0.154	-0.038	0.008	0.360	0.012	0.097	0.915	-1.920	0.015	0.463
Model 2	0.332	0.131	0.162	-0.040	0.039	0.332	0.008	0.117	0.945	-1.862	0.023	0.477
Model 3	0.356	0.109	0.139	-0.037	0.010	0.367	0.019	0.144	0.867	-1.947	0.017	0.459
Fat (g/day)												
Model 1	-0.014	0.137	**0.004**	33.608	0.001	0.966	-0.003	0.106	0.287	0.011	0.011	0.833
Model 2	-0.014	0.184	**0.004**	5.799	0.030	0.945	-0.002	0.127	0.294	0.011	0.018	0.843
Model 3	-0.013	0.152	**0.007**	8.178	0.002	0.924	-0.002	0.149	0.427	0.009	0.012	0.873
Protein (g/day)												
Model 1	-0.003	0.067	0.575	<0.001	0.003	0.618	-0.001	0.097	0.824	-0.029	0.012	0.638
Model 2	-0.009	0.135	0.124	<0.001	0.030	0.890	-0.002	0.123	0.404	-0.010	0.018	0.875
Model 3	-0.004	0.093	0.498	<0.001	0.004	0.639	-0.001	0.145	0.698	-0.027	0.014	0.659
Carbohydrate (g/day)												
Model 1	0.005	0.129	**0.007**	-7.912	0.001	0.823	0.001	0.103	0.395	<0.001	0.010	0.993
Model 2	0.006	0.197	**0.002**	9.396	0.030	0.979	0.001	0.127	0.276	-0.003	0.018	0.890
Model 3	0.005	0.147	**0.010**	-9.155	0.003	0.797	0.001	0.148	0.498	0.001	0.012	0.980
Fiber (g/day)												
Model 1	-0.010	0.072	0.379	-0.003	0.019	0.170	-0.005	0.104	0.362	0.077	0.014	0.515
Model 2	-0.006	0.117	0.584	-0.002	0.042	0.258	-0.004	0.121	0.487	0.063	0.021	0.602
Model 3	-0.009	0.096	0.395	-0.003	0.020	0.175	-0.005	0.150	0.378	0.076	0.016	0.522
Alcohol (g/day)												
Model 1	-0.024	0.072	0.358	-0.003	0.005	0.493	-0.005	0.098	0.674	-0.148	0.013	0.596
Model 2	-0.031	0.127	0.226	-0.004	0.038	0.373	-0.007	0.120	0.551	-0.122	0.020	0.664
Model 3	-0.024	0.097	0.350	-0.003	0.007	0.494	-0.005	0.146	0.660	-0.147	0.014	0.598
SFA (g/day)												
Model 1	-0.036	0.145	**0.003**	-4.915	0.020	0.310	<0.001	0.097	**0.032**	-0.002	0.010	0.430
Model 2	-0.037	0.198	**0.002**	0.003	0.048	0.166	-0.001	0.117	0.870	0.029	0.018	0.828
Model 3	-0.035	0.162	**0.004**	0.003	0.023	0.146	<0.001	0.144	0.939	0.021	0.012	0.874
MUFA (g/day)												
Model 1	-0.022	0.132	**0.006**	1.076	<0.001	0.781	<0.001	0.105	**0.002**	-0.001	0.014	0.664
Model 2	-0.023	0.185	**0.004**	<0.001	0.031	0.741	-0.004	0.127	0.297	-0.051	0.021	0.557
Model 3	-0.021	0.148	**0.009**	<0.001	0.002	0.820	-0.003	0.149	0.453	-0.058	0.016	0.508
PUFA (g/day)												
Model 1	-0.006	0.066	0.745	5.043	<0.001	0.893	<0.001	0.098	**0.004**	-0.003	0.013	0.257
Model 2	-0.005	0.115	0.762	-3.640	0.030	0.990	-0.003	0.118	0.692	0.094	0.021	0.607
Model 3	-0.006	0.090	0.742	<0.001	0.002	0.972	-0.003	0.145	0.671	0.096	0.014	0.600
Cholesterol (mg/day)												
Model 1	0.001	0.072	0.378	2.095	0.013	0.536	<0.001	0.096	**0.004**	<0.001	0.046	0.921
Model 2	0.001	0.120	0.427	<0.001	0.045	0.215	-1.121	0.117	0.976	-0.016	0.053	0.058
Model 3	0.001	0.099	0.303	<0.001	0.014	0.267	8.552	0.144	0.813	-0.016	0.049	**0.048**

Linear regression analyses were performed adjusting for fat free mass (*, only for the energy intake analyses) or energy intake (for all variables except energy intake) (Model 1), model 1 and sex (Model 2) and model 1 and body mass index (Model 3). Non-Standardized β coefficient, R^2 and P values are provided. Bold values mean P<0.05. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

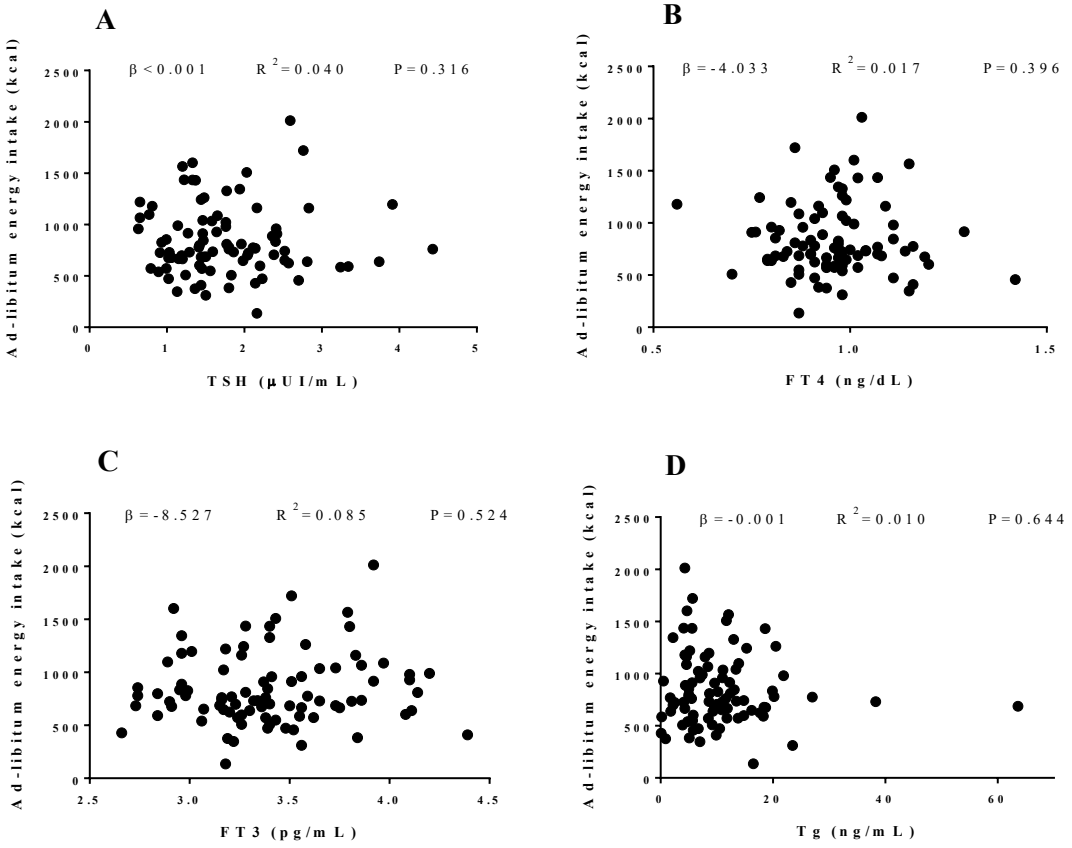

Figure 2. Associations between *ad libitum* energy intake and thyroid function. Non- Standardized β coefficient, R^2 and P value from multiple linear regression analyses adjusting for fat free mass. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin.

MED-P was negatively associated with TSH (β=-0.034; R^2=0.113; P=0.020), independently of energy intake, sex and BMI (P≤0.037, Table 5). Furthermore, MED-S was negatively associated with FT4 (β=-0.021; R^2=0.071; P=0.007) and the results also persisted after adjusting for energy intake, sex and BMI (P≤0.009, Table 5). On the other hand, no associations were found between any dietary pattern with FT3 or Tg (P≥0.051, Table 5). No *Sex* x *TSH*/*THs*/*Tg* interactions effect on dietary patterns were detected.

Table 5. Associations between dietary patterns and thyroid function.

	TSH (µUI/ml)			FT4 (ng/dl)			FT3 (pg/ml)			Tg (ng/mL)		
	β	**R^2**	**P**	**B**	**R^2**	**P**	**β**	**R^2**	**P**	**β**	**R^2**	**P**
MeD-P (A priori Mediterranean diet pattern)												
Model 1	-0.034	0.113	**0.020**	-0.002	0.007	0.392	-0.012	0.123	0.098	0.051	0.011	0.750
Model 2	-0.030	0.150	**0.037**	-0.002	0.034	0.519	-0.010	0.140	0.141	0.032	0.020	0.842
Model 3	-0.032	0.132	**0.027**	-0.002	0.009	0.411	-0.010	0.168	0.131	0.045	0.013	0.776
MeD-S (Mediterranean diet score)												
Model 1	-0.021	0.066	0.678	-0.021	0.071	**0.007**	-0.033	0.114	0.169	-0.029	0.011	0.957
Model 2	-0.014	0.111	0.773	-0.021	0.090	**0.009**	-0.031	0.128	0.192	-0.069	0.027	0.897
Model 3	-0.031	0.086	0.546	-0.022	0.071	**0.008**	-0.040	0.166	0.087	0.043	0.022	0.936
MeD-DQI (Dietary quality index for a Mediterranean diet)												
Model 1	0.062	0.093	0.079	0.009	0.023	0.128	0.012	0.094	0.468	-0.027	0.007	0.945
Model 2	0.052	0.142	0.140	0.008	0.043	0.183	0.009	0.119	0.613	0.016	0.014	0.967
Model 3	0.069	0.129	0.051	0.009	0.026	0.121	0.016	0.152	0.326	-0.041	0.009	0.916
DASH (Dietary Approaches to Stop Hypertension)												
Model 1	-0.029	0.096	0.064	-0.002	0.010	0.338	-0.007	0.104	0.370	0.062	0.013	0.716
Model 2	-0.019	0.129	0.242	-0.001	0.027	0.630	-0.004	0.116	0.626	-0.001	0.024	0.996
Model 3	-0.028	0.111	0.073	-0.003	0.011	0.332	-0.006	0.146	0.419	0.052	0.026	0.757
DQI (Diet quality index)												
Model 1	-0.054	0.082	0.170	0.006	0.008	0.373	-0.006	0.097	0.753	0.123	0.011	0.774
Model 2	-0.048	0.127	0.220	0.007	0.040	0.300	-0.004	0.117	0.840	0.094	0.018	0.826
Model 3	-0.043	0.099	0.284	0.007	0.012	0.321	0.003	0.144	0.875	0.091	0.012	0.835
DII (Dietary inflammatory index)												
Model 1	0.046	0.071	0.404	0.011	0.014	0.238	0.051	0.130	0.051	0.041	0.010	0.945
Model 2	0.034	0.118	0.538	0.010	0.040	0.308	0.048	0.145	0.070	0.096	0.018	0.873
Model 3	0.056	0.098	0.310	0.012	0.017	0.221	0.058	0.186	**0.024**	0.016	0.012	0.978

Linear regression analyses were performed, adjusting for energy intake (Model 1), energy intake and sex (Model 2) and energy intake and body mass index. (Model 3). Non- Standardized β coefficient, R^2 and P values are provided. Bold values mean P<0.05. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin.

Descriptive parameters of micronutrient intake are presented in Table 6. Selenium intake was positively associated with TSH (β=0.008; R^2=0.120; P=0.012), whereas folate and vitamin C intake were negatively related to FT3 (β=-0.001; R^2=0.149; P= 0.014 and β=-0.002; R^2=0.137; P=0.032, respectively). The results remained or were attenuated after adjusting for energy intake, sex and BMI (P≤0.057) (Table 7). No other associations were found between self-reported micronutrient intake and thyroid function (P≥0.060, Table 7). However, *Sex* x *TSH, FT3 and Tg* interactions effect was found on tiamine, Vitamin B6, Vitamin D, Niacine and vitamin B5 intake. These significant associations were observed in women, not in men, except for Niacine that was observed in male but not in female (P< 0.032).

Table 6. Self-reported micronutrient intake.

	All (n=105)		Men (n=34)		Women (n=71)		P value
Vitamin A (µg retinol eq)	692.0	(379.8)	653.3	(308.4)	709.6	(408.8)	0.476
Vitamin D (µg)	4.5	(7.9)	3.9	(4.7)	4.8	(9.0)	0.582
Vitamin E (mg a-tocoferol)	11.0	(3.8)	10.7	(3.5)	11.1	(3.9)	0.647
Biotin (µg)	0.7	(1.5)	0.8	(1.7)	0.7	(1.4)	0.708
Folate (µg)	216.0	(90.5)	209.2	(79.6)	219.1	(95.4)	0.601
Niacin equivalents (mg)	24.9	(7.8)	29.2	(9.1)	23.0	(6.3)	<0.001
Pantothenic acid (mg)	0.9	(0.8)	1.1	(0.9)	0.9	(0.7)	0.080
Riboflavin (mg)	1.6	(0.6)	1.8	(0.6)	1.5	(0.5)	0.024
Thiamine (mg)	1.5	(1.6)	1.9	(2.5)	1.3	(0.9)	0.090
Vitamin B12 (µg)	4.9	(3.8)	5.9	(5.6)	4.5	(2.5)	0.074
Vitamin B6 (mg)	2.0	(0.8)	2.3	(1.0)	1.9	(0.6)	0.007
Vitamin C (mg)	97.2	(53.0)	89.7	(46.7)	100.6	(55.6)	0.321
Calcium (mg)	697.4	(259.8)	760.0	(300.2)	669.0	(235.9)	0.090
Iron (mg)	11.1	(3.7)	12.1	(3.7)	10.7	(3.7)	0.072
Potassium (mg)	2555.5	(724.4)	2730.2	(778.8)	2476.4	(589.2)	0.090
Magnesium (mg)	246.1	(69.2)	259.4	(72.5)	240.1	(67.3)	0.179
Sodium (mg)	2011.9	(713.3)	2321.9	(727.6)	1871.4	(665.0)	0.002
Phosphorus (mg)	1151.1	(292.3)	1279.6	(357.7)	1092.8	(238.0)	0.002
Iodine (µg)	78.9	(32.5)	79.2	(34.0)	78.7	(32.1)	0.942
Selenium (µg)	91.2	(28.0)	100.4	(32.1)	87.0	(25.1)	0.019
Zinc (mg)	7.8	(2.6)	9.0	(3.3)	7.2	(1.9)	0.001

Data are presented as mean (standard deviation). P value is from an independent samples *t*-test comparing men vs women.

Table 7. Associations between self-reported micronutrient intake and thyroid function.

	TSH (µUI/ml)			FT4 (ng/dl)			FT3 (pg/ml)			Tg (ng/mL)		
	β	**R^2**	**P**	**β**	**R^2**	**P**	**β**	**R^2**	**P**	**β**	**R^2**	**P**
Vitamin A (µg retinol eq)												
Model 1	-8.287	0.066	0.688	-2.649	0.006	0.450	<0.001	0.107	0.278	-0.003	0.026	0.206
Model 2	-2.227	0.114	0.913	-1.902	0.033	0.587	-8.914	0.124	0.366	-0.003	0.037	0.164
Model 3	-6.888	0.090	0.736	-2.593	0.007	0.461	-9.748	0.153	0.312	-0.003	0.028	0.201
Vitamin D (µg)												
Model 1	-0.005	0.067	0.614	-0.002	0.021	0.142	-0.008	0.124	0.077	-0.140	0.029	0.167
Model 2	-0.004	0.116	0.684	-0.002	0.049	0.160	-0.008	0.143	0.087	-0.144	0.038	0.154
Model 3	-0.005	0.091	0.620	-0.002	0.023	0.144	-0.008	0.171	0.073	-0.139	0.032	0.167
Vitamin E (mg a-tocoferol)												
Model 1	-0.013	0.067	0.577	-0.003	0.005	0.489	-0.003	0.097	0.284	-0.083	0.011	0.734
Model 2	-0.004	0.115	0.849	-0.002	0.032	0.675	<0.001	0.117	0.977	-0.121	0.020	0.623
Model 3	-0.009	0.091	0.684	-0.003	0.006	0.514	-0.001	0.144	0.957	-0.140	0.030	0.167
Biotin (µg)												
Model 1	-0.040	0.069	0.473	0.006	0.004	0.541	-0.031	0.109	0.233	-0.024	0.010	0.968
Model 2	-0.040	0.119	0.458	0.006	0.034	0.541	-0.032	0.130	0.227	-0.022	0.018	0.971
Model 3	-0.037	0.093	0.502	0.006	0.006	0.534	-0.030	0.155	0.253	-0.032	0.012	0.958
Folate (µg)												
Model 1	-0.001	0.081	0.175	<0.001	0.034	0.060	-0.001	0.149	**0.014**	-0.005	0.013	0.618
Model 2	-0.001	0.125	0.260	<0.001	0.058	0.087	-0.001	0.163	**0.021**	-0.006	0.022	0.547
Model 3	-0.001	0.101	0.256	<0.001	0.035	0.067	-0.001	0.183	**0.029**	-0.005	0.015	0.573
Niacin equivalents (mg)												
Model 1	0.004	0.066	0.745	0.001	0.002	0.668	-0.005	0.103	0.394	-0.035	0.011	0.773
Model 2	-0.004	0.116	0.694	<0.001	0.030	0.924	-0.008	0.134	0.167	-0.003	0.018	0.983

Model 3	0.002	0.089	0.841	0.001	0.003	0.692	-0.006	0.153	0.290	-0.031	0.012	0.797
Pantothenic acid (mg)												
Model 1	0.022	0.065	0.829	0.007	0.002	0.695	-0.017	0.098	0.728	1.330	0.025	0.216
Model 2	-0.020	0.115	0.838	0.001	0.030	0.933	-0.031	0.121	0.526	1.552	0.038	0.155
Model 3	-0.002	0.089	0.987	0.006	0.003	0.737	-0.033	0.148	0.484	1.422	0.028	0.192
Riboflavin (mg)												
Model 1	-0.104	0.068	0.551	-0.011	0.002	0.710	-0.040	0.098	0.633	0.233	0.010	0.901
Model 2	-0.143	0.120	0.403	-0.016	0.033	0.588	-0.052	0.121	0.531	0.394	0.018	0.835
Model 3	-0.079	0.091	0.648	-0.010	0.003	0.738	-0.023	0.145	0.780	0.167	0.012	0.961
Thiamine (mg)												
Model 1	-0.003	0.065	0.950	-0.007	0.006	0.427	-0.011	0.098	0.664	-0.100	0.010	0.856
Model 2	-0.012	0.115	0.806	-0.008	0.038	0.347	-0.014	0.120	0.578	-0.063	0.018	0.910
Model 3	-0.006	0.089	0.907	-0.007	0.008	0.421	-0.013	0.146	0.602	-0.093	0.012	0.867
Vitamin B12 (µg)												
Model 1	-0.016	0.070	0.429	-0.002	0.002	0.648	0.008	0.102	0.436	-0.292	0.028	0.177
Model 2	-0.023	0.125	0.257	-0.002	0.035	0.485	0.006	0.120	0.557	-0.271	0.033	0.215
Model 3	-0.014	0.093	0.492	-0.001	0.004	0.669	0.009	0.152	0.337	-0.300	0.030	0.169
Vitamin B6 (mg)												
Model 1	-0.064	0.068	0.556	0.004	0.001	0.809	-0.050	0.105	0.335	-0.150	0.010	0.997
Model 2	-0.114	0.124	0.287	-0.002	0.030	0.933	-0.067	0.131	0.200	0.046	0.018	0.969
Model 3	-0.076	0.094	0.479	0.004	0.002	0.831	-0.058	0.155	0.250	-0.119	0.012	0.919
Vitamin C (mg)												
Model 1	<0.001	0.065	0.922	<0.001	0.022	0.136	-0.002	0.137	**0.032**	0.016	0.020	0.313
Model 2	0.001	0.117	0.581	<0.001	0.044	0.232	-0.001	0.148	0.057	0.014	0.025	0.393
Model 3	4.257	0.089	0.977	<0.001	0.024	0.133	-0.002	0.188	**0.021**	0.016	0.022	0.307
Calcium (mg)												
Model 1	<0.001	0.078	0.226	2.537	0.002	0.684	<0.001	0.100	0.522	0.002	0.012	0.674
Model 2	<0.001	0.078	0.226	2.269	0.031	0.713	<0.001	0.120	0.545	0.002	0.020	0.658
Model 3	<0.001	0.099	0.308	2.909	0.004	0.645	<0.001	0.152	0.336	0.001	0.013	0.711
Iron (mg)												
Model 1	-0.010	0.066	0.677	-0.008	0.031	0.074	-0.009	0.102	0.449	-0.292	0.022	0.276
Model 2	-0.013	0.117	0.593	-0.008	0.064	0.059	-0.010	0.123	0.405	-0.282	0.029	0.295
Model 3	-0.008	0.090	0.734	-0.007	0.032	0.078	-0.008	0.148	0.510	-0.298	0.024	0.269
Potassium (mg)												
Model 1	1.409	0.065	0.913	-2.433	0.012	0.266	-8.693	0.114	0.157	<0.001	0.010	0.880
Model 2	1.409	0.065	0.913	-2.518	0.043	0.244	-8.904	0.136	0.144	<0.001	0.018	0.864
Model 3	2.184	0.089	0.864	-2.403	0.014	0.274	-8.180	0.160	0.174	<0.001	0.012	0.892
Magnesium (mg)												
Model 1	-0.002	0.077	0.245	<0.001	0.025	0.112	-0.001	0.110	0.220	-0.016	0.023	0.249
Model 2	-0.002	0.126	0.244	<0.001	0.054	0.112	-0.001	0.130	0.222	-0.017	0.031	0.246
Model 3	-0.001	0.099	0.305	<0.001	0.025	0.121	-0.001	0.153	0.297	-0.017	0.026	0.234
Sodium (mg)												
Model 1	<0.001	0.076	0.266	3.021	0.018	0.179	1.682	0.097	0.791	0.002	0.030	0.151
Model 2	<0.001	0.138	0.097	2.323	0.040	0.307	-2.837	0.117	0.965	0.002	0.044	0.098
Model 3	<0.001	0.102	0.236	2.990	0.019	0.186	1.131	0.144	0.856	0.002	0.032	0.148
Phosphorus (mg)												
Model 1	<0.001	0.079	0.215	-7.674	<0.001	0.907	<0.001	0.105	0.339	<0.001	0.010	0.957
Model 2	-0.001	0.141	0.077	-3.072	0.032	0.642	<0.001	0.131	0.199	0.001	0.018	0.817
Model 3	<0.001	0.101	0.256	-5.787	0.002	0.930	<0.001	0.149	0.418	<0.001	0.012	0.980
Iodine (µg)												
Model 1	0.001	0.066	0.664	<0.001	0.011	0.308	<0.001	0.097	0.908	-0.027	0.021	0.296
Model 2	0.002	0.118	0.516	<0.001	0.038	0.371	<0.001	0.118	0.804	-0.030	0.030	0.261
Model 3	0.002	0.093	0.517	<0.001	0.011	0.325	<0.001	0.145	0.678	-0.029	0.024	0.272
Selenium (µg)												
Model 1	0.008	0.120	**0.012**	<0.001	0.006	0.450	<0.001	0.097	0.828	-0.009	0.011	0.778
Model 2	0.007	0.160	**0.021**	-0.001	0.039	0.342	-0.001	0.118	0.705	-0.006	0.018	0.849
Model 3	0.007	0.139	**0.017**	<0.001	0.008	0.430	-0.001	0.145	0.674	-0.008	0.012	0.804
Zinc (mg)												
Model 1	-0.043	0.078	0.223	0.003	0.003	0.588	0.001	0.096	0.958	-0.288	0.016	0.447
Model 2	-0.065	0.144	0.063	0.001	0.030	0.887	-0.005	0.118	0.755	-0.221	0.021	0.572
Model 3	-0.047	0.105	0.179	0.003	0.004	0.608	-0.002	0.144	0.918	-0.279	0.017	0.464

Linear regression analyses were performed, adjusting for energy intake (Model 0), energy intake and sex (Model 1) and energy intake and body mass index. (Model 2). Non- Standardized β coefficient, R^2 and P values are provided. Bold values mean P<0.05. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin.

Descriptive parameters for the frequency of food groups consumption are presented in Table 8. The consumption of vegetables, olive oil, and canned fish were negatively associated with TSH levels, which was positively related to soft drinks consumption (all P≤0.048, Table 9). Meat, processed meat, and margarine intake were positively associated with FT4 concentration, while it was negatively associated with eggs and canned fish consumption (all P≤0.031). Lastly, negative associations were found between meat, white meat and fish with Tg (all P≤0.043). All these associations remained after adjusting for energy intake, sex and BMI (Table 9). No *Sex x TSH/THs/Tg* interactions effect on food groups consumption was detected.

Table 8. Frequency of food groups consumption.

	All (n=105)		Men (n=34)		Women (n=71)		P value
Dairy products (s/d)	1,6	(1,0)	1,7	(1,0)	1,6	(1,1)	0.484
Vegetables (s/d)	2,7	(1,6)	2,3	(1,3)	2,8	(1,7)	0.108
Fruits (s/d)	2,0	(1,5)	1,7	(1,0)	2,1	(1,7)	0.199
Cereals (s/d)	2,4	(1,3)	2,6	(1,4)	2,3	(1,2)	0.271
Fat (s/d)	2,3	(1,0)	2,5	(1,3)	2,2	(0,9)	0.136
Olive oil (s/d)	2,0	(1,1)	2,2	(1,3)	1,9	(0,9)	0.353
Vegetable oils (s/w)	0,7	(1,6)	0,5	(1,0)	0,7	(1,7)	0.575
Eggs (s/w)	2,9	(2,5)	3,0	(2,8)	2,8	(2,3)	0.674
Meat (s/w)	10,6	(6,6)	13,6	(7,8)	9,3	(5,4)	0.001
White meat (s/w)	2,6	(1,7)	3,0	(1,7)	2,4	(1,7)	0.069
Red meat (s/w)	2,2	(1,7)	3,2	(1,8)	1,7	(1,4)	<0.001
Processed meat (s/w)	5,8	(5,0)	7,2	(6,5)	5,1	(4,1)	0.041
Fish (s/w)	5,1	(2,9)	4,7	(2,9)	5,3	(2,9)	0.324
White fish (s/w)	1,1	(1,1)	0,9	(1,0)	1,2	(1,1)	0.297
Blue fish (s/w)	0,4	(0,7)	0,6	(0,9)	0,4	(0,5)	0.104
Canned fish (s/w)	1,9	(1,8)	1,5	(1,3)	2,1	(1,9)	0.122
Crustacean (s/w)	0,3	(0,3)	0,3	(0,3)	0,2	(0,3)	0.399
Cephalopod (s/w)	0,4	(0,4)	0,3	(0,3)	0,	(0,4)	0.071
Decaphodos (s/w)	0,2	(0,3)	0,2	(0,2)	0,2	(0,3)	0.506
Legumes (s/w)	1,6	(1,2)	1,7	(1,3)	1,5	(1,1)	0.553
Nuts (s/w)	1,6	(1,9)	1,4	(1,7)	1,7	(2,0)	0.498
Margarine (s/w)	0,7	(2,0)	1,0	(3,2)	0,6	(1,2)	0.260
Butter (s/w)	0,6	(1,3)	1,0	(1,9)	0,5	(0,9)	0.063
Sweets (s/w)	10,0	(11.0)	11,3	(13,2)	9,5	(9,9)	0.425
Alcoholic beverages (s/w)	2,6	(3,0)	2,9	(2,4)	2,4	(3,2)	0.374
Soft drinks (s/w)	5,4	(6,8)	8,7	(8,8)	3,9	(5,0)	<0.001

Data are presented as mean (standard deviation). Abbreviations: s/d, Servings/day; s/w, Servings/week.

Table 9. Associations between frequency of food groups consumption and thyroid function.

	TSH (µUI/ml)			FT4 (ng/dl)			FT3 (pg/ml)			Tg (ng/mL)		
	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P
Dairy products (s/d)												
Model 1	0.008	0.064	0.917	0.006	0.003	0.630	0.002	0.099	0.957	-0.833	0.022	0.285
Model 2	-0.001	0.112	0.991	0.005	0.032	0.691	-0.001	0.121	0.978	-0.798	0.030	0.307
Model 3	0.025	0.089	0.732	0.007	0.005	0.585	0.014	0.150	0.681	-0.903	0.025	0.253
Vegetables (s/d)												
Model 1	-0.091	0.100	**0.048**	<0.001	<0.001	0.987	-0.004	0.099	0.853	0.103	0.011	0.838
Model 2	-0.075	0.136	0.102	0.003	0.031	0.746	0.002	0.122	0.927	0.022	0.019	0.966
Model 3	-0.106	0.134	**0.022**	<0.001	0.002	0.961	-0.013	0.152	0.563	0.141	0.013	0.782
Fruits (s/d)												
Model 1	-0.082	0.089	0.102	-0.013	0.023	0.129	-0.031	0.113	0.201	-0.482	0.018	0.375
Model 2	-0.072	0.131	0.149	-0.012	0.048	0.173	-0.027	0.133	0.257	-0.538	0.029	0.324
Model 3	-0.086	0.114	0.087	-0.013	0.025	0.127	-0.033	0.166	0.160	-0.473	0.020	0.386
Cereals (s/d)												
Model 1	-0.042	0.069	0.478	8.140	<0.001	0.994	0.011	0.100	0.702	-1.069	0.038	0.091
Model 2	-0.057	0.121	0.325	-0.002	0.031	0.855	0.006	0.122	0.831	-1.016	0.044	0.110
Model 3	-0.057	0.096	0.334	-0.001	0.002	0.957	0.001	0.149	0.974	-1.051	0.039	0.102
Fat (s/d)												
Model 1	-0.140	0.096	0.061	0.012	0.008	0.360	-0.004	0.099	0.907	-0.430	0.013	0.596
Model 2	-0.161	0.154	0.029	0.009	0.036	0.460	-0.011	0.122	0.770	-0.352	0.021	0.667
Model 3	-0.136	0.118	0.067	0.012	0.010	0.354	-0.001	0.149	0.971	-0.443	0.015	0.587
Olive oil (s/d)												
Model 1	-0.148	0.103	**0.038**	0.004	0.001	0.745	0.003	0.099	0.941	-0.246	0.011	0.752
Model 2	-0.161	0.159	**0.021**	0.002	0.031	0.845	-0.002	0.121	0.962	-0.191	0.020	0.806
Model 3	-0.139	0.122	**0.050**	0.004	0.003	0.720	0.009	0.149	0.782	-0.276	0.014	0.724
Vegetable oils (s/w)												
Model 1	0.017	0.065	0.718	-0.004	0.002	0.643	0.023	0.107	0.323	-0.244	0.013	0.637
Model 2	0.022	0.114	0.634	-0.003	0.032	0.698	0.024	0.132	0.284	-0.267	0.022	0.606
Model 3	0.008	0.088	0.860	-0.004	0.005	0.608	0.017	0.153	0.465	-0.220	0.014	0.674
Eggs (s/w)												
Model 1	0.037	0.079	0.210	-0.013	0.061	**0.012**	-0.001	0.099	0.951	-0.189	0.014	0.558
Model 2	0.034	0.124	0.244	-0.013	0.095	**0.009**	-0.002	0.122	0.888	-0.174	0.022	0.590
Model 3	0.030	0.097	0.327	-0.013	0.068	**0.009**	-0.007	0.151	0.605	-0.168	0.015	0.610
Meat (s/w)												
Model 1	-0.002	0.064	0.870	0.004	0.046	**0.031**	0.011	0.134	**0.045**	-0.262	0.055	**0.032**
Model 2	-0.011	0.121	0.336	0.003	0.058	0.089	0.009	0.143	0.115	-0.250	0.056	0.053
Model 3	-0.005	0.089	0.688	0.004	0.046	**0.035**	0.009	0.173	0.087	-0.260	0.055	**0.036**
White meat (s/w)												
Model 1	-0.039	0.071	0.396	0.009	0.012	0.271	0.039	0.127	0.072	-1.072	0.057	**0.028**
Model 2	-0.069	0.132	0.132	0.006	0.035	0.490	0.032	0.140	0.148	-1.019	0.059	**0.043**
Model 3	-0.052	0.100	0.255	0.008	0.013	0.297	0.031	0.167	0.145	-1.064	0.057	**0.032**
Read meat (s/w)												
Model 1	0.025	0.067	0.587	0.002	0.001	0.773	0.021	0.106	0.351	-0.551	0.023	0.266
Model 2	-0.020	0.114	0.680	-0.004	0.032	0.652	0.008	0.122	0.748	-0.432	0.026	0.426
Model 3	0.024	0.090	0.598	0.002	0.003	0.778	0.020	0.156	0.355	-0.548	0.024	0.270
Processed meat (s/w)												
Model 1	-0.002	0.064	0.910	0.006	0.055	**0.018**	0.012	0.125	0.087	-0.274	0.039	0.088
Model 2	-0.009	0.116	0.547	0.005	0.071	**0.039**	0.010	0.139	0.154	-0.253	0.042	0.123
Model 3	-0.005	0.089	0.745	0.006	0.055	**0.020**	0.010	0.167	0.148	-0.269	0.039	0.098
Fish (s/w)												
Model 1	-0.045	0.093	0.078	-0.007	0.025	0.111	0.005	0.100	0.670	-0.556	0.050	**0.043**
Model 2	-0.040	0.134	0.114	-0.006	0.050	0.149	0.007	0.124	0.557	-0.587	0.063	**0.034**
Model 3	-0.043	0.113	0.096	-0.007	0.026	0.119	0.007	0.152	0.542	-0.568	0.053	**0.040**
White fish (s/w)												
Model 1	-0.073	0.091	0.271	-0.002	<0.001	0.847	0.040	0.109	0.228	-0.596	0.017	0.427
Model 2	-0.064	0.122	0.331	-0.001	0.036	0.960	0.045	0.140	0.175	-0.651	0.026	0.387

Model 3	-0.076	0.097	0.254	-0.003	0.005	0.818	0.035	0.174	0.273	-0.582	0.018	0.440
Blue fish (s/w)												
Model 1	0.019	0.064	0.861	0.020	0.011	0.299	0.009	0.099	0.863	-1.668	0.030	0.158
Model 2	-0.025	0.113	0.823	0.014	0.036	0.448	-0.005	0.122	0.918	-1.520	0.035	0.206
Model 3	0.019	0.088	0.864	0.020	0.013	0.301	0.009	0.149	0.865	-1.666	0.032	0.160
Canned fish (s/w)												
Model 1	-0.090	0.106	**0.032**	-0.017	0.057	**0.015**	-0.033	0.123	0.097	-0.601	0.028	0.186
Model 2	-0.076	0.142	0.068	-0.016	0.076	**0.028**	-0.029	0.139	0.154	-0.689	0.041	0.134
Model 3	-0.081	0.121	0.055	-0.017	0.057	**0.017**	-0.026	0.164	0.187	-0.651	0.032	0.159
Crustacean (s/w)												
Model 1	0.117	0.066	0.628	0.023	0.003	0.572	-0.045	0.100	0.695	-1.858	0.016	0.473
Model 2	0.090	0.114	0.704	0.020	0.033	0.629	-0.054	0.123	0.635	-1.739	0.024	0.503
Model 3	0.109	0.090	0.649	0.023	0.005	0.580	-0.051	0.151	0.652	-1.834	0.017	0.481
Cephalopod (s/w)												
Model 1	-0.079	0.066	0.688	0.001	<0.001	0.966	0.177	0.131	0.056	-0.646	0.011	0.758
Model 2	<0.001	0.112	0.998	0.012	0.032	0.718	0.210	0.165	**0.024**	-1.031	0.022	0.629
Model 3	-0.114	0.091	0.560	-8.436	0.002	0.998	0.154	0.173	0.090	-0.551	0.013	0.795
Decaphodos (s/w)												
Model 1	0.049	0.064	0.872	-0.071	0.019	0.168	-0.015	0.099	0.919	-4.147	0.027	0.199
Model 2	0.101	0.112	0.998	-0.077	0.053	0.128	-0.031	0.122	0.829	-3.952	0.034	0.223
Model 3	0.033	0.088	0.913	-0.071	0.021	0.165	-0.026	0.149	0.853	-4.104	0.028	0.206
Legumes (s/w)												
Model 1	0.082	0.079	0.211	-0.016	0.020	0.158	-0.003	0.099	0.932	-0.190	0.011	0.787
Model 2	0.073	0.124	0.251	-0.017	0.053	0.126	-0.005	0.122	0.861	-0.153	0.020	0.828
Model 3	0.095	0.107	0.144	-0.015	0.021	0.171	0.006	0.149	0.845	-0.229	0.013	0.747
Nuts (s/w)												
Model 1	-0.054	0.081	0.181	0.008	0.012	0.271	0.013	0.103	0.485	-0.195	0.012	0.653
Model 2	-0.042	0.122	0.291	0.009	0.048	0.172	0.018	0.129	0.351	-0.255	0.023	0.559
Model 3	-0.055	0.105	0.167	0.007	0.014	0.277	0.012	0.153	0.506	-0.191	0.014	0.660
Margarine (s/w)												
Model 1	-0.005	0.064	0.866	0.021	0.108	**0.001**	-0.011	0.102	0.563	0.091	0.011	0.825
Model 2	-0.009	0.113	0.806	0.021	0.133	**0.001**	-0.012	0.125	0.514	0.107	0.020	0.793
Model 3	-0.009	0.088	0.819	0.021	0.108	**0.001**	-0.013	0.153	0.472	0.100	0.013	0.807
Butter (s/w)												
Model 1	0.078	0.081	0.172	-0.013	0.017	0.194	-0.037	0.115	0.177	-0.518	0.017	0.402
Model 2	0.061	0.123	0.284	-0.015	0.054	0.114	-0.044	0.144	0.107	-0.443	0.024	0.479
Model 3	0.073	0.103	0.198	-0.013	0.019	0.188	-0.041	0.168	0.127	-0.504	0.019	0.418
Sweets (s/w)												
Model 1	-0.004	0.066	0.619	<0.001	0.001	0.831	0.002	0.102	0.571	-0.017	0.011	0.832
Model 2	-0.004	0.115	0.586	<0.001	0.031	0.808	0.002	0.124	0.584	-0.015	0.020	0.844
Model 3	-0.001	0.088	0.872	<0.001	0.002	0.898	0.004	0.160	0.260	-0.025	0.013	0.755
Alcoholic beverages (s/w)												
Model 1	-0.039	0.087	0.118	0.001	0.001	0.815	-0.003	0.099	0.811	-0.209	0.016	0.439
Model 2	-0.044	0.141	0.070	<0.001	0.030	0.934	-0.005	0.123	0.702	-0.188	0.024	0.489
Model 3	-0.039	0.111	0.112	0.001	0.002	0.819	-0.003	0.149	0.788	-0.208	0.018	0.444
Soft drinks (s/w)												
Model 1	0.029	0.129	**0.007**	0.002	0.012	0.267	0.007	0.113	0.204	-0.074	0.014	0.540
Model 2	0.024	0.150	**0.038**	0.001	0.034	0.557	0.004	0.127	0.421	-0.041	0.020	0.744
Model 3	0.027	0.139	**0.017**	0.002	0.013	0.298	0.004	0.154	0.431	-0.065	0.015	0.599

Linear regression analyses were performed, adjusting for energy intake (Model 1), energy intake and sex (Model 2) and energy intake and body mass index. (Model 3). Non- Standardized β coefficient, R^2 and P values are provided. Bold values mean P<0.05. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin; s/d, Servings/day; s/w, Servings/week.

Associations between sedentary behaviour and physical activity with thyroid function

Time spent on vigorous and overall physical activity were negatively related to FT4 levels (β=-0.009; R^2=0.052; P=0.022 and β=-0.003; R^2=0.042; P=0.041, respectively), which was attenuated after adjusting for sex and BMI (all P≤0.104, Table 10). No associations were found between sedentary time or physical activity and TSH, FT3, or Tg (all P≥0.062, Table 10). No *Sex x TSH/THs/Tg* interaction effect on physical activity levels was detected.

Table 10. Associations between thyroid function, sedentary time, and physical activity levels.

	TSH (µUI/ml)			FT4 (ng/dl)			FT3 (pg/ml)			Tg (ng/mL)		
	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P
Sedentary time (min)												
Model 1	0.001	0.011	0.294	4.335	<0.001	0.827	-0.001	0.014	0.232	-0.014	0.013	0.253
Model 2	<0.001	0.081	0.845	-6.894	0.032	0.738	-0.001	0.089	0.043	-0.011	0.019	0.393
Model 3	0.001	0.031	0.349	3.595	0.003	0.857	-0.001	0.060	0.164	-0.014	0.014	0.267
Light PA (min)												
Model 1	-0.005	0.035	0.062	-0.001	0.033	0.070	0.001	0.007	0.422	0.009	0.001	0.763
Model 2	-0.003	0.089	0.345	-0.001	0.047	0.199	0.002	0.079	0.081	-0.003	0.012	0.933
Model 3	-0.005	0.058	0.055	-0.001	0.036	0.069	0.001	0.047	0.444	0.009	0.003	0.757
Moderate PA (min)												
Model 1	-0.004	0.026	0.106	-0.001	0.028	0.096	0.001	0.011	0.290	0.009	0.001	0.755
Model 2	-0.003	0.093	0.258	-0.001	0.049	0.172	0.002	0.072	0.122	0.003	0.012	0.912
Model 3	-0.004	0.048	0.102	-0.001	0.030	0.097	0.001	0.052	0.288	0.009	0.003	0.754
Vigorous PA (min)												
Model 1	-0.026	0.012	0.279	-0.009	0.052	**0.022**	-0.011	0.009	0.343	-0.147	0.003	0.563
Model 2	-0.014	0.084	0.570	-0.008	0.070	**0.046**	-0.006	0.052	0.589	-0.207	0.018	0.423
Model 3	-0.024	0.032	0.327	-0.009	0.053	**0.025**	-0.009	0.047	0.418	-0.155	0.005	0.544
Moderate-Vigorous PA (min)												
Model 1	-0.004	0.027	0.100	-0.001	0.033	0.069	0.001	0.008	0.366	0.006	0.001	0.813
Model 2	-0.003	0.093	0.257	-0.001	0.053	0.132	0.002	0.069	0.158	0.001	0.012	0.983
Model 3	-0.004	0.049	0.098	-0.001	0.035	0.071	0.001	0.049	0.356	0.006	0.002	0.815
Overall PA												
Model 1	-0.016	0.027	0.100	-0.003	0.042	**0.041**	0.002	0.002	0.669	0.007	<0.001	0.944
Model 2	-0.009	0.089	0.361	-0.003	0.057	0.104	0.005	0.061	0.265	-0.025	0.012	0.811
Model 3	-0.015	0.047	0.108	-0.003	0.043	**0.043**	0.002	0.043	0.615	0.006	0.002	0.955

Linear regression analyses (Model 1), and multiple regression analyses adjusting for sex (Model 2) and for BMI (Model 3) were performed. Non- Standardized β coefficient, R^2 and P values are provided. Bold values mean P<0.05. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin; PA, Physical activity.

Associations between sleep habits and thyroid function

No associations were found between sleep-related outcomes and TSH, THs, or Tg (all P≥0.145, Table 11). Likewise, no *Sex x TSH/THs/Tg* interaction effects on sleep outcomes were found.

Table 11. Associations between thyroid function and sleep habits.

	TSH (µUI/ml)			FT4 (ng/dl)			FT3 (pg/ml)			Tg (ng/mL)		
	β	**R^2**	**P**	**B**	**R^2**	**P**	**β**	**R^2**	**P**	**β**	**R^2**	**P**
Night onset (hh:mm)												
Model 1	-0.068	0.007	0.413	-0.010	0.005	0.472	-0.025	0.004	0.536	-0.367	0.002	0.673
Model 2	-0.099	0.095	0.221	-0.013	0.040	0.342	-0.036	0.058	0.359	-0.256	0.012	0.770
Model 3	-0.065	0.028	0.429	-0.010	0.007	0.480	-0.023	0.044	0.559	-0.375	0.003	0.668
Wake up (hh:mm)												
Model 1	0.066	0.006	0.424	0.016	0.013	0.249	-0.038	0.009	0.337	0.567	0.004	0.512
Model 2	0.037	0.083	0.645	0.013	0.039	0.343	-0.050	0.065	0.201	0.695	0.018	0.425
Model 3	0.064	0.028	0.440	0.015	0.015	0.255	-0.040	0.051	0.308	0.574	0.006	0.509
Sleep time (min)												
Model 1	<0.001	<0.001	0.913	<0.001	0.021	0.145	<0.001	<0.001	0.901	0.017	0.008	0.363
Model 2	0.001	0.084	0.554	0.001	0.068	0.051	<0.001	0.051	0.679	0.013	0.016	0.499
Model 3	<0.001	0.022	0.914	<0.001	0.026	0.124	<0.001	0.041	0.862	0.016	0.009	0.391
Sleep efficiency												
Model 1	-2.030	0.016	0.211	-0.126	0.002	0.635	-0.809	0.011	0.301	-16.684	0.010	0.325
Model 2	-1.058	0.085	0.510	-0.024	0.031	0.929	-0.444	0.053	0.573	-21.822	0.028	0.208
Model 3	-1.945	0.036	0.228	-0.122	0.004	0.649	-0.752	0.050	0.329	-16.949	0.012	0.319

Linear regressions (Model 1), and multiple linear regressions adjusting for sex (Model 2) and for BMI (Model 3) were performed. Non- Standardized β coefficient, R^2 and P values are provided. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; Tg, Thyroglobulin.

DISCUSSION

The results of this study show that self-reported habitual energy intake is positively associated with TSH and FT3, but energy intake in an ad libitum meal test is not related to TSH, THs or Tg in young euthyroid adults. Additionally, self-reported carbohydrate intake was positively associated with TSH, while fat intake (total fat, SFAs, and MUFAs) was negatively related to TSH levels. Moreover, adherence to the Mediterranean diet was also negatively associated with TSH and FT4 levels. On the other hand, physical activity levels were negatively associated with FT4 levels. However, no associations were found between sedentary time or sleep habits and thyroid function. These findings suggest that lifestyle factors such as dietary habits and physical activity levels might modify the thyroid function, even when circulating levels of TSH and THs are within the normal range, in euthyroid young adults.

Several studies support that decreased THs circulating levels in response to fasting is a relevant adaptive mechanism to preserve the energy during food shortage (43–45). However, the mechanisms involved in the thyroid metabolism regulation during caloric deprivation have not been clarified. In our study, we observed a positive association of self-reported energy intake with TSH and FT3, which concur with others (46). Basolo et al. (46) observed a decrease in serum TSH and FT3 concentrations in response to 36-hours fasting. Therefore, the changes produced in TSH and FT3 levels might indicate an effect of decreased energy intake on both central (TSH response) and peripheral (lower conversion of FT4 to FT3) mechanisms to preserve the energy. Nonetheless, the associations found in our study should be considered with caution, since self-reported energy intake suffers from a very relevant measurement error (47) and we failed to see any association between energy intake, assessed by an *ad libitum meal test*, and thyroid function.

Variations of diet composition, even in isocaloric conditions, can alter peripheral thyroid hormone metabolism (48,49). Although dietary fat is a crucial component of the human diet (50), excess dietary fat intake has been reported to interfere with the endocrine system (51). We observed a negative association between fat intake (total fat, SFAs, and MUFAs) and TSH levels. A similar trend was observed with FT3 although these results disappeared after adjusting for sex and BMI. This is in accordance, at least in part, with the observations by Otten et al. (49), who documented a decrease of T3 serum levels after high-fat diet intervention in

humans. The secretion of some gastrointestinal hormones has been documented to be stimulated by dietary fat, amino acids, or glucose (49), which has been speculated to mediate the relation between macronutrient intake and THs metabolism (52). However, no clear relationship between these gastrointestinal hormones and peripheral THs metabolism has yet been established (49). In addition, a high-fat diet rich in saturated and mono-unsaturated fatty acids produce hypothyroxinemia, affecting the lipid profile of the thyroid gland (53). Therefore, dietary fatty acids might influence the fatty acid pool in the thyroid gland, impacting its function.

In addition, studies analyzing the effect of low carbohydrate diets on THs levels have provided heterogeneous results (11,48,54,55), likely because energy intake was not usually controlled in these studies. A very low carbohydrate diet is known to produce alterations in thyroid hormone metabolism, decreasing the conversion of T4 to T3, and therefore, THs serum levels (56). Contrary, high carbohydrate diets prevent these changes (48,57). We observed a positive association between carbohydrate intake and TSH levels, which remained after adjusting for energy intake, sex, and BMI. Dietary carbohydrates have been demonstrated to increase the sympathetic nervous system activity (58). Therefore, it is plausible that a high carbohydrate intake could influence the central nervous system activity, stimulating the hypothalamic-pituitary-thyroid axis and increasing the TRH production. Consequently, a rise in TSH levels production might occur, followed by a higher conversion of T4 to T3.

Adherence to the Mediterranean diet has been shown to reduce the risk of suffering cardiometabolic diseases (59). Some reports in the literature have described goitrogenic foods that can affect thyroid function by inhibiting the synthesis of THs (60), such as nuts and vegetables, and specifically cruciferous, or greens, which are consumed abundantly in the Mediterranean diet. In this study, we observed a negative association between adherence to the Mediterranean diet, assessed by two different dietary patterns, and TSH and FT4 levels. We also observed similar associations with some food groups related to Mediterranean dietary pattern such as olive oil and vegetables. These observations are in agreement with the ones reported by Zupo et al. (13), who demonstrated that higher adherence to the Mediterranean diet was independently associated with a reduced thyroid function in euthyroid subjects.

Several micronutrients such as selenium, zinc, iron, copper, vitamin A, Vitamin C, and folate, are involved in regulating thyroid function (14). We found a positive association between selenium intake TSH levels, and a negative association between folate and vitamin C intake with FT3 levels. These results are in agreement with the previous evidence that support that several micronutrients are involved in the regulation of THs synthesis and secretion, and protecting the thyroid gland from oxidative stress, interacting with iodine during conversion of the T4 hormone to the metabolically active T3 hormone (14,15,61). However, our results might seem paradoxical since deficiencies of multiple micronutrients such as iodine, iron, zinc, vitamin A, and folate, seems to exacerbate iodine deficiency and contribute to altered thyroid function. Nevertheless, the paradoxical results and the lack of association between iodine intake and thyroid function, may be due to the limitations of self-reported intake methodology being the urinary iodine concentration the barometer recommended to assess the iodine status (62).

THs have an important effect on numerous systemic actions including muscular and cardiorespiratory function (63). It has been extensively reported that the hypothalamic-pituitary-adrenal axis is stimulated in response to physical activity, and different exercise intensities were also demonstrated to have an important role on thyroid function (19). However, there are few studies exploring the relationship between physical activity levels and thyroid function in euthyroid adults. We observed a negative association between vigorous and overall physical activity levels and FT4 concentrations. These results contrast with those reported by Roa Dueñas et al (63), who found no associations between physical activity and TSH or FT4 levels in adults. More studies are needed to clarify the lack of association between physical activity levels and thyroid function in euthyroid individuals.

TSH and THs secretion can be affected by sleep-wake state (23). In fact, subjects with hyperthyroidism or hypothyroidism suffer numerous sleep disorders, such as insomnia (64). Kim et al. (64), observed an increased risk of subclinical hyperthyroidism (suppression of TSH secretion) in short sleepers, evaluated by a self-reported questionnaire, which contrasts with the lack of association between sleep habits and thyroid function observed in our study. This discrepancy might be explained by the different methodology used to measure the sleep duration, because sleep deprivation was not performed in this study, and due to the

participants involved in our study were euthyroid, while in the studies that observed associations between THs and sleep habits, a relatively sleep deprivation was applied to the participants. Moreover, the accelerometers use could be underestimating/overestimating the sleep outcomes, as compared to polysomnography, which is the gold-standard method to properly assess sleep duration and efficiency.

Limitations

These results need to be interpreted with caution because some limitations are present. First, it is a cross-sectional study and, consequently, no causality can be established. Additionally, only euthyroid young adults are involved in the study, so the results are not extrapolatable to older, younger, or people with thyroid disorders. In addition, blood samples were taken at different times of the day, and due to the circadian variations of THs levels, part of our results could be explained by these variations throughout the day. Moreover, despite the largest proportion of the study sample were women, we did not control the menstrual cycle, which might have influenced the results observed. Furthermore, the self-reported dietary intake presents a large measurement error, so the risk of underreporting or misclassification needs to be considered.

Conclusion

In summary, self-reported energy and carbohydrates intake are positively associated with thyroid function, while lipid intake and adherence to the Mediterranean diet are negatively associated with thyroid function, even in young euthyroid adults. Overall and vigorous physical activity are also negatively related to thyroid function, and no relationship seems to exist between sedentarism and sleep habits and thyroid function in young euthyroid adults.

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CHAPTER 2:

Circulating concentrations of free triiodothyronine are associated with central adiposity and cardiometabolic risk factors in young euthyroid adults

ABSTRACT

Purpose: Thyroid dysfunction is associated with classic cardiometabolic risk factors in humans. However, this relationship remains unclear in young euthyroid adults. The present work examines the associations of circulating thyroid hormones (THs) and thyroid-stimulating hormone (TSH) concentrations with body composition and cardiometabolic risk factors in young euthyroid adults.

Methods: A total of 106 sedentary, euthyroid adults (72 women; 22±2 years old) participated in this cross-sectional study. THs and TSH serum concentrations were determined in fasting conditions (6 h). Body composition (Fat mass (FM), lean mass (LM) and visceral adipose tissue (VAT)) was determined by dual-energy X-ray absorptiometry, anthropometric parameters (weight, height and waist circumference) were measured, and neck adipose tissue mass was quantified trough computed tomography (CT) scanning. Cardiometabolic risk factors including fasting glucose and lipid metabolism markers, hepatic phosphatase and transaminases and blood pressure were also assessed.

Results: Free triiodothyronine (FT3) concentration was positively associated with body mass index, LM, VAT and waist circumference (all $P \leq 0.038$). FT3 was also associated with glucose, insulin, HOMA-IR, fatty liver index, and blood pressure (all $P < 0.024$). All the associations were attenuated when adjusting for sex. In contrast, we found no associations of TSH or free thyroxine with any body composition parameter or cardiometabolic risk factors.

Conclusions: FT3 is associated with central adiposity and cardiometabolic risk factors including insulin resistance, fatty liver index and mean, systolic and diastolic blood pressure in young euthyroid adults.

BACKGROUND

Obesity is related to increased risk of developing cardiovascular disease (1). Thyroid function plays an important role on energy homeostasis and cardiovascular health (2), and consequently, several studies have investigated its relationship with obesity and cardiometabolic risk factors in euthyroid subjects with contrary results (3–6). Moreover, despite thyroid function has been related to classic cardiometabolic risk factors in euthyroid adults (7), whether it is also true in young euthyroid adults (18-25 years) remains to be investigated.

This study investigated the association of circulating THs and TSH concentrations with body composition and cardiometabolic risk factors in young euthyroid adults.

MATERIALS AND METHODS

Participants

A total of 106 young adults (34 men, 72 women) aged 22 ± 2 years were included in this cross-sectional study (Table 12). All of them participated in the ACTIBATE study (8), an exercise-based randomized controlled trial (ClinicalTrials.gov ID: NCT02365129), from which baseline data were obtained to conduct this secondary analysis. All participants were young (18-25 years old) euthyroid adults (FT3, FT4 and TSH levels within the normal range: 2.5-4.94 pg/ml, 0.38-1.5 ng/dL and 0.34-5.6 µUI/ml and, respectively) who reported: i) being sedentary (less than 20 minutes on less than 3 days per week of physical activity) ii) having a stable body weight (changes < 3 kg in the last 3 months), iii) not being smoker, iv) not having acute or chronic diseases, and v) not being pregnant. The study protocol and written informed consent were performed in accordance with the Declaration of Helsinki. Ethics Committee of Human Research of the University of Granada (nº 924) and the *Servicio Andaluz de Salud* (Centro de Granada, CEI-Granada) approved the study. For logistic reasons, the measurements were conducted in eight evaluation waves, which means that participants were measured in different groups during the months of October, November and December in 2015 and 2016.

Table 12. Characteristics of participants.

	All (n=106)		Men (n=34)		Women (n=72)		P
Age (years)	22.0	(2.1)	22.2	(2.1)	22.0	(2.1)	0.546
Thyroid hormones							
FT3 (pg/mL)	3.4	(0.4)	3.5	(0.3)	3.3	(0.4)	0.029
FT4 (ng/dL)	0.9	(0.1)	1.0	(0.1)	0.9	(0.1)	0.077
TSH (µUI/mL)	1.7	(0.8)	2.0	(0.9)	1.6	(0.6)	0.004
PTFQI	0.2	(0.3)	0.3	(0.2)	0.2	(0.2)	0.002
Body composition and anthropometric measurements							
Weight (kg)	70.6	(16.6)	83.7	(17.3)	64.7	(12.5)	<0.001
Height (cm)	167.7	(8.9)	176.3	(6.9)	163.8	(6.7)	<0.001
BMI (kg/m²)	24.9	(4.6)	26.9	(5.5)	24.0	(3.8)	0.002
Lean mass (kg)	41.4	(9.8)	52.8	(7.4)	36.3	(5.4)	<0.001
Fat mass (kg)	25.2	(8.8)	25.5	(10.9)	24.9	(7.7)	0.511
Fat mass (%)	36.2	(7.2)	30.8	(7.2)	38.6	(5.7)	<0.001
VAT mass (g)	340.6	(174.5)	432.4	(180.9)	299.6	(156.0)	<0.001
WC (cm)	80.9	(13.8)	89.8	(15.1)	76.8	(11.1)	<0.001
Total NAT mass (g)	4.0	(1.9)	4.3	(2.4)	3.8	(1.6)	0.231
Cardiometabolic risk factors							
Glucose (mg/dL)	87.5	(6.7)	89.6	(7.7)	86.5	(6.0)	0.027
Insulin (µUI/mL)	8.5	(5.0)	9.5	(6.7)	8.1	(4.0)	0.196
HOMA-IR	1.9	(1.3)	2.2	(1.7)	1.8	(1.0)	0.124
Total-Cholesterol (mg/dL)	164.5	(29.4)	163.4	(32.4)	164.9	(28.2)	0.806
HDL-C (mg/dL)	52.3	(11.0)	45.3	(7.9)	55.5	(10.8)	<0.001
LDL-C (mg/dL)	96.2	(24.6)	99.8	(27.1)	94.5	(23.4)	0.302
Triglycerides (mg/dL)	83.2	(47.5)	91.4	(48.3)	79.5	(47.0)	0.228
LDL-C/HDL-C ratio	1.9	(0.7)	2.3	(0.8)	1.8	(0.5)	<0.001
TG/HDL-C ratio	1.7	(1.3)	2.2	(1.6)	1.5	(1.0)	0.003
TC/HDL-C ratio	3.2	(0.9)	3.7	(1.1)	3.0	(0.6)	<0.001
APOA1 (mg/dL)	144.5	(27.8)	128.2	(18.1)	151.8	(28.4)	<0.001
APOB (mg/dL)	70..4	(20.3)	73.9	(25.1)	68.8	(17.8)	0.239
C-reactive-protein (mg/L)	2.5	(3.3)	2.1	(2.3)	2.7	(3.6)	0.406
Homocysteine (µmol/L)	10.9	(3.4)	12.8	(3.9)	10.0	(2.7)	<0.001
Leptin (µg/l)	6.2	(4.0)	4.7	(4.0)	6.9	(3.9)	0.006
Adiponectin (mg/l)	11.1	(8.0)	7.6	(5.7)	12.7	(8.4)	0.002
GGT (U/L)	18.8	(18.9)	28.9	(30.8)	14.1	(4.7)	<0.001
ALP (U/L)	72.5	(18.9)	79.7	(19.5)	69.2	(17.9)	0.007
CMR Score	0.0	(0.7)	-0.0	(0.7)	0.0	(0.6)	0.839
Fatty liver index	20.5	(24.7)	36.8	(31.7)	12.8	(15.5)	<0.001
Systolic BP (mmHg)	116.2	(11.7)	124.7	(11.3)	112.4	(9.7)	<0.001
Diastolic BP (mmHg)	71.0	(7.5)	72.8	(8.8)	70.1	(6.8)	0.094
Mean BP (mmHg)	85.9	(8.0)	89.9	(8.6)	84.1	(7.1)	<0.001
VO_2max (ml/kg/min)	40.9	(8.1)	44.8	(8.8)	39.1	(7.1)	0.001

Data are presented as mean and standard deviation. P value is from an independent samples *t*-test comparing men vs women. Abbreviations: FT3, free triiodothyronine; FT4, free Thyroxine; TSH, Thyroid-Stimulating Hormone; PTFQI, Parametric Thyroid Feedback Quantile based Index; BMI, Body mass index; LMI, Lean mass index, FMI, Fat mass index; VAT, visceral adipose tissue; NAT, Neck adipose tissue; HOMA-IR, homeostatic model assessment-Insulin resistence; HDL-C, High density lipoprotein choesterol; LDL-C, low density lipoprotein cholesterol; TG, Triglycerides; TC, total cholesterol; APOA1, Apolipoprotein A-1; APOB, Apolipoprotein B; GGT, gamma-glutamyltransferase; ALP, *Alkaline phosphatase;* CRM Score, Cardiometabolic risk score; BP, blood pressure; VO_2max, maximum oxygen consumption; WC, Waist Circumference.

Thyroid function

A blood draw was taken after a 6-hour fast and after avoiding moderate (within 24 hours) and vigorous (within 48 hours) physical activity in the previous days. The blood draw took place between 8am and 6.30pm (no association was found between the time of the day when the samples were collected and the concentrations of FT3, FT4 and TSH, data not shown). Upon collection, blood was centrifuged and stored at 4 °C until analyses. FT3, FT4 and TSH circulating levels were determined using a Beckman Coulter DXI (33880) chemiluminescent immunoassay system. In addition, we calculated the PTFQI – an indicator of resistance to thyroid hormones - which oscillates between -1 to 1 (9). Hence, negative values of this index indicate low TSH values by high inhibition of FT4, which indicates high sensitivity to FT4. In contrast, positive PTFQI values indicate high TSH by low inhibition of FT4, which means low sensitivity to FT4. Therefore, PTFQI evaluate the set point of the central regulation of THs concentration. PTFQI was calculated following Excel spreadsheet formula created by Laclaustra et al. (9).

Body composition and anthropometric measurements

A whole-body DXA scan (Discovery Wi, Hologic, Inc., Bedford, MA, USA) was used to determine FM, LM and VAT mass. Data were obtained from the Hologic APEX 4.0.2. (Hologic, Inc., Bedford, MA, USA) software. Weight and height of participants were measured without shoes and wearing light clothes using a SECA scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany), and body mass index was calculated (kg/m2). Waist circumference (cm) was measured twice at the minimum perimeter, or at the medium point between the inferior rib and the iliac crest when the minimum perimeter was not apparent, with an inextensible metallic tape, in the standing position, and the average value were considered for further analyses.

NAT volume was determined by the analyses of the CT being part of a PET/CT [Siemens 16 PET/CT scanner (Siemens, Erlangen, Germany)] that was carried out in the ACTIBATE study for quantifying brown adipose tissue volume (8). The CT scans were performed from the atlas vertebra to the mid chest region, and all of them were analysed using the FIJI software (http://sourceforge.net/projects/bifijiplugins/)(10). NAT volume was determined at the level of C5, as previously described (11). We obtained the total NAT

volume applying a radiodensity range of -300 to -10 Hounsfield Units. NAT volume was then multiplied by a fat density coefficient of 0,9 g/ml to obtain the total NAT mass (12).

Circulating cardiometabolic risk markers

Blood samples for determining cardiometabolic risk factors were obtained in the morning after an overnight fast (>10 hours), in resting conditions. Blood was later centrifuged, and serum aliquots were stored at -80 °C until analyses. Fasting blood glucose concentration was determined by Beckman Coulter reagent OSR6521 in a Beckman Coulter AU5832 analyzer. Insulin concentration was determined by Chemiluminescent immunoassay of Beckman Coulter (33410) with a DXI analyzer. The homeostatic model assessment index of insulin resistance (HOMA-IR) was calculated (13). Concentrations of total cholesterol, triglyceride, Apolipoprotein A (APO A), and high-density lipoprotein cholesterol (HDL-C) were obtained with routine enzymatic methods with a Beckman Coulter AU5832 analyzer. To determinate low-density lipoprotein cholesterol (LDL-C) concentration, the Friedewald formula was used (14). Lipoprotein ratios (LDL-C/HDL-C, TG/HDL-C and TC/HDL-C) were calculated as markers of dyslipidemia (15).

To measure the concentrations of C-reactive protein (CRP) Beckman Coulter reagent OSR6299 was used with an immunoturbidimetric method and then samples were processed in a Beckman Coulter AU5833 analyzer. Reagent from Axis-Shield Diagnostics Ltd (B08176) was used in a colorimetric method to determinate homocysteine concentration in Beckman Coulter AU5833 analyzer. The Beckman Coulter 447730 reagent was used with an immunoturbidimetric method to determine the Apolipoprotein B (APO B) levels in the Beckman Coulter AU5833 analyzer. To measure gamma-Glutamiltransferasa (GGT) and Alkaline phosphatase (ALP) concentrations, Beckman Coulter reagent was used with a colorimetric method of International Federation of Clinical Biochemistry (IFCC) in a Beckman Coulter AU5832 analyzer. Adiponectin concentration was determined with the MILLIPLEX MAP Human Adipokine Magnetic Bead Panel 2 (Catalogue # HADK2MAG-61K; Intra-assay CV= 9%) and leptin concentration was determined with the MILLIPLEX MAP Human Adipokine Magnetic Bead Panel 1 (Catalogue # HADK1MAG-61K; Intra-assay CV= 9%), both using Luminex MAP Technology platform (Luminex Corporation, Austin, TX, USA).

Blood pressure

Blood pressure was assessed with an automatic sphygmomanometer Omrom M2 (Omron Healthcare, Kyoto, Japan) on three different days, while the participant rested seated. The average of systolic and diastolic pressure on the three different days was calculated. Later, mean blood pressure (MBP) was calculated as follow (16):

$$MBP = Diastolic\ blood\ pressure + 0.33 \times (Sistolic\ blood\ pressure - Diastolic\ blood\ pressure).$$

Combined cardiometabolic risk factors

Clustered score for cardiometabolic risk factors

A clustered score for cardiometabolic risk factors, based in diagnostic criteria of metabolic syndrome, was calculated (17). Firstly, sex-specific Z-scores were obtained for WC, MBP, HDL-C, triglycerides, and glucose. The Cardiometabolic risk factors score was the average of the inverse Z-score of HDL-C and Z-scores of triglycerides, glucose, WC and MBP.

Fatty liver index

The fatty liver index (FLI) was calculated with the following formula proposed by Bedogni et al. (18):

$$FLI = (e^{0.953*\log e\ (\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e\ (\text{GGT}) + 0.053*\text{waist circumference} - 15.745})/(1 + e^{0.953*\log e\ (\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e\ (\text{GGT}) + 0.053*\text{waist circumference} - 15.745}) \ x\ 100$$

Cardiorespiratory fitness assessment

Maximum oxygen consumption (VO2max) was determined by an incremental maximal graded treadmill (H/P/Cosmos Sport & Medical GMBH, Germany) walking test applying the modified Balke protocol (19,20). Participants did not consume stimulants 24 hours before the test, fasted 3 to 5 hours and did not perform vigorous or moderate physical activity (48-h and 24-h, respectively) before the test (8,21). The gas exchange was continuously registered by indirect calorimetry (Ultima CardiO2 cart, Medgraphics Corp, Minnesota, USA) with an oronasal mask (model 7400, Hans Rudolph Inc, Kansas City, MO, USA). VO_2 was averaged every 5 s employing the Breeze Suite software (version 8.1.0.54 SP7,

MGC Diagnostic®) and the highest observed VO_2 after removing apparent artifacts were defined as the VO_2max.

Statistical analyses

Data are reported as mean and standard deviation. To study the association of FT3, FT4 and TSH, and serum levels and PTFQI, with body composition parameters and cardiometabolic risk factors, we conducted simple linear regressions. All variables were transformed (square root transformation), except PTFQI, to make normalize its distribution. Moreover, we also tested these associations using multiple linear regressions adjusting for sex. The sex x TSH/THs interaction effect on body composition/cardiometabolic risk factors was also analysed for all the associations. Error alpha propagation by multiple comparisons correction (Benjamini-Hochberg procedure (22)) was performed. The Statistical Package for Social Sciences (SPSS, v. 22.0, IBM SPSS Statistics, IBM Corporation) was used to perform the analyses. The GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used to build the graphical plots. Statistical significance was set at $P<0.05$.

RESULTS

FT3 was positively associated with BMI (β=0.201; R^2=0.041; P=0.038), LM (β=0.244; R^2=0.059; P=0.014), VAT mass (β=0.240; R^2=0.058; P=0.016) and WC (β=0.221; R^2=0.049; P=0.025) (Figure 3). Despite there were not sex interaction effects, the associations between FT3 and VAT were attenuated after adjusting for sex (β=0.174; R^2=0.150; P=0.072), and the association with BMI, WC or LM disappeared (all P>0.13) (data not shown).

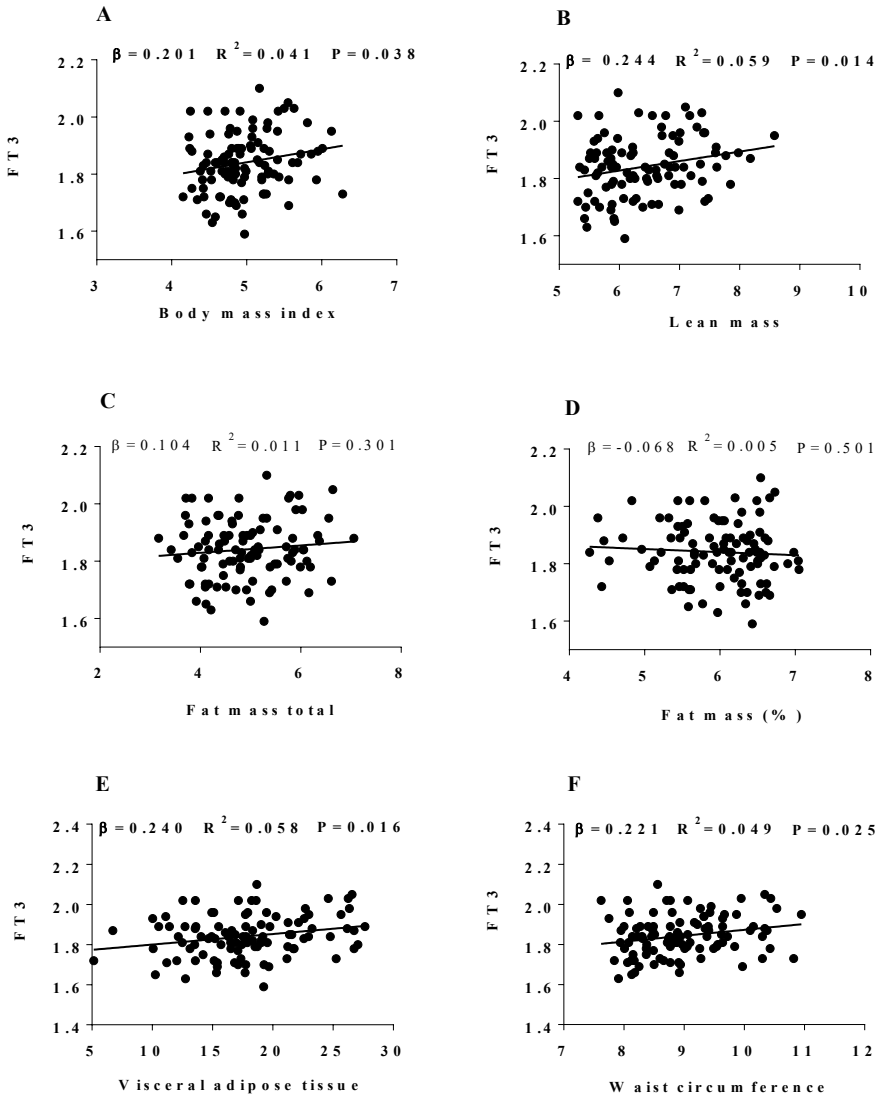

Figure 3. Association between serum levels of free triiodothyronine (FT3) and body composition parameters. Standardized β coefficient, R^2 and P value from linear regression analyses. All variables were square root transformed (SQRT).

In contrast, FT4 was not associated with any body composition parameter (all P>0.080) (Figure 4).

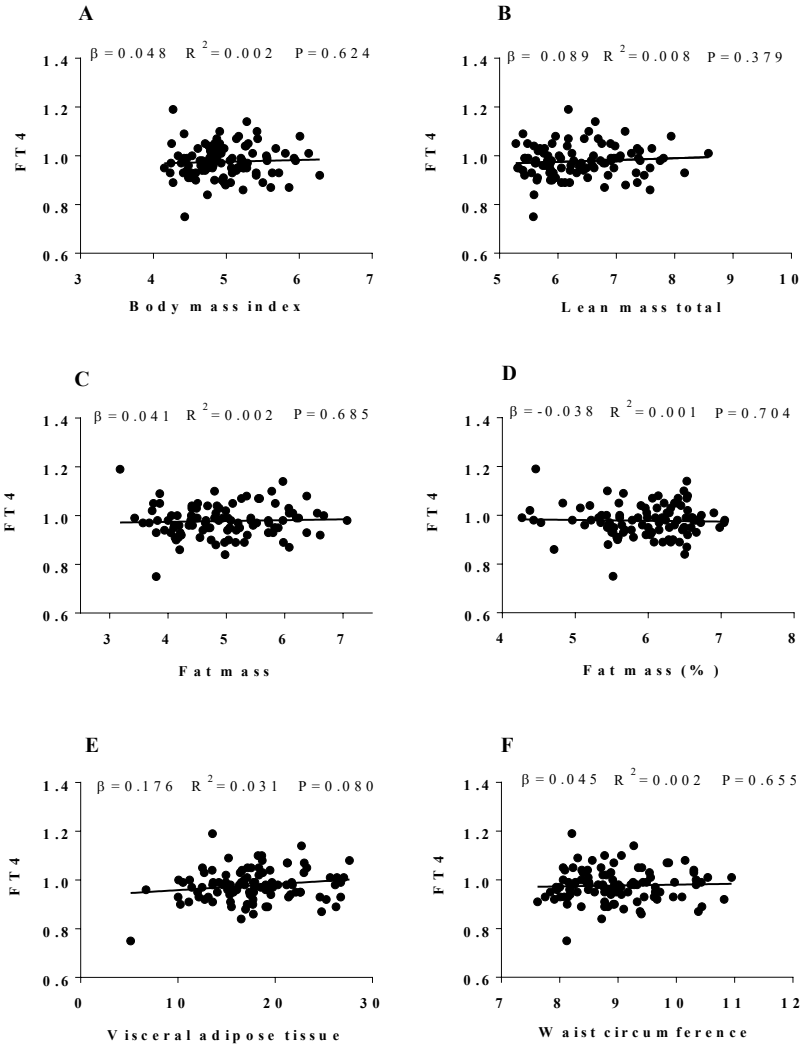

Figure 4. Association between serum levels of free thyroxine (FT4) and body composition parameters. Standardized β coefficient, R^2 and P value from linear regression analyses. All variables were transformed with square root transformation (SQRT).

TSH was positively associated with LM (β=0.268; R^2=0.072; P=0.007) (Figure 5), but the association disappeared after adjusting for sex (β=0.072; R^2=0.619, P=0.673, data not shown).

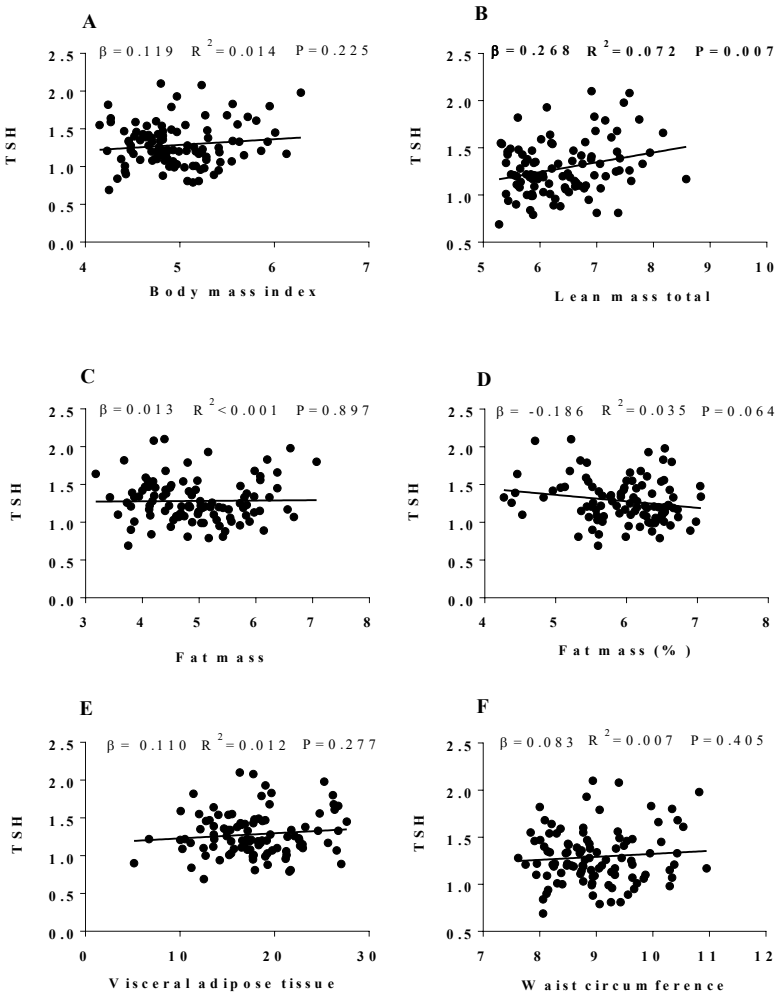

Figure 5. Association between serum levels of Thyroid-Stimulating Hormone (TSH) and body composition parameters. Standardized β coefficient, R^2 and P value are from linear regression analyses. All variables were transformed with square root transformation (SQRT).

PTFQI was also positively associated with LM (β=0.243; R^2=0.059; P=0.015) and VAT mass (β=0.210; R^2=0.044; P=0.036 (Figure 6), but these associations disappeared after adjusted for sex (P>0.271, data not shown).

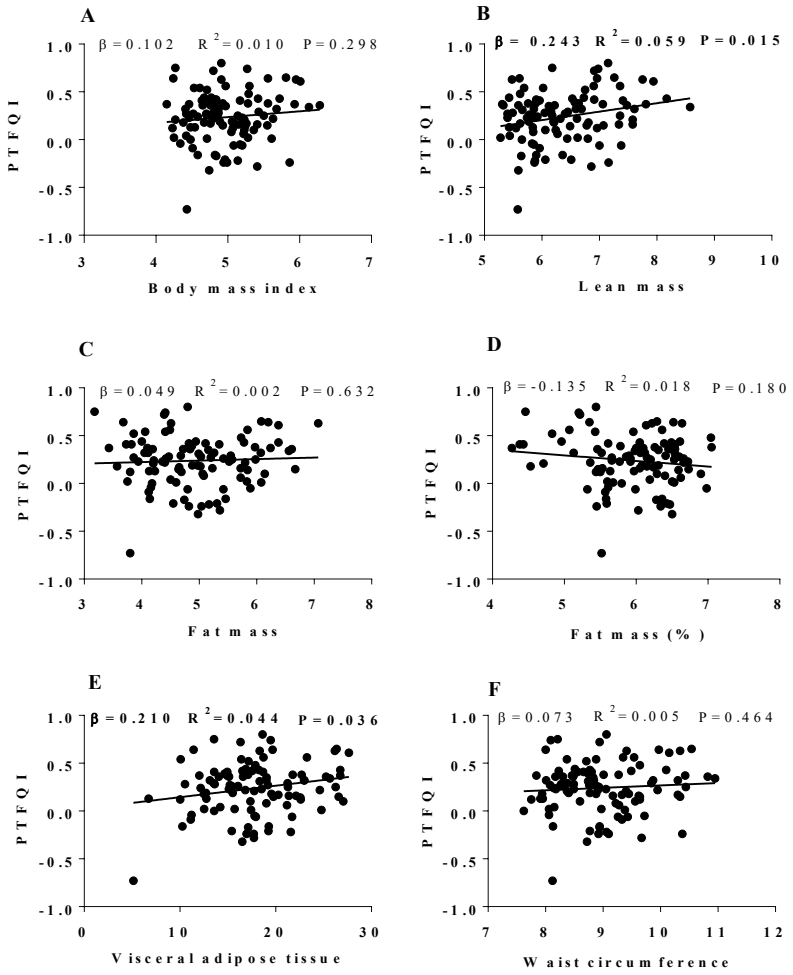

Figure 6. Association between Parametric Thyroid Feedback Quantile based Index (PTFQI) and body composition parameters. Standardized β coefficient, R^2 and P value from linear regression. All variables were transformed with square root transformation (SQRT), except PTFQI.

No associations were found between FT3, FT4, TSH, or PTFQI and total NAT mass (Figure 7). The association analyses in men and women separately can be found in table 13.

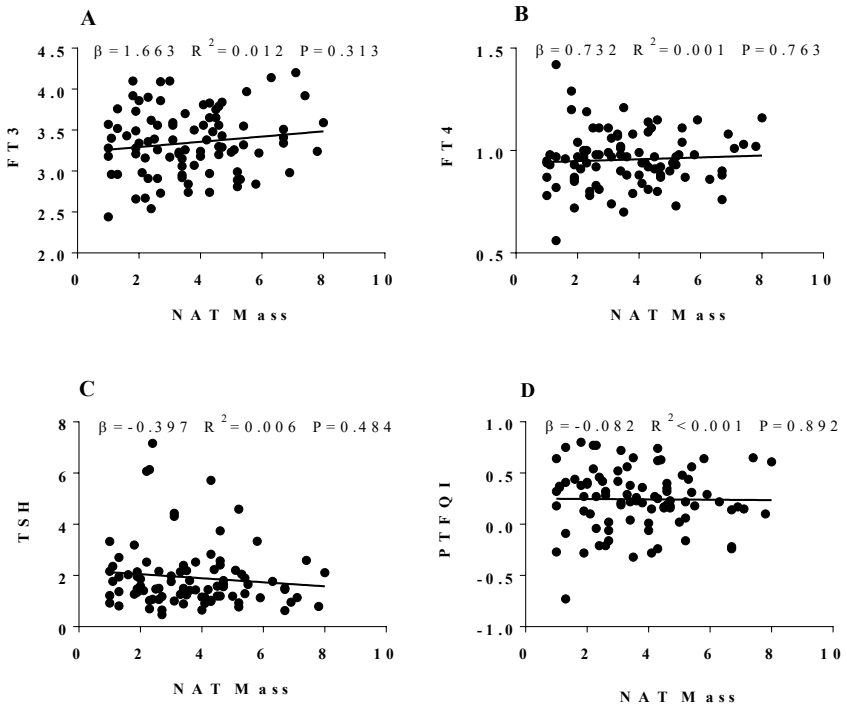

Figure 7. Association between serum levels of thyroid hormones and Parametric Thyroid Feedback Quantile based Index (PTFQI) with neck adipose tissue (NAT) mass. Standardized β coefficient, R^2 and P value from linear regression. All variables were transformed with square root transformation (SQRT), except PTFQI. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, Free Thyroxine; FT3, Free Triiodothyronine.

Table 13. Association between serum levels of thyroid hormones and Parametric Thyroid Feedback Quantile based Index (PTFQI) with body composition by sex.

	FT3 (pg/mL)						FT4 (ng/dL)						TSH (µUI/mL)						PTFQI					
	Men			Women			Men			Women			Men			Women			Men			Women		
	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P
BMI	0.059	0.003	0.741	0.201	0.040	0.091	-0.226	0.051	0.198	0.136	0.018	0.256	0.191	0.036	0.279	-0.070	0.005	0.559	0.033	0.001	0.852	-0.005	<0.001	0.968
Lean mass	0.101	0.010	0.581	0.141	0.020	0.251	-0.290	0.084	0.107	0.080	0.006	0.519	0.096	0.009	0.600	0.006	<0.001	0.961	-0.030	0.001	0.869	-0.001	<0.001	0.993
Fat mass	0.070	0.005	0.705	0.126	0.016	0.307	-0.211	0.045	0.246	0.211	0.044	0.084	0.095	0.009	0.603	-0.074	0.006	0.546	-0.019	<0.001	0.918	0.085	0.007	0.490
Fat mass (%)	0.058	0.003	0.753	0.061	0.004	0.619	-0.170	0.029	0.352	0.213	0.046	0.081	0.052	0.003	0.779	-0.092	0.008	0.457	-0.050	0.002	0.786	0.114	0.013	0.355
VAT	0.224	0.050	0.217	0.166	0.028	0.175	-0.157	0.025	0.391	0.271	0.073	**0.025**	0.107	0.011	0.560	-0.062	0.004	0.615	0.037	0.001	0.841	0.146	0.021	0.236
WC	0.041	0.046	0.818	0.214	0.047	0.078	-0.201	0.040	0.254	0.078	0.006	0.526	0.043	0.002	0.810	-0.089	0.008	0.465	-0.087	0.007	0.626	-0.048	0.002	0.698
NAT mass	0.152	0.023	0.449	0.137	0.019	0.300	0.062	0.004	0.757	0.055	0.003	0.678	-0.093	0.009	0.644	-0.043	0.002	0.747	0.020	<0.001	0.921	0.010	<0.001	0.937

Standardized β coefficient, R^2 and P value from linear regression analyses. All variables were transformed with square root transformation (SQRT), except PTFQI.
Abbreviations: FT3, free Triiodothyronine; FT4, free Thyroxine; TSH, Thyroid-Stimulating Hormone; BMI, Body mass index; VAT, visceral adipose tissue; WC, waist circumference; NAT, Neck adipose tissue.

Figure 8 and table 14 show the associations of THs and TSH with cardiometabolic risk factors. FT3 was positively associated with glucose, insulin, HOMA-IR, GGT, fatty liver index, systolic blood pressure (SBP), diastolic blood pressure (DBP) and MBP (all P<0.024, table 2). However, the associations between FT3 and cardiometabolic risk factors disappeared after adjusting for VAT mass (data not shown), except for the association between FT3 and SBP and MBP (β=0.170; R^2=0.373; P=0.038 and β=0.171; R^2=0.347; P=0.041 respectively). FT4 was negatively associated with adiponectin, and positively associated with SBP, DBP and MBP (all P<0.047), but not with the rest of cardiometabolic risk factors (all P>0.075; table 2). A negative association was observed between TSH and HDL-C, and positive associations were found between TSH and LDL-C/HDL-C ratio, TG/HDL-C ratio, TC/HDL-C ratio, homocysteine and

SBP (all P<0.039, Table 2), but no other association was observed between TSH and other cardiometabolic risk factors (all P>0.051, Table 2). Cardiorespiratory fitness was not associated with any thyroid metabolism variables (P≥0.662, data not shown). All the associations between FT4, TSH and cardiometabolic risk factors remained after adjusting for VAT mass (data not shown). PTFQI was negatively associated with adiponectin, and was positively associated with homocysteine, SBP, DBP and MBP (all P<0.023, table 14).

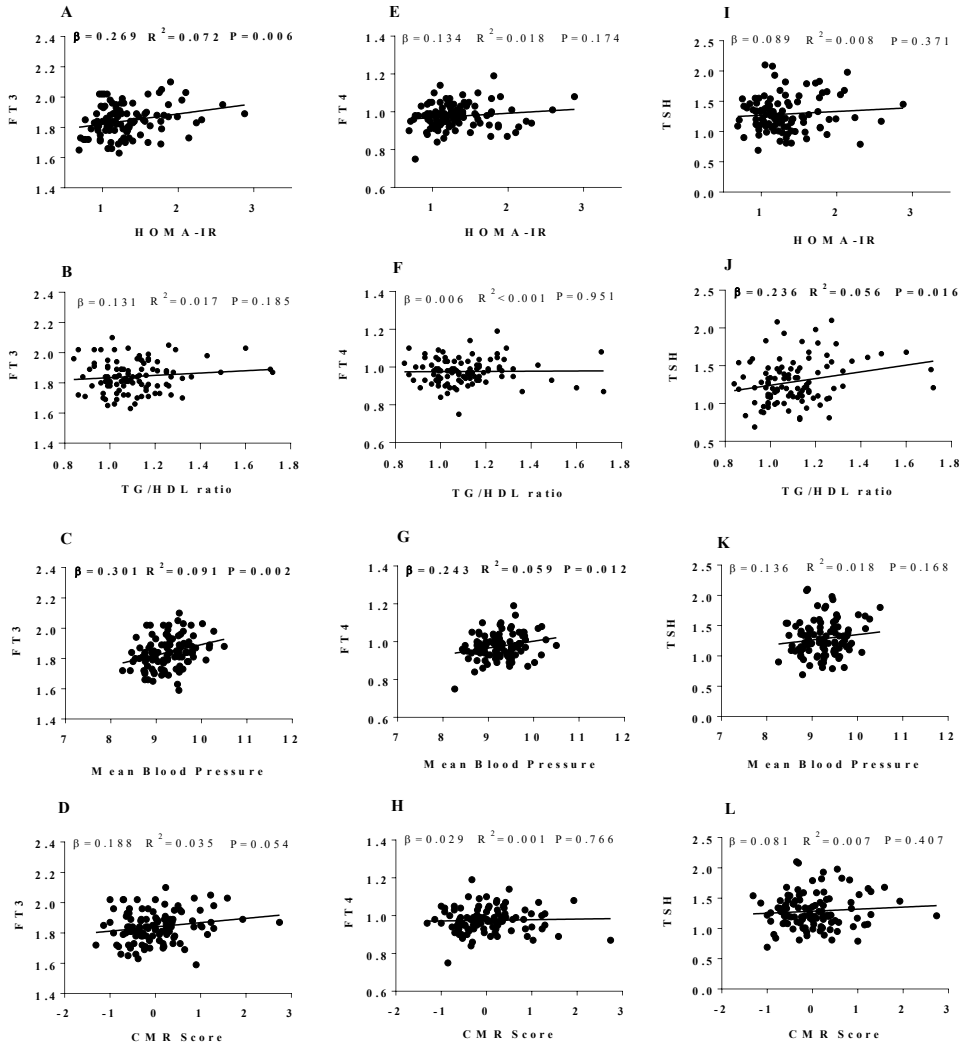

Figure 8. Association between serum levels of TSH, thyroid hormones and Parametric Thyroid Feedback Quantile based Index (PTFQI) with homeostatic model assessment of insulin resistance (HOMA-IR), Triglycerides (TG) / High-density lipoproteins cholesterol (HDL-C) ratio, mean blood pressure and cardiometabolic risk score (CMR Score). Standardized β coefficient, R^2 and P value from linear regression analyses. All variables were transformed with square root transformation (SQRT), except PTFQI. Abbreviations: FT3, free Triiodothyronine; FT4, free thyroxine; TSH, Thyroid-Stimulating Hormone.

All the associations between THs and cardiometabolic risk factors were attenuated when adjusting for sex (Table 15).

A significant *sex x FT3* interaction effect was found on homocysteine (P=0.002) levels. The *sex x FT4* interaction was significant on leptin levels (P=0.036). A significant *sex x TSH* interaction effect was also observed on total cholesterol (P=0.017) and APOB (P=0.014) levels. The association analyses in men and women separately can be found in table 4. In addition, the association analyses adjusting for sex are showed in table 16.

None of the associations previously reported between thyroid function and body composition and cardiometabolic risk factors remained after error alpha propagation correction (data not shown).

Table 14. Association between serum levels of thyroid hormones and Parametric Thyroid Feedback Quantile based Index (PTFQI) with cardiometabolic risk factors.

	FT3 (pg/mL)			FT4 (ng/dL)			TSH (µUI/mL)			PTFQI		
	β	**R^2**	**P**	**B**	**R^2**	**P**	**β**	**R^2**	**P**	**β**	**R^2**	**P**
Glucose	0.224	0.050	**0.022**	0.075	0.006	0.450	0.095	0.009	0.340	0.106	0.011	0.283
Insulin	0.263	0.069	**0.007**	0.141	0.020	0.153	0.082	0.007	0.409	0.120	0.014	0.225
HOMA-IR	0.269	0.072	**0.006**	0.134	0.018	0.174	0.089	0.008	0.371	0.122	0.015	0.218
Total Cholesterol	-0.117	0.014	0.237	-0.019	<0.001	0.848	0.060	0.004	0.543*	0.050	0.002	0.617
LDL - C	-0.073	0.005	0.461	-0.031	0.001	0.754	0.095	0.009	0.336	0.061	0.004	0.540
HDL-C	-0.192	0.037	0.051	-0.038	0.001	0.704	-0.225	0.051	**0.021**	-0.175	0.031	0.075
Triglycerides	0.051	0.003	0.610	-0.022	<0.001	0.826	0.188	0.035	0.056	0.092	0.009	0.351
LDL-C/HDL-C ratio	0.072	0.005	0.468	0.004	<0.001	0.970	0.206	0.043	**0.035**	0.152	0.023	0.124
TG/HDL-C ratio	0.131	0.017	0.185	0.006	<0.001	0.951	0.236	0.056	**0.016**	0.145	0.021	0.141
TC/HDL-C ratio	0.095	0.009	0.336	0.026	0.001	0.793	0.239	0.057	**0.015**	0.191	0.036	0.052
APOA1	-0.124	0.015	0.211	-0.040	0.002	0.690	-0.193	0.037	0.051	-0.158	0.025	0.111
APOB	<0.001	<0.001	1.000	0.007	<0.001	0.948	0.069	0.005	0.486*	0.063	0.004	0.528
C Reactive protein	0.109	0.012	0.270	0.121	0.015	0.219	-0.092	0.008	0.352	-0.018	<0.001	0.854
Homocysteine	0.152	0.023	0.123*	0.024	0.001	0.810	0.250	0.062	**0.011**	0.222	0.049	**0.023**
Leptin	-0.008	<0.001	0.937	-0.175	0.031	0.075*	-0.118	0.014	0.233	-0.189	0.036	0.054
Adiponectin	-0.129	0.017	0.193	-0.218	0.047	**0.027**	-0.168	0.028	0.089	-0.260	0.068	**0.008**
GGT	0.263	0.069	**0.007**	0.072	0.005	0.472	-0.025	0.001	0.805	0.044	0.002	0.657
ALP	0.149	0.022	0.131	0.064	0.004	0.518	-0.013	<0.001	0.894	0.007	<0.001	0.947
CMR Score	0.188	0.035	0.054	0.029	0.001	0.766	0.081	0.007	0.407	0.062	0.004	0.527
Fatty liver index	0.260	0.067	**0.009**	0.051	0.003	0.615	0.126	0.016	0.210	0.115	0.013	0.252
Systolic BP	0.306	0.094	**0.001**	0.194	0.038	**0.047**	0.202	0.041	**0.039**	0.283	0.080	**0.003**
Diastolic BP	0.246	0.061	**0.011**	0.238	0.057	**0.014**	0.061	0.004	0.534	0.237	0.056	**0.015**
Mean BP	0.301	0.091	**0.002**	0.243	0.059	**0.012**	0.136	0.018	0.168	0.285	0.081	**0.003**
VO_2max	0.008	<0.001	0.938	-0.044	0.002	0.662	0.030	0.001	0.766	0.019	<0.001	0.848

Standardized β coefficient, R^2 and P value from simple linear regression analyses. *Significant sex interaction. All variables were transformed (square root transformation), except PTFQI. Abbreviations: FT3, free Triiodothyronine; FT4, free Thyroxine; TSH, Thyroid-Stimulating Hormone; HOMA-IR, Homeostatic model assessment index of insulin resistance; HDL-C, High density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; APOA1, Apolipoprotein A-1; APOB, Apolipoprotein B; GGT, gamma-glutamyltransferase; ALP, Alkaline phosphatase; CRP: C reactive protein; CMR Score, Cardiometabolic risk score; FLI, Fatty liver index; BP, blood pressure; VO_2max, maximum oxygen consumption.

Table 15. Association between serum levels of thyroid hormones and Parametric Thyroid Feedback Quantile based Index (PTFQI) with cardiometabolic risk factors separating for sex.

	FT3 (pg/mL)						FT4 (ng/dL)						TSH (µUI/mL)						PTFQI					
	Men			Women			Men			Women			Men			Women			Men			Women		
	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P	β	R^2	P
Glucose	0.014	<0.001	0.936	0.285	0.082	**0.017**	-0.132	0.017	0.458	0.146	0.021	0.229	0.059	0.004	0.739	0.026	0.001	0.832	-0.097	0.009	0.585	0.129	0.017	0.288
Insulin	0.289	0.084	0.097	0.234	0.055	0.052	0.190	0.036	0.281	0.086	0.007	0.481	0.207	0.043	0.240	-0.076	0.006	0.534	0.263	0.069	0.133	-0.014	<0.001	0.905
Homa	0.265	0.070	0.129	0.252	0.063	**0.036**	0.157	0.025	0.374	0.089	0.008	0.464	0.198	0.039	0.261	-0.071	0.005	0.562	0.235	0.055	0.181	-0.008	<0.001	0.950
TC	0.034	0.001	0.848	-0.195	0.038	0.105	-0.201	0.040	0.254	0.093	0.009	0.446	0.341	0.116	**0.049**	-0.139	0.019	0.252	0.141	0.020	0.426	0.012	<0.001	0.919
LDL-C	-0.017	<0.001	0.922	-0.140	0.019	0.249	-0.191	0.036	0.280	0.035	0.001	0.772	0.290	0.084	0.097	-0.093	0.009	0.443	0.094	0.009	0.596	-0.001	<0.001	0.993
HDL-C	-0.093	0.009	0.601	-0.121	0.015	0.317	-0.174	0.030	0.324	0.126	0.016	0.299	0.004	<0.001	0.982	-0.200	0.040	0.096	-0.096	0.009	0.588	-0.035	0.001	0.771
Triglycerides	0.205	0.042	0.244	-0.066	0.004	0.589	0.009	<0.001	0.958	-0.079	0.006	0.517	0.328	0.108	0.058	0.040	0.002	0.743	0.264	0.070	0.131	-0.057	0.003	0.642
LDL-C/HDL-C ratio	0.045	0.002	0.800	-0.027	0.001	0.823	-0.055	0.003	0.757	-0.062	0.004	0.611	0.216	0.047	0.219	0.054	0.003	0.658	0.129	0.017	0.469	0.009	<0.001	0.939
TG/HDL-C ratio	0.208	0.043	0.239	0.001	<0.001	0.993	0.067	0.005	0.706	-0.119	0.014	0.327	0.261	0.068	0.136	0.100	0.010	0.408	0.252	0.064	0.150	-0.051	0.003	0.673
TC/HDL-C ratio	0.098	0.010	0.508	-0.035	0.001	0.775	-0.023	0.001	0.896	-0.055	0.003	0.652	0.236	0.056	0.179	0.081	0.007	0.503	0.173	0.030	0.329	0.034	0.001	0.779
APOA1	0.025	0.001	0.890	-0.067	0.004	0.583	-0.264	0.070	0.137	0.096	0.009	0.429	-0.021	<0.001	0.907	-0.146	0.021	0.226	-0.130	0.017	0.471	-0.026	0.001	0.833
APOB	0.052	0.003	0.773	-0.067	0.005	0.580	-0.232	0.054	0.194	0.119	0.014	0.327	0.305	0.093	0.084	-0.177	0.032	0.142	0.106	0.011	0.556	-0.007	<0.001	0.955
C Reactive protein	0.000	<0.001	0.999	0.159	0.025	0.187	0.372	0.138	**0.030**	0.043	0.002	0.723	-0.030	0.001	0.865	-0.114	0.013	0.349	0.148	0.022	0.402	-0.061	0.004	0.618
Homocysteine	0.462	0.213	**0.006**	-0.117	0.014	0.335*	0.020	<0.001	0.912	-0.079	0.006	0.517	0.130	0.017	0.465	0.202	0.041	0.093	0.166	0.028	0.347	0.104	0.011	0.390
Leptin	0.034	0.001	0.851	0.081	0.007	0.507	-0.365	0.133	**0.034**	0.013	<0.001	0.913	0.034	0.001	0.850	-0.096	0.009	0.429	-0.205	0.042	0.244	-0.044	0.002	0.718
Adiponectin	0.075	0.006	0.674	-0.113	0.013	0.356	0.109	0.012	0.538	-0.296	0.087	**0.014**	-0.177	0.031	0.315	-0.030	0.001	0.808	-0.001	<0.001	0.997	-0.243	0.059	**0.044**
GGT	0.286	0.082	0.102	0.159	0.025	0.192	-0.063	0.004	0.724	0.072	0.005	0.558	-0.220	0.048	0.211	-0.134	0.018	0.273	-0.203	0.041	0.249	-0.042	0.002	0.733
ALP	0.051	0.003	0.774	0.125	0.016	0.303	0.088	0.008	0.620	-0.006	<0.001	0.961	-0.013	<0.001	0.940	-0.126	0.016	0.300	0.053	0.003	0.768	-0.128	0.016	0.290
CMR Score	0.200	0.040	0.257	0.196	0.038	0.099	-0.016	<0.001	0.929	0.058	0.003	0.629	0.133	0.018	0.454	0.057	0.003	0.632	0.107	0.012	0.545	0.051	0.003	0.671
FLI	0.141	0.020	0.426	0.234	0.055	0.056	-0.178	0.032	0.313	0.084	0.007	0.501	0.093	0.009	0.601	-0.075	0.006	0.544	-0.012	<0.001	0.948	-0.038	0.001	0.758
Systolic BP	0.263	0.069	0.139	0.226	0.051	0.057	-0.08	<0.001	0.922	0.191	0.036	0.109	0.085	0.007	0.637	0.084	0.007	0.484	0.135	0.018	0.453	0.168	0.028	0.157
Diastolic BP	0.374	0.140	**0.032**	0.150	0.022	0.210	0.152	0.023	0.397	0.254	0.064	**0.031**	0.017	<0.001	0.925	0.024	0.001	0.842	0.214	0.046	0.232	0.198	0.039	0.096
Mean BP	0.370	0.137	**0.034**	0.197	0.039	0.097	0.098	0.010	0.588	0.248	0.061	**0.036**	0.247	0.002	0.794	0.054	0.003	0.655	0.206	0.043	0.249	0.202	0.041	0.088
VO2max	0.075	0.006	0.678	-0.140	0.020	0.252	-0.040	0.002	0.824	-0.139	0.019	0.256	-0.074	0.005	0.683	-0.045	0.002	0.711	-0.071	0.005	0.695	-0.086	0.007	0.483

Standardized β coefficient, R^2 and P value from simple linear regression analyses. All variables were transformed with square root transformation (SQRT), except PTFQI. Abbreviations: FT3, free Triiodothyronine; FT4, free Thyroxine; TSH, Thyroid-Stimulating Hormone; HOMA-IR, Homeostatic model assessment index of insulin resistance; TC, Total cholesterol; HDL-C, High density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; APOA1, Apolipoprotein A-1; APOB, Apolipoprotein B; GGT, gamma-glutamyltransferase; ALP, Alkaline phosphatase; CRP: C reactive protein; CMR Score, Cardiometabolic risk score; FLI, Fatty liver index; BP, blood pressure; VO_2max, maximum oxygen consumption.

Table 16. Association between serum levels of thyroid hormones and Parametric Thyroid Feedback Quantile based Index (PTFQI) with cardiometabolic risk factors adjusting for sex.

	FT3 (pg/mL)			FT4 (ng/dL)			TSH (µUI/mL)			PTFQI		
	β	**R^2**	**P**	**B**	**R^2**	**P**	**β**	**R^2**	**P**	**β**	**R^2**	**P**
Glucose	0.187	0.081	0.058	0.040	0.049	0.686	0.041	0.049	0.684	0.046	0.049	0.651
Homa- IR	0.252	0.078	**0.011**	0.116	0.030	0.247	0.059	0.020	0.567	0.091	0.025	0.378
Insulin	0.252	0.072	**0.012**	0.127	0.027	0.205	0.058	0.015	0.573	0.097	0.020	0.351
Total Cholesterol	-0.118	0.014	0.246	-0.016	0.001	0.874	0.070	0.005	0.496	0.061	0.004	0.559
LDL-C	-0.098	0.019	0.333	-0.049	0.012	0.628	0.075	0.0.015	0.467	0.035	0.011	0.739
HDL-C	-0.105	0.206	0.252	0.036	0.197	0.688	-0.119	0.209	0.197	-0.049	0.198	0.601
Triglycerides	0.019	0.024	0.849	-0.049	0.026	0.625	0.158	0.047	0.119	0.051	0.026	0.620
APOA1	-0.042	0.170	0.654	0.013	0.169	0.889	-0.098	0.171	0.297	-0.048	0.171	0.612
APOB	-0.023	0.012	0.823	-0.007	0.011	0.943	0.046	0.013	0.654	0.036	0.012	0.725
C Reactive protein	0.123	0.016	0.226	0.132	0.019	0.190	-0.087	0.009	0.396	-0.007	0.002	0.948
Homocysteine	0.077	0.146	0.412	-0.039	0.142	0.676	0.164	0.166	0.085	0.122	0.154	0.205
Leptin	0.061	0.105	0.526	-0.124	0.117	0.196	-0.038	0.103	0.700	-0.102	0.111	0.303
Adiponectin	-0.057	0.133	0.549	-0.162	0.155	0.085	-0.080	0.136	0.410	-0.167	0.155	0.087
GGT	0.172	0.252	0.055	-0.007	0.224	0.934	-0.159	0.248	0.080	-0.108	0.235	0.243
ALP	0.102	0.071	0.301	0.024	0.062	0.808	-0.083	0.068	0.407	-0.073	0.066	0.471
CMR Score	0.200	0.038	**0.046**	0.033	0.001	0.745	0.091	0.008	0.371	0.073	0.005	0.480
Fatty liver index	0.171	0.245	0.059	-0.025	0.218	0.784	0.007	0.217	0.942	-0.024	0.218	0.795
Systolic BP	0.211	0.281	**0.016**	0.108	0.250	0.218	0.076	0.244	0.397	0.145	0.258	0.110
Diastolic BP	0.223	0.071	**0.024**	0.217	0.069	**0.028**	0.021	0.024	0.833	0.210	0.064	**0.040**
Mean BP	0.240	0.166	**0.011**	0.188	0.145	**0.046**	0.050	0.113	0.608	0.201	0.147	**0.039**
VO2max	-0.071	0.096	0.471	-0.101	0.102	0.300	-0.056	0.095	0.573	-0.081	0.098	0.423

Linear regression analyses were performed. Standardized β coefficient, R^2 and P value are provided. All variables were transformed with square root transformation (SQRT), except PTFQI. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free Thyroxine; FT3, free Triiodothyronine; HOMA-IR, Homeostatic model assessment index of insulin resistance; HDL-C, High density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; APOA1, Apolipoprotein A-1; APOB, Apolipoprotein B; GGT, gamma-glutamyltransferase; ALP, Alkaline phosphatase; CRP: C reactive protein; CMR Score, Cardiometabolic risk score; FLI, Fatty liver index; BP, blood pressure; VO_2max, maximum oxygen consumption.

DISCUSSION

The results of this study show that FT3 is positively associated with BMI, lean mass, central adiposity and several cardiometabolic risks factors in young euthyroid adults. However, these associations were attenuated when adjusting for sex, and only some of them remained when analysing men and women separately, which might point to a relevant role of sex in this relationship. On the other hand, we found no associations between FT4, TSH and PTFQI and body composition parameters or cardiometabolic risk factors. These findings suggest that thyroid function, even when circulating levels of THs and TSH are within the normal range, might be related to body composition and cardiovascular risk in euthyroid young adults.

Several studies have analysed the relationship between FT3 and adiposity (6,23), but few have evaluated the connection between thyroid function and central adiposity in euthyroid young adults. Central adiposity is an important risk factor in the development of metabolic syndrome and CVD (24). We observed a positive association of FT3 with WC and VAT mass in young euthyroid adults. These results are in agreement with previous studies in euthyroid older obese subjects (25,26). Despite VAT is commonly associated to ectopic fat deposition, we found no association of FT3, FT4 or TSH concentrations with total NAT mass. Moreover, we found no association of any body composition variable with FT4 or TSH, in agreement with the results obtained by Manji et al. (27). Future studies should evaluate the relationship between thyroid function and central adiposity to understand the potential causes underlying the positive associations observed in studies (6).

Metabolic syndrome includes several risk factors for cardiovascular disease. Namely, central obesity, elevated blood pressure, atherogenic dyslipidaemia (high triglycerides and low HDL-C cholesterol) and hyperglycaemia. We observed the cardiometabolic risk score being positively associated with FT3 in euthyroid young adults. The relationship between thyroid function, insulin resistance and metabolic syndrome is unknown as contradictory results have been published (28). Indeed, De Pergola et al. (29), in line with our results, showed that FT3 was associated with WC, hyperinsulinemia and other components of metabolic syndrome in overweight and obese euthyroid women. These findings suggest that thyroid function could predict the metabolic syndrome risk in euthyroid young adults. Furthermore, we observed that FT3 is associated with fasting glucose, insulin and HOMA-IR. These findings have been widely

reported in subjects with pathological circulating levels of THs (30,31). Ferranini et al. (32), showed that normal FT3 levels were associated with insulin resistance and glucose intolerance in subjects with BMI between 17–44 kg/m2.

The relationship between THs and blood pressure has also been extensively studied in pathological states, although this relationship still remains unclear in euthyroid subjects. In our study, THs and PTFQI were positively associated with systolic, diastolic and mean blood pressure, and these results persist after adjusting for sex. However, no association was found between TSH and blood pressure, which concur with the finding by Roos et al. (33). THs are important for vascular function. Both T3 and T4 acts as vasodilator on vascular smooth muscle cells (34). Therefore, an increase of THs concentration in euthyroid subjects might represent a compensation for high blood pressure values. Alternatively, elevated concentrations of THs might itself contribute to increase blood pressure. Hyperthyroidism can produce tachycardia, increased heart contractility, elevated cardiac output, high systolic pressure, increased pulse pressure, and muscle weakness (35). Therefore, elevated THs within the normal range could have the same effect than in hyperthyroid state, increasing blood pressure.

THs are important regulators of various metabolic processes, such as lipid metabolism (36). The association between THs concentrations and serum lipids in pathological states has for long been studied (37,38). However, this relationship in euthyroid subjects is not clear yet. We observed a tendency to negative association of FT3, TSH and PTFQI with HDL-C, which is in agreement with Bakker et al. (39). Hypothyroidism had been reported to be associated with an increased risk for dyslipidemia (especially higher serum levels of cholesterol, whereas their levels are reduced in hyperthyroidism) and atherosclerotic cardiovascular disease (40). This phenomenon appears to be related to the cholesterol ester transfer protein (CEPT) activity. CEPT is positively regulated by THs, regulating the exchange of cholesteryl-ester between HDL-C and very-low-density lipoprotein (VLDL). Higher activity of CEPT would imply an increased cardiovascular risk, related to elevation of VLDL and decreased of HDL-C serum levels (36). Thus, it is plausible that the tendency to inverse association that we found of FT3, TSH and PTFQI with HDL-C, is mediated by this mechanism, even in euthyroid subjects.

Cardiorespiratory fitness is currently recognized as an important marker of cardiovascular health, as it integrates the function of many physiological systems (41). Therefore, we also investigated whether THs are associated with cardiorespiratory

fitness. Despite FT3 was associated to many cardiometabolic risk factors, no association was found between TSH/THs and cardiorespiratory fitness in euthyroid adults.

Limitations

The present results need to be interpreted with caution, since some limitations are present. First, it is a cross-sectional study and, therefore, no causality can be established. In addition, only healthy young adults are included in the study, so the results are not extrapolatable to older, younger, or unhealthy people. Moreover, despite roughly two thirds of the study sample were women, we did not standardize neither controlled the menstrual cycle, which might have contributed to bias the results. Finally, despite DXA is valid instrument to assess body composition, it is limited to accurately assess VAT mass. Other more precise techniques such as magnetic resonance might have been more informative concerning the relationship between thyroid function and body composition. Likewise, other techniques such as lipoprotein profile analyses or hyperinsulinemic clamps for determining insulin resistance might be able to better detect relationships between thyroid function and cardiometabolic risk.

Conclusion

In summary, FT3 seems to be associated with central adiposity and metabolic syndrome factors such as insulin resistance, mean, systolic and diastolic blood pressure and fatty liver in euthyroid young adults. More studies are needed in this population to clarify the role of thyroid function in the development and prevention of cardiometabolic diseases at early ages.

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CHAPTER 3

Thyroid function is not associated with brown adipose tissue volume and ^{18}F-Fluorodeoxyglucose uptake in young euthyroid adults

ABSTRACT

Purpose: Thyroid hormones (THs) are important mediators of brown adipose tissue (BAT) differentiation. However, the association of TH concentrations with human BAT in euthyroid individuals is unclear. The present work examines the associations between circulating thyroid-stimulating hormone (TSH) and THs concentrations (i.e. free tri-iodothyronine, FT3, and free thyroxine, FT4), under thermoneutral [22-23ºC] and cold-induced conditions, and BAT volume, ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake and mean radiodensity.

Methods: A total of 106 young healthy, euthyroid adults (34 men/72 women; 22 ± 2 years old; 24.9 ± 4.6 kg/m2) participated in this cross-sectional study. BAT volume, ^{18}F-FDG uptake and mean radiodensity were assessed after 2 h of personalized (i.e., contemplating each individual's shivering threshold) cold exposure via positron emission tomography/computerized tomography (PET/CT) static scanning. TSH and THs levels were determined before (thermoneutral) and 1 h after the cold exposure.

Results: Cold exposure increased circulating FT4 (P=0.038) and reduced of TSH levels (P≤0.001). Conversely, the FT3 serum concentration was not modified by cold exposure (P=0.435). No associations were found between the TSH and THs thermoneutral (all P>0.289) or cold-induced levels (all P>0.067) and BAT volume,^{18}F-FDG uptake and mean radiodensity. These findings were independent of sex and body mass index.

Conclusions: Thyroid function is modulated by cold exposure, yet it is not associated with BAT volume or glucose metabolism assessed after 2h of cold exposure in young healthy, euthyroid adults.

BACKGROUND

BAT plays an important role in the energy homeostasis acting as a key thermogenic tissue. This thermogenic activity is possible through the activity of the uncoupling protein 1 (UCP1) (1). Similarly, the THs play also an important role in energy homeostasis and body temperature regulation. Previous studies support that THs contribute to BAT recruitment and differentiation (2,3), suggesting that the expression of UCP1 is partially due to the presence of T3 (2,4).

Interestingly, TSH-receptors are also expressed in different tissues, such as BAT (3), meaning that cold-induced might increase the secretion of TSH and THs in humans, contributing to BAT recruitment. However, in euthyroid subjects, the results are contradictory (5–10)

The aims of the present study were: (i) to analyse the effect of a personalized cold exposure on the circulating levels of TSH, FT4 and FT3 in young, healthy, euthyroid adults; (ii) to analyse the association of thermoneutral and cold-induced levels of circulating TSH, FT4 and FT3 levels, with BAT volume, ^{18}F-FDG uptake and mean radiodensity, assessed after a 2 h cold exposure.

MATERIAL AND METHODS

Study subjects

The study subjects were 106 young adults (34 men, 72 women) aged 22 ± 2 years (Table 17). All were enrolled in the ACTIBATE study (11), an exercise-based randomized controlled trial (ClinicalTrials.gov ID: NCT02365129). All data included in the present study were obtained from the baseline evaluation of the ACTIBATE study, which was carried out in October to December 2015 and 2016 at the University of Granada, Granada (Spain). All subjects reported themselves as healthy (confirmed by comprehensive medical examination), sedentary (< 20 min moderate-vigorous intensity physical activity on < 3 days/ week), non-smokers, and not to be taking any medication that might influence their response to cold exposure. The study protocol adhered to the Declaration of Helsinki, and written informed consent to be included was provided by all subjects. The study was approved by the Human Research Ethics Committees of the University of Granada (n◦924) and the *Servicio Andaluz de Salud*.

Table 17. Characteristics of participants.

	All n=106*		Men n=34		Women n=72	
Age (years)	22.0	(2.1)	22.2	(2.1)	21.9	(2.1)
Body composition parameters						
BMI (kg/m^2)	24.9	(4.6)	26.9	(5.5)	24.0	(3.8)
LMI (kg/m^2)	14.5	(2.4)	16.9	(2.2)	13.4	(1.5)
FMI (kg/m^2)	9.1	(2.9)	8.5	(3.5)	9.3	(2.7)
Fat mass (%)	36.6	(7.2)	31.1	(7.2)	39.0	(5.7)
BAT ^{18}F-FDG uptake parameters						
BAT volume (ml)	67.8	(55.5)	75.5	(62.7)	64.4	(52.1)
BAT SUVmean	3.7	(1.8)	3.3	(1.4)	3.9	(1.9)
BAT SUVpeak	10.9	(7.9)	9.8	(7.3)	11.4	(8.1)
BAT mean radiodensity	-59.8	(11.0)	-56.6	(11.7)	-61.4	(10.4)
Thyroid hormones						
TSH (µUI/ml)	1.7	(0.8)	2.0	(0.9)	1.6	(0.6)
FT4 (ng/dl)	0.9	(0.1)	1.0	(0.1)	0.9	(0.1)
FT3 (pg/ml)	3.4	(0.4)	3.5	(0.3)	3.3	(0.4)
PTFQI	0.2	(0.3)	0.3	(0.2)	0.2	(0.2)

Data are presented as mean and standard deviation. *N for mean BAT mean radiodensity=78 (23 men, 55 women). Abbreviations: BMI, Body mass index; LMI, Lean mass index, FMI, Fat mass index; ^{18}F-FDG, ^{18}F-Fluorodeoxyglucose; BAT, Brown adipose tissue; SUV Standardized uptake value; TSH, Thyroid-Stimulating Hormone; FT4, free thyroxine; FT3, free triiodothyronine; PTFQI, Parametric Thyroid Feedback Quantile based Index.

Procedures

The measurements recorded in this study were collected on three different days. On the first day, a STT was performed. Some 48–72 h later, a PET/CT scan was performed immediately after subjecting each individual to a personalized cooling protocol (12). Blood samples were collected before (thermoneutral) and 1 h after starting the personalized cold exposure (cold-induced). Upon arrival, subjects entered in a room (22.1 ± 1.6 °C) where they remained seated for 30 min. They were then conducted to a mild-cold room (19.8 ± 0.5°C) where they put on a cooling-vest (Polar Products Inc, Ohio, USA) covering the clavicular, chest, abdominal and back areas; this was connected to a temperature-controlled water circuit. The water temperature was reduced progressively every 10 min from the initial temperature (16.6°C) until shivering started or a temperature of 3.8°C was reached. The participants who did not shiver continued in the cold room for another 45 min when the test was terminated. Throughout the test, participants remained seated. Shivering was determined visually and by self-reporting. The temperature at which participants started to shiver was established as the individual shivering threshold. On the final day, 48–72 h after the PET/CT scan, CIT was assessed. For all visits, subjects arrived at the research centre in a motorized vehicle, having fasted for at least 6 h. All subjects were asked to follow their normal sleep routine, not to perform moderate physical activity 24 h before the tests, or vigorous activity in the 48 h before the tests. They were also requested to refrain from alcohol and stimulant beverages for 6 h before the tests. Subjects wore standardized clothing-sandals, T-shirt and shorts (clo-value = 0.20 (ISO-standard 9920, 2009)) – for all assessments.

Personalized cooling protocol before ^{18}F-FDG-PET/CT scanning

The procedure started in a room (22.2 ± 0.5°C) with a 30 min rest. A peripheral vein catheter was then inserted into the antecubital vein, and a first blood sample was taken. Later, participants were transferred to a mild-cold room (20.2 ± 0.3°C) where remaining seated, they put on the same cooling vest used in the STT, with the temperature of the circulating water set at 4°C above the individual´s shivering threshold. For those subjects who did not shiver in the STT, the water temperature was set at 3.8°C (13). After 60 min of cold exposure, a second peripheral blood sample was taken, and a bolus of ^{18}F-FDG (185 MBq; ~ 2.8 MBq/kg) was injected. At this point, the water temperature was increased by 1°C and maintained at that temperature during

the second hour of cold exposure. Nonetheless, the participants were instructed to immediately inform the evaluators if they began to shiver; in this event, the water temperature was immediately increased by 1°C and the subject was covered with a bathrobe until the tremors disappeared. Promptly after cold exposure, participants were accompanied to another room, where PET/CT scanning using a Siemens Biograph 16 PET/CT apparatus (Siemens) was performed. For the PET acquisition, two bed positions (6 min each one) from atlas vertebrae to mid-chest were analysed. CT was obtained by applying 120 kV (12).

Cold-induced thermogenesis

Cold-induced thermogenesis was assessed in a subgroup of 33 subjects. Before starting the assessment, the subjects laid on a bed for 20 min in a room at 23.2 ± 0.7ºC. Their RMR was then assessed over 30 min by indirect calorimetry, using a CCM Express or Ultima CardiO2 cart (Medgraphics Corp, Minnesota, USA) (11). The average value for the most stable 5 min period was taken as the RMR (14). Immediately after RMR assessment, subjects were accompanied to the cold room (19.7 ± 0.4ºC) where they put on the water perfused cooling vest once again, this time set at a temperature 4ºC above the shivering threshold, and laid on a bed for 65 min. CIT was assessed by indirect calorimetry over two periods of 30 min separated by 5 min, during which time the gas analysers were recalibrated. To obtain a single representative value for CIT, the recorded 60 min were divided into four 15 min periods, and the most stable 5 min period in every 15 min period selected for averaging. Finally, these four 5 min periods, together with the RMR, were used to calculate the area under the curve (trapezoidal rule), expressing it as a percentage of RMR (14).

Thyroid function

After blood extraction, serum samples were separated by centrifugation and refrigerated until analysis. TSH, FT4 and FT3 were determined by a chemiluminescent immunometric assay using a Beckman Coulter apparatus (33880) (Beckman Coulter Inc, Brea CA, USA) with a DXI analyser. The normal ranges considered for TSH, FT3 and FT4 to define the euthyroid condition were 0.34–5.6 µUI/mL, 2.5–4.94 pg/mL and 0.38–1.5 ng/dL, respectively.

The Parametric Thyroid Feedback Quantile-based Index (PTFQI), which oscillates in the range of 1 to −1, was also calculated. Positive values indicate higher TSH values due to

reduced inhibition by FT4 (lower sensitivity to FT4), while negative values mean lower TSH levels due to greater inhibition by FT4 (greater sensitivity to FT4). The PTFQI was calculated using the Excel spreadsheet formula reported by Laclaustra et al. (15) and using the FT4 and TSH values for the thermoneutral and cold exposure periods.

PET/CT analysis

All PET/CT scans were analysed using FIJI software (16) (http://sourceforge.net/projects/bifijiplugins (17)). The region of interest was set from the atlas vertebrae to the thoracic vertebra 4. The standardized uptake value (SUV) was calculated as (^{18}F-FDG uptake (kBq/mL)/ (injected dose (kBq)/patient weight (g))). The SUV threshold (SUVt) for BAT was established as (1.2/ (lean body mass/body mass)) (18). SUVt of 1.5 and 2 was also used for conducting sensitivity analyses (19). A fixed radiodensity range (−190 to −10 Hounsfield units) was used for BAT assessment (18). BAT volume, SUV mean, SUV peak and mean radiodensity were calculated using the above software. Twenty-one individuals presented some voxels classified as BAT out of the anatomical areas where BAT is located and were, therefore, excluded from BAT mean radiodensity analyses.

Body composition and anthropometric measurements

Fat and lean masses were assessed by dual-energy X-ray absorptiometry using a Discovery Wi device (Hologic, Inc., Bedford, MA, USA) and the data were processed using Hologic APEX v.4.0.2. software (Hologic, Inc., Bedford, MA, USA). Weight and height were measured using a model 799 electronic column scale and stadiometer (SECA, Hamburg, Germany). Body, lean and fat mass indices were calculated as body/lean/fat mass (kg)/height (m^2).

Statistical analysis

Descriptive variables are reported as means and standard deviations unless otherwise stated. The effect of cold exposure on circulating levels of TSH, FT4 and FT3, and the PTFQI, was examined by repeated measures of analyses of variance (ANOVA), comparing the thermoneutral condition values with those obtained 1 h after cold exposure. Analysis of covariance (ANCOVA) was used to explore the effect of the interaction *sex* × *time* on circulating TSH, FT4 and FT3 and the PTFQI.

Simple linear regression was used to examine the association between the thermoneutral circulating concentrations of TSH, FT4 and FT3, PTFQI and BAT-related

variables. These analyses were repeated adjusting for sex and BMI in separate models. To test whether the cold-induced changes in TSH, FT4 and FT3 levels and PTFQI were associated with BAT-related variables, the difference between the baseline and 1 h-post-cold exposure circulating levels of TSH and THs were determined and expressed as a percentage of the baseline values. The associations of these changes with BAT-related variables were examined by simple linear regression and also adjusted for sex and BMI. Finally, the association of both thermoneutral and cold-induced circulating concentrations of TSH, FT4 and FT3 and PTFQI with CIT was studied using simple linear regression.

All analyses were performed with the Statistical Package for the Social Sciences v.21.0 software (IBM Corporation). GraphPad Prism Software (GraphPad) was used for plots. Significance was set at $P < 0.05$.

RESULTS

FT4 concentrations were increased after cold exposure (0.95 ng/dL ± 0.01 vs 0.97 ng/dL ± 0.01, *P* = 0.038, Fig. 9C) whereas FT3 concentrations did not change (*P* = 0.435, Fig. 9B). TSH serum concentrations and the PTFQI decreased after cold exposure (TSH: 1.75 µUI/mL ± 0.08 vs 1.38 µUI/mL ± 0.07, *P* < 0.001, Fig. 9A; PTFQI: 0.24 ± 0.03 vs 0.15 ± 0.02, *P* < 0.001, Fig. 9D).

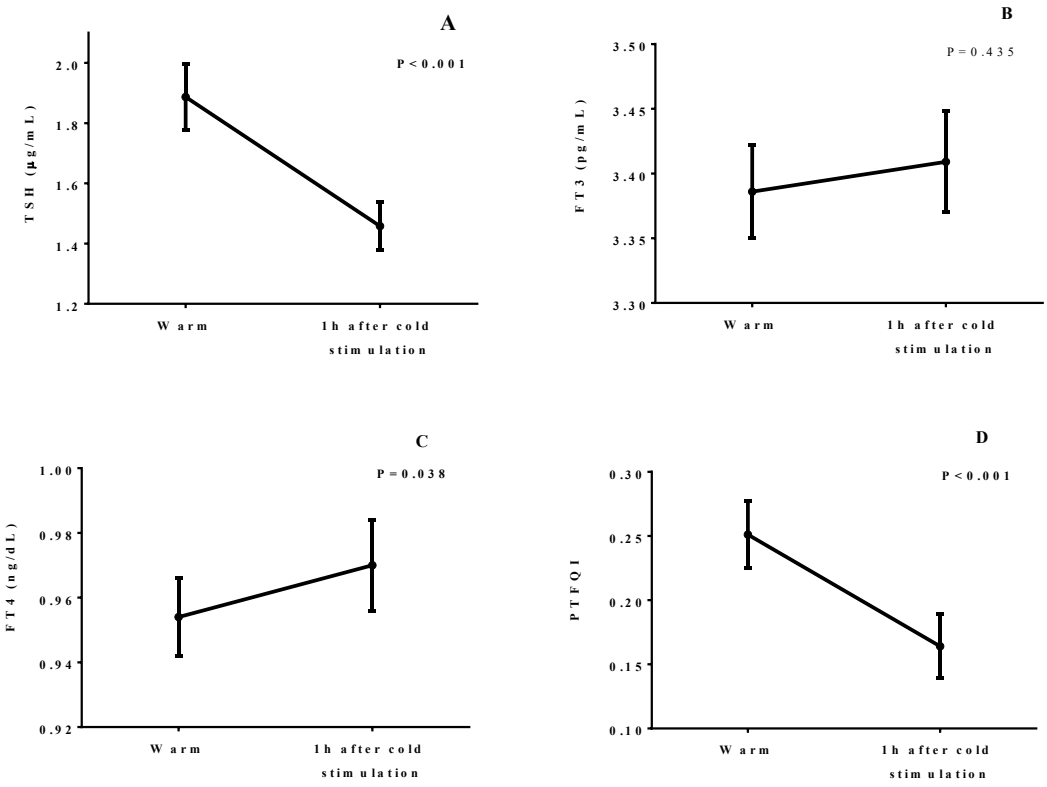

Figure 9. Cold-induced changes in circulating levels of thyroid-stimulating hormone (TSH) (A), free tri-iodothyronine (FT3) (B) and free thyroxine (FT4) (C), and the parametric thyroid feedback quantile-based index (PTFQI) (D), in young, healthy, euthyroid adults (n=99). P value are for repeated measures ANOVA.

There was no *sex* × *time* interaction on the cold-induced changes in TSH, FT4 or PTFQI (all *P* ≥ 0.470, Fig. 10). A sensitivity analysis excluding subjects with TSH circulating levels above 4.5 µUI/mL (5) and below 0.5 µUI/mL (1) was also performed, and the results remained similar (data not shown). Additional analyses were conducted after excluding participants with low BAT volume (i.e. < 8 mL, *n* = 23) and the results did not change (data not shown).

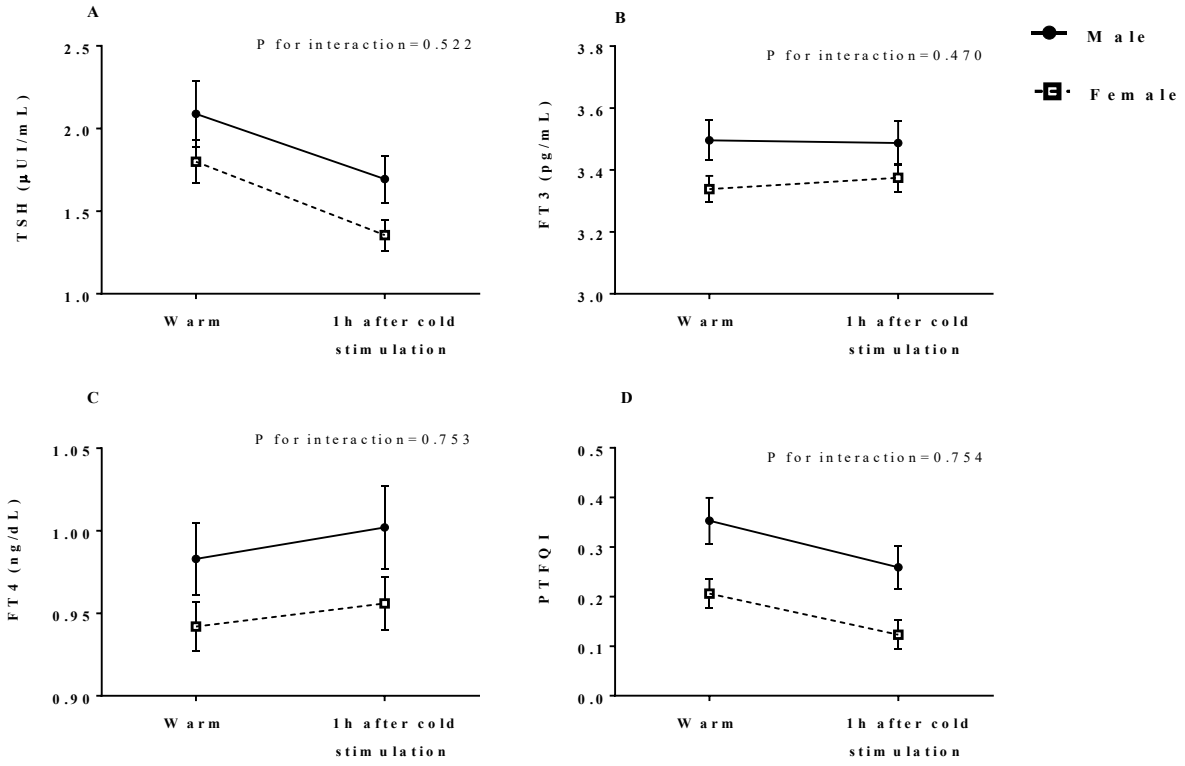

Figure 10. Cold-induced changes on Thyroid-Stimulating Hormone (TSH) (A), free Thyroxine (FT4) (B), free triiodothyronine (FT3) (C) circulating levels and Parametric Thyroid Feedback Quantile based Index (PTFQI) (D) by sex after one hour of cold stimulation in young euthyroid adults (n=102, 71 female). Analyses of variance (ANOVA) were performed.

TSH, FT4, FT3 and PTFQI values for the thermoneutral condition were not associated with any BAT-related variable (all $P \geq 0.111$, Fig. 11) or CIT ($n = 33$; Table 18) (all $P \geq 0.277$, Table 19). In sensitivity analyses, we conducted additional regression models after adjusting for sex and BMI and the results persisted, except for the association between TSH and BAT mean radiodensity which became significant when adjusted for BMI ($P = 0.019$). We also conducted sensitivity analyses excluding participants presenting with BAT volume < 8 mL, observing similar results except for a negative association that was found between PTFQI and BAT SUVmean ($\beta = -1.415$; $R2 = 0.070$; $P = 0.024$). Similar results were also observed in sensitivity analyses using different SUVt of 1.5 and 2 (data not shown).

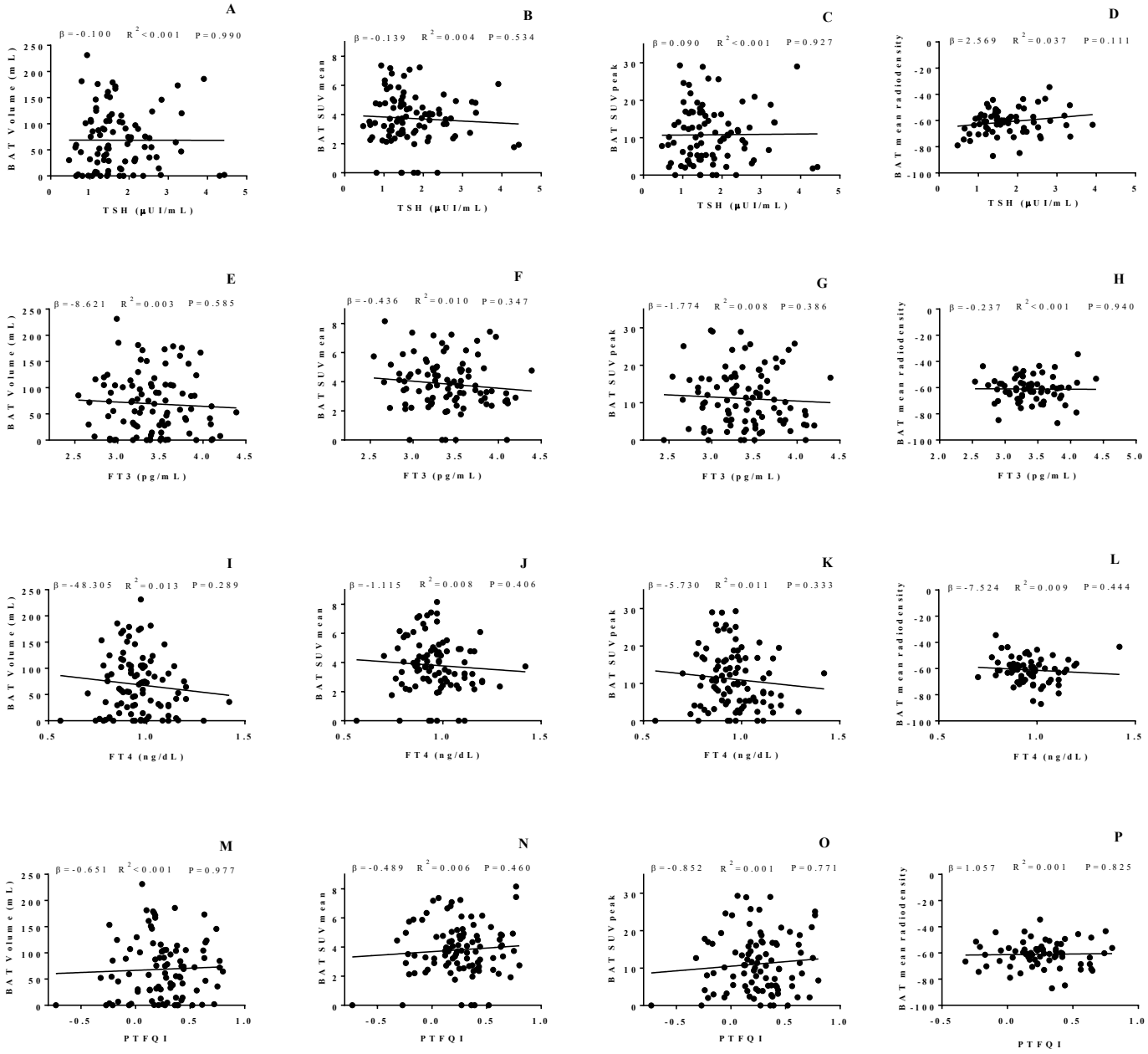

Figure 11. Associations between circulating levels of thyroid stimulating hormone (TSH) (A-D), free triiodothyronine (FT3) (E-H), and free thyroxine (FT4) (I-L), as well as the parametric thyroid feedback quantile-based index (PTFQI) (M-P) under thermoneutral conditions and brown adipose tissue (BAT) volume, ^{18}F-fluorodeoxyglucose uptake and mean radiodensity after individualized cold exposure, in young, healthy, euthyroid adults (n=91). Unstandardized β, R^2 and P values are for simple linear regression analyses. Abbreviations: SUV, Standardized uptake value.

The cold-induced changes in TSH, FT4, FT3 and PTFQI were not associated with any BAT-related variable (all $P \geq 0.067$, Fig. 12), or with CIT (n = 33; Table 18) (all $P \geq 0.480$, Table 19). In addition, these findings persisted after adjusting for sex and BMI (data not shown). Moreover, neither the TSH, FT4, FT3 nor PTFQI level was

associated with RMR (data not shown). Similar results were observed when excluding BAT-negative (i.e. BAT volume < 8 mL) individuals (data not shown). Similarly, modifying the SUVt did not impact the results.

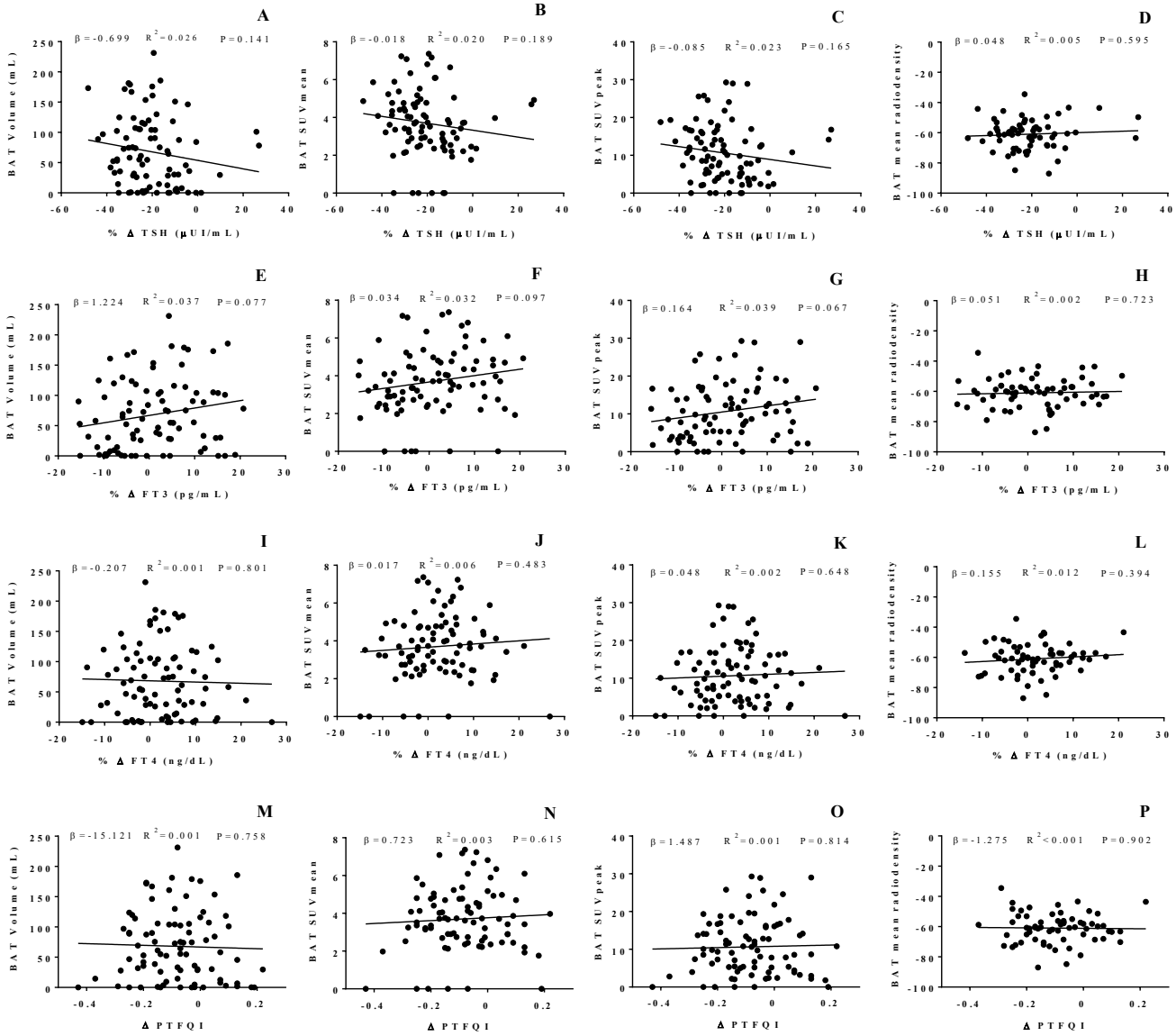

Figure 12. Association between cold-induced changes in circulating levels of thyroid-stimulating hormone (TSH) (A-D), free tri-iodothyronine (FT3) (E-H) and free thyroxine (FT4) (I-L), as well as the parametric thyroid feedback quantile-based index (PTFQI) (M-P) (expressed as a percentage of baseline values), and brown adipose tissue (BAT) volume, ^{18}F-fluorodeoxyglucose uptake and mean radiodensity after individualized cold exposure in young, healthy euthyroid adults (n=86). Unstandardized β, R^2 and P values are for simple linear regression analyses. Abbreviations: BAT, Brown adipose tissue; SUV, Standardized uptake value.

Table 18. Characteristics of participants included in the analyses of cold-induced thermogenesis.

	All n=33		Men n=12		Women n=21	
Age (years)	21.0	(2.1)	22.2	(2.3)	21.7	(2.0)
Body composition parameters						
BMI (kg/m^2)	25.2	(4.5)	26.4	(4.8)	24.6	(4.3)
LMI (kg/m^2)	14.5	(2.2)	16.5	(1.7)	13.4	(1.7)
FMI (kg/m^2)	9.4	(3.2)	8.5	(3.5)	9.9	(3.0)
Fat mass (%)	37.5	(7.9)	31.9	(8.0)	40.4	(6.2)
BAT ^{18}F-FDG uptake parameters						
BAT volume (ml)	77.6	(55.2)	83.7	(57.4)	74.5	(54.8)
BAT mean radiodensity	-57.4	(11.2)	-52.6	(9.9)	-60.0	(11.2)
BAT SUVmean	4.2	(1.7)	3.7	(1.4)	4.4	(1.9)
BAT SUVpeak	13.2	(8.6)	11.8	(7.6)	13.9	(9.1)
Thyroid hormones						
TSH (µUI/ml)	1.8	(0.8)	1.8	(0.8)	1.8	(0.9)
FT4 (ng/dl)	0.9	(0.1)	0.9	(0.1)	0.9	(0.1)
FT3 (pg/ml)	3.4	(0.3)	3.5	(0.4)	3.3	(0.3)
PTFQI	0.2	(0.3)	0.2	(0.3)	0.2	(0.2)

Data are presented as mean and standard deviation. Abbreviations: BMI, Body mass index; LMI, Lean mass index, FMI, Fat mass index; ^{18}F-FDG, ^{18}F-Fluorodeoxyglucose; BAT, Brown adipose tissue; SUV, Standardized uptake value; TSH, Thyroid-Stimulating Hormone; FT4, free thyroxine; FT3, free triiodothyronine; PTFQI, Parametric Thyroid Feedback Quantile based Index.

Table 19. Associations of warm period (WP) and cold-induced (CI) circulating levels of TSH, FT4 and FT3 with cold-induced thermogenesis (CIT) (n=33).

	β	**R^2**	**P**
WP TSH	-0.005	0.008	0.610
WP FT4	-0.001	0.010	0.575
WP FT3	0.005	0.037	0.277
WP PTFQI	-0.003	0.021	0.417
CI TSH	0.001	0.001	0.866
CI FT4	0.001	0.016	0.480
CI FT3	-0.001	0.001	0.879
CI PTFQI	0.001	0.009	0.584

Non-standardized β coefficient, R^2 and P value from linear regression analyses. Abbreviations: TSH, Thyroid-Stimulating Hormone; FT4, free thyroxine; FT3, free triiodothyronine; PTFQI, Parametric Thyroid Feedback Quantile based Index; CI, Cold-induced.

DISCUSSION

The present study shows that personalized mild-cold exposure increases FT4 levels and reduces serum TSH and PTFQI in young, healthy, euthyroid adults. The results also reveal that neither the TSH, FT4 or FT3 level nor the PTFQI, under either thermoneutral conditions or after cold exposure, is associated with BAT volume, ^{18}F-FDG uptake and mean radiodensity or whole-body CIT. These findings indicate that THs concentrations are modified by mild-cold exposure, yet their concentration is not associated with BAT glucose metabolism when assessed after a relatively short cold exposure in young, euthyroid, healthy adults.

The effect of cold exposure on circulating THs in euthyroid adults has been investigated in several studies with contradictory results (20,21). The observed discrepancies might be partially due to the diversity of cold exposure procedures used. It is now accepted that a personalized cold exposure is necessary if the effect of mild-cold exposure in humans is to be properly studied (13). In the present work, after personalized cold exposure inducing non-shivering thermogenesis and BAT activation (12,22), an increase in FT4 was seen, but with no change in FT3. This increase in FT4 is likely due to increased thyroid secretion. If this is the case, it might seem paradoxical that FT3 levels are not modified after cold exposure. It should be noted, however, that appreciable changes in T3 function in euthyroid humans are the consequence of the DIO2- catalyzed conversion of T4 to T3, rather than the thyroid gland's secretion of T3. However, most T4 deiodination is likely to occur within tissues, which might not be reflected in circulating levels as reported by Maia *et al.* (23). Thus, it is plausible that the observed increase in circulating FT4 contributes to the induction of the thermogenic machinery via local conversion to T3.

In the present work, a reduction in the circulating levels of TSH was seen following the cold exposure, which contrasts with reports indicating that TSH and THs not to be affected by cold exposure lasting 2 h (6) or longer (20). TSH is the main hormone inducing THs secretion by the thyroid gland (24), and the marked reduction observed after cold exposure might, therefore, be expected to be coupled with reduced THs levels. This contrasts with the observed increase in FT4 but this increase in the latter might indeed explain why TSH is reduced. A negative feedback loop aiming to maintain THs levels stable is activated when THs concentrations rise, leading to a reduction in the secretion of TSH (25). In fact, and according to the interpretation of the

PTFQI, this indicates an inhibition of TSH serum levels by FT4 (15). Thus, the initial increase in FT4 levels could cause the reduction seen in TSH after 1 h of cold exposure. It is of note that this negative anticipatory response might also be reflected in the TRH level, which might itself contribute to a reduction in TSH. However, since TSH receptors are located in tissues other than the thyroid gland, it cannot be ruled out that the peripheral uptake of TSH also contributes to falling circulating concentrations of TSH (26).

BAT is present and metabolically active in most human adults and is regarded as a promising therapeutic target in strategies to combat obesity and related comorbidities (27,28). Identifying the factors regulating human BAT recruitment is, therefore, a promising avenue of research. T3 is a well-known contributor to BAT recruitment (2). Moreover, TSH receptors have been identified in brown adipocytes, suggesting a direct signalling effect of TSH in thermogenesis (26). However, it is not clear whether the variation of circulating levels of THs and TSH within their physiological ranges contributes significantly to BAT recruitment in young, euthyroid humans (3). Given that THs are involved in thermogenic gene transcription rather than in regulating UCP1 thermogenic activity (2), we hypothesized that baseline, but not cold-induced THs, would be correlated with BAT-related variables. However, TSH, FT4, FT3 concentrations and the PTFQI under thermoneutral conditions were not associated with BAT volume, ^{18}F-FDG uptake or mean radiodensity after a personalized cold exposure. Similarly, cold-induced changes in TSH, FT3, FT4, and PTFQI were found not to be associated with BAT-related outcomes. These results are in agreement with the conviction of that TH effects on BAT mass are not to be expected in short-term cold exposures, like the one applied in this study (29). Nonetheless, it does not rule out the possibility that chronic or long-term cold exposure modifies the TSH and THs cold-induced levels, which might promote BAT recruitment or white adipose tissue browning. In addition, the lack of associations seen in the present work might be partially explained by the intracellular deionization of T4 being the main factor contributing to BAT recruitment rather than any change in circulating TSH and/or THs.

BAT is an important endocrine organ (30), with the capacity of secreting hormones (31), metabolites (32) and nucleic acids (33) that are able to regulate the function of central and peripheral tissues. Therefore, it is plausible that besides the regulation of BAT recruitment/activation by the thyroid axis, reverse endocrine communication might exist as well. It is tempting to speculate with some BAT secreted factors (i.e. batokines)

being in part responsible for the changes in TSH and FT4 concentration observed in our study. It is biologically plausible that BAT recruitment secretes molecules that enhance THs secretion, which in turn will act on the pituitary gland to suppress TSH secretion. Indeed, a previous study has suggested the existence of such a feedforward mechanism, with factors emerging from activated BAT that are able to regulate thyroid function (34).

The role of THs in energy expenditure has been widely studied, especially in thyroid disease (35,36). In the hyperthyroid state, circulating levels of THs are elevated, leading to an increase in the basal metabolic rate. The opposite occurs in hypothyroidism, in which lower circulating levels of T4 and T3 result in reduced energy expenditure (37). The direct contribution of BAT to human energy expenditure seems to be quantitatively small; indeed, muscle thermogenesis seems to play the main role in human non-shivering thermogenesis (38). Therefore, even if TSH and THs are not associated with BAT-related outcomes, they could theoretically be associated with whole-body CIT. However, in the present work, no association was seen between TSH, FT4 and FT3 levels and CIT, suggesting that tissue T4-to-T3 conversion is the key thyroid metabolism-related factor affecting euthyroid human CIT rather than circulating THs or TSH levels. The lack of association might also be explained because cold-induced responses in TSH and THs and CIT were determined on different days. Therefore, day-to-day biological variability might have precluded us to detect an association between thyroid function and CIT. Nonetheless, these results are in agreement with Maushart et al. (39), who did not find associations between cold-induced responses in TSH and THs and CIT having both assessed at the same time.

Limitations

The present results need to be understood with some caution. Although the anatomical region analysed by the PET/CT scan (from the cerebellum to thoracic vertebra 4) covers the main BAT deposits (40), others, such as the suprarenal BAT deposits, were excluded. Further, this work involved young, healthy adults, so these findings cannot be extrapolated to older or unhealthy populations. The static PET/CT scan used only reflects the accumulated ^{18}F-FDG uptake over a 120 min period; it is possible that a dynamic PET/CT scan might detect associations between THs and ^{18}F-FDG uptake during cold-stimulation. Although personalized cold exposure plus ^{18}F-FDG PET/CT scanning is currently the best available option for assessing human BAT volume *in vivo*,

^{18}F-FDG is not an adequate method for assessing BAT activity (41). Thus, future studies are needed to investigate whether thyroid function and its cold-induced changes are related to BAT thermogenic or endocrine activity. The present work should be replicated using other radiotracers such as [15O] O_2 or [11C] acetate (42) and repeated using better technological options for BAT activity assessment as they become available. On the other hand, CIT was only assessed in a subgroup of 33 subjects, which diminishes the statistical power. In addition, the mild and short-term cold exposure (~2 h) applied in our study prevent extrapolating our results to more intense or longer cooling stimulus. Finally, TH levels and BAT activity/volume were measured at different times of the day, and circadian rhythmicity could partially compromise the results of this study (43).

Conclusion

In summary, we observed that the circulating concentrations of TSH and THs are modified by a short personalized mild cold exposure, but neither the thermoneutral nor the cold-induced TSH and THs concentrations are associated with BAT volume, ^{18}F-FDG uptake or mean radiodensity in young, healthy, euthyroid adults.

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GENERAL DISCUSSION

General discussion

In this Doctoral Thesis, we analysed whether several lifestyle factors are related to thyroid function, and whether it is associated to adiposity and cardiometabolic health in young euthyroid adults. Finally, we explore the association between thyroid function and BAT volume and metabolism, as potential link between thyroid function and cardiometabolic health. Data obtained from the present Doctoral Thesis show that thyroid function is associated with several lifestyle factors even in euthyroid young adults. In addition, the results also show that FT3 is related to central adiposity and cardiometabolic risk. However, no relationship between thyroid function and BAT was observed.

Thyroid dysfunction and obesity are common disorders in humans. A direct link between thyroid function and obesity has been hypothesized (1–5). Importantly, thyroid disorders are closely related to metabolic disturbances (6), and even in the absence of thyroid dysfunction (i.e., euthyroid individuals), slight differences in THs may influence energy metabolism and cardiometabolic health (7). Therefore, is crucial explore the possible factor involved on thyroid metabolism, and their implication on cardiometabolic health, even in euthyroid adults.

LIFESTYLE FACTORS AND THYROID FUNCTION IN YOUNG EUTHYROID ADULTS

Several studies have hypothesized that dietary intake (8–14), physical activity (15,16), sleep habits (17,18) and cold exposure (19,20) modulate TSH and THs circulating levels. However, the relationship between these factors and thyroid function in healthy and euthyroid adults is still unknown, and the potential mechanisms underlying such relationship are still to be explored. It is well known that fasting induce a decrease of THs circulating levels as an adaptive mechanism to preserve energy during food restriction (8–10). In the **study 1** of the present Doctoral Thesis, positive associations between energy intake and TSH and FT3 were found. These results might indicate an effect of decreased energy intake on both central (TSH response) and peripheral (lower conversion of FT4 to FT3) mechanisms to preserve energy. Previous studies also observed that diet composition, even in isocaloric conditions, can alter thyroid function, and therefore, peripheral TH metabolism (21–23). In the **study 1**, negative associations between fat intake and TSH levels were observed. In contrast, a positive association between carbohydrate intake and TSH levels was found. These results support the hypothesis that excess of dietary fat intake interfere with the endocrine system (23),

possibly altering the lipid profile of thyroid gland (11). In addition, these results are also in agreement with the evidence that high carbohydrate diet increase the sympathetic nervous system activity, which might stimulate the HPT axis increasing the conversion of T4 to T3 (12,13).

Adherence to the Mediterranean diet has been demonstrated to reduce the risk of suffering cardiometabolic diseases (24). Relevantly, some reports in literature have described that some food items frequently consumed in the Mediterranean diet can affect thyroid function by inhibiting the synthesis of THs (25). In this doctoral thesis **(study 1),** negative associations were observed between the adherence to the Mediterranean diet and TSH and FT4 circulating levels. Similar associations were found with the intake of some food groups related to Mediterranean dietary pattern, such as olive oil and vegetables. These results are in agreement with the reported by Zupo et al. (14), who showed that higher adherence to the Mediterranean diet was associated to a reduced thyroid function in euthyroid subjects.

On the other hand, several micronutrients are involved in the regulation of THs metabolism, protecting the thyroid gland from oxidative stress by interacting with iodine during conversion of the T4 to T3 (26–28). In this doctoral thesis **(study 1),** a positive association between selenium intake and TSH, and a negative association between folate and vitamin C intake with FT3 were found**.** These results suggest that deficient intakes of some micronutrients seem to exacerbate iodine deficiency and contribute to altered thyroid function.

The hypothalamic-pituitary-adrenal axis is stimulated in response to physical activity, and different exercise intensities have been reported to have an important role on thyroid function (15). In the **study 1,** negative associations between vigorous and overall physical activity levels and FT4 were found. These results contrast with the reported by Roa Dueñas et al (16), who found no associations between TSH or FT4 levels and physical activity levels. However, there are few studies exploring the relationship between physical activity levels and thyroid function in euthyroid adults and, therefore, more studies are needed to clarify this relationship. Cardiorespiratory fitness is currently recognized as an important marker of cardiovascular health, as it integrates the function of many physiological systems (29). However, in the **study 2**, despite FT3 was associated to many cardiometabolic risk factors, no association was found between TSH/THs and cardiorespiratory fitness in euthyroid adults.

It is known that circadian rhythms and sleep-wake state can affect TSH and THs secretion (17). Subjects with thyroid disfunction have been reported suffering different sleep disorders (18). Nevertheless, in the **study 1**, no associations were found between sleep habits and thyroid function, which might be explained by the indirect methodology used and the euthyroid and good sleepers (6-7 hours/day) state of the participants. Of note, in contrast to the **study 1**, the studies observing associations between sleep habits and THs included a relatively sleep deprivation.

Cold exposure is another external stimulus that modulate the THs circulating levels due to the role of thyroid function in thermogenesis (20). However, the effect of cold exposure in euthyroid adults has been investigated with inconclusive results (19,20). In the **study 3** of this Doctoral Thesis a reduction on the TSH circulating levels and an increase in FT4 levels was observed following the cold exposure. It contrasts with reports indicating that TSH and THs are not affected by acute cold exposure (19,30). There is clear evidence showing that TSH is the main hormone inducing THs secretion by the thyroid gland (31), and therefore, the marked reduction on circulating TSH levels observed in **study 3** after cold exposure would anticipate reduced THs levels. This negative anticipatory response might also affect the TRH concentration, contributing to the reduction of TSH levels. Additionally, since TSH receptors have been found in tissues other than the thyroid gland, it is possible that the peripheral uptake of TSH also contributes to decreased circulating concentrations of TSH (32).

THYROID FUNCTION, BODY COMPOSITION AND CARDIOMETABOLIC HEALTH IN YOUNG EUTHYROID ADULTS

Several studies have analysed the relationship between thyroid function and adiposity (33,34), but few have evaluated the connection with central adiposity in euthyroid young adults. Central adiposity is an important risk factor in the development of metabolic syndrome and cardiovascular disease (35). In the present Doctoral Thesis **(study 2)**, a positive association of FT3 with WC and VAT mass was observed in young euthyroid adults. These results are in agreement with previous studies in euthyroid older obese subjects (36,37). Despite VAT is commonly associated to ectopic fat deposition, we found no association of FT3, FT4 or TSH concentrations with total NAT mass. Moreover, no associations of FT4 or TSH with any body composition variable were found, in agreement with the results obtained by Manji et al. (38). Future studies should evaluate the relationship between thyroid function and central adiposity to understand

the potential causes underlying the positive associations observed here and elsewhere (34).

The relationship between thyroid function, insulin resistance and metabolic syndrome is unknown as contradictory results have been published (39). Metabolic syndrome is defined and diagnosed by the presence of several risk factors for cardiovascular disease. Namely, central obesity, elevated blood pressure, atherogenic dyslipidaemia (high triglycerides and low HDL-C cholesterol) and hyperglycaemia. We observed that a cardiometabolic risk score including the metabolic syndrome risk factors is positively associated with FT3 in euthyroid young adults. In line with the results obtained on the **study 2** of this doctoral thesis, De Pergola et al. (6) showed that FT3 was associated with WC, hyperinsulinemia and other components of the metabolic syndrome in overweight and obese euthyroid women. These findings suggest that thyroid function could predict the metabolic syndrome risk in euthyroid young adults. Furthermore, we observed that FT3 is associated with fasting glucose, insulin and HOMA-IR. These findings have been widely reported in subjects with pathological circulating levels of THs (40,41). Ferranini et al. (42), showed that normal FT3 levels were associated with insulin resistance and glucose intolerance in subjects with BMI ranging between 17–44 kg/m^2.

Despite the relationship between THs and blood pressure has been extensively studied in pathological states, this relationship still remains unclear in euthyroid subjects. In the **study 2**, THs and PTFQI were positively associated with systolic, diastolic and mean blood pressure. However, no association was found between TSH and blood pressure, which concur with the finding by Roos et al. (43). THs are important regulators of vascular function. Both T3 and T4 acts as vasodilators on vascular smooth muscle cells (44). Therefore, an increase of THs concentration in euthyroid subjects might represent a compensation for high blood pressure values. Alternatively, elevated concentrations of THs might itself contribute to increase blood pressure. Hyperthyroidism can produce tachycardia, increased heart contractility, elevated cardiac output, high systolic pressure, increased pulse pressure, and muscle weakness (45). Therefore, elevated THs within the normal range could have the same effect than in hyperthyroid state, increasing blood pressure.

THs are important regulators of lipid metabolism (46). The association between THs concentrations and serum lipids in pathological states has been for long documented (47,48). However, whether this relationship exist in euthyroid subjects is not clear yet. A

tendency towards a negative association of FT3, TSH and PTFQI with HDL-C was observed in the **study 2**, which is in agreement with Bakker et al. (49). Hypothyroidism has been reported to be associated with higher serum levels of cholesterol, whereas their levels are reduced in hyperthyroidism, and atherosclerotic cardiovascular disease (50). This phenomenon appears to be related to the cholesterol ester transfer protein (CEPT) activity. CEPT is positively regulated by THs, regulating the exchange of cholesteryl-ester between HDL-C and very-low-density lipoprotein (VLDL). Higher activity of CEPT would imply an increased cardiovascular risk, related to elevation of VLDL and decreased of HDL-C serum levels (46). Thus, it is plausible that the tendency towards an inverse association that we found of FT3, TSH and PTFQI with HDL-C is mediated by this mechanism, even in euthyroid subjects.

After observing a link between thyroid function and cardiometabolic health in young euthyroid individuals, we ought to explore whether BAT metabolism could be indeed explaining such association. BAT is involved in non-shivering thermogenesis, through the energy dissipation as heat (51). BAT is present and metabolically active in most human adults and is regarded as a promising therapeutic target in strategies to combat obesity and related comorbidities (52,53). T3 is a well-known contributor to BAT recruitment (54). Moreover, TSH receptors have been identified in brown adipocytes, indicating a direct signalling effect of TSH in thermogenesis (32). Therefore, given that THs are involved in thermogenic gene and in regulating UCP1 thermogenic activity (54), it is plausible to think that TSH and THs would be correlated with BAT-related variables within the normal range, and that contributes significantly to BAT recruitment in euthyroid humans (55).

However, the results obtained in this doctoral thesis **(study 3)** showed that TSH, FT4, FT3 concentrations and the PTFQI under thermoneutral conditions were not associated with BAT volume, glucose uptake and radiodensity, when assessed after a personalized cold exposure. Similarly, cold-induced changes in TSH, THs, and PTFQI were found not to be associated with BAT-related outcomes. These findings indicate that despite THs concentrations are modified by mild-cold exposure, their concentration seems not be associated with BAT metabolism in young, euthyroid, healthy adults.

The role of thyroid function in energy expenditure has been extensively studied, particularly in thyroid dysfunction (56,57). In the hyperthyroid state, circulating levels of THs are elevated, increasing the basal metabolic rate. Contrary, in hypothyroidism,

circulating levels of THs result in reduced energy expenditure (58). The direct contribution of BAT to human energy expenditure seems to be quantitatively small; indeed, muscle thermogenesis seems to play the main role in human non-shivering thermogenesis (59). Therefore, even if TSH and THs are not associated with BAT-related outcomes, they could theoretically be associated with whole-body CIT. However, no association was seen between TSH, FT4 and FT3 levels and CIT in the **study 3**, suggesting that peripheral T4-to-T3 conversion is the key thyroid metabolism-related factor affecting euthyroid human CIT rather than circulating THs or TSH levels. These results obtained in the **study 3** accord with Maushart et al. (60), who did not find associations between cold-induced responses in TSH and THs and CIT.

In summary, the results of the present Doctoral Thesis suggest that thyroid function is associated with lifestyle factors such as diet, physical activity and cold exposure. On the other hand, thyroid function (i.e. FT3) seems to be related to central adiposity and cardiometabolic risk even in young euthyroid adults, suggesting that thyroid function may be related to development of non-communicable disease. Finally, even if the changes observed on THs levels after cold exposure evidence the role of TSH and THs in thermogenesis, no associations were found between thyroid function and BAT metabolism, which suggest that BAT metabolism does not explain the relationship between thyroid function and cardiometabolic risk**.**

GENERAL LIMITATIONS

The results presented in this Doctoral Thesis need to be considered with some caution since there are some limitations:

- This Doctoral Thesis is composed by three cross-sectional studies and, therefore, no causality can be established.
- Only young, healthy adults are involved in the present Doctoral Thesis, so the results obtained are not extrapolatable to older or unhealthy populations.
- We did not standardize neither controlled the menstrual cycle, which might have affected the results.
- The self-reported dietary intake quantification presents a large measurement error, so risk of underreporting or misclassification need to be considered.
- The accelerometers used to assess physical activity and sleep habits could be underestimating/overestimating the sleep outcomes, as compared to polysomnography, which is the gold-standard method to properly assess sleep duration and efficiency.
- Despite DXA is valid instrument to assess body composition, it is limited to accurately assess VAT mass. Other more precise techniques such as magnetic resonance might have been more informative concerning the relationship between thyroid function and body composition.
- Other techniques such as lipoprotein profile analyses or hyperinsulinemic clamps for determining insulin resistance might be able to better detect relationships between thyroid function and cardiometabolic risk.
- Although the anatomical region analysed by the PET/CT scan (from the cerebellum to thoracic vertebra 4) covers the main BAT deposits (61), others, such as the suprarenal BAT deposits, were excluded.
- The static PET/CT scan used (study 2), only reflects the accumulated ^{18}F-FDG uptake over a 120 min period; it is possible that a dynamic PET/CT scan might detect associations between THs and ^{18}F-FDG uptake during cold-stimulation.
- Although personalized cold exposure plus ^{18}F-FDG PET/CT scanning is currently the best available option for assessing human BAT volume *in vivo*, ^{18}F-FDG is not an adequate method for assessing BAT activity (62). The present work should be replicated using other radiotracers such as [^{15}O] O_2 or [^{11}C] acetate (63) and

repeated using better technological options for BAT activity assessment as they become available.

- CIT was only assessed in a subgroup of 33 subjects, which diminishes the statistical power.
- The mild and short-term cold exposure (~2 h) applied (study 3), prevent extrapolating our results to more intense or longer cooling stimulus.
- THs levels and BAT activity/volume were measured at different times of the day, and circadian rhythmicity could partially compromise the results of the study 2 (64).

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CONCLUSIONS AND FUTURE PERSPERTIVES

CONCLUSIONS AND FUTURE PERSPERTIVES

General conclusion

The results of the present Doctoral Thesis show that, in euthyroid young adults, several lifestyle factors are associated with thyroid function. Moreover, circulating FT3 levels are related to central adiposity and cardiometabolic risk. Finally, no relationship was observed between thyroid function and BAT in young adults.

Specific conclusions

Conclusion 1 (Study 1)

Self-reported energy is positively associated with TSH and FT3 levels. Carbohydrate intake is positively associated with TSH levels, and total fat intake, SFA, MUFA and PUFA, is negatively associated also with TSH levels. Moreover, the adherence to the MED is negatively related to TSH and FT4. Further, vigorous-intensity and overall physical activity levels are negatively related to FT4 even in young euthyroid adults. In contrast, no associations between sleep parameters and thyroid function have been observed.

Conclusion 2 (Study 2)

FT3 is positively associated with central adiposity and metabolic syndrome factors such as insulin resistance, blood pressure and fatty liver in euthyroid young adults.

Conclusion 3 (Study 3)

Thyroid function is modulated by cold exposure, but neither the thermoneutral nor the cold-induced TSH and THs concentrations are associated with BAT volume, ^{18}F-FDG uptake or mean radiodensity in young, healthy, euthyroid adults.

FUTURES PERSPECTIVES

- Future randomized controlled trials with nutritional intervention, such as dietary Mediterranean Pattern, should be conducted to assess its influence the thyroid function. To this end, secondary analyses of previously conducted studies might offer an efficient manner of achieving so. Likewise, analyses of blood samples collected during previous experimental studies applying physical activity interventions, manipulating sleep time and/or assessing it with polysomnography are warranted to understand the impact of this lifestyle factors on the thyroid function of euthyroid adults.

- The results of the present International Doctoral Thesis support that thyroid function is related to energy homeostasis and cardiometabolic health, even in young euthyroid adults. To further understand this relationship, further studies should focus on investigating the predictive capacity of thyroid function on future cardiometabolic health (i.e. observational longitudinal studies).

- Moreover, the aforementioned link between thyroid function and cardiometabolic health worth being investigated in children and older adults.

- We detected a cold-induced decrease in TSH in response to cold-exposure in euthyroid adults. It is still to be determined whether a parallel decrease occur in the hypothalamic-produced TRH.

- Finally, data obtained in the present International Doctoral Thesis suggests that BAT volume and metabolism are not related to thyroid function in young euthyroid adults. Future studies should revisit this issue using dynamic PET/CT scans, other more adequate radiotracers for assessing BAT activity, as well as other imaging technologies able to detect widespread small depots of BAT, should these technologies become available.

ANNEXES

PAPERS DERIVED FROM THE PRESENT DOCTORAL THESIS

1. **Merchan-Ramirez E**, Sanchez-Delgado G, Jurado-Fasoli, L, Acosta Francisco M, Muñoz-Torres M, Llamas-Elvira Jose M., Ruiz Jonatan R. Association between lifestyle factors and thyroid function in young euthyroid adults. ***Submitted.***

2. **Merchan Ramirez E**, Sanchez-Delgado G, Arrizabalaga-Arriazu C, Acosta F.M, Arias-Tellez M.J., Muñoz-Torres Manuel, Garcia-Lario J.V, Llamas-Elvira J.M, Ruiz, JR. Circulating concentrations of free triiodothyronine are associated with central adiposity and cardiometabolic risk factors in young euthyroid adults. ***Submitted.***

3. **Merchan-Ramirez E**, Sanchez-Delgado G, Arrizabalaga-Arriazu C, Martinez-Tellez B, Mendez-Gutierrez A, Muñoz-Torres M, Llamas-Elvira J.M, Ruiz JR. Thyroid function is not associated with brown adipose tissue volume and 18F-Fluorodeoxyglucose uptake in young euthyroid adults. Eur J Endocrinol. 2021 Jul 1;185(2):209-218.

CURRICULUM VITAE

1. Personal information

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2. Academic profiles

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3. Actual position

PhD student and researcher of Department of Physical and Sports Education, Sport and Health University Research Institute (iMUDS), Faculty of Sports Science, University of Granada, Granada, Spain.

4. Previous positions

Jan 2020-Dic 2021	Spanish Ministry of Economy and Competitiveness. Fondo social Europeo e iniciativa de Empleo Juvenil (PEJ2018-003843-A).
Oct 2018-Oct 2019	Research contract. Danone Institute. Ref. Danone, 2017.
Jan 2017-Dic 2017	Research contract charged to the project ("ACTIBATE" project). Excellence actions: Units of Excellence; Unit of Excellence on Exercise and Health (UCEES). University of Granada
Oct 2016-Dic 2016	Research contract. University of Granada. ("ACTIVEBRAINS" project).

5. Education

2017-2018	Master´s degree in Clinical Nutrition, "Universidad Católica de San Antonio" (UCAM), Murcia.
2015-2016	Master´s degree in Kinanthropometry and sports nutrition, University of Valencia.
2011-2015	Bachelor's degree in Human Nutrition and Dietetics, University of Granada.

6. Courses and extracurricular activities

2021	Updates in the Nutritional Treatment of patients with cancer. Online modality (IFOA).
2021	Course "Avances en Nutrición pediátrica" (FINUT)
2020	Course "Estrategias para modificar la redacción, publicación y comunicación de artículos científicos". Universidad de Granada, España.
2017	Expert in clinical nutrition and hospital dietetics. Online Modality. (Universidad Camilo José Cela).

2015 Course "Nutrición Deportiva, Farmacología y Suplementación aplicada al deporte". Online modality (Egea Granada).

7. Research projects

2019-2020 **ACTIFOX Project:** Effect of capsinoids intake and physical exercise on energy metabolism and brown adipose tissue in adults.

Principal Investigator: Jonatan R. Ruiz

Funding: Spanish Ministry €25,000.

2015 – 2017 **ACTIBATE Project:** Activating Brown Adipose Tissue through Exercise.

Effects of an exercise intervention on activity and quantity of Brown adipose tissue: A Randomized Controlled Trial.

Principal Investigator: Jonatan R. Ruiz.

Funding: Spanish Ministry €175,000; others: €150,000.

2015 – 2016 **ActiveBrains Project:** An exercise-based randomized controlled trial on brain, cognition, physical health and mental health in overweight/obese children

Principal Investigator: Francisco B. Ortega Porcel

Funding: Spanish Ministry €120,000.

8. Divulgation activities

2020/2021 Contents elaboration in "Máster en Nutrición Clínica y Deportiva" (MEDAC).

2020/2021 Internal research seminars to share scientific knowledge (methodological issues, statistical analysis...).

2016 Nutrition education workshop for children (Material and content elaboration, and realization of the sessions). "Activebrains project".

2016 Nutrition education workshop for adults (Material and content elaboration, and realization of the sessions) "Activebrains projects".

9. Publications list

1. Thyroid function is not associated with brown adipose tissue volume and ^{18}F-Fluorodeoxyglucose uptake in young euthyroid adults. **Merchan-Ramirez E**, Sanchez-Delgado G, Arrizabalaga-Arriazu C, Martinez-Tellez B, Mendez-Gutierrez A, Muñoz-Torres M, Llamas-Elvira JM, Ruiz JR. Eur J Endocrinol. 2021 Aug 1;185(2):209–18.
    - doi: 10.1530/EJE-21-0192.
    - IF JCR: 6.66
    - Ranking: 20/145. Q1 [ENDOCRINOLOGY & METABOLISM].
2. Association between dietary factors and brown adipose tissue volume/18F-FDG uptake in young adults. Jurado-Fasoli L, **Merchan-Ramirez E**, Martinez-Tellez B,

Acosta FM, Sanchez-Delgado G, Amaro-Gahete FJ, Muñoz Hernandez V, Martinez-Avila WD, Ortiz-Alvarez L, Xu H, Arias Téllez MJ, Ruiz-López MD, Llamas-Elvira JM, Gil Á, Labayen I, Ruiz JR. Clin Nutr. 2021 Apr;40(4):1997-2008.

- doi: 10.1016/j.clnu.2020.09.02
- IF JCR: 7.324.
- Ranking: 7/88. Q1 [NUTRITION & DIETETICS].
- Ranking: 40/145. Q2 [ENDOCRINOLOGY & METABOLISM].

3. Neck adipose tissue accumulation is associated with higher overall and central adiposity, a higher cardiometabolic risk, and a pro-inflammatory profile in young adults. Arias Téllez MJ, Acosta FM, García-Rivero Yolanda, Pascual-Gamarra JM, **Merchán Ramírez E**, Martínez-Téllez B, Silva MA, Almanza López J, Llamas-Elvira JM, Ruiz JR. Int J Obes (Lond). 2021 Apr;45(4):733-745.

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- IF JCR: 4.222.
- Ranking: 32/88. Q2 [NUTRITION & DIETETICS].

5. Higher Physical Activity Is Related to Lower Neck Adiposity in Young Men, but to Higher Neck Adiposity in Young Women: An Exploratory Study. Arias-Tellez MJ, Acosta FM, Migueles JH, Pascual-Gamarra JM, **Merchan-Ramirez E**, de Lucena Martins CM, Llamas-Elvira JM, Martinez-Tellez B, Ruiz JR. Int J Sport Nutr Exerc Metab. 2021 Mar;31(3):250–8.

- doi: 10.1123/ijsnem.2020-0193.
- IF JCR: 4.599.
- Ranking: 15/88. Q1 [SPORT SCIENCES].

6. Energy Expenditure and Macronutrient Oxidation in Response to an Individualized Nonshivering Cooling Protocol. Sanchez-Delgado G, Alcantara JMA, Acosta FM, Martinez-Tellez B, Amaro-Gahete FJ, **Merchan-Ramirez E**, Löf M, Labayen I, Ravussin E, Ruiz JR. Obesity. 2020 Nov 27;28(11):2175–83.

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7. Skin temperature response to a liquid meal intake is different in men than in women. Martinez-Tellez B, Ortiz-Alvarez L, Sanchez-Delgado G, Xu H, Acosta FM, **Merchan-Ramirez E**, Muñoz-Hernandez V, Martinez-Avila WD, Contreras-Gomez MA, Gil A, Labayen I, Ruiz JR. Clin Nutr. 2019 Jun;38(3):1339-1347.

- doi: 10.1016/j.clnu.2018.05.026.

- IF JCR: 7.324.
- Ranking: 7/88. Q1 [NUTRITION & DIETETICS].

8. Associations of dietary energy density with body composition and cardiometabolic risk in children with overweight and obesity: role of energy density calculations, under-reporting energy intake and physical activity. Gomez-Bruton A, Arenaza L, Medrano M, Mora-Gonzalez J, Cadenas-Sanchez C, Migueles JH, Muñoz-Hernández V, **Merchan-Ramirez E**, Martinez-Avila WD, Maldonado J, Oses M, Tobalina I, Gracia-Marco L, Vicente-Rodriguez G, Ortega FB, Labayen I. Br J Nutr. 2019 May;121(9):1057-1068.

- doi: 10.1017/S0007114519000278.
- IF JCR: 3.718.
- Ranking: 44/88. Q3 [NUTRITION & DIETETICS].

9. Association of Objectively Measured Physical Activity with Brown Adipose Tissue Volume and Activity in Young Adults. Acosta FM, Martinez-Tellez B, Sanchez-Delgado G, Migueles JH, Contreras-Gomez MA, Martinez-Avila WD, **Merchan-Ramirez E**, Alcantara JMA, Amaro-Gahete FJ, Llamas-Elvira JM, Ruiz JR. J Clin Endocrinol Metab. 2019 Feb 1;104(2):223-233

- doi: 10.1210/jc.2018-01312.
- IF JCR: 5.958.
- Ranking: 28/145. Q1 [ENDOCRINOLOGY & METABOLISM].

10. Congruent Validity of Resting Energy Expenditure Predictive Equations in Young Adults. Amaro-Gahete FJ, Sanchez-Delgado G, Alcantara JMA, Martinez-Tellez B, Muñoz-Hernandez V, **Merchan-Ramirez E**, Löf M, Labayen I, Ruiz JR. Nutrients. 2019 Jan;11(2).

- doi: 10.3390/nu11020223.
- IF JCR: 5.717.
- Ranking: 24/103. Q1 [NUTRITION & DIETETICS].

11. Energy expenditure differences across lying, sitting, and standing positions in young healthy adults. Amaro-Gahete FJ, Sanchez-Delgado G, Alcantara JMA, Martinez-Tellez B, Acosta FM, **Merchan-Ramirez E**, Löf M, Labayen I, Ruiz JR. PLoS One. 2019;14(6): e0217029.

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- IF JCR: 3.240.
- Ranking: 26/73. Q2 [MULTIDISCIPLINARY SCIENCES].

12. Congruent validity and inter-day reliability of two breath by breath metabolic carts to measure resting metabolic rate in young adults. Alcantara JMA, Sanchez-Delgado G, Martinez-Tellez B, **Merchan-Ramirez E**, Labayen I, Ruiz JR. Nutr Metab Cardiovasc Dis. 2018 Sep;28(9):929–36.

- doi: 10.1016/j.numecd.2018.03.010.
- IF JCR: 4.22.
- Ranking: 33/88. Q2 [NUTRITION & DIETETICS]

13. Influence of Physical Activity on Bone Mineral Content and Density in Overweight and Obese Children with Low Adherence to the Mediterranean Dietary Pattern. Muñoz- Hernandez V, Arenaza L, Gracia-Marco L, Medrano M, **Merchan-Ramirez E**,

Martinez-Avila WD, Oses M, Ruiz JR, Ortega FB, Labayen I. Nutrients. 2018 Aug 12;10(8):1075.

- doi: 10.3390/nu10081075.
- IF JCR: 5.717.
- Ranking: 24/103. Q1 [NUTRITION & DIETETICS].

14. Association of Breakfast Quality and Energy Density with Cardiometabolic Risk Factors in Overweight/Obese Children: Role of Physical Activity. Arenaza L, Muñoz-Hernández V, Medrano M, Oses M, Amasene M, **Merchán-Ramírez E**, Cadenas-Sanchez C, Ortega FB, Ruiz JR, Labayen I. Nutrients. 2018 Aug;10(8).

- doi: 10.3390/nu10081066.
- IF JCR: 5.717.
- Ranking: 24/103. Q1 [NUTRITION & DIETETICS].

15. An exercise-based randomized controlled trial on brain, cognition, physical health and mental health in overweight/obese children (ActiveBrains project): Rationale, design and methods. Cadenas-Sánchez C, Mora-González J, Migueles JH, Martín-Matillas M, Gómez-Vida J, Escolano-Margarit MV, Maldonado J, Enriquez GM, Pastor-Villaescusa B, de Teresa C, Navarrete S, Lozano RM, de Dios Beas-Jiménez J, Estévez-López F, Mena-Molina A, Heras MJ, Chillón P, Campoy C, Muñoz-Hernández V, Martinez-Avila WD, **Merchan ME**, Perales JC, Gil Á, Verdejo-García A, Aguilera CM, Ruiz JR, Labayen I, Catena A, Ortega FB. Contemp Clin Trials. 2016 Mar; 47:315-24.

- doi: 10.1016/j.cct.2016.02.007.
- IF JCR: 2.226
- Ranking: 110/140. Q4 [MEDICINE, RESEARCH & EXPERIMENTAL].

10. Accepted congress communications as first author

Title: Association between body composition and cognitive performance in obese children: Preliminary results from ActiveBrains Project.

Congress: International Symposium Active brains for all: Exercise, Cognition and Mental Health.

Date and place: June 2017. Granada, España.

Type: Abstract, poster

Autores: Elisa Merchán, Pontus Henriksson, Hanna Henriksson, Victoria Muñoz-Hernández, Martinez-Avila WD, José Mora-González, Cristina Cadenas-Sánchez, Irene Estaban-Cornejo, Jairo H. Migueles, Sanchez-Delgado G, María Rodriguez-Ayllón, Pablo Molina-García, Martín-Matillas M, Lide Arenaza, Idoia Labayen, Francisco B. Ortega.

11. Other merits:

2017 Member of the organizing committee of the international symposium: "Active brains for all: exercise, cognition and mental health". Granada, Spain.

2018 Member of the organizing committee of the international symposium: "Role of Brown adipose tissue in human health". Granada, Spain.

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