JUAN MANUEL ALCÁNTARA ALCÁNTARA

PROGRAMA DE DOCTORADO EN BIOMEDICINA

ASSESSMENT OF RESTING ENERGY EXPENDITURE AND NUTRIENT OXIDATION BY INDIRECT CALORIMETRY:

METHODOLOGICAL IMPLICATIONS

UNIVERSIDAD DE GRANADA

ASSESSMENT OF RESTING ENERGY EXPENDITURE AND NUTRIENT OXIDATION BY INDIRECT CALORIMETRY:

METHODOLOGICAL IMPLICATIONS

ASSESSMENT OF RESTING ENERGY EXPENDITURE AND NUTRIENT OXIDATION BY INDIRECT CALORIMETRY: METHODOLOGICAL IMPLICATIONS

Cover design & Layout: Manuel Padilla Jódar - www.padillax.com

International Doctoral Thesis / Tesis Doctoral Internacional

Assessment of resting energy expenditure and nutrient oxidation by indirect calorimetry: methodological implications

UNIVERSIDAD DE GRANADA

Determinación del gasto energético y oxidación de nutrientes en reposo mediante calorimetría indirecta: implicaciones metodológicas

PROGRAMA DE DOCTORADO EN BIOMEDICINA

DEPARTAMENTO DE EDUCACIÓN FÍSICA Y DEPORTIVA
FACULTAD DE CIENCIAS DEL DEPORTE
UNIVERSIDAD DE GRANADA

Juan M. Alcántara Alcántara

2020

Editor: Universidad de Granada. Tesis Doctorales
Autor: Juan Manuel Alcántara Alcántara
ISBN: 978-84-1306-740-7
URI: http://hdl.handle.net/10481/65383

DEPARTAMENTO DE EDUCACIÓN FÍSICA Y DEPORTIVA
FACULTAD DE CIENCIAS DEL DEPORTE
UNIVERSIDAD DE GRANADA

Assessment of resting energy expenditure and nutrient oxidation by indirect calorimetry: methodological implications

Determinación del gasto energético y oxidación de nutrientes en reposo mediante calorimetría indirecta: implicaciones metodológicas

Juan M. Alcántara Alcántara

Directores de la Tesis Doctoral [Doctoral Thesis Supervisors]

Jonatan Ruiz Ruiz
PhD
Prof. Titular de Universidad
Universidad de Granada

Guillermo Sánchez Delgado
PhD
Postdoctoral Researcher
Pennington Biomedical
Research Center

Miembros del Tribunal [Doctoral Thesis Comittee]

María Belén Feriche Fernández-Castanys
PhD
Catedrática de Universidad
Universidad de Granada, España

Alfredo Santalla Hernández
PhD
Profesor Titular de Universidad
Universidad Pablo de Olavide,
España

Virginia Arianna Aparicio García-Molina
PhD
Profesora Titular de Universidad
Universidad de Granada, España

Rodrigo Fernández Verdejo
PhD
Profesor Asistente
Pontificia Universidad Católica
de Chile, Chile

Pedro José Benito Peinado
PhD
Profesor Titular de Universidad
Universidad Politécnica de Madrid, España

Granada, 11 de diciembre de 2020

PLUS
ULTRA

PLUS
ULTRA

El doctorando D. Juan M. Alcántara Alcántara ha realizado la presente Tesis Doctoral Internacional siendo beneficiario de un contrato con cargo al programa de Formación de Profesorado Universitario (referencia: **FPU 15/04059**) del Ministerio de Educación, Cultura y Deporte (actualmente Ministerio de Universidades), por resolución de 19 de noviembre de 2015, de la Secretaría de Estado de Educación, Formación Profesional y Universidades (BOE-A-2015-12849, publicado el 27 de noviembre de 2015).

RESEARCH PROJECTS AND FUNDING

The present International Doctoral Thesis was performed under the framework of the ACTIBATE and the FITAGEING studies, which were funded by the following organizations and/or institutions:

- Spanish Ministry of Economy and Competitiveness via the *Fondo de Investigación Sanitaria del Instituto de Salud Carlos III* (PI13/01393).

- *Retos de la Sociedad* (DEP2016-79512-R) and European Regional Development Funds (ERDF).

- Spanish Ministry of Education (FPU 13/04365, 14/04172, 15/04059).

- *Fundación Iberoamericana de Nutrición* (FINUT).

- *Redes Temáticas de Investigación Cooperativa* RETIC (Red SAMID RD16/0022).

- AstraZeneca HealthCare Foundation.

- University of Granada *Plan Propio de Investigación* 2016 Excellence Actions: Unit of Excellence on Exercise and Health (UCEES) and *Plan Propio de Investigación* 2018 and 2019 *Programa Contratos-Puente* and *Plan Propio de Investigación* 2018 *Programa Perfeccionamiento de Doctores*

- *Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades* (ERDF: ref. SOMM17/6107/UGR).

- Fundación *Alfonso Martín Escudero*.

A mi segunda madre,
mi madrina,
La Madrina

A mis padres, hermana, y novia,
gracias por todo vuestro amor y apoyo

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

^{18}O: Oxygen-18
24hEE: 24-hour energy expenditure
^{2}H: Deuterium
$^{2}H_2{}^{18}O_2$: Doubly-labelled water
Acetyl-CoA: Acetyl coenzyme A
ACTIBATE: acronym for the 'Activating brown adipose tissue through exercise' study
ADMR: Average daily metabolic rate
AM: Ante meridiem
ANOVA: Repeated-measures analyses of variance
ATP: Adenosine triphosphate
BIPM: International Bureau of Weights and Measures – French acronym for – '*Bureau International des Poids et Mesures*'
BMI: Body mass index
BMR: Basal metabolic rate
B×B: breath-by-breath metabolic cart (system and/or approach)
Cal: Calorie
CCM: CCM Express metabolic cart (Medgraphics Corp, Minnesota, USA)
CHO: Carbohydrate
CHOox: Carbohydrate oxidation
CIT: Cold-induced thermogenesis
CO_2: Carbon dioxide
CV: Coefficient of variation
$CV_{D\text{-}to\text{-}D}$: Day-to-day coefficient of variation
DC: Direct Calorimetry
DIT: Diet induced thermogenesis
DTC: Deltatrac metabolic cart (Datex Instrumentarium Corp, Helsinki, Finland)
DXA: Whole-body dual-energy X-ray absorptiometry (Discovery Wi, Hologic, Inc., Bedford, MA, USA)
EAT: Exercise activity thermogenesis
EE: Energy expenditure
EI: Energy intake
Eq.: Equation
FATox: Fat oxidation
$FeCO_2$: Fraction of expired carbon dioxide
FeO_2: Fraction of expired oxygen
$FiCO_2$: Fraction of inspired carbon dioxide
FiO_2: Fraction of inspired oxygen
FIT-AGEING: acronym for 'Physical Fitness as Klotho Protein Stimulator' study
g: Gram
h: Hour
H^+: Hydrogen ions concentration
IC: Indirect Calorimetry
J: Joule
K: Kelvin degree
kcal: Kilocalorie
kcal/day: Kilocalories per day
kg: Kilogram
L: Liter
m: meter
MC: metabolic cart
Mean CVs: average of diverse coefficient of variations (i.e. coefficient of variation for volume of whole-body oxygen consumption, volume of whole-body carbon dioxide production, respiratory exchange ratio [or respiratory quotient], and/or minute ventilation)

Mean$_{25\text{-}min}$ RMR: Resting metabolic rate values (i.e. resting energy expenditure) obtained from a pre-determined period of 25 minutes
mg: Milligram
MGU: CPX Ultima CardiO2 metabolic cart (Medgraphics Corp, Minnesota, USA)
min: minute
MIT: Meal induced thermogenesis
ml: milliliters
mmHg: Millimeter of mercury
N: Nitrogen
N_2: Molecular nitrogen
NEAT: Non-exercise activity thermogenesis
NPRQ: Non-protein respiratory quotient
$NPVO_2$: Non-protein oxygen consumption
O_2: Oxygen
°C: Celsius degree
Omnical: Omnical metabolic cart (Maastricht Instruments, Maastricht, The Netherlands)
P_a: Ambient atmospheric pressure
PAEE: Physical activity related energy expenditure
PCCP: Post-calorimetric correction procedure
***p*MVD:** Per minute ventilation data
PROox: Protein oxidation
PROT: Protein
P_s: Standard pressure criterion
PVO_2: Protein oxygen consumption
P_w: Partial pressure of water vapor
Q: Heat
Q-NRG: Q-NRG metabolic cart (Cosmed, Rome, Italy)
R^2: Adjusted R-squared
REE: resting energy expenditure
RER: Respiratory exchange ratio
RICORS 1.0: Room Indirect Calorimetry Operating and Reporting Standards 1.0
RMC Study: Acronym for the 'Reproducibility of metabolic carts' study
RMR: Resting metabolic rate
RQ: Respiratory quotient
s: second
SD: Standard deviation
SEM: Standard error of the mean
SMR: Sleeping metabolic rate
SPA: Spontaneous physical activity
SPSS: Statistical Package for Social Sciences (IBM SPSS Statistics, IBM Corporation)
SS: Steady state
SSt: Steady state time method
STPD: Standard Temperature Pressure and Dry conditions
T_a: Ambient atmospheric temperature
TDEE: Total daily energy expenditure
TEF: Thermic effect of food
TI: time interval method
T_s: Standard temperature criterion
Ultima: Ultima CardiO2 metabolic cart (Medgraphics Corporation, St. Paul, MN, USA)
VCO_2: Volume of whole-body carbon dioxide production
VE: minute ventilation
V_{in}: Air volume flowing into from the Indirect Calorimetry system
VN_2: Volume of mollecular nitrogen
VO_2: Volume of whole-body oxygen consumption
V_{out}: Air volume flowing out from the Indirect Calorimetry system
V_S: Standard volume
Vyntus: Vyntus CPX metabolic cart (Jaeger-CareFusion, Höchberg, Germany)
ΔCO_2: Difference between inhaled and exhaled carbon dioxide
ΔO_2: Difference between inhaled and exhaled oxygen

ABSTRACT

Indirect calorimetry (IC) is the reference method to assess human resting metabolic rate (RMR), the largest component of total energy expenditure, except in extremely active individuals. In addition to RMR, IC also allows to determine the respiratory exchange ratio (RER), which gives valuable information about the type of energy substrates (fat vs. carbohydrate) being metabolized. Among the available IC systems, metabolic carts are the most extended in both research and clinical settings. The Deltatrac metabolic cart (Datex Instrumentarium Corp, Helsinki, Finland), which has been for long considered the gold standard for assessing RMR, is no longer manufactured. Numerous research groups around the world are deeply studying human RMR and RER and its regulation. Therefore, the identification of valid metabolic carts and data analysis methodology is of great importance. On the other hand, the method for IC data analysis commonly differs across studies, and the use of different methods might result in different estimates of RMR and RER.

The main aims of this Doctoral Thesis are to study the performance of six commercially available metabolic carts for assessing RMR and RER in healthy humans (**Section 1**), and to determine the most suitable method for IC data analysis when assessing RMR and RER by metabolic carts (**Section 2**). In **Section 1**, the accuracy, precision, and the day-to-day biological reproducibility of the Q-NRG (Cosmed, Rome, Italy), the Vyntus CPX (Jaeger-CareFusion, Höchberg, Germany), the Omnical (Maastricht Instruments, Maastricht, The Netherlands), and the Ultima CardiO2 (Medgraphics Corporation, St. Paul, MN, USA) were assessed, while the day-to-day biological reproducibility was assessed for the CCM Express and the CPX Ultima CardiO2 (Medgraphics Corporation, St. Paul, MN, USA). Lastly, the effect of a post-calorimetric correction procedure, based on the infusion of pure gases after the individual measurement (developed to correct the metabolic cart data using its previously assessed 'error'), was also tested in the Q-NRG, the Vyntus CPX, the Omnical and the Ultima CardioO2. In **Section 2**, three methods for IC data analysis were analyzed: a) the steady state time method (i.e. the analysis of a period remarkably stable), b) the time interval method (i.e. the analysis of a pre-defined period – short or long time intervals) and c) the filtering method (i.e. data above or below a given threshold are discarded). The impact of these methods on the RMR and RER estimations and its day-to-day biological reproducibility was assessed.

The results of the present Doctoral Thesis show that all the metabolic carts yielded different RMR and RER measures. The Omnical metabolic cart presented better accuracy and precision than the rest of metabolic carts, although the day-to-day biological reproducibility achieved by the Q-NRG and the Vyntus CPX was similar to the one achieved by the Omnical. The post-calorimetric correction procedure did not improve neither the comparability nor the day-to-day RMR and RER biological reproducibility in the four analyzed metabolic carts. On the other hand, we observed a strong association between the day-to-day biological reproducibility assessed with the CCM Express and the CPX Ultima CardiO2 metabolic carts. Finally, the long-time interval method for IC data analysis presented the best day-to-day RMR and RER biological reproducibility in four metabolic carts.

Collectively, the results of this Doctoral Thesis suggest that the Omnical is the best metabolic cart for assessing RMR and RER among the six metabolic carts analyzed. Moreover, this Doctoral Thesis suggest that the day-to-day biological reproducibility is largely attributable to the individual's characteristics and is not improved by the application of a post-calorimetric procedure based on the infusion of pure gases after the individual measurement. Finally, among the methods for IC data analysis, the long-time interval method seems to be the most adequate for analyzing the data provided by the analyzed metabolic carts.

RESUMEN

La calorimetría indirecta (CI) es el método de referencia para determinar el gasto metabólico en reposo (GMR) humano, el mayor componente del gasto energético total, excepto en sujetos extremadamente activos. Además del GMR, la CI también permite determinar la relación de intercambio respiratorio (RER), la cual proporciona información valiosa sobre el tipo de sustratos energéticos (grasas vs. carbohidratos) que están siendo metabolizados. Entre los sistemas de CI disponibles, los carros metabólicos son los más extendidos en entornos de investigación y clínicos. El carro metabólico Deltatrac (Datex Instrumentarium Corp, Helsinki, Finlandia), que ha sido durante mucho tiempo considerado el "dispositivo de referencia" (del inglés, *Gold Standard*) para determinar el GMR y el RER, no se fabrica ni se comercializa en la actualidad. Numerosos grupos de investigación en todo el mundo están investigando el GMR y el RER, así como su regulación. Por lo tanto, identificar carros metabólicos válidos, así como una metodología de análisis de datos válida es de gran relevancia. Por otro lado, el método utilizado para analizar los datos de CI difiere comúnmente entre estudios, y el uso de diferentes métodos podría resultar en diferentes estimaciones del GMR y RER.

Los objetivos principales de la presente Tesis Doctoral son estudiar el rendimiento de seis carros metabólicos, actualmente disponibles en el mercado, para determinar el GMR y RER en humanos sanos (**Sección 1**), y determinar el método más adecuado para analizar los datos de CI cuando se determina el GMR y RER mediante la utilización de carros metabólicos (**Sección 2**). En la **Sección 1**, la validez, precisión y reproducibilidad biológica entre días de los carros metabólicos Q-NRG (Cosmed, Roma, Italia), Vyntus CPX (Jaeger-CareFusion, Höchberg, Alemania), Omnical (Maastricht Instruments, Maastricht, Holanda) y Ultima CardiO2 (Medgraphics Corporation, St. Paul, MN, Estados Unidos) fueron determinadas, mientras que la reproducibilidad biológica entre días fue determinada para los carros metabólicos CCM Express y CPX Ultima CardiO2 (Medgraphics Corporation, St. Paul, MN, Estados Unidos). Por último, el efecto de un procedimiento de corrección post-calorimétrica, basado en la infusión de gases puros inmediatamente después de la medida del individuo (desarrollado para corregir los datos de los carros metabólicos usando su "error" previamente determinado), fue testado en los carros metabólicos Q-NRG, Vyntus CPX, Omnical y Ultima CardiO2. En la **Sección 2**, tres métodos para el análisis de los datos de CI fueron investigados: a) el método de estados estables (i.e. el análisis de un periodo de tiempo marcadamente estable), b) el método de intervalos de tiempo (i.e. el análisis de un tiempo pre-determinado – ya sean intervalos largos o cortos), y c) el método de filtrado (i.e. los datos por encima o por debajo de un umbral determinado son descartados). Se determinó el impacto de esos métodos en la estimación de GMR y RER, así como en su reproducibilidad biológica entre días.

Los resultados de la presente Tesis Doctoral muestran que todos los carros metabólicos obtuvieron diferentes valores de GMR y de RER. El carro metabólico Omnical presentó mejor exactitud y precisión que el resto de carros metabólicos, aunque la reproducibilidad biológica entre días mostrada por el Q-NRG y el Vyntus CPX fue similar a la mostrada por el Omnical. El procedimiento de corrección post-calorimétrica no mejoró ni la comparabilidad ni la reproducibilidad biológica entre días del GMR y del RER en los cuatro carros metabólicos analizados. Por otro lado, se observó una asociación fuerte entre la reproducibilidad biológica entre días determinada con el CCM y la determinada con el CPX Ultima CardiO2. Finalmente, el método de análisis de datos de CI basado en intervalos de tiempo largos presentó la mejor reproducibilidad biológica entre días en el GMR y RER en cuatro carros metabólicos.

Colectivamente, los resultados de esta Tesis Doctoral sugieren que el carro metabólico Omnical es el mejor para determinar el GMR y el RER entre los seis carros metabólicos analizados. Además, esta Tesis Doctoral sugiere que la reproducibilidad biológica del GMR y RER es fundamentalmente atribuible a las características del individuo y que no es mejorada por la aplicación de un procedimiento de corrección post-calorimétrica basado en la infusión de gases puros inmediatamente después de la medida del individuo. Finalmente, entre los métodos para analizar los datos de CI, el método basado en intervalos de tiempo largos parece ser el más adecuado para analizar los datos obtenidos mediante los carros metabólicos analizados.

GENERAL INTRODUCTION

1. CALORIMETRY, A TOOL FOR STUDYING ANIMAL METABOLISM

The word '*calorimetry*' derives from the Latin word '*calor*' (heat) and the Greek word '*metrion*' (measurement) [1]. It refers to the measurement of the heat energy released (or expended) by the body. This technique is fundamental for a better understanding of the animal energy costs of living – in either health or disease conditions – [2], as all life processes requires energy. The energy cost of living has been studied from different points of view [3], such as a basic biological [4–6], military [7], and sports performance [8] perspectives, and even to study the feeding efficiency in farm animals [9–11], among others.

Energy can be converted, stored and transferred from one form into another involving chemical reactions and/or mechanical work. It should be noted that '*energy production*' refers to the conversion of metabolizable energy into the chemical energy of adenosine triphosphate (ATP; plus loss of some of that energy during it is metabolism), however, sooner or later all energy will be transformed into '*heat*' (Q) [12,13]. This is the main reason why Q, '*energy*' and '*work*' can be used exchangeable [3]. The International System of Units defined the *Joule* (J) as the unit for reporting energy [14]. The energy exchanged per unit of time, e.g. Joule per second, exchanged between two different 'systems' represents the Q. Therefore, by using a *calorimeter* (i.e. instrument that allows the Q determination) such energy exchanged can be measured. The *calorie* (Cal) can also be used for expressing Q (4.184 J = 1 Cal) [15]. In the present Doctoral Thesis, the Q will be expressed as Cal instead of J – concretely as kilocalories per day (kcal/day) – unless otherwise stated.

The Q produced by a system (e.g. an animal) can be either *measured* using physical methods (i.e. *Direct Calorimetry*) or *estimated* (i.e. *Indirect Calorimetry*) from the measurement of the chemical by- and end-products of metabolism (e.g. oxygen, carbon dioxide, water, etc.) as is presented in **Figure 1**. Two laws of thermodynamics allow the use of both techniques for Q determination. These laws are the *Principle of Conservation of Energy* (also known as the First Law of Thermodynamics) and the *Hess Law of Constant Heat Summation* [16]. The first law mandated that '*energy cannot be created or destroyed, only changed in form*', while the second '*the heat released by a chain of reactions is independent of the chemical pathways, and dependent only on the end-products*'. In other words, quoting McLean and Tobin [2], both laws guarantee that *'the heat evolved in the enormously complex cycle of biochemical reactions that occur in the body is exactly the same as that which is measured when the same food is converted into the same end-products by simple combustion'*.

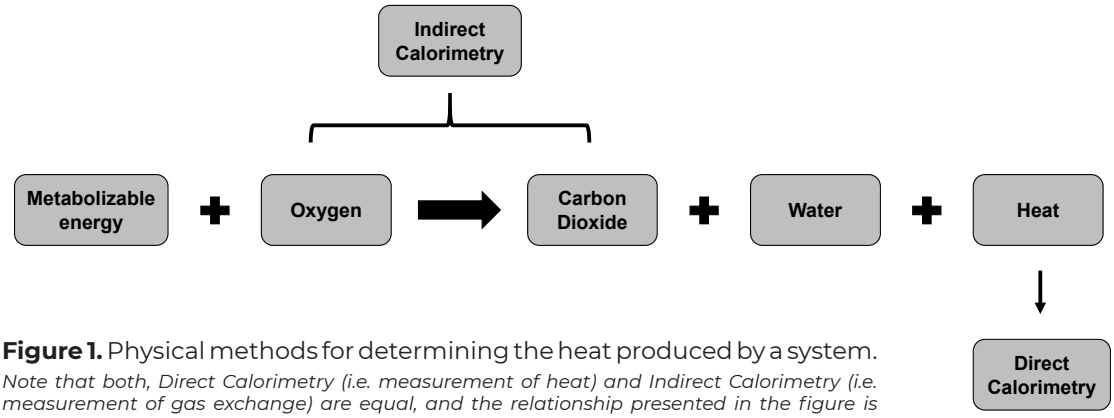


Figure 1. Physical methods for determining the heat produced by a system.
Note that both, Direct Calorimetry (i.e. measurement of heat) and Indirect Calorimetry (i.e. measurement of gas exchange) are equal, and the relationship presented in the figure is valid, if the external work is zero.

As aforementioned, a living organism may be considered as a *'system*', concretely an *'open*' system [1], which continuously exchanges Q with it is environment. In animals, the amount of energy required to maintain the life constants is obtained through food ingestion and its digestion (commonly named as '*energy intake*'; EI). In other words, the energy contained in the food is metabolized in the body – via cellular metabolism – into other energy forms (e.g. ATP) which are useful for the body cells [12]. Then, the energy obtained after the digestion is used for all the necessary life processes, homeostasis, and growth [12]. Importantly, a considerable amount of energy is given off from the cells as Q [2]. Therefore, this 'wasted' energy, or in other words, this energy exchanged between systems (e.g. animal and environment) allows the measurement of Q by using a calorimeter.

Although animals obtained their energy through EI, not all the energy contained in the food can be metabolized, and therefore, used by the body cells. In fact, some cannot be digested (e.g. fiber) by non-ruminants animals resulting in a loss of energy in the form of feces (i.e. 'rejecta' or waste energy). The term *digested energy of the food* [2] is defined as the difference between the energy contained in the food ingested vs. the energy contained in the feces [13]. Except those excreted in feces, nutrients (i.e. carbohydrates, fats, and proteins and amino acids, commonly named *metabolizable energy*) are absorbed into the bloodstream [12]. This *metabolizable energy* provides the necessary amount of energy for maintaining the requirements of all body cells [2], whole-body homeostasis, and growth [12]. Others derived products (waste products) from nutrients' metabolism than the ones found in feces exists and are excreted in body liquids such as urine (e.g. urea and creatinine).

All energy requirements of the body represent the *total daily energy expenditure* (TDEE), which can be divided in different components. Of note, the following definitions and percentages refers to humans rather to others animals, although most of the definitions may be used for different species [13]. The relationship between the metabolizable energy and the TDEE, is commonly known as *energy balance* [17–19]. If any metabolizable energy remained after meeting all the body cells requirements, this 'excess' of energy will be stored [2] in the body (*retained energy*). For instance, an excess of energy, usually refers as '*positive energy balance*' (i.e. energy intake higher than energy expenditure), may implicate to retain that energy as a new tissue (or tissue-growth), production of milk (during pregnancy), etc. On the other hand, if the metabolizable energy is not enough to meet all the energy requirements, usually referred as '*negative energy balance*', the body will consume its own energy depots. Noteworthy, the amount of EI is regulated by internal mechanisms to meet the energy requirements of maintenance [20] or *vice versa* (meet the energy requirements regulates the EI). All the aforementioned process is summarized in **Figure 2**. It should be noted that the explanation of the energy balance process has been simplified for explanatory purposes, as other elements should be also considered [17–19].

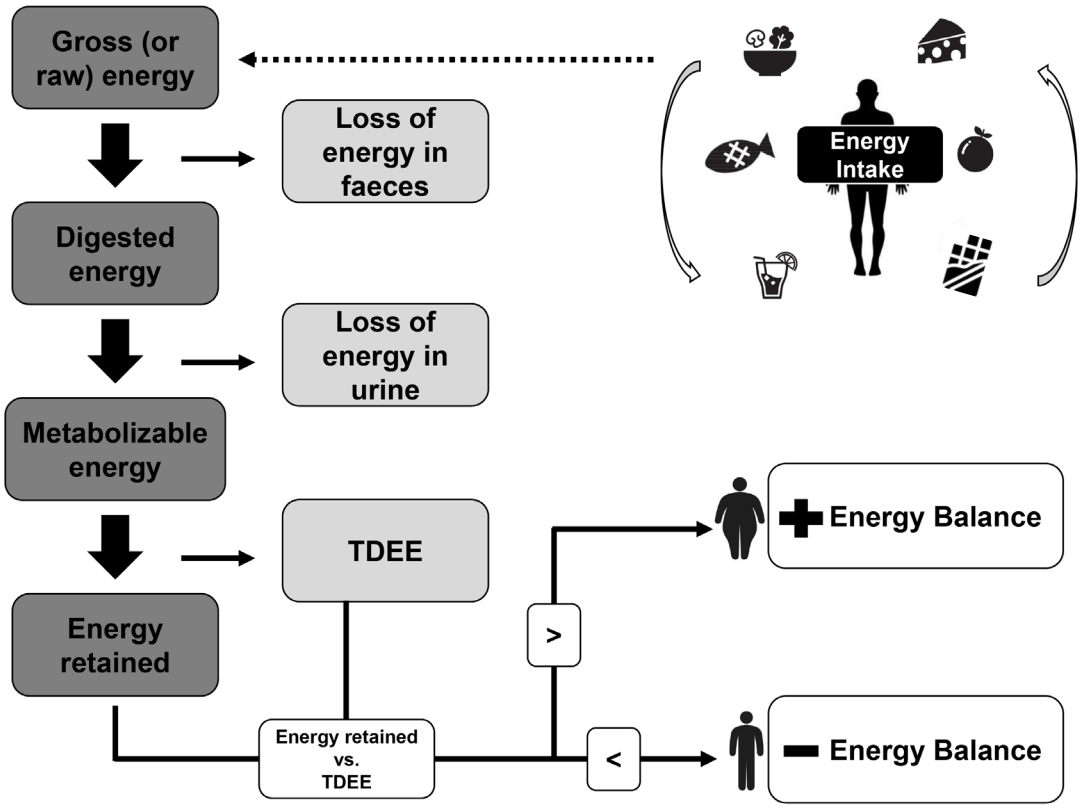


Figure 2. Gross energy process: summary of the gross energy process from the beginning, i.e. the moment in which the energy is consumed through food intake, until the end, i.e. the moment in which the energy is retained. *TDEE: total daily energy expenditure.*

In humans, the TDEE components are commonly divided as follows. Firstly, the *sleeping metabolic rate* (SMR), which is commonly defined as the energy expenditure (EE) during the night, while the person is sleeping [17]. Adding the energy cost of *arousal*, or in other words, adding the energy cost of *'wakefulness'* to the SMR [17] we obtain the *basal metabolic rate* (BMR) or the *resting metabolic rate* (RMR). Although they are not exactly the same (the BMR is assessed under much controlled, specific and strict conditions than the RMR; this will be extensively described later **Section 5.2**), commonly both are defined as the energy needed for maintaining normal body homeostasis, in the resting state, and in thermoneutrality in an awake person [21]. Therefore, BMR and RMR represent the minimum EE in an awake person (while resting), and usually accounts for 60-70% of the TDEE [17]. The *thermic effect of food* (TEF). TEF may be divided into *obligatory* and *facultative* responses [22]. The obligatory response is composed by all the EE associated with the digestion process and absorption into the bloodstream and storage [23–26]. On the other hand, the facultative response is the excess EE above the expected (calculated) requirements for digestion [23–26]. Usually, the TEF accounts for the 5-15% of the TDEE during energy balance conditions [27,28]. The *cold-induced thermogenesis* (CIT) may also play an important role under certain situations (e.g. mild-cold exposure). Importantly, CIT can be divided into non-shivering (i.e. non involuntary muscle contractions are involved) and shivering (i.e. involuntary muscle contractions are involved) thermogenesis responses [29], albeit both can occur concomitantly [30]. Thus, CIT is a highly variable TDEE component, which can increase EE up to, approximately, ≈40% of the BMR/RMR [31]. The last component is the EE from the movement or the *activity thermogenesis* [17]. On one

hand, this EE can be employed during non-volitional activities, which is commonly defined as *spontaneous physical activity* (SPA) and during *non-exercise activity thermogenesis* (NEAT). The latter includes the energy cost of maintaining posture, fidgeting, washing the dishes, carrying the shopping bags, etc. On the other hand, this energy can also be expended during well-structured or defined exercise (i.e. volitional activities or *exercise activity thermogenesis* [EAT]). Usually, the activity thermogenesis component accounts for the 20-30% of the TDEE [17]. Importantly, this range reported for the activity thermogenesis component represents an 'average', but it should be pointed that this component is, as well as the CIT, highly variable. In fact, activity thermogenesis may range from ≈0% (e.g. intensive care patients) to even three or fourfold the BMR/RMR (e.g. ultra-endurance athletes during a competition) [32,33].

Lastly, it should be highlighted that some confusion may arise as different notations for the aforementioned EE components exists in literature (e.g. meal or diet induced thermogenesis [MIT or DIT respectively] vs. TEF; physical activity related energy expenditure [PAEE] vs. activity thermogenesis; average daily metabolic rate [ADMR] vs. TDEE; etc.) although all are referred to the same concept [3].

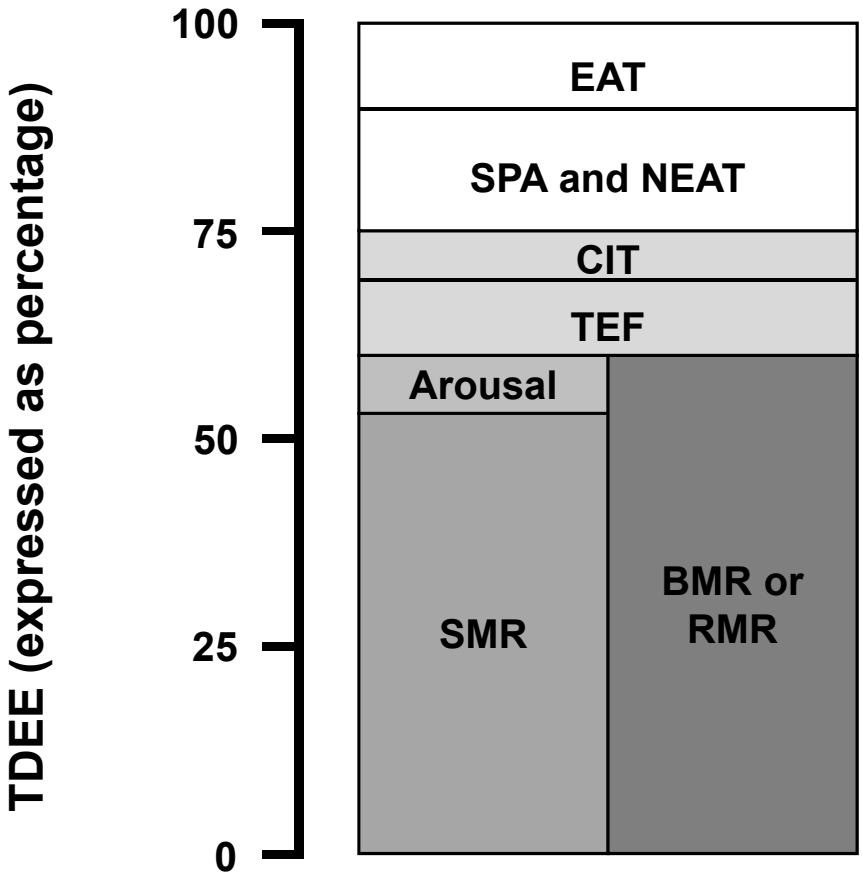
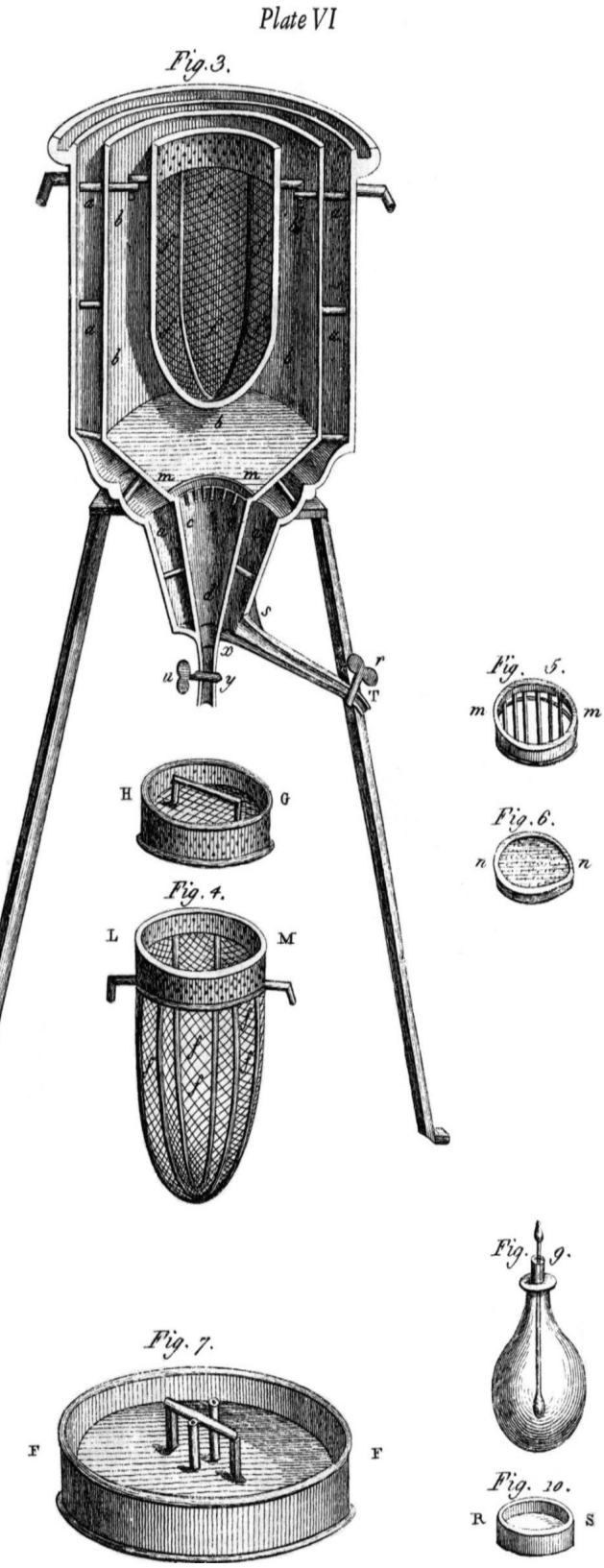


Figure 3. Example of the different components which make-up the total daily energy expenditure. *TDEE: total daily energy expenditure; BMR: basal metabolic rate; RMR: resting metabolic rate; SMR: sleeping metabolic rate; TEF: thermic effect of food; SPA: spontaneous physical activity; NEAT: non-exercise activity thermogenesis. EAT: exercise activity thermogenesis (i.e. volitional activities).*



2. ORIGINS AND HISTORY OF CALORIMETRY

Since the time of the ancient Greeks, the fact that human body released heat was a matter of captivation. Ancient Greeks were convinced that the heart produced the Q, which was 'vital' for maintaining life, and even that 'vital' Q was produced thanks to respiration of air and its circulation via the blood. Aristotle was the first to note the 'enigmatic' relationship between food, energy and respiration [34]. More than a millennium later, Santorio Sanctorius (1561-1636), observed that the weight of food ingested and the weight of feces did not coincide [5]. Inadvertently, he discovered that carbon leaved the body as carbon dioxide (CO_2), but he was not able to explain this fact at that moment. Later, Joseph Priestley (1733-1804) found that gas concentrations in the air (i.e. atmospheric air) varies [35]. During the following years, Antoine-Laurent Lavoisier (1743-1794), Marie-Anne Pierrette Paulze (1758-1836) – known as 'Marie Lavoisier' – and colleagues studied the respiration of humans and other animals [6]. Around that time, they were capable to measure gas exchange [36].

The actual first measurement of Q took place just over two hundred and fifty years ago. The first calorimeter for measuring the Q from a small animal was built in 1780 by Lavoisier and his colleague Pierre-Simon de Laplace (1749-1827) [4,37–39]. Their calorimeter was known as the '*ice-calorimeter*' (**Figure 4**). Although the Lavoisier and Laplace calorimeter is widely recognized as the first one, some have argued that Adair Crawford (1748-1795) might have measured the Q of an animal (using a water jacked surrounding a chamber containing the animal) before Lavoisier and Laplace. However, his work was published later [1,2] and thus, the credit was given to Lavoisier and Laplace [40,41].

Figure 4. Representation of the ice-calorimeter developed by Lavoisier and Laplace as was drawn by Marie-Anne Lavoisier [3,38,39].

Importantly, the calorimeter built by Lavoisier and Laplace allow them to refute an extended theory at their time, the *phlogiston theory*. The phlogiston theory, postulated by Georg Ernest Stahl (1660-1734), stated that an invisible element (the phlogiston) was present in all the combustible bodies and was 'liberated' or released during its combustion producing 'phlogisticated' air [1,2]. From previous studies by Robert Boyle (1627-1691), Joseph Black (1728-1799), Joseph Priestley, and Giovanni Francesco Cigna (1734-1791), it was known that pure air (i.e. not 'phlogisticated' air) was necessary to support life [2], and also that the respiration had a direct influence on surrounding air (changing its composition, volume, etc.). Crucially, Lavoisier and Laplace's calorimeter proved that a 'new element' (the oxygen), not the '*phlogiston*', was necessary to support animal life. At the beginning, both Crawford and Lavoisier compared the quantity of Q released by the animal placed inside the calorimeter vs. that released by a small flame (e.g. a candle) [2]. Nevertheless, it was Lavoisier who made the stunning-discovery, as he understood that the process occurring inside the calorimeter was not the production of 'phlogisticated' air, but the consumption of an element which he named '*oxygene*'. Lavoisier came up to a conclusion summarized in his, maybe most famous, statement: '[...] *La respiration n'est qu'une combustion lente de carbone et d'hydrogène [...]*' (i.e. the respiration, or breathing, is a slow combustion of carbon and hydrogen) [38].

The main limitation of the ice-calorimeter was that all the experiments had to be carried-out during winter [1], when air temperature was cold enough – in fact had to be close to freezing. Importantly, this ambient temperature may have influenced the animal physiology, increasing their EE [42–49]. Notwithstanding, the mechanism used in the ice-calorimeter was both, thorough and elegant. In brief, the Q melted the ice, and the melt-water was totally captured and measured (weighted). Then, the Q was calculated by using the latent Q of the ice and/or melted-ice. Besides refuting the phlogiston theory, the ice-calorimeter was used to further analyze the energy contained in food. All his discoveries and advances elevated Lavoisier to the figure of 'Father of Modern Chemistry' [1]. Unfortunately, Lavoisier was executed in Paris, and another century was necessary until new considerable contributions were done.

Following Lavoisier discoveries, a question remained unsolved: was the Q produced just by the combustion of carbon itself or anything else was involved in the process? César-Mansuéte Despretz (1791-1863) and Pierre Louis Dulong (1785-1838) tried to answer these questions [2]. Although they worked in different research groups and laboratories, both built almost identical calorimeters in which the Q of small animals warmed water that surrounded the calorimeter. Importantly, the gas exchange was collected for its posterior analysis. Therefore, we could say that both Despretz [50] and Dulong [51] built the firsts *respiration calorimeters*. The calorimeter developed by Dulong [51] is presented (**Figure 5**) as an example of their devices. Briefly, their rationale was to measure simultaneously the oxygen (O_2) and the CO_2 concentrations allowing the calculation of the amounts of hydrogen and carbon being oxidized, and therefore, the Q released by the animals.

A few years later, a ships' doctor named Julius Robert von Mayer (1814-1878) formulated, in 1842, the *Principle of Conservation of Energy*. One of its applications is the *Energy Balance Equation* (Eq. 1), maybe underlining the most important principle of Indirect Calorimetry [2].

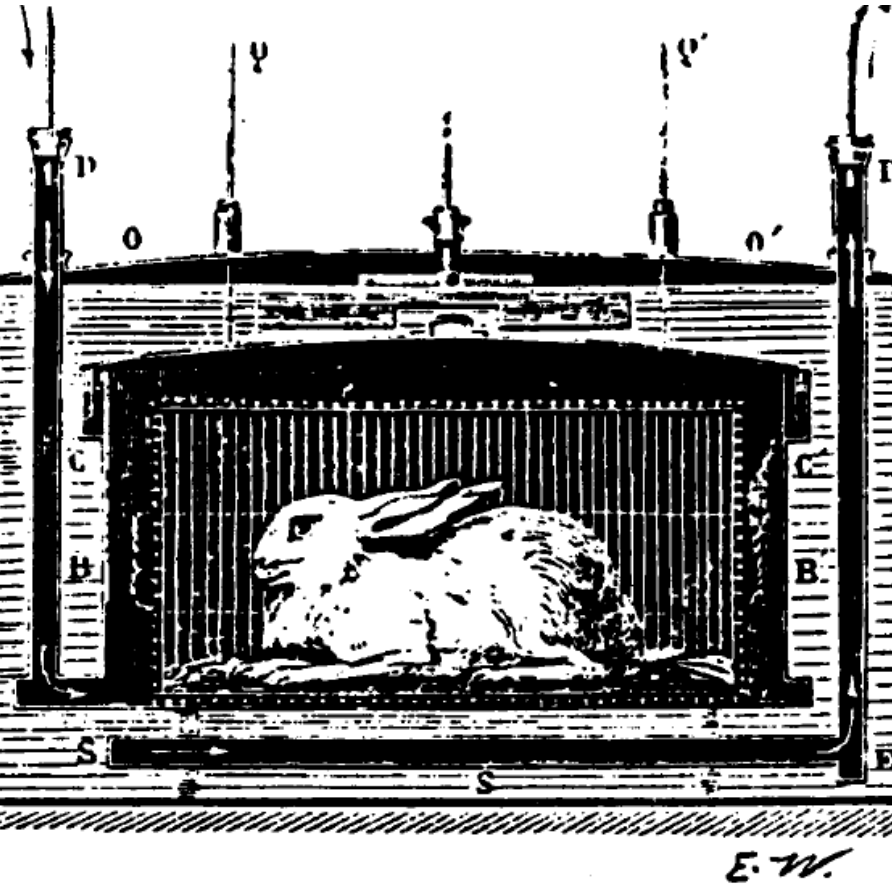
Gross (or raw) energy = energy loss in feces + energy loss in urine + energy retained + internal and external work (Eq. 1)

where gross or raw energy represent all the EI (energy contained in food) by the animal, energy loss in feces and urine are the waste energy excreted from the nutrients' metabolism, and the energy retained is the energy stored in the body after meeting all the body cells energy requirements. Lastly, the internal and external work represent the necessary energy

to maintain vital process or functions (e.g. body temperature [internal work], and movement [external work]). Importantly, if applicable (e.g. in large-ruminants animals) in Eq. 1, the energy loss in methane can be also considered and added to the equation. Another example of the aforementioned law is the *Heat Balance Equation* (Eq. 2), which manifest the principle of Direct Calorimetry [2].

Heat exchanged (or released) = heat loss by conduction + heat loss by radiation + heat loss by convection + heat loss by evaporation *(Eq. 2)*

where the heat exchanged or released represent the Q released by the animal in its different manifestations, i.e., the Q loss by conduction (Q transferred through direct or physical contact), Q loss by radiation (Q transferred through thermal or infrared emission), Q loss by convection (Q transferred through the movement of a gas or a liquid), Q loss by evaporation (Q transferred as water changed its state from a liquid to a gas).



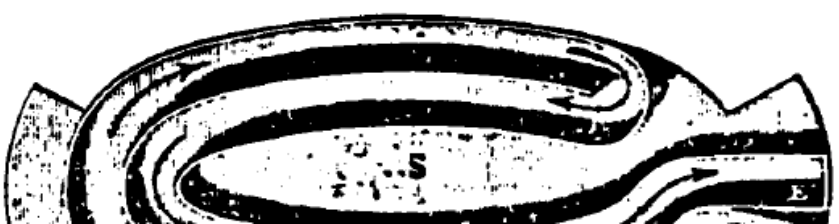


Figure 5.
Representation of the calorimeter and gas exchange measurement developed by Dulong as was depicted in Lefèvre's work [52].

The next step forward was made in 1849 [53] by Henri Victor Regnault (1810–1878) and Jules Reiset (1818-1896). They designed, for first time, a *closed-circuit system* for measuring gas exchange (**Figure 6**), based on a 'simple' mechanism. Firstly, the animal was placed inside the chamber. Then, pipettes placed on the right part (which contained a potassium hydroxide solution) pumped air in and out of the chamber. During this pull process, the CO_2 from inside the chamber was absorbed. Lastly, the O_2 necessary for the animal was replenished from one (out of the three) volumetric containers placed on the left part of the system (**Figure 6**). The basic principle of their closed-circuit system was similar to the one used in the closed-circuit respiration chambers of nowadays [2]. Nonetheless, its main limitation was the insufficient control of the temperature and thus, its possible influence on EE [42–49].

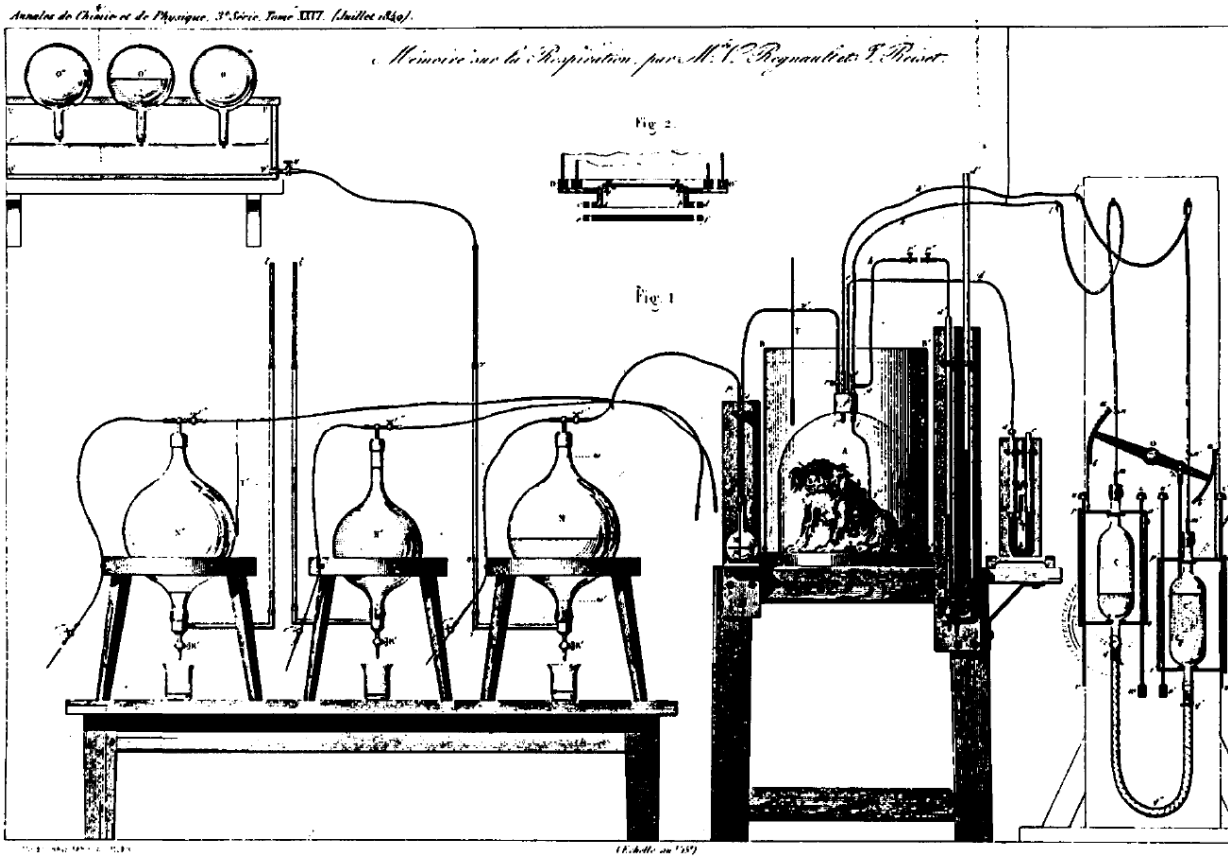


Figure 6. Representation of the closed-circuit system for gas exchange measurement developed by Regnault and Reiset as was depicted in their own work [53].

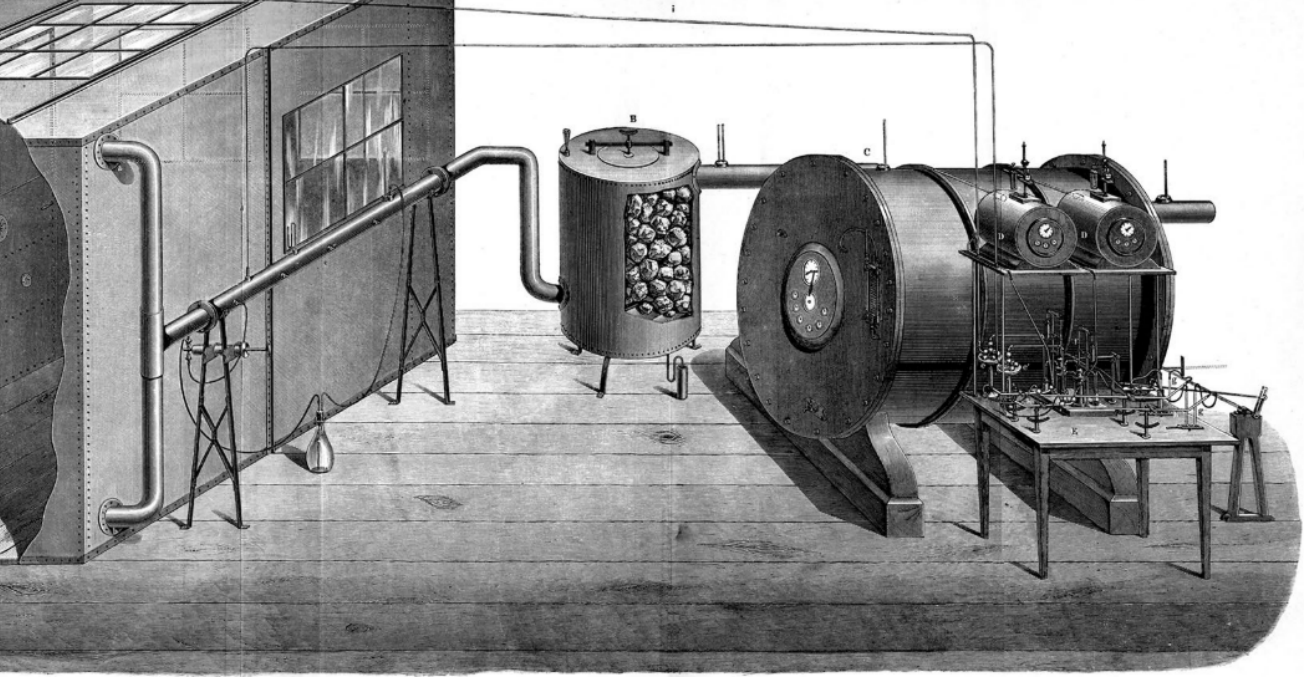


Figure 7. Representation of the open-circuit respiration chamber developed by Pettenkofer as was depicted in the work of Gorup-Besanez [59–61].

In 1862 [54], Max Joseph von Pettenkofer (1818-1901) and Carl von Voit (1831-1908) built the first *open-circuit respiration chamber* (**Figure 7**), using a sophisticated mechanism. Firstly, using a gas-meter they were able to measure the air drawn inside the chamber, while a sample of that air was collected for its latter analysis. Then, weight changes of the subject (a human) as well as the water and food consumed and the feces and urine excreted were collected and measured. Analyzing the composition of the food ingested and excreta (i.e. feces and urine) they calculated the total quantity of carbon and nitrogen using the *carbon-nitrogen balance method*. Of note, this carbon-nitrogen balance method consisted in measuring the amounts of both, carbon and nitrogen stored in the body. Using these measured quantities, the 'amount' of energy could be inferred by assuming that whole-body tissue is make-up of proteins and fats of well-known compositions and the Q released by its combustion [2]. Therefore, Pettenkofer and Voit were able to quantify the ratios of oxygen consumption (VO_2), carbon dioxide production (VCO_2) and the excreted nitrogen (N) involved in the metabolism of their subjects vs. different test-meals [55]. A few years later, Max Rubner (1854-1932) built a Direct Calorimeter for measuring the Q [56] that was also connected to a Pettenkofer-Voit system, allowing therefore both, the direct measurement of the Q as well as the gas exchange.

Based on a previous work [57] – a calorimeter for rabbits – carried-out by Jacques-Arsène d'Arsonval (1851-1940), Wilbur Olin Atwater (1844-1907) and Edward Bennett Rosa (1873-1921) built their calorimeter for large farm-animals (and humans) in which both, the Q (released by the animal) and the gas exchange could be measured and collected (using a Pettenkofer-Voit system) respectively. Therefore, Q could be determined using both techniques – i.e. Direct and Indirect Calorimetry – [58]. Later, their calorimeter, funded by the United States Department of Agriculture Office of Experimental Stations, was 'up-graded' several times [2]. An important consequence of these improvements was that the 'new' calorimeter [8] allowed the calculation of Q using the much easier respiratory quotient method (RQ; or the ratio between VCO_2 and VO_2), instead of the previously used carbon-nitrogen balance method (analyzing the composition of the food ingested and excreta). Nonetheless, the RQ calculation method depended on knowing the exact amount of the gas exchanged (especially the VO_2). Unfortunately, about that period only the Regnault-Reiset [53] closed-circuit system for gas exchange measurement was able to record the rate of VO_2, and was not very accurate [2].

Others Direct Calorimeters were also developed during the same period. For example the Lefèvre's *convection calorimeter* (called the 'air' calorimeter; i.e. a ventilated tunnel) [52]. Another *confinement system*, as the convection calorimeter, was the one developed by Ferdinand Laulanié (1850-1906). In this system, the animal was limited in an airtight chamber, and changes in the composition of indoor-air were calculated from analysis of samples obtained at different intervals [62]. This method, although was not commonly used at the beginning, was updated later by others researchers [2,63–65], to measure larger farm-animals.

Well into the nineteenth and at the beginning of the twentieth century, scientists performed important advances into the relationship between internal and external work, Q and gas exchange [7–10,54]. The ratios between the VO_2 and the VCO_2 – named RQ by Eduard Friedrich Wilhelm Pflüger (1829-1910) – as well as the amount of Q, were deeply studied using diverse meals and thus, different nutritional compositions. The amount of protein metabolized was measured from the nitrogen excreted in urine [3]. Then, the non-protein RQ (i.e. the VO_2 and VCO_2 that remained after the protein metabolism) could be used to determine the Q from carbohydrates and fat [3]. Following the work of Atwater and Benedict [8], the focus was established on the development of Indirect Calorimetry rather than Direct Calorimetry systems. In this regard, a considerable number of respirations chambers were built – although mostly focused on the measurement of large farm-animals [66–71]. Those chambers were based on the Pettenkofer-Voit system. Nonetheless, they implemented an innovative technique [72] introduced by Klas Gustaf Anders Sondén (1859-1940) and Robert Adolph Armand Tigerstedt (1853-1923), consisting on the analysis of the gases concentrations (O_2, CO_2 and methane) from aliquots samples of the outlet air.

Despite of those important innovations a limitation still existed, as the animal had to be confined in a respiration chamber. Therefore, the necessity of developing a portable device which allow the measurement in different places and conditions was evident. Jules Tissot (1870-1950) in 1904 developed a spirometer [73], equipped with a face-mask (that used lightweight valves to separate inspired from expired gases) for gas exchange collection, in which all the air expired was collected in a metal bell. A few years later, Claude Gordon Douglas (1882-1963) invented the '*Douglas bag*' (**Figure 8A**) for collecting total gas exchange [74]. This device could be carried on the back of the subject, allowing the measurement of gas exchange on different conditions, from resting to exercising [75]. However, these '*total collection methods*' were limited by their gas-recipient capacity, and therefore the duration of the measurement was relatively short [75]. Trying to overcome this limitation, these face-mask methods were updated using the Pettenkofer-Voit open-circuit principle [55]. This was done by Nathan Zuntz (1847-1920) in 1906 [76], who designed an open-circuit system that could be carried on the back (**Figure 8B**), as the Douglas bag, but allowing long-term gas exchange measurements during a wide range of activities [75].

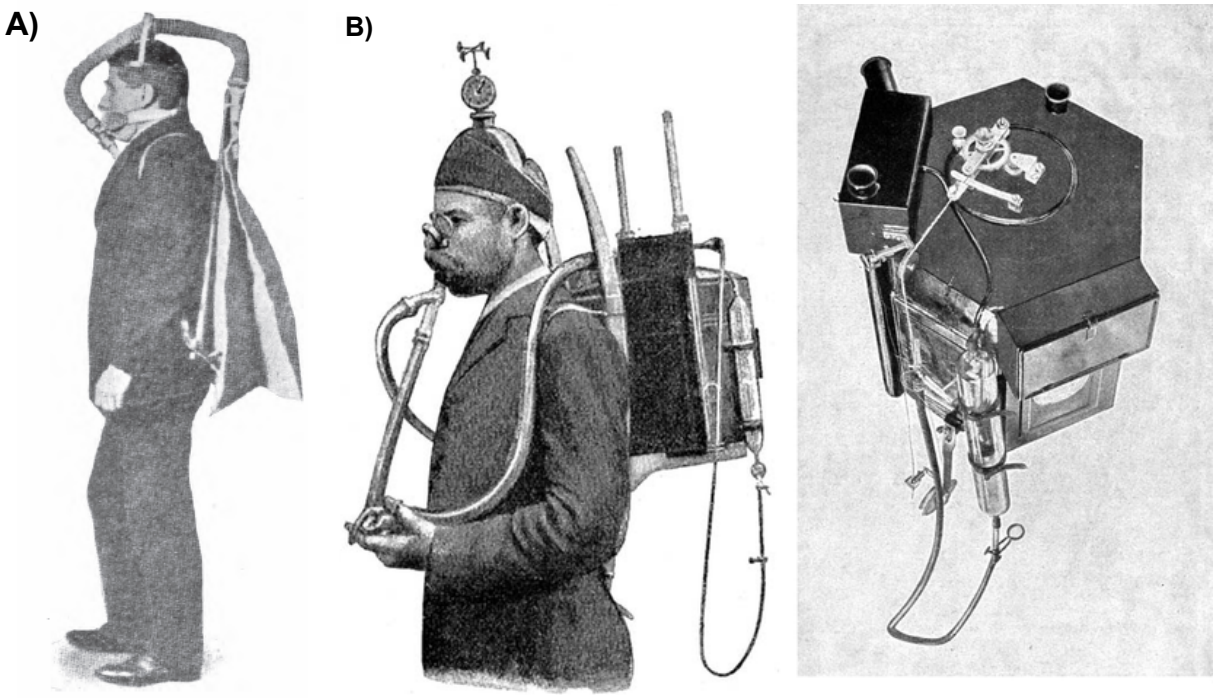


Figure 8. Representation of the Douglas bag system, a total collection gas exchange measurement method (Panel A; extracted from [74]), and the Zuntz open-circuit system for gas exchange measurement (Panel B; extracted from [76]).

All these systems were built before the days of electronics, informatics, computers and automatic control. Thus every single step needed to be manually operated by researchers. Gas samples had to be aliquot manually over mercury and transferred to other recipients (e.g. volumetric containers) for it is later chemical analysis. It was not until the Second World War when the major advances in electronics and engineering appeared, radically transforming the calorimetry [2]. The application of the basic calorimetric principles developed during the past centuries together with the implementation of electronic methods for gas and data analysis and computerization, significantly improved the quality of the calorimetry.

The most relevant advance (for Direct Calorimetry) probably was the implementation of the thermoelectric measurement of the mean temperature gradient using a plexiglass plastic interconnected-network membrane [77]. Other technologies such as the Integrating Motor Pneumotachograph [78] or the Metabolic Rate Monitor [79] were also implemented (for Indirect Calorimetry). The first, used an amplified pneumatic signal proportional to the flow (volume) of expired gases. Thus, the volume was estimated from the revolutions of the turbine-engine, while the gas analysis was performed later. The latter was an open-circuit face-mask device which was ventilated by a servo-controlled blower. Flow-rate was then inferred from the speed of the blower (as is linearly related). Therefore, using the flow-rate and air concentration difference, this device eliminated the need for gas exchange samples collection or aliquots [2] as were needed for example by the Douglas bag system [74].

If possible, EE should be determined using both VO_2 and VCO_2. This is of vital importance since the EE equivalents of VO_2 and VCO_2 are not constant if carbohydrates, fats or proteins are oxidized [80]. Nevertheless a relatively recent technique, totally different to both Direct and Indirect Calorimetry, was developed in the late 40's and early 50's [81–86]. Using this technique, the VCO_2 could be inferred and thus, the EE estimated. This method is called the '*doubly-labelled water*' ($^2H_2{}^{18}O_2$). This method uses deuterium (^2H) and oxygen-18 (^{18}O) for estimating the VCO_2 of the subject [81]. Of note, the ^2H is only eliminated as water while the ^{18}O is also eliminated as VCO_2 and thus might be diluted faster than the ^2H. Since both isotopes are stable, the $^2H_2{}^{18}O_2$ remains in the body until their natural elimination or dilution. The rates of dilution of both isotopes are calculated after mass-spectrometric analysis of urine samples [81]; from these results the amount of CO_2 elimination might be calculated. This method has the great advantage that it is non-invasive and also allows the subject to be measured in free-living conditions over weekly intervals [3,87–92], without for example, wearing face-mask or being confined into a chamber. However, a possible limitation is that this technique measures only the VCO_2, which is not as accurate as VO_2 for estimating EE (because as aforementioned, the EE equivalents are not constant for the different macronutrients being oxidized) [80]. Lastly, the obtained results are means of EE over intervals ranging (commonly) from one to two weeks [3], and thus, there is not possible to differentiate among the different TDEE components (**Figure 3**).

In summary, calorimetry has been developed – and keeps evolving and changing – in different forms and ways since the first measurements performed by Lavoisier and Crawford. However, the main innovations have been made in the last century thanks to the advances in engineering and informatics [2,3]. The chemical reactions of the three basic nutrients (carbohydrates, fat and proteins) with VO_2 (after their combustion) and the VCO_2 and Q released by them were deeply studied [2,3]. As a consequence of studying the link between VO_2 and VCO_2 that allowed the accurate estimation of EE from gas exchange, after a correction for loss of energy in urine [7,8,93–99]. In this regard, the amount of EE per each gram of every metabolized nutrient could be assessed and are presented in **Table 1**.

Table 1. Oxygen consumption, carbon dioxide production and energy released per 1 gram of metabolized nutrient.

Author (reference)	Nutrient	VO_2	VCO_2	EE
Zuntz et al. [7]	CHO	828.8	828.8	4.182
Cathcart et al. [100]	FAT	2019.3	1431.1	4.316
Lusk et al. [101]	PROT	967.0	775.2	9.461

CHO: carbohydrate; PROT: protein; Excreted N: nitrogen catabolized and excreted in urine; Oxygen consumption (VO_2) in milliliters; carbon dioxide production (VCO_2) in milliliters; Energy expenditure (EE) is presented as kilocalories (kcal), further, EE is presented considering the energy loss in urine (i.e. -2.17 kcal per gram of excreted urinary nitrogen catabolized) as was proposed by Weir et al. [93].

3. SYSTEMS FOR DETERMINING ANIMAL HEAT PRODUCTION

3.1. DIRECT CALORIMETRY SYSTEMS

Devices based on the Direct Calorimetry (DC) technique particularly measures the Q released by the body (i.e. energy dissipated by radiation, evaporation and convection or conduction). Human body cells consume the energy available, in the form of ATP, for performing both internal and external work [3,37,102,103]. Internal work represents the total energy of every single internal processes and body functions, and finally set out the body in the form of Q. External work represents all the non-heat energy which the body transferred to the environment (e.g. the energy transmitted to the pedals of a bike while pedaling). Therefore, it should be highlighted that DC exclusively measures internal work. While resting, as no external work is occurring, the EE is equal to the internal work [2,37]. On the other hand, while exercising (e.g. pedaling) part of the energy produced by the metabolism processes is transmitted into performing the required external work. In this scenario, around ≈70-80% of the EE is released as Q since humans are at best ≈20-30% efficient in converting energy into external work [1,3,104].

As previously mentioned, the DC technique 'forces' to place the subject into an insulated chamber. To be valid, a DC system must be a closed system which guarantee that all the Q can be 'captured' inside the calorimeter and thus, there is no Q transference between the calorimeter and the environment [1,37]. The simplest DC system is able to measure exclusively the non-evaporative or 'dry' Q loss (i.e. convective, conductive and radiant). The next step is to 'upgrade' the system [2,37] allowing to distinguish between the non-evaporative and the evaporative Q loss (e.g. from the skin and sweating, and even from the respiratory tract). Therefore, DC becomes irreplaceable for studying human thermoregulation [1]. As the DC requires the confinement of the subject into the insulated chamber, engineers and researchers had designed large chambers in which different activities, simulating the free-living conditions [1], may be done by the subject. Nevertheless, the DC should not be the first option if the primary aim is to determine EE as the circadian variations of body temperature [105] might dissuade its use in periods shorter than 24 hours. In any case, DC is still considered the 'gold standard' for assessing EE [60,80], although it is obsolete and seldom used nowadays [80,106].

Three are the primary varieties of DCs – although more types, as well as extended explanations about them, can be found elsewhere [1]. The DC systems can be grouped on adiabatic, convection and isothermal depending on its approach for Q measurement. A DC may be upgraded to measure 'different types' of Q, and thus, these three approaches can be combined in the same DC system. In the adiabatic system (also called *heat sink*) the Q released by the subject is transferred to a liquid-cooled heat exchanger [107]. The rate of Q extraction from the chamber is continuously regulated by using the interior and exterior chamber walls, maintaining an equal inner to outer wall temperature, which produces a zero temperature gradient wall [108]. In the convection system air is ventilated into the chamber in a known flow-rate and conditions. Thus, the Q released by the subject is calculated using the flow-rate, the increase in temperature of ventilating outlet air and the specific heat capacity of the inlet air [109–111].The isothermal approach, consists of an insulated chamber equipped with a special band of insulating material [112]. The inner part of the band is in thermal-equilibrium with the interior of the chamber while the outside part of the band is in thermal-equilibrium with the chamber wall. The chamber wall temperature is kept steady using a circulating liquid [112]. Thus, the Q released by the subject is proportional to the temperature gradient over the insulating band.

3.2. INDIRECT CALORIMETRY SYSTEMS

Systems based on the Indirect Calorimetry (IC) technique inferred EE from the measured gas exchange rates of O_2 and CO_2 [2,3,22,60,113–115]. In simple terms, the IC systems are able to measure VO_2 and VCO_2 using the difference between these gases concentrations in the inspired and expired air. As VO_2 and VCO_2 are measured, the aforementioned RQ method (i.e. VCO_2/VO_2 ratio) can be used, allowing the substrate utilization estimation [2,3,22,60,113–115].

Different equations have been proposed, using VO_2, VCO_2 and excreted urinary N to estimate EE, fat and carbohydrates oxidation (FATox and CHOox respectively). The most used equations for estimate EE, FATox and CHOox are presented in **Table 2**. These equations have been derived from the stoichiometry (i.e. the relationship between the number of moles [and thus mass] of diverse substances [products and reactants] involved in a chemical reaction) of EE equivalents of VO_2 and VCO_2 after the combustion of diverse macronutrients. Importantly, considering that urinary excretion is the major mechanism of N excretion [116,117], mainly in the form of urea (>90% in healthy subjects), the amount of protein oxidation may be inferred from urinary N. In this regard, a total of 6.25 g of protein oxidation per each gram of urinary N is widely accepted. That 'historical factor' (can be traced back to the 19th century) postulate that the N content of most mixed proteins is ≈16% of its total composition [118]. Although it is widely accepted, the fact that pure proteins differ on their N content has been highlighted more recently [119], as well as that this historical factor may be significantly deviate from the net protein oxidation in some cases [118].

The stoichiometry of a biomolecule (or nutrient) oxidation does not directly depend on its intermediate metabolic process nor in the tissue in which this oxidation occurs [99]. For that reason, the equations for estimating EE, FATox and CHOox are valid independently of the metabolic process (or metabolic pathways) by which this biomolecule is oxidized (although some considerations, that are described later, should be take into account). However, it is important to note that all this equations rely on different assumptions, as well as the IC technique itself. Therefore, caution is needed when either EE or nutrient oxidations rates are inferred, as these equations may produce erroneous inferences when are applied in situations different than those for which were specifically designed [22,115,120].

Table 2. Some of the most used equation for inferring energy expenditure (EE), fat oxidation (FATox) and carbohydrate oxidation (CHOox).

Author (reference)	Equation
Energy expenditure considering urinary N excretion	
Weir [93]	EE = 3.941 × VO_2 (l/min) + 1.106 × VCO_2 (l/min) – 2.17 × N (g/min)
Brouwer [96]	EE = 3.866 × VO_2 (l/min) + 1.2 × VCO_2 (l/min) – 1.43 × N (g/min)
Ferrannini [115]	EE = 3.91 × VO_2 (l/min) + 1.1 × VCO_2 (l/min) – 3.34 × N (g/min)
Consolazio [95]	EE = 3.78 × VO_2 (l/min) + 1.16 × VCO_2 (l/min) – 2.98 × N (g/min)
Jéquier [105]	EE = [4.686 + 1.096 × (NPRQ – 0.707)] × $NPVO_2$ (l/min) + 4.6 × PVO_2 (l/min)
Fat substrate oxidation	
Frayn [99]	FATox = 1.67 × VO_2 (l/min) – 1.67 × VCO_2 (l/min) – 1.92 × N (g/min)
Jéquier [105]	FATox = 1.689 × VO_2 (l/min) – 1.689 × VCO_2 (l/min) – 0.324 × PROox (g/min)
Carbohydrate substrate oxidation	
Frayn [99]	CHOox = 4.55 × VCO_2 (l/min) – 3.21 × VO_2 (l/min) – 2.87 × N (g/min)
Jéquier [105]	CHOox = 4.113 × VCO_2 (l/min) – 2.907 × VO_2 (l/min) – 0.375 × PROox (g/min)

EE: energy expenditure expressed as kcal/min. VO_2: oxygen consumption. VCO_2: carbon dioxide production. N: nitrogen. NPRQ: non-protein respiratory quotient. $NPVO_2$: non-protein oxygen consumption. PVO_2: protein oxygen consumption. PROox: protein oxidation. Adapted from [80].

All the IC systems are based on various fundamental principles and assumptions. One of them is that all the measured VO_2 is used in the complete oxidation of substrates (i.e. carbohydrates, fat and protein) and then, the VCO_2 thereby evolved is fully recovered [115]. Although this assumption is true for healthy subjects, in the presence of certain metabolic situations as for example alcohol ingestion, lactic acidosis, diabetic ketoacidosis, hyperventilation or hypoventilation, among others, it might not be correct; extended and detailed examples are illustrated elsewhere [115]. Further, it is assumed that on resting conditions, the measurement is performed during a 'metabolic steady-state'. In that situation, the gas exchange measurements made at a whole-body level (i.e. from gases measured at the mouth) are correlated to the gas exchange made at a tissue or organ level (i.e. 'cellular respiration') [61]. In other words, it assumes that the respiratory exchange ratio (RER) is equal to the RQ. In the present Doctoral Thesis, RER rather than RQ will be used to refer the VCO_2/VO_2 ratio, although otherwise stated.

Later, once VO_2 and VCO_2 have been measured, these data can be employed to determine the net rate of '*disappearance*' of lipids, carbohydrates and proteins from their metabolic pools. Importantly, under most situations the '*oxidation*' is the primary pathway by which a biomolecule disappears from its respective pool. Nevertheless, both terms are used interchangeably as synonyms which it is not always correct [22]. In fact, IC determines the net substrate disappearance rates (i.e. difference between the oxidized and synthetized substrates) and under certain 'special' situations (see **Section 3.2.1**) the rates of disappearance and the rates of oxidation might differ considerably among them. Thus, from knowledge of VO_2 and VCO_2 as well as the N excreted in urine, one can calculate the glucose, lipid and protein disappearance rates (commonly known as oxidation rates) using the aforementioned equations (**Table 2**). Importantly, for comparability purposes, thereinafter the term *'oxidation'* will be used in the present Doctoral Thesis.

As was previously noted, the energy (i.e. kcal) released by the oxidation of each gram of the different substrates are known, and therefore, the 'energy production' (i.e. EE) can be determined using the equations showed in **Table 2**. Nonetheless, some assumptions support these calculations and should be highlighted to correctly interpret the VO_2 and VCO_2 data obtained by IC [22,115]. In this regard, the value for protein oxidation obtained from urinary N excretion may not be representative during short-term assessments [115], but it becomes important during long-term assessments, since the size of the body's urea pool may fluctuate [22]. Importantly, the error introduced in the EE estimation by either missing or ignoring the fact of taking into account N excretion in urine is negligible; however, the impact on substrate oxidation may be significant. Nonetheless, one might correct that by measuring the N concentration in the urine [121,122]. Generally, an estimation of the error produced by missing the protein oxidation rates can be calculated using the non-protein RQ (NPRQ; i.e. ratio between the non-protein VCO_2 and the non-protein VO_2), or in other words, the portion of total VCO_2 and VO_2 that is related to fat and carbohydrate oxidation. Therefore, when the RQ ≈ 0.80, the NPRQ and RQ are (practically) equals, and the protein oxidation rate is not crucial to estimate either the fat or carbohydrates oxidation rates [22,115]. On the contrary, when the RQ is far from 0.80, the difference between RQ and NPRQ becomes evident and thus, the errors when estimating either the fat or carbohydrates oxidation rates will significantly increase, as the difference between them becomes larger.

In summary, the fundamental principles and assumptions in which IC is based are: (a) all the VO_2 is used in the complete oxidation of substrates and the VCO_2 is fully recovered, independently of the intermediate metabolic pathways; (b) under the majority of situations, the direct oxidation of a biomolecule represent the principal route by which a substrate disappears from its pool; and, (c) although N excretion in urine (and thus, protein oxidation) did not significantly influence the EE estimations, its proper determination may be fundamental for substrate oxidation rates.

3.2.1. Special metabolic situations

All the equations presented in **Table 2** apply to the specific situation in which all the O_2 is used for the complete oxidation of substrates and all the CO_2 released is produced by these chemical reactions. For that reason, in situations where there is a post-exercise excess of oxygen consumption, hypo- or hyperventilation, etc. the gas exchange will represents other non-metabolic processes and the IC assessment might be compromised [115]. Thus, an equilibrium of O_2 and CO_2 gases in body pools is necessary when using IC [22].

Importantly, there is no O_2 reservoirs within the body, and thus all the VO_2 is used after its uptake [115,123]. On the contrary, the endogenous cellular produced CO_2 may be retained into the bicarbonate pool under certain situations (although is not very common), and that may produce a delay in the rise of expired CO_2 (i.e. VCO_2). So, the interpretation of gas exchange patterns should be considered with caution, and it is very important to perform the IC measurements under metabolic-steady state conditions.

On the other hand, other metabolic processes different that such fluctuations on gas exchange may also influence the interpretation of the obtained data (especially the nutrient oxidation rates). These metabolic processes, which are described below, could be the lipogenesis, the gluconeogenesis, the lactate metabolism, and the alcohol oxidation, among others.

Lipogenesis: the acetyl coenzyme A (acetyl-CoA) pool in the mitochondria is an important 'turning point'. In fact, oxidation of the three basic nutrients feed this acetyl-CoA pool, and thus, lipid synthesis is produced from it. Certainly, lipogenesis may be produced from glucose or amino acids happening simultaneously with the oxidative reactions [120]. Lipogenesis is postulated to occur coupled with glucose oxidation, since certain amount of energy is needed for producing that synthesis process [120] – the same rationale applies to lipogenesis from amino acids. Thus, as both processes are occurring concomitantly (i.e. lipogenesis and glucose oxidation) the solution of FATox equations may produce positive or negative results. The first denotes a '*net fat synthesis*' (i.e. lipid synthesis is higher than lipid oxidation) while the latter a '*net fat oxidation*' (i.e. lipid oxidation is higher than lipid synthesis). Importantly, although the nutrient oxidation rates may significantly vary, the EE production would not be highly influenced by the presence of net lipid synthesis. The extended rationale can be found elsewhere [22,115].

Gluconeogenesis: pyruvate, lactate and glycerol are possible substrates for this process, but without involving gas exchange [120]. On the contrary, alanine may be also converted into glucose in the liver, and when this occurs, the amino group is transferred to urea (through urea cycle). In this final process both, CO_2 and energy are required. If gluconeogenesis process is occurring, several issues should be considered. For example, the N excreted in urine will reflect both, alanine deamination and protein oxidation. Furthermore, the CHOox will be underestimated in the same amount as new glucose is synthetized from amino acids (i.e. *net glucose oxidation* vs. *net glucose synthesis*). Lastly, the FATox rate will be underestimated in an important amount, while the EE will be also influenced (but less than FATox rate). The extended rationale can be found elsewhere [22,115].

Ketone body metabolism: the production of these molecules is an oxygen-demanding process, and because of that, if ketone bodies are produced in excess of their metabolism, they will impact the gas exchange. The process of synthesis for acetoacetic acid and β-hydroxybutyric acid from lipids is related with an increase of hydrogen ions concentration (H^+). Thus, this H^+ may displace equivalent values of CO_2

from the bicarbonate whole-body pool, and then the measured VCO_2 will increase. On the contrary, during their oxidation some CO_2 is retained as bicarbonate to account for the consumption of H^+ associated with the oxidative process [115]. Importantly, ketone bodies negatively influences the gas exchange either if they are oxidized or formed. Therefore, whenever the circulation concentration of ketone bodies change, the gas exchange measurements will change and the gas exchange should be corrected accordingly (e.g. measuring ketone bodies in urine). The extended rationale can be found elsewhere [22,115].

Lactate metabolism: accumulation of lactate will origin the addition of H^+ and the possibility of displace metabolic CO_2. On the other hand, net loss of lactate will consume H^+ and traps CO_2 in the form of bicarbonate. When this occur, the RER falls as an artifact produced by that trapped CO_2. Thus, if the retained CO_2 is added to the measured VCO_2 ('artificial' VCO_2), one will get the 'real' VCO_2 and thus, the RER which would be similar to the RER in a metabolic steady-state [120]. This situation is, in a greater or lesser extent, almost identical to the one produced by ketone bodies. The extended rationale can be found elsewhere [115].

3.3. DIRECT VERSUS INDIRECT CALORIMETRY SYSTEMS

It is important to not forget that DC and IC determine energy but following different approaches. As described previously, DC measures the Q released by the body while IC measures the gas exchange (VO_2 and VCO_2); but at the end both determine EE.

For that reason the aforementioned relationship depicted in **Figure 1** does not completely applies, unless the subject is in a steady (resting) state and in thermoneutrality air atmospheric conditions [2,37]. Given that energy can be stored in the body as different forms (e.g. as body tissue-growth, as an increase of whole-body temperature, among others) aiming to make easier the representation of the relationship between DC and IC the body energy storage was considered to be null (i.e. the system was in energy balance). Therefore, the relationship depicted in **Figure 1** should be completed adding the remaining elements which were not previously included, the internal and external work, to 'solve the complete puzzle' as illustrated in **Figure 9**.

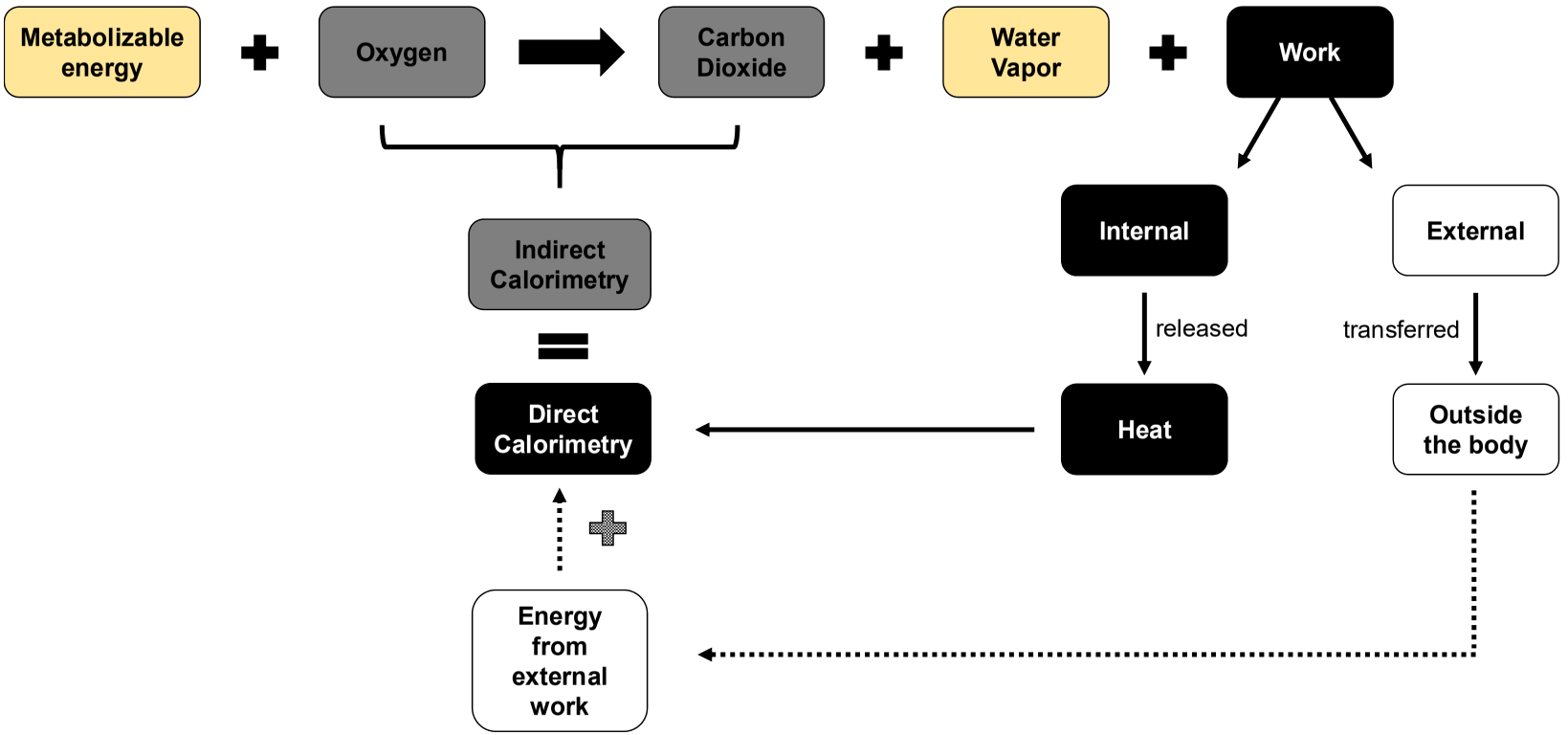


Figure 9. Physical methods for determining the heat produced by a system, and the relationship between Direct Calorimetry and Indirect Calorimetry. *This figure illustrates how the external work processes are determined by Indirect Calorimetry through gas exchange measurements, how the heat produced after bodily functions (internal work) is released in the form of heat and measured by Direct Calorimetry, and how the external work is transferred outside from the body not producing heat inside the body. Adapted from* [3]. *Gray boxes represent the volume of oxygen and the volume of carbon dioxide measured using Indirect Calorimetry; Black boxes represent the internal work (in the form of heat) measured using Direct Calorimetry.*

Both systems present advantages and disadvantages, which are presented below in **Table 3**.

Table 3. Main advantages and disadvantages of Direct Calorimetry (DC) and Indirect Calorimetry (IC) systems.

	Measurement duration	Advantages	Disadvantages
DC	From one to several days	- Measures heat production (and thus, EE) directly. - Offers a total control of environmental factors.	- Technically demanding. - It is not possible to determine substrate oxidation rates. - It is not able to detect acute changes. - Requires the confinement of the subject and the mobility of the subject. - Bedside measurements are not possible. - Measurement accuracy is high only on thermoneutrality situations
IC	Hours to various days*	- Real-time data. - Allows the measurement of components of EE and macronutrient utilization. - It is able to detect acute changes. - Easy to operate and suitable in clinical settings. - Allows to bedside measurements.	- Technically demanding. - Requires the confinement of the subject. - Restricts mobility of the subject. - Requires the application of formulas or stoichiometric equations (e.g. EE equation).

EE: energy expenditure; *: measurements lasting more than several hours are mostly performed using whole-room calorimeters instead of metabolic carts. Adapted from [80].

The best option (if possible) would be to combine both systems, and thus, obtaining the main advantages from DC and IC systems [3,102,124–127]. Nevertheless, due to the aforementioned advantages (and the 'acceptable' disadvantages), IC has been largely accepted for assessing EE – mostly in form of metabolic carts – rather than DC [3].

4. INDIRECT CALORIMETRY: OPEN-CIRCUIT SYSTEMS

The first developed IC were basically closed-circuit systems, and thus, rates of VO_2 and VCO_2 were measured by changes in either volume or pressure [2,128]. Nowadays these systems are still in use but primarily for small animal assessments [2]. Conversely, and using a completely different principle, we can find the open-circuit systems. In these systems, both ends of the IC are exposed to the surrounding environment [2,3,113,128,129]. Importantly, subject's gas exchange is maintained separated (i.e. inspired from expired gases) by using different non-rebreathing parts (e.g. two- or three-way respiratory valves, non-rebreathing face-masks, etc.).

Furthermore, the gas exchange may be either collected in a recipient (e.g. Douglas bag) [74] or be sampled for its posterior analysis without collecting gas exchange (e.g. whole-room calorimeter). As it was mentioned, the total collection systems are mainly limited by the recipient capacity. Other limitations should be also acknowledged. These devices usually are equipped with a mouth-piece plus a nose-clip system for gases collection, which in some cases might produce hyperventilation, and therefore biased the obtained VO_2 and VCO_2 values [130,131]. However, a face-mask could be used to overcome that problem. But importantly, these devices might result infeasible for measurements lasting more than 30 minutes, since they might not be comfortable for the subjects [131].

Trying to circumvent these limitations, systems that did not require to collect all subject's gas exchange into a container were developed, as for example, the *whole-room calorimeter* or the 'newer' *metabolic carts* [3,22,60,105,132]. The different gases exchange collection systems are extensively detailed later (**Section 4.3.2**). The mechanism is relatively simple. The gas is collected and drawn into the IC system by a fan. Although the atmospheric gases concentrations are constant (20.94% O_2 and 0.03% CO_2), when measurements are performed indoor the air concentration must be regularly measured [3], since the indoor O_2 and CO_2 concentrations may show a high minute-to-minute variation, introducing considerable errors in both VO_2 and VCO_2 measurements [3,22,60]. Importantly, at this point the '*Haldane correction*' must be acknowledged. Haldane [10] postulated that the volume of inert gases (i.e. not reactive gases) given off from, or absorbed by, the animal is constant as are not used in the body. These gases contained in fresh-air are primarily N_2 (78%) and noble gasses like Argon and Helium (0.9%). Using the Haldane correction (will be presented below in Eq. 5) the changes in VO_2 and VCO_2 can be calculated, as inert gases do not vary and remains unaltered during breathing.

In open-circuit systems the volume of inlet air (i.e. atmospheric air flowing through the canopy or the whole-room calorimeter) is measured using a pressure transducer, a turbine, a bellows device, or any other system able to measure the flow-rate. Then, the measured volumes must be converted to *Standard Temperature Pressure and Dry* (STPD) units by using the equation presented below (Eq. 3),

$$V_S = \frac{(P_a - P_w) \times V_a \times T_s}{T_a \times P_s} \quad \text{(Eq. 3)}$$

where V_s is the standard volume (i.e. STPD units; L), P_a is the ambient atmospheric pressure (mmHg), P_w is the partial pressure of water vapor present in the expired air (mmHg) at the ambient atmospheric temperature (T_a; in K), V_a is the ambient air volume (L) measured by the flow-rate monitor, T_s is the standard temperature criterion (273K), and P_s is the standard pressure criterion (760 mmHg). Once the volume has been measured, a subsample of the

collected gas exchange is pumped (by an uninterrupted low-flow) for it is analysis, and thus, obtaining VO_2 and VCO_2 values (in STPD units) as well as RER. It is of vital importance that the pumped gas sample be totally dry before it is analysis, as the presence of water vapor (even 1-2 mmHg) will negatively influence the VCO_2 and, specially, the VO_2 measurement [22,133]. Nevertheless, some devices measures the amount of water contained in the gas sample instead of dry it and then, used this measured water concentration to correct all the calculations.

The VO_2 is measured by the difference between the O_2 flowing into the IC system (fraction of inspired O_2 [FiO_2]) and the O_2 flowing out from it (fraction of expired O_2 [FeO_2]). Thus, VO_2 should be calculated as follows (Eq. 4):

$$VO_2 = V_{in} \times Fi_{O_2} - V_{out} \times Fe_{O_2} \quad \text{(Eq. 4)}$$

where V_{in} and V_{out} represent the air volume flowing into or out from the IC system respectively. Importantly, unless the RER = 1.0, the volume flowing into the canopy and the volume flowing out from the canopy are not the same [115]. For that reason (i.e. different flow volumes), the Haldane correction must be used to accurately calculate VO_2 [95] using the equation below (Eq. 5), instead of the previous one (Eq. 4),

$$VO_2 = \frac{V_{out}\,(Fi_{O_2} - Fe_{O_2} - Fe_{CO_2} \times Fi_{O_2} + Fi_{CO_2} \times Fi_{O_2})}{1 - Fi_{O_2}} \quad \text{(Eq. 5)}$$

where $FeCO_2$ is the fraction of expired CO_2, and $FiCO_2$ is the fraction of inspired CO_2.

Regarding the VCO_2 calculation (Eq. 6), a similar equation that the one used for VO_2 (Eq. 4) is applied,

$$VCO_2 = volume_{out} \times Fe_{CO_2} - volume_{in} \times Fi_{CO_2} \quad \text{(Eq. 6)}$$

however, as the $FiCO_2$ value is negligible because the ambient CO_2 concentration is close to 0 (concretely 0.03%), the Haldane correction it is not necessary [22,134]. Thus, the equation for calculate the VCO_2 (Eq. 7) can be simplify as follows,

$$VCO_2 = volume_{out} \times Fe_{CO_2} \quad \text{(Eq. 7)}$$

Lastly, with the calculated VO_2 and VCO_2 the RER can be then calculated using the Eq. 8,

$$RQ = \frac{VCO_2}{VO_2} \quad \text{(Eq. 8)}$$

Note that if the Haldane correction is not used (i.e. Eq. 5), the errors in the resulting VO_2 *per se* will negatively influence the RER. Moreover, this error will eventually become greater as the RER deviates from 1.0 [22].

It should be pointed that the present Doctoral Thesis will be focus on metabolic carts systems, devices that are described below (**Section 4.3**). Nonetheless, a summary of the different IC systems has been done. Depending on their approach for gas exchange measurement, IC systems can be classified as: (a) *confinements systems*, in which the subject is placed inside of an airtight chamber, with a fixed amount of air, and the subject's gas exchange is measured by the changes of concentrations between indoor-air and outdoor-air [2,128]; (b) *closed-circuit systems*, the subject is placed inside of a closed chamber equipped with both, water vapor and CO_2 absorbers, and thus the amount of O_2 uptake can be measured; (c) *total collection systems*: all exhaled gases are collected and both, their volume and composition are measured. An example of this IC system is the Douglas bag, which is considered a gold standard for measuring gas exchange [2,128] although have a main limitation related with the measurement length (limited to the size of the bag); and lastly, (d) *open-circuit systems*, where the subject's gas exchange is collected and analyzed. Further, the open-circuit systems may also be classified depending on their mobility and on how the subject is connected to the IC system.

Depending on their mobility, two different types of open-circuit systems exist. The 'fixed placed' ventilated open-circuit IC systems (e.g. whole-room calorimeters) and the 'portable' gas analysis IC systems (e.g. metabolic carts) [60,61]. However, regardless of their mobility, the basis and most of the components are similar. The subject normally breathes and the expired air is pumped out of the collection device (flow-rate is measured). Then, the expired air is mixed using a fan and/or a mixing chamber (i.e. a recipient in which the gas sample is mixed to ensure a representative sample of the whole gas exchange process), and a sample of this expired air is dried (if the system has a dryer) and analyzed to measure O_2 and CO_2 concentrations [2,3,60,113,128,129]. Lastly, depending on the hardware and software characteristics, air mixing rate and room or gases collection systems, the response time could differ from ≈1 to 15 minutes for a whole-room calorimeter, ≈1-2 minutes for a ventilated hood canopy (metabolic cart), and ≤30 seconds for a face-mask or a mouth-piece plus a nose-clip systems (metabolic cart) [108,135]. On the other hand, the portable gas analysis IC systems are commonly configured as a metabolic cart, by which the system can be easy transported to a different place using a wheeled cart. Importantly in metabolic carts rather than in whole-room calorimeters, $FiCO_2$ should not outreach 1% [136], because concentrations exceeding that percentage might influence the respiratory effort reducing it is efficiency and biasing the results. In whole-room calorimeters it is difficult to outreach that percentage as the air volume is much higher than inside, for example, a ventilated hood canopy [61]. To overcome this 'problem' it is important to ensure a proper ventilation of the gas collection system.

The second classification for open-circuit IC systems is based on how subjects are connected to the device [60]. In this regard, we could differentiate between systems that did not require the connection to the subject's face (i.e. whole-room calorimeters; **Figure 10A**) – from those that need it, as an IC system equipped with ventilated hood canopy (**Figure 10B**), with a face-mask (**Figure 10C** and **D**) or a mouth-piece plus a nose-clip (**Figure 10E**). Among them (i.e. these system that did not require the connection to the subject's face and these that need it) we may differentiate them depending on the approach followed by the IC system for the exhaled gases analysis [60]. Therefore, we may differentiate among: (a) the full capture of diluted exhaled air (**Figure 10A, B,** and **C**); (b) the breath-by-breath (B×B) analysis (**Figure 10D**); and lastly, (c) the full capture of non-diluted exhaled air approach (**Figure 10E**).

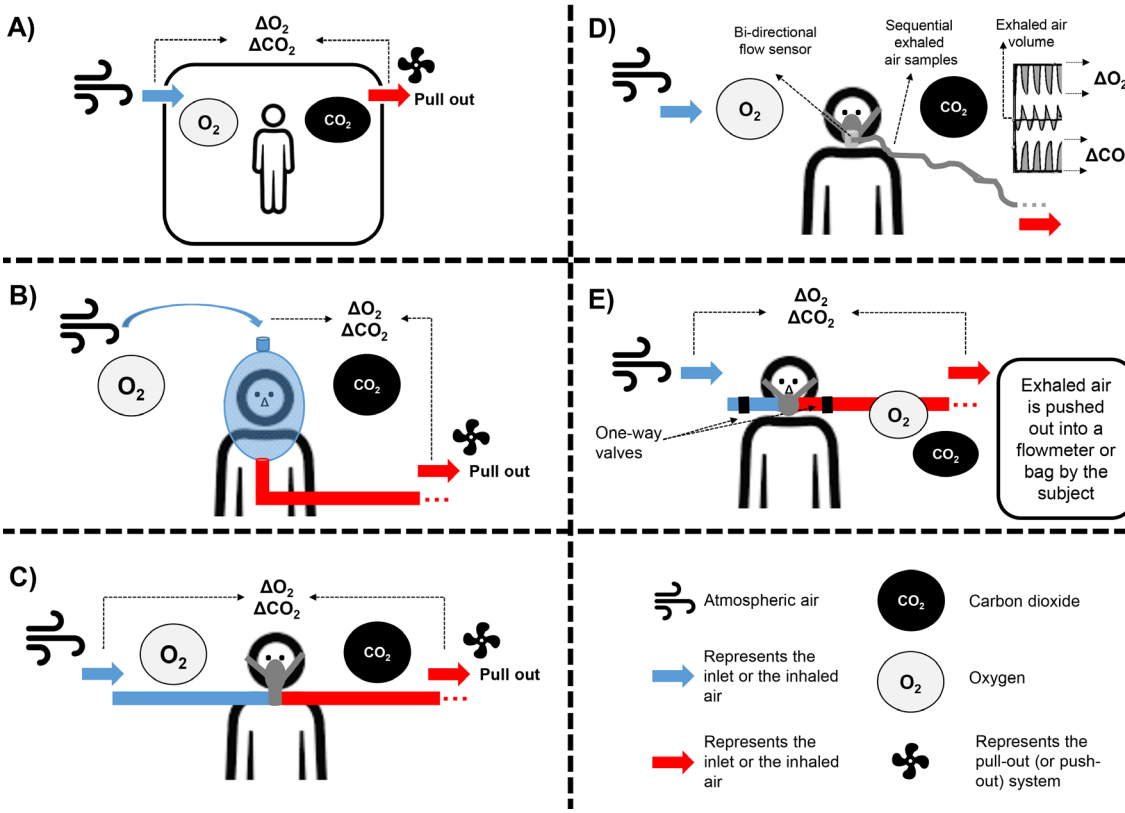


Figure 10. Summary of open-circuit approach Indirect Calorimetry systems. *A pull whole-room calorimeter, in which the inlet air is drawn in and out of the room, is presented in Panel A; a similar procedure (but in a smaller device) is followed for the ventilated hood canopy (Panel B) or the face-mask systems (Panel C) equipped in the metabolic carts. A bi-directional flow sensor system, in which sequential samples of either the exhaled or inhaled air (i.e. breath-by-breath) are transported through a single sample line (or tube), is presented in Panel D. Classic one-way respiratory valves systems to collect the total gas exchange into a Douglas bag [74] or a flow-meter [7,73] is presented in Panel E. ΔO_2 and ΔCO_2 represents the difference between inhaled O_2 and exhaled O_2 or between inhaled CO_2 and exhaled CO_2 respectively. Adapted from [60].*

In the subsequent sections, the characteristics of the IC systems (i.e. whole room calorimeters and metabolic carts) will be described in detail. Of note, the most important parts and subsystems will be described below. Regarding the metabolic carts, in depth information will be exposed about their (a) gas exchange collection systems (i.e. mouth-piece plus a nose-clip, face-mask or ventilated hood canopy) and (b) gas exchange analysis approaches (i.e. mixing chamber or B×B). Lastly, validation and calibration of IC systems, tests that can be performed using them and how to select and to analyze the obtained data will also be described.

4.1. PARTS AND SUBSYSTEMS OF MOST INDIRECT CALORIMETERS

The parts and subsystems of the IC systems may be classified either into hardware components – i.e. those physical, electrical and electronic sensors or devices which compose the metabolic cart – or software components – i.e. those programs and routines that allow the metabolic cart to properly work as a whole. Their adequate interconnections, suitability, calibration, validation, accuracy and precision will be determinant for the IC system performance [2,3,60,61,113,128,129].

The most important **hardware components** are: (a) the gas analyzers, (b) the flow-meter, (c) the dryer system (if the system incorporate it), (d) the pressure, temperature, and humidity sensors, and (e) the computer (or laptop).

Gas analyzers: The analyses of gas exchange in the past were done manually by using complex chemical analysis and techniques [2,3,102,137–139], while nowadays it is done (in an automatic way) by electronic devices (i.e. the gas analyzer) [2,3,145–147,113,129,137,140–144]. Commonly, IC systems uses sensors ranging from small gas analyzer cells to considerable mass-spectrometers. The firsts are usually implemented in metabolic carts while the seconds are used in whole-room calorimeters [3,113]. Most common O_2 analyzers are the paramagnetic O_2 analyzers, zirconia-cell O_2 analyzers and the fuel-cell (or electrochemical) O_2 analyzers. Extended information about their advantages, disadvantages and applications can be found elsewhere [113]. Regarding CO_2, the most common is the infra-red CO_2 analyzer [113].

Flow-meters: the measurement of air flow-rate can be done either measuring the volume of air over a determined period of time or by the constant measurement of flow-rate through a pneumotachometer. Between the possible options for measuring flow-rate of air, the most common are the rotameters and the mass flow-meters [113]. The latter (maybe the most used in IC systems) can supply continuous information (by an electrical output). Mass flow-meters allows both, the continuous recording of flow-rate information, as well as the control of the flow-rate itself (through a variable-aperture solenoid or a variable-drive pump to create a 'mass flow-controller') [113].

Dryer systems: the chemical scrubbers – particularly before the arrival of accurate and precise gas analyzers – were one of the most important parts of IC systems. As the mathematical compensation for the different interactions between water vapor, O_2 and CO_2 was almost impossible, chemical scrubbers were the easiest way of eradicating such troubles from the equations [113]. Different chemical water vapor scrubbers exist, and while some may be relatively easy restored to their 'dry state', others cannot. The most common chemical dryers are the drierite, the silica gel and the magnesium perchlorate. On the other hand, there also exists the 'thermal scrubbers' for removing water vapor. These scrubbers uses the dew point at which water vapor naturally condense into liquid water to eradicate water vapor from the system. Lastly, another dryer system is the 'selective membrane' scrubbers for water vapor [113]. Such selective membrane is permeable exclusively to water vapor and is known as 'nafion'. The nafion membrane is maybe the most used dryer in the gas exchange sample-line tubes employed in most B×B systems. This is mainly because the reaction is exclusively specific to water vapor and thus, the other gases (e.g. N_2, O_2 and CO_2) passing through the nafion membrane are unbiased [113]. Extended information about the advantages, disadvantages and applications of the aforementioned dryers systems can be found elsewhere [113].

Pressure, temperature, and humidity sensors: both atmospheric pressure and temperature can be accurately measured using a barometer and a thermometer respectively. In fact, most of the newest IC systems incorporate them as well as humidity sensors to create an 'atmospheric ambient unit' sensor, which recorded them and automatically save the data in the system (for further analysis and calculations). The proper measurement of atmospheric relative humidity or ambient water vapor is more complex than atmospheric pressure and temperature. Nonetheless, the aim of measuring the water vapor present in air is to obtain the partial pressure of water vapor [113], because its direct influence in gas exchange calculations (particularly negative in VO_2; see **Section 3.2.3**). Most water vapor sensors do not offers direct values about water vapor pressure, they provide data that can be transformed (re-calculated) into water vapor pressure values. Last, the capability to analyze the water vapor present in air in a continuous flow-rate system is crucial [133]. Of note, most of metabolic carts do

not ensure accurate gas exchange values if the ambient conditions change by more than 2ºC and 10% (for ambient temperature and relative humidity respectively) from the last record – mainly because their influence on inspiratory related variables, i.e. FiO_2 and $FiCO_2$.

Computers: with the development of potent computers it has become plausible to determine different kinds of responses as well as to reduce the necessary time of measurements [102,140,148,149]. A few examples are the 'instantaneous transformation' or Z-transformation, which is used to reduce the electrical noise from the gas analyzers [113]; the response time of the system, which can be calculated dividing the room (or mixing chamber) volume by the flow-rate of air across it [113]; the zeroing-phase filters; simple or complex statistics, etc. Regarding the response time of the system, if a very fast-fluctuating event is going to be studied (e.g. during exercise) the system had to be capable of ramp-up flow-rates as high as possible, or in other words, to reduce the response time of the system; on the other hand, if the study is focused on resting measurements, a longer time will be enough. Be aware of that, in some systems, reducing the response time of the system (i.e. increase the flow-rate) may implicate a poor mixing of the gas sample, and thus, inaccurate values and results.

Besides the hardware components, another key element of the IC systems is the *software* installed on the computer. These routines and programs allow the IC systems to functioning as a whole, integrating the information from the gas analyzers, flow-meter, and the aforementioned subsystems. Thus, just as important as the hardware components mentioned above is the software. The software (usually specifically designed by the IC systems brands) is the responsible of executing the tasks mentioned above (e.g. Z-transformation, zeroing-phase filters, modifying the flow-rate, among others), and for that reason a potent and well-designed software is needed for a good performance of the IC system.

4.2. WHOLE-ROOM CALORIMETERS SYSTEMS

Whole-room calorimeters systems were developed in 1875 by Pettenkoffer and Voit [55] but the method fell into disuse due to its difficulty of analyzing large amount of expired gases accurately, and because at that period existed others methods easier than this one [2]. Later, thanks to advances in electrical and automated gas analyzers, the interest on whole-room calorimeters systems raised. In fact, nowadays they are used to measure gas exchange during longer periods of time, normally ranging from 12 hours to several days. As mentioned above, whole-room calorimeters are based on the open-circuit principle in which the room is ventilated using atmospheric outdoor-air for 'refreshing' the indoor-air. In other words, the expired air is diluted, and thus, that air had to be collected and sampled for it is posterior analysis determining VO_2 and VCO_2.

The main advantage of whole-room calorimeters vs. the metabolic cart systems is that it allows the subject to move freely inside the room [3,60,61]. Nonetheless, the movements are recorded by using a radar and/or motion sensor systems [150] or optionally by using wearable devices (e.g. accelerometer, heart rate monitor, etc.) or force platforms [151]. As measurements may last several days, different components of TDEE (i.e. SMR, BMR, TEF, CIT and activity thermogenesis) and the TDEE itself, can be accurately measured. Of note, when IC is used the TDEE is normally called *24-hour EE*, although at the end both refers to the same concept. However, the activity thermogenesis is somehow limited because the confined and restricted space and thus, it is not representative of the free-living activities. To circumvent such limitation, and trying to simulate free-living conditions, a physical activity procedure might be used by adding a treadmill or a cycloergometer into the room. In this regard, a typical whole-room calorimeter includes a bed, a 'work station' (i.e. a table and a chair), a toilet

(which may be also used to collect samples), a sink, television, intercommunication, phone, computer, airlocks and samples ports, windows, etc. Although in the majority of whole-room calorimeters the ambient conditions may be modified [61] these are normally maintained relatively constant during measurements.

In the first whole-room calorimeters systems, the response time was slow, measuring VO_2 and VCO_2 in time periods ranging from 10 to 15 minutes [102,141]. Technology improvements (e.g. components, signal processing, etc.) have directly influenced to the development of new whole-room calorimeters systems in which the response time may vary from 1 to very few minutes [61,108,135,145,152,153]. Whole-room calorimeter systems can either pull air from or push air into the room (i.e. pull or push systems) [60], although others employ a 'push-pull' system which is thought to reduce the fluctuation in CO_2 concentration inside the room [152]. In any case, leaks of air had to be as less as possible to obtain unbiased data. In this sense, it is of vital importance to either have a unique inlet-air point or be sure that all inlet-air have exactly the same composition. Further, all whole-room calorimeters need an optimal air mixing system, ensuring that the collected and sampled (a sub-sample of the entire air room) air is representative of the whole room gas concentrations and conditions.

4.3. METABOLIC CARTS SYSTEMS

Although both systems are based on the same rationale, the metabolic carts systems are much smaller and 'practical' than the whole-room calorimeters. Metabolic carts systems can be equipped with a ventilated hood canopy (**Figure 10B**), a face-mask (**Figure 10C** and **D**) or a mouth-piece plus a nose-clip (**Figure 10E**) for gas exchange collection. VO_2 and VO_2 measures can be obtained either by the open-circuit approach (i.e. the procedures would be similar to those performed by the aforementioned whole-room calorimeters) or by the B×B approach [134]. Of note, these IC systems are the most commonly used for assessing human resting EE and are a noninvasive method which can be relatively easy employed either in healthy (i.e. non-critically ill and/or spontaneously breathing subjects) or in ventilated subjects.

Although metabolic carts are inexpensive and user-friendly compared with whole-room calorimeters systems, the metabolic carts limit the locomotion and movement, limiting the measurements up to, as much, a few hours [80]. Normally, measurements using metabolic carts last from 5 to 30 minutes, from which an even shorter period of time is selected and analyzed (see **Section 6**). The Deltatrac metabolic cart (Datex Instrumentarium Corp, Helsinki, Finland), has been for long considered the gold standard for assessing RMR, however this metabolic cart is no longer manufactured [154–162]. Although some have suggested the Vmax Encore 2900 (SensorMedics, Yorba Linda, CA, USA) could be the 'successor' to the Deltatrac as the new gold standard [163], currently, there is not unanimity in scientific community. Therefore, there is a real necessity of identifying a valid, accurate and precise metabolic cart that could be considered as the new gold standard by the scientific community. In this regard, studies aiming to determine the preferred metabolic cart among the different commercially available metabolic carts are currently being performed. Sadly, most of them have shown an unacceptable (day-to-day) biological reproducibility, which is in clear contrast with the Deltatrac. In fact, the Deltatrac metabolic cart had showed a day-to-day coefficient of variation ($CV_{D\text{-}to\text{-}D}$) close to 4% [160], which is thought to be close to the physiological variability of human RMR [28,164–166]. It is important to mention that most of the studies examining the validity, accuracy, and precision of the different commercially available metabolic carts have not compared them within the same settings, conditions and/or cohorts (e.g. [155]). Moreover, most of them have not used recently manufactured metabolic carts (e.g. [167]), which may, somehow, bias the results due the deterioration of the systems.

Achieving a high precision and accuracy is of great importance as otherwise effects of interventions may be 'masked' by the measurement noise [159]. Another important aspect

is to determine the biological variability (i.e. inherent physiological differences [healthy subjects] among subjects [inter-subject variability] and within the same subject [intra-subject variability] over time) and the comparability (i.e. how similar are the same measurements obtained by different systems) between different metabolic carts. Thus, doubts remain to better understand if some metabolic carts systems are more precise than others, and if there are, it is necessary to determine and identify the most precise metabolic carts which are commercially available on the market.

4.3.1. Mixing chamber and breath-by-breath metabolic carts

The way in which mixing chamber and breath-by-breath metabolic carts analyze the gas concentrations differ, although both are based on the open-circuit principle.

For mixing chamber metabolic carts (**Figure 11**), the expired gas is pumped and collected into the mixing chamber, and then, the gas analyzers sampled the collected gas at a factory-fixed intervals which may range from ≤1 to 5 minutes [134]. Further, if the system is also a dilution system (i.e. operates with diluted samples), the system collects the expired gas, dilute it with atmospheric air, and then bypass these diluted gases into the mixing chamber for its posterior analysis [60,61]. Importantly, this system works best for spontaneously breathing subjects (i.e. healthy non-ventilated subjects), while the subject is resting and in a steady-state [134]. As aforementioned, in mixing chamber metabolic carts (normally when a ventilated hood canopy system is equipped) the $FiCO_2$ should be maintained in a range of ≈0.65-0.85% (without outreach 1%), a level which is supposed to be enough to avoid the external (non-physiological) stimulation of ventilation [22]. This proper $FiCO_2$ is produced by a constant air-flow (commonly 4-5 times the subject's minute ventilation) through the canopy system to induce a slight negative pressure gradient from the canopy to the mixing chamber system [22].

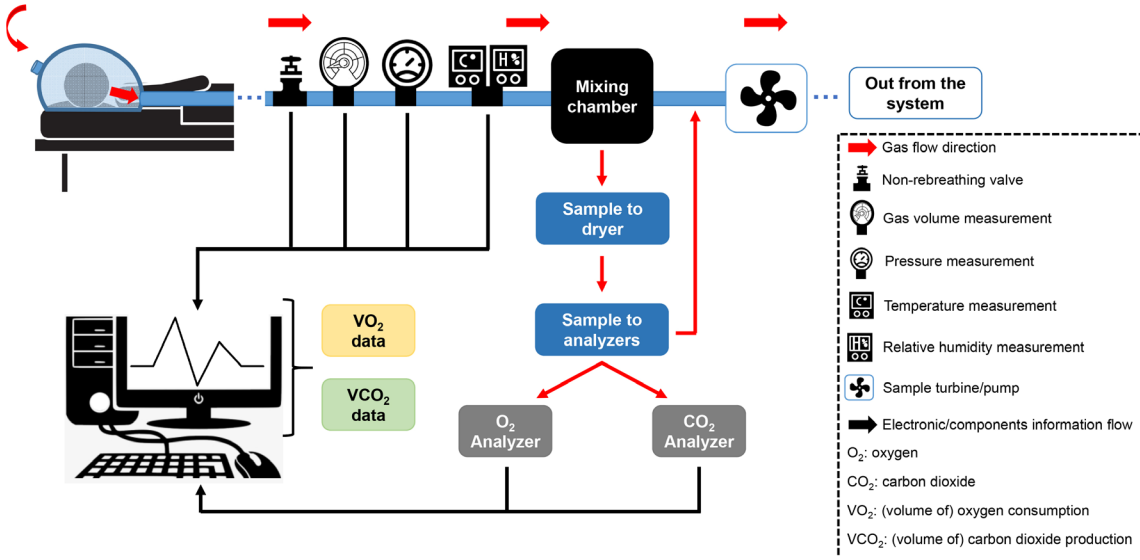


Figure 11. Schematic representation of a generic mixing chamber metabolic cart.

On the other hand, the B×B metabolic cart (**Figure 12**) samples the VO_2 and VCO_2 concentrations every breath, and subsequently, the metabolic cart averages and calculates

the data obtained over a reduced time (commonly ranging between ≈5–7 seconds). Unlike the mixing chamber system, B×B systems are supposed to be more appropriate for subjects experiencing problems of partial mixing of inspired gas or presenting a significant dead space (e.g. mechanically ventilated subjects), those with unstable FiO_2 or elevated $FiCO_2$, and elevated concentrations of water vapor in expired gases [168] as the B×B metabolic carts are supposed to both, detect such variations quicker than mixing chamber devices and, not be biased by 'unstable' conditions.

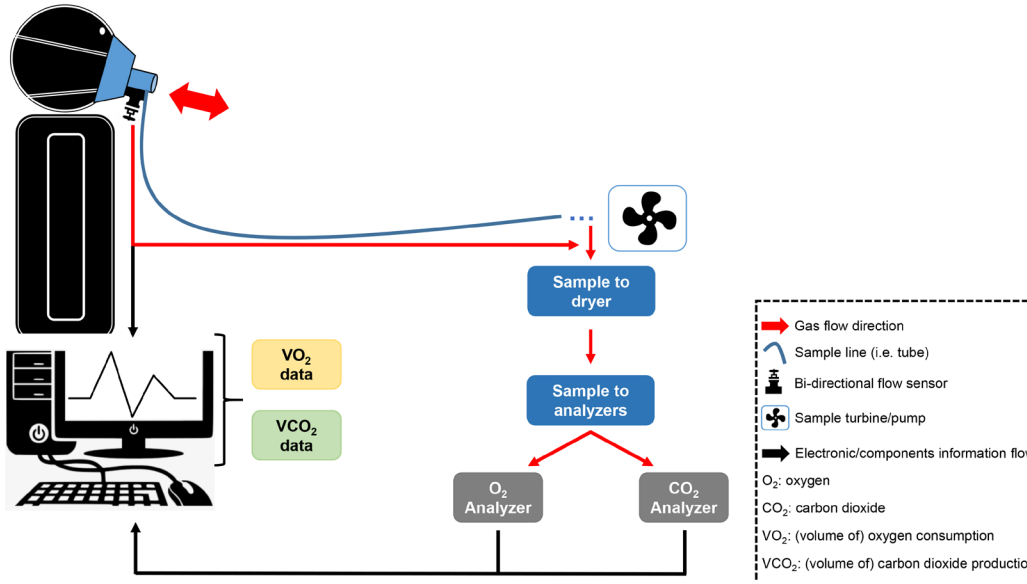


Figure 12. Schematic representation of a generic breath-by-breath metabolic cart.

With the presumed exception of B×B metabolic carts, most IC systems would need to measure during longer aggregation interval [60]. This longer aggregation interval also applies for full capture of the subject exhaled air systems, doing unnecessary the assumptions for the synchronization of gas analysis and breathing [60]. A longer aggregation interval, could be considered as an advantage because such synchronization is not effortlessly validated, especially for fast breathing patterns (e.g. during maximal exercise) [169–175]. On the contrary, a disadvantage of a longer aggregation interval may be that measuring a number of breaths superior to such aggregation interval during, for example, slow breathing patterns (i.e. small gas exchange volume) this may introduce errors [3,60] due to desynchronizations (except if the breathing rhythm is measured for 'manual' synchronization). Mainly, for that reasons, slow breathing patterns during resting gas exchange measurements may results in noticeable hills and valleys of measured gases and thus, estimated EE.

Lastly, because tidal volume (i.e. lung volume characterized by the normal volume of ambient air displaced between a normal relaxed-breathing [normal inhalation and exhalation] when extra-effort is not performed) of breathing is not steady or constant [172,176,177], even measurements carried-out using B×B metabolic carts will show variability and will need various breaths above the aggregation interval. Nevertheless, for whole-room calorimeters the influence of breathing 'intensities' is practically negligible, as the subject's breaths are diluted into a significantly larger volume (compared to ventilated hood canopy or face-mask systems) of air contained in the room [60].

4.3.2. Gas exchange collection systems

Depending on the form in which the metabolic cart analyzes the gas exchange collected, different gas exchange collection systems may be equipped. Three different systems for gas exchange collection, exists: (a) the ventilated hood canopy system, (b) the face-mask system, and (c) the mouth-piece plus nose-clip system. Their use may also depend on the measurement or the test that is made (e.g. a face-mask is usually the one selected for exercise testing).

Ventilated hood canopy system: this system has been broadly used either in humans and other animals [2,178,179]. The canopy is made of plastic, perspex, or polythene and cover the head, the superior part of the body or even the whole body (**Figure 13A**). Atmospheric air enters the canopy either through one (or more) inlet. In most open-circuit IC systems equipped with a ventilated hood canopy the air is drawn through the canopy by negative pressure induced by either a pump or a fan, thus reducing considerably the possibility of losing the subject's expired gases [180]. Canopy systems are considered the best option for assessing the RMR using metabolic carts [181], and have been previously validated [182–184]. This is mainly because this system allows the subjects to breathe freely under the canopy [185], which is in clear contrast with the constriction commonly produced when using either face-mask or mouth-piece plus nose-clip system [186]. Of note, other systems which are between canopy and face-mask are known as *face-tent systems* (**Figure 13B**). Brands using this system (e.g. Medical Graphics Diagnostics®) argue that the face-tent system has the following advantages compared to canopies: (a) face-tent removes the feeling of claustrophobia which may alter breathing patterns, (b) face-tent eliminates the possibility of a rising in $FiCO_2$ which may bias the assessments, (c) there is no possibility of small leaks, which are really difficult (or impossible) to detect when using canopies, and (d), face-tent allows assessing BMR or RMR in B×B approach, which (theoretically) will result in a faster steady-state achievement than canopies. Nonetheless, this gas collection system is not commonly used, and whether these abovementioned advantages are real remains unknown.

Face-mask and *mouth-piece plus nose-clip systems*: these systems for gas exchange collection are commonly used for exercise measurements [187] or others that requires the ability of measuring and analyzing the gas exchange every breath or close to it (i.e. using B×B systems). These systems are connected to the metabolic cart through either a hose tube or a Nafion/Permapure desiccant tube [188] equipped with a flow-meter (e.g. pneumotachograph, turbine, etc.). Although previous studies have suggested possible problems regarding clenching the teeth and the resulting low air-passage when using a mouth-piece plus a nose-clip, and thus, negatively influencing the VO_2 determination [187], it has been shown that neither wearing a face-mask (**Figure 13C**) nor a mouth-piece plus a nose-clip (**Figure 13D**) significantly influences the running style of subjects running at submaximal and comfortable running speeds [187]. Despite of these system are mainly used during exercise, there are studies using either the face-mask or the mouth-piece plus a nose-clip for assessing RMR [130,156,162,184,189–191]. Regarding the influence it might have on the RMR measurement, disagreements exists between studies (which are supposed to be produced by the subjects discomfort), as some studies did not find differences between canopy and the others methods for gas exchange collection for assessing RMR [190,192,193]. However, consistently higher RMR values were observed using the mouth-piece plus nose-clip system (1687±271 kcal/day; this value is a mean of 5 days) vs. the ventilated hood canopy (1593±294 kcal/day; this value is a mean of 5 days) system [131]. Similar results were found in a different study comparing the face-mask (1762±66 kcal/day) and the mouth-piece plus nose-clip systems (1788±63 kcal/day) vs. the ventilated hood canopy (1644±60 kcal/day) system [130]. Lastly, the $CV_{D\text{-}to\text{-}D}$ (mean of 5 days) was 2.28% for the ventilated hood and 3.11% for mouth-piece plus nose-clip system [131].

Figure 13. Different gas exchange collection systems that may be equipped to the metabolic cart. *Panel A represents a plastic ventilated hood canopy system; Panel B represents the face-tent system; Panel C represents two different face-mask that differ in the material, i.e. silicon (upper picture; blue face-mask) and neoprene (lower picture; black face-mask) which they are made; and Panel D represents the mouth-piece plus nose-clip system.*

4.4. CALIBRATION AND VALIDATION OF INDIRECT CALORIMETRY SYSTEMS

It must be taken into account that *calibration* and *validation* of IC systems [60,61] are two completely different processes. Following the definition by the International Bureau of Weights and Measures (i.e. BIPM – French acronym for the 'Bureau International des Poids et Mesures'), under specified and well-controlled conditions, the calibration process *'establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, [...], uses this information to establish a relation for obtaining a measurement result from an indication'* [194]. Thus, in other words, the calibration represents the act of testing whether the different components of the IC system are suitable (or not) for it is purposeful use.

Regarding the validation, and following the definition proposed by the BIPM, organization which defines validation as *'verification [i.e. provision of objective evidence that a given item fulfils specified requirements], where the specified requirements are adequate for an intended use'* [194]. Importantly, the same organization highlighted the importance of known that validation should not be confounded with calibration, as both process are not equals [194]. Lastly, the calibration process does not examine the well-functioning of the IC system as 'a whole', or in other words, that all subsystems and parts are working properly and synchronized [3,60].

4.4.1. Calibration of systems

The calibration processes of the different parts and subsystems previously mentioned (**Section 4.1**) are of vital importance for the suitability of the IC system as a whole [3]. In fact, calibration processes are mandatory requirements for achieving accurate gas exchange data. The gas analyzers (VO_2 and VCO_2) and the flow-meter must be calibrated separately – considering the atmospheric conditions (i.e. the ambient pressure, temperature, and humidity). Nonetheless, in most of the new IC systems most (if not all) of the calibration processes are performed automatically (e.g. the calibration of the flow-meter, introduction of atmospheric conditions into the IC software, etc.). The calibration processes of most of the IC systems can be summarized as follows:

Calibration of flow-rate: the process consists on performing different injections of air using a certified syringe of a well-known volume capacity. The manufacturer's recommendations or most of the brands is to perform different injections – ranging from 3 to 7 injections depending on the IC system and/or brand (although most of them allows to change the number of injections) – at different rhythms trying to mimic 'slow' breathings (i.e. mimicking resting conditions) and 'fast' or intense breathings (i.e. mimicking exercise conditions). Normally the calibration of flow-rate is performed once per day, although the manufacturer's recommendations are to calibrate the flow-rate every single time after replacing the flow-meter (e.g. replacing the turbine, the pneumotachograph, etc.).

Calibration of gas analyzers: this type of calibration normally consist of infusing two different gases mixtures of a well-known concentration of O_2 and CO_2 across the gas analyzers. This calibration process may be done by the consecutively infusion (i.e. dual span) of two gases or using just one gas mixture and ambient air [60]. The general manufacturer's recommendations for gas analyzers calibrations are once per day, except if ambient conditions vary more than 2°C and 10% for ambient temperature and relative humidity respectively.

4.4.2. Validation of systems

Once the IC system has been fully calibrated and every single calibration has been successful, the system is ready to be validated. Five different approaches may be used to validate an IC system [60,61]: (a) using a 'zero test', (b) using burning test, (c) using pure gas infusion, (d) using the (day-to-day) biological reproducibility, and (e) using parallel validation.

Zero test: this validation test (**Figure 14**) consists on recording gas exchanges rates in an empty and air-calm room (or canopy) over the duration of a routine measurement [61]. This test provides information about the system bias, air-stream and or drift, and thus intrinsic measurement 'noise' [148].

Burning test: this validation method is widely employed [60,61,145,153], and it is based on the combustion of a flammable substance like propane, butane, methanol, or ethanol (**Figure 14**). After its combustion, depending on the amount of substance burned and the duration of the test, the expected VO_2 and VCO_2 values can be calculated based on stoichiometry equations [167,195]. This method has the advantages of generating water vapor and Q, as well as simulating a determined RER, furthermore, it does not imply complex procedures (i.e. is user-friendly). However, certain limitations should be acknowledged, as for example assumes the combustion purity and efficiency (e.g. is free of contaminants and/or water) although are 'open flames' [61]. Further, the yielded RER is outside the physiological range (i.e. below 0.70), being 0.667 for alcohols and 0.60 for alkanes [61]. Of note, the physiological within limits of RER are usually considered to be 0.70 – 1.0 [21]. Lastly, the combustion rate cannot be modified during the test, thus producing a fixed gas exchange rate.

Gas infusion: this validation method is based on the direct infusion of pure gases (**Figure 14**), typically N_2 and CO_2 (concentration and purity > 99.99%), using mass-flow controllers to mimic EE and RER patterns over the physiological range [60,61,152,161,196–199]. Of note, the N_2 (i.e. volume of N_2 – VN_2) is used to dilute ambient O_2 based on the theoretical approach done by Haldane (i.e. Haldane Correction, see **Section 4**). This method has the great advantage of plasticity, allowing different controlled VO_2 and VCO_2 rates infusions and therefore, changes in both EE and RER can be simulated. Nonetheless, an important culprit of this method is that it relies on others devices than the IC system itself. Secondly, the infused gases are dry and cold, which do not unlike expired air. In fact, the measurement error of IC system commonly is directly related to the system's ability to dry (or to 'manage' the humidity) the expired gases [133]; on the other hand, cooling (and even freezing of external pieces) may occurs due to adiabatic expansion (i.e. process that occurs in a system in which the temperature decreases while the pressure remains unaltered) [60]. Lastly, the mass-flow controllers used for controlling the infused gases are susceptible to drift, thus periodical verification of the systems are needed [61].

Biological reproducibility: this validation uses the (theoretical) within known limits of biological day-to-day variability in healthy subjects [28], to determine the suitability of the IC system to assess such biological variability [60]. In other words, this validation uses the repeated measures approach to assess the biological reproducibility. Thus, if the IC system is able to 'detect' such variability, we may consider that is valid and capable to test subjects. Importantly, for referring to an IC system as a 'totally valid' device must be previously validated using both approaches, i.e. '*ex vivo'* using simulations methods (i.e. zero test, burning test, and/or gas infusion) and '*in vivo'* using real subjects and its within known limits of biological variability and reproducibility [60]. Repeated measures using healthy subjects are usually followed to use this validation approach. Components of 24hEE as SMR, BMR, 24hEE itself and exercise has been previously studied. High intensity exercise [200] have shown the lowest $CV_{D\text{-}to\text{-}D}$ compared to, maybe its contrary 24hEE component while awaking as

is the BMR ($CV_{D\text{-}to\text{-}D}$ of 1.2% and 3.3% respectively) [201]. Sleeping metabolic rate ($CV_{D\text{-}to\text{-}D}$ of 2.4%) [202] and TDEE ($CV_{D\text{-}to\text{-}D}$ of 1.9%) [141] have shown lower $CV_{D\text{-}to\text{-}D}$ than BMR. Of note, all the measurements were carried-out in healthy subjects, some of them were high-level athletes evaluated using a metabolic cart equipped with a face-mask [200], a canopy [201] or were confined into a whole-room calorimeter [141,202].

Parallel validation: this validation method is based on testing one IC system using another one as the reference method or gold standard. For a correct application of this approach, both systems must measure the exactly the same air source. Although this validation method seems easy, a few considerations should be highlighted [3,60]: (a) the gold standard system has to be previously validated *in situ* – i.e. one IC system which has been validated in literature at some point may not be a valid gold standard system at that moment; (b) interactions between systems had to be prevented (e.g. air leaks), and (c) this validation method will introduce the abovementioned biological variability, as a subject is used (i.e. measured) for the validation process. An example of a validation of two systems using the parallel validations is presented below in **Figure 14**.

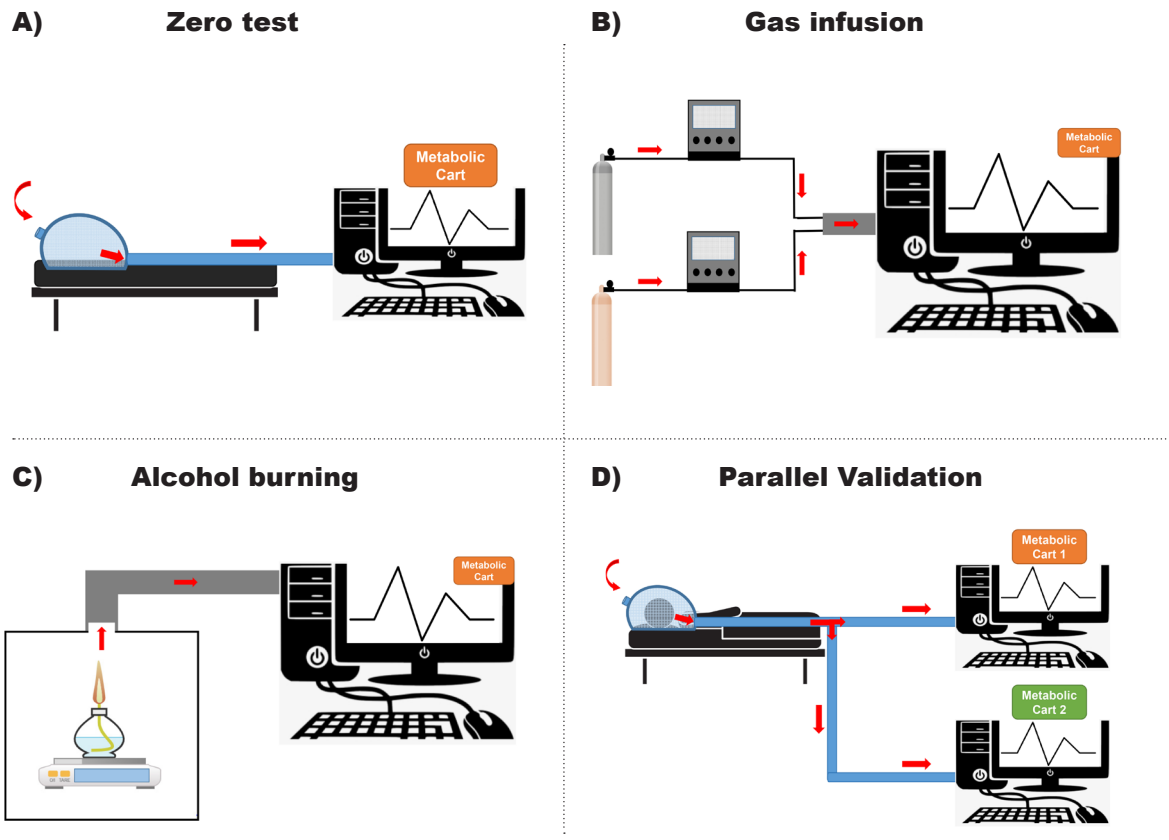


Figure 14. Validation of Indirect Calorimetry systems. *Panel A represents the 'zero test' (i.e. recording gas exchanges rates in an empty and air-calm canopy). Panel B represents the alcohol burning test (i.e. combustion of a flammable substance like methanol or ethanol; depending on the amount of substance burned and the duration of the test, the expected oxygen consumption and carbon dioxide values can be calculated and the error of the system determined). Panel C represents the gas infusion test (i.e. direct infusion of pure gases, typically nitrogen and carbon dioxide using mass-flow controllers to mimic energy expenditure and respiratory exchange patterns). Panel D represents the parallel validation test (i.e. testing one IC [e.g. metabolic cart 1] system using another one [metabolic cart 2] as the reference method or gold standard). Red arrows represent the gas flow direction.*

5. APPLICATION OF INDIRECT CALORIMETRY TO THE STUDY OF 24-HOURS ENERGY EXPENDITURE COMPONENTS

Human EE is not unaltered or unchanged over time, as it varies depending on the time of the day, the physical activity level of the subject and/or the exercise performed, the amount of food and its composition, etc. Normally, when we are measuring human EE it is practically impossible to separate or even to individually measure different components of 24hEE. However we may identify a specific component during a particular period of time [60]. Thus, these different components can be identified while the subject is resting, sleeping, doing physical activity or during a period of 24h.

As most other physiological mechanisms, the different EE components do not merely commence and cease, as each 24hEE component exhibits an individualized response and rhythm [61]. Given those particularities and depending on the component which is going to be measured (e.g. BMR vs. RMR), the standard operating protocols are of vital importance and will have their particularities. Moreover, depending on the operating protocols design, the obtained IC data may be compromised or enhanced, or in other words, obtaining precise and accurate EE data from the VO_2 and VCO_2 measurements or not [61]. In the following sections, the possibilities offered by metabolic carts for measuring VO_2 and VCO_2, while the subject is in a physically resting state (i.e. primarily lying or sitting), will be introduced.

5.1. SLEEPING OR OVERNIGHT METABOLIC RATE

Although some studies have assessed SMR using metabolic carts equipped with a canopy – see an example [203]; SMR is commonly assessed using whole-room calorimeters, as these IC systems have all the technical facilities and instruments for assessing them under the best possible conditions [60,61].

Before any other comment, it should be pointed that SMR does not have a clear definition yet [61] as the criteria for its determination may be different between researchers and studies. On the one hand, some researchers have proposed that SMR is represented by the lowest assessed EE during the night and over 3 consecutive hours [202]; on the other hand, other researchers have proposed that SMR is characterized by the average EE assessed during the night and during the 3 consecutive hours presenting the lowest observed physical activity (i.e. movements) – this approach requires the implementation of either accelerometers or indoor motion sensor systems [202,204,205]. In theory, the lowest physical activity may be produced earlier in the night while TEF remains [61]. Attending to this hypothesis, the best SMR definition should be 'the period of 3 consecutive hours presenting the lowest physical activity (movements) that take place with no less than 6 hours after the last meal' [61]; or even, 'the averaged EE from minutes of minimal physical activity during a pre-established (fix) post-TEF time period [61].

Regardless the lack of consensus among scientific community, SMR is sometimes assessed instead of BMR or RMR, because SMR present a better biological reproducibility than them – expressed as intra-subject $CV_{D\text{-}to\text{-}D}$ [202,206]. A possible limitation regarding SMR is that the EE while sleeping decreases over time, and thus, SMR assessment varies from the early phase of biological night (higher EE) to the later phase (lower EE). This gradual reduction

is mainly promoted by the decrease in the TEF component as well as by the circadian rhythm of the BMR (or RMR). In this regard, BMR (or RMR) reaches its minimum EE value in the biological night (≈4-5 AM) [61] that also matches the minimum value in endogenous core body temperature [207]. On the other hand, another possible limitation is that for ensuring accurate SMR data, the subject has to sleep in the whole-room calorimeter at least 2 days, due to the 'first night effect' [61]. Such negative effects may produce, for example, a delay in the onset of deep sleep stages and more awake periods, among others. Nonetheless, it has been proposed that after the first night, this disruptions in sleep quality disappears [61].

Taking all together, although SMR is a high reproducible 24hEE component (presenting the lowest intra-subject $CV_{D\text{-}to\text{-}D}$), caution is needed due to the possible mentioned limitations as well as the absence of a clear definition and consensus among researchers. Nonetheless, the panel of experts which composed the 'Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0)' [61] has recently proposed two (slightly) different definitions (mentioned above) trying to overcome this fact.

5.2. BASAL METABOLIC RATE OR RESTING METABOLIC RATE

Both, BMR and RMR are commonly measured using a ventilated hood canopy system while the subject is resting in a thermo-neutral environment [21,208]. Theoretically, both represents the necessary EE for maintaining vital functions and whole-body homeostasis on an awake person; although following the strictest definition, such 'necessary EE' would be represented by the BMR rather than by the RMR [3,60]. In fact, the BMR is obtained under much controlled, specific and strict conditions than the RMR. In this regard, the BMR implies that the subject be in a fasting state (post-absorptive state of 10 to 12 hours) without consuming any kind of caloric beverage or food, calm, avoiding fidgeting, awake, breathing normally, laying on a bed, and in a thermo-neutral environment (controlling both, temperature and relative humidity) with dim lighting and without any external stimuli [21,208]. Importantly, the assessment has to be carried-out early in the morning, after the subject sleeps and wake-up in the room in which the assessment will take place [61]. Therefore, the BMR requires an overnight stay for avoiding the possible EE derived of transportation to the center. In any other way, and if the resting assessment is performed under less restrictive conditions (e.g. different fasting, positioning, time of the day, etc.) this assessment should be referred as RMR. Both, BMR or RMR are influenced by diverse physiological and/or situational characteristics, which may include age [209], sex [210], body composition [211], ethnicity [212], genetics [18,213], metabolic disorders, cold-exposure [31,46,214,215] and food intake and composition [26,216–219] among others.

Brief comments should be included regarding the interpretation of either BMR or RMR values. The most important may be that either a low BMR or RMR is (in simple terms) an indicator of potential weight gain (or weight re-gain) in the future because it is important contribution on 24hEE [220]. Of note, although obese subjects have a higher RMR compared to their counterpart, i.e. normal weight subjects [221,222], this may be not totally true, as such differences are mainly explained because exists a direct (and positive) association between total body weight and resting EE [223]. Thus, for a proper identification of subjects presenting low resting EE values it is necessary to adjust for either lean or fat-free mass and fat mass, as the variance in BMR (or RMR) explained by these tissues may account for up to 60-70% [145,211,224,225]. A common correction is to 'relativize' or 'normalize' the BMR (or RMR) by the simply ratio between resting EE (expressed, for example, as kcal/day) and total body weight or lean (fat-free) mass (expressed as kg) [226]. However, as their association has not a zero intercept (e.g. see Figure 5 in [61]), such correction may induce misunderstandings [227,228]. For instance, comparisons between groups which present different body compositions, or after significantly body composition changes (e.g. after an exercise-based intervention), the regression-based methods are considered to be the best option for that 'normalization' process [229].

5.3. THERMIC EFFECT OF FOOD

The TEF (also called meal- or diet- induced thermogenesis [MIT or DIT respectively]), represents the increase in EE subsequent to the consumption of a meal [26] or the intravenous infusion of nutrients [22]. The TEF traditionally has been separated into two different components, the obligatory and the facultative (or regulatory) component [22,230–232]. The obligatory component is supposed to represent the minimum energy requirements to transform a nutrient into its primary storage form within the body [22]. Although the theoretical cost values of nutrients storage within the body have been known since many years ago [22], it was appreciated that the measured TEF always exceeded the theoretical cost of its storage [22,230–232]. This phenomenon was primarily called '*luxuskonsumption*' and later the 'facultative component' of the TEF response. Theoretically, this facultative response is thought to represent a kind of adaptive mechanism by which the body expended the excess of EI (i.e. caloric intake) trying to preserve the normal body weight [233–236].

In brief, TEF indicate the energy requirements for both, the processing and the digestion of the meal [26], process which may last for 8-10 hours [237,238] following the consumption of large meals (≥1000 kcal). Importantly, TEF is influenced by the size of the meal and the macronutrient composition [239]. In this regard, the TEF is often expressed as percentage of EI, by dividing the increase in EE subsequent to the consumption of the meal by the EI [226,239]. The contribution of TEF to 24hEE is commonly declared to account for ≈10% [27,28]. However, this value may differ between subjects or within subjects whose energy balance has changed. Thus, that percentage is close to the reality when healthy subjects are in (or close to) energy balance and consuming a mixed diet [61]. In whole-room calorimetry studies it is difficult to discern between increases in BMR (or RMR) produced by TEF or by the activity thermogenesis except if one of these components is minimized as much as possible by design. To overcome these problems three alternatives have been proposed, the first is to fast subjects for one day and feeding them on another day while the activity thermogenesis is reduced by limiting the physical activity [240–242]. The other method is to estimate TEF by using measurements of physical activity [240,241,243,244]. With the latter, the measured physical activity (*y*-axis) is regressed against the EE (*x*-axis) per unit of time (see Figure 4 in [61]). Therefore, the y-intercept represents the EE of the subject in an inactive status, and the difference between the y-intercept and the BMR (or RMR) represents the cumulative TEF. Nonetheless, this method has shown poor biological reproducibility when subjects have been measured more than once [241]. Lastly, the other feasible alternative is to assess TEF while the subject is laying on bed, and thus, there is neither physical activity nor movements influencing the assessment (at least in theory).

The biological reproducibility of TEF using metabolic carts (normally are mixing chamber systems equipped with a canopy) and expressed as $CV_{D\text{-}to\text{-}D}$, range from 15 to 33% [26,166,245–248]. Although this elevated $CV_{D\text{-}to\text{-}D}$ might be induced (at least in part) by the method used to calculate the TEF [26]. The TEF is usually calculated as the difference between the RMR (assessed immediately before the meal) and the total post-meal EE [26,243,249–254], for that reason, even if the TEF is (almost) identical from one day to another, the variability in the pre-meal RMR may negatively affect the calculated value for TEF. Trying to overcome that issue, previous studies used a 'fixed-RMR' value to calculate each consecutive TEF. However, the improvements in between days precision or biological reproducibility (expressed as $CV_{D\text{-}to\text{-}D}$), if existed, were minimal [26,245,247,255]. Thus, improvements in methodology to either measure TEF or to select (and analyze) TEF data when using metabolic carts are still needed.

5.4. COLD-INDUCED THERMOGENESIS

The CIT is a physiological process regulated by both, non- and shivering physiological mechanisms [29]. The shivering thermogenesis represents the EE necessary to sustain involuntary skeletal muscle contractions which are produced in response to cold stimuli [31]. Conversely, the non-shivering thermogenesis refers to the EE or consumed in other process different than muscle contractions [30] as for example the uncoupling respiration produced in brown adipocytes (in the mitochondria). Some interest rely on CIT as a possible 'clinical tool' to induce a negative energy balance during acute periods. In fact, previous studies have shown that shivering thermogenesis may produce increases in EE up to five times above the resting EE (i.e. RMR), however, the loss of motor coordination as well as the uncomfortable nature of shivering (and intense cold exposure) make shivering thermogenesis a unsuitable option [30]. Regarding the non-shivering thermogenesis, it has been previously showed that may induce moderate increases on EE (although there is large inter-individual differences; ranging from 0 to ≈40% above resting EE in young healthy adults) [31]. Importantly, both, shivering and non-shivering thermogenesis are supposed to occur concomitantly (even during light cold exposure) [256,257] and whether shivering or non-shivering thermogenesis has a greater impact may vary within-subjects [31,258,259].

Regarding the 'cold tolerance' (important aspect if cold exposure is going to be used as a clinical tool) there is exhibit also a great within-subjects heterogeneity. Thus, to observe the mechanisms and responses produced by mild (or moderate) cold exposure the cold stimuli had to be individualized depending on the subject's cold tolerance [31,260–262]. Thus, an assessment of the 'shivering threshold' (i.e. the lowest external temperature without inducing external, and therefore, observable or perceived muscle shivering) is necessary as a start or reference point to adjust and establish the individualized cold exposure temperature [31] – normally a few degrees above their shivering threshold (e.g. 4°C; [31,46,214,215]). Of note, this protocol (i.e. firstly determining the shivering threshold and then, individualized the cool exposure) has been used and accepted as an approach to induce CIT [46,214]. However, it is important to highlight that the shivering threshold approach may have a limitation that should be acknowledged, and is that the shivering threshold might not totally dismiss the shivering thermogenesis produced by the skeletal muscle (e.g. deep muscles which its contractions/shivering may not be 'visually' detected). In any case, the potential utilization of CIT as a clinical tool should be take into consideration.

6. GENERAL RECOMMENDATIONS FOR OBTAINING AND MANAGE GAS EXCHANGE DATA FROM RESTING METABOLIC RATE ASSESSMENTS

The assessment of the BMR or RMR using metabolic carts are normally performed during a 10–30 min period. Moreover, the combination of measured VO_2 and VCO_2 data using IC together with the measured values of excreted urinary N is the exclusive technique that enable accurate inferences of the utilization of diverse substrates [17,18]. Importantly, accurate and reproducible assessments of BMR/RMR in healthy (i.e. non-critically ill) as well as in intensive care unit subjects using IC is of vital importance for both, researchers and clinicians [2,3,22,60,113–115].

In 2006, The Academy of Nutrition and Dietetics Evidence Analysis Library published a systematic review [208] of best practices to assess RMR, aiming to help researchers and clinicians to select the best procedures to accurately assess RMR using IC. A few years later, in 2015, an update of these recommendations was published [21] and these recommendations are broadly used. Importantly, the latter recommendations are focused in 'healthy' and 'non-critically ill' subjects (defined as those subjects that do not have any dysfunction of one [or more] organs or systems requiring dependence on advanced instruments and/or therapy to survive) [21]. Thus, to identify the best practices for assess RMR in critically ill, others [263,264] but not this previous mentioned work should be consulted. Below, based on the work published by Fullmer et al. [21], a detailed description about the best practices for assessing RMR (in healthy subjects) using IC will be provide. Later, information and recommendations regarding the methods for gas exchange data selection (see **Section 6.1**) will be also detailed.

Resting period prior the RMR assessment (i.e. acclimation period): as most of assessments are not performed in an overnight metabolic unit (i.e. is a RMR assessment rather than a BMR, as was detailed in the **Section 5.2**), this 'acclimation period' is a vital step when conducting RMR assessments using IC [21]. Thus, once subjects arrive to the research center (or the clinic), it is important for the resting EE to return to baseline values (i.e. to resting conditions) before assessing the RMR. In adults, different studies [265–269] have evaluated this issue and have determined (or at least suggested) how much time may be needed prior the assessment (i.e. acclimation period). In one study [266], no differences were observed in mean RMR assessed after 30 minutes of acclimation period (the subjects slept at home) vs. the mean RMR assessed directly after slept at the overnight metabolic unit. Similar results were obtained in another study [265] assessing the RMR after 30 minutes of acclimation period. These results were in agreement with those obtained in a different study [268], in which they observed that after 10 minutes of walking, the mean RMR measured after 15 minutes of acclimation period were significantly higher than the obtained after a 30 minutes [268]. On the other hand, studies evaluating the effect of a shorter acclimation period have shown that 20 minutes may be enough, but importantly, if no movement is allowed [267]. Nonetheless, the EE continued to gradually decline during the subsequent period (i.e. the remaining acclimation period) and the RMR assessment itself. Similar results were found in a different study [269]. They observed that 20 minutes of acclimation were sufficient, as no mean RMR differences between measurements performed after awakening, being transported in a wheeled chair (to the measurement room) and allowing an acclimation period after light physical activity were found [269]. Therefore, in summary, if 30 minutes of acclimation period are not possible there is necessary at least 20 minutes (not permitting movements) prior the RMR assessment to ensure that the subject is in a 'resting state'. Of note, regarding children, no differences in RMR measured at minutes 10, 15, 20 or 25 of the RMR assessment were observed compared to the last 5 minutes of measurement (i.e. 30) [270]. That may suggest that in children, if an acclimation period prior the RMR assessment is not possible, the data obtained after the minute 10 can be considered as 'valid' as no differences were observed from this time period to the end [270].

Activities during the acclimation period (i.e. prior the RMR assessment): during this period, some studies have shown that activities as reading or listening to music, as well as laughing or coughing during a RMR assessment increase the resting EE [271,272]. Regarding physical activities during the acclimation period, in the systematic review performed by Fullmer et al. [21], they stated that no studies were found determining which type of activities (if there are any) may be performed. However, due to the aforementioned reasons, one might extrapolate that any kind of physical activity should be avoid during this acclimation period. Thus, based on literature, the recommendations to subjects would be to rest as quiet and calm as possible during both, the acclimation and the RMR assessment periods.

Steady State during the RMR assessment: Steady state (SS) has different definitions, however, in the present Doctoral Thesis the definition proposed by Fullmer et al. [21] in their recommendations will be followed. Such definition is the following [21]: '*a pre-determined criterion that defines a minimum variation in gas exchange variables from one minute to the next*'. Moreover, the SS is closely linked to its method for gas exchange data selection (i.e. the steady state time period [SSt]), as the criteria used to define the SS will establish the criteria for the SSt method. This fact will be extensively describe below (see **Section 6.1**). In brief, RMR data obtained before the achievement of the SS criterion are often discarded [21,208], as certain artifacts (e.g. non-metabolic variations on the gas exchange) may be introduced in the RMR assessment (even if all the recommendations have been strictly followed). Importantly, there is not published studies [21] determining the duration of a RMR assessment (to ensure accurate resting EE values) that is needed when a SS is not achieved in either healthy or non-critically ill individuals.

Body positioning during the RMR assessment: the body positioning and its impact in EE has been studied [273,274]. In fact, in a recent study [275] differences in mean RMR were found between standing vs. lying and sitting positions (being ≈10% higher for standing compared to the other positions), while, no differences were observed between sitting and lying. Importantly, all the participants were motionless during each positioning [275], as fidgeting (regardless the positioning) further increase the EE [276,277]. Another study [277] also found that mean RMR when sitting and standing were 3.7% and 13% higher than lying respectively (participants were also motionless in each positioning). Lastly, other studies have also shown similar results (although the differences are larger), in these studies [278,279] mean RMR while sitting was 11% higher than while lying, and mean RMR while sitting or standing was 6% and 14% greater than while lying respectively. In summary, and based on these studies, the RMR assessments should be performed while the subject is lying down [21], and fidgeting during the RMR assessments must be avoided.

Gas exchange collection systems for assessing the RMR: as aforementioned (see **Section 4.3.2**) different systems for gas exchange collection can be equipped to the metabolic cart and thus, used to assess the RMR. In this sense, the RMR parameters (i.e. VO_2, VCO_2, resting EE, etc.) may be influenced depending on the system equipped [130,131,190,191]. Nonetheless, some did not observed statistically significant differences in the assessed RMR (higher values when using a face-mask or a mouth-piece plus nose-clip system compared to a ventilated hood canopy system). Additional research is needed comparing the aforementioned gas exchange collection systems as not enough literature is still available [21]. Nonetheless, whenever possible, the ventilated hood canopy should be used to assess RMR rather than the other systems.

Time of the day for assessing the RMR: one study [164] have evaluated the effect of the time of the day (i.e. diurnal variation) on RMR assessed on 4 occasions, however, morning RMR vs. afternoon RMR were not assessed on the same day in all occasion (but were obtained within a two-week period). In their study [164], they observed that the within-day morning RMR (i.e. $CV_{D\text{-}to\text{-}D}$) was 4.5% while the afternoon was 2.8%. Regarding the differences between morning RMR assessments vs. the afternoon RMR assessments the difference, expressed as coefficient of variation (CV), was 4.6%. Such difference was of ≈110 kcal/day, being the RMR assessed during the afternoon higher than the RMR assessed during the morning [164]. Conversely, other two studies [192,280] also evaluated that diurnal variation on RMR but did not find significant differences. Importantly, in both studies [192,280] the subjects stayed at the research center (resting or avoiding physical activity during the whole day), which may somehow be biasing the results. Nevertheless, the common recommendation is to assess the RMR early in the morning [21], to overcome the possible influence of diurnal variation.

Room conditions for assessing the RMR: it is well known that as ambient temperature decreases the EE increases as was previously described in the CIT assessment (**Section 5.4**). In fact, has been previously determined that ambient room temperature directly influences the RMR assessments [268,281,282]. They observed differences between the RMR assessed in a room at an ambient temperature of ≤20°C compared to a room at ≈22-25°C – being the RMR assessed at the room at lower ambient temperature higher. Importantly, the use of a blanket to cover the subjects minimized the increase in the assessed RMR [268,281]. However, no studies [21] have determined the influence of ambient room temperature higher than 25°C on RMR. Furthermore, studies addressing other ambient room conditions (e.g. lighting, noise and humidity) that might be required for accurate RMR assessments have not been performed yet [21]. In any case, the usual recommendations are to assess the RMR in a controlled ambient temperature room (22-25°C), with dim lighting, as quiet as possible, and covering the subject with a blanket during the entire assessment [21].

Fasting period prior to assess the RMR: as was described in the TEF assessment (**Section 5.3**), there is exists a period of time (depending on the composition of the meal and/or the EI) in which EE is increased over the RMR as a product of such TEF process itself. Thus, if RMR assessments are carried-out while the TEF process is 'active', the obtained RMR values will be influenced. Different studies have used different meals, compositions, and duration of the IC assessment to determine the 'end' of such increase in EE derived by the TEF [219,280,283–287]. However, most of studies did not measure the total TEF response, as the assessment period was not long enough to determine when the RMR returned to baseline values. However, based on these studies as well as in the current recommendations [21] the minimum fasting time would be 7 hours (if EI ≈1300 kcal); nonetheless, others authors recommend at least 10-12 hours [61].

Caffeine and other stimulants prior to assess the RMR: although it is known that caffeine as well as other stimulants increase the RMR, the duration and the magnitude of the increase is not totally clear yet [21], as the effects are mainly product- and/or dose-dependent. In this regard, the influence of caffeine (50 mg) increasing the RMR (≈6%) remained for at least 4 hours [288–290], however, the concrete duration of such effect is unclear as any study assesses RMR beyond this 4 hour time period [21]. In agreement with these results, doses of 5 mg of caffeine per kilogram of fat-free mass increased the RMR ≈7-15% [288]. On the other hand, results regarding tea consumption as well as other stimulants remained unclear. While some studies did not observe influence of green tea on RMR, one found an effect of oolong tea (increasing RMR) during more than 2 hours [289,291]. Lastly, products with mixed stimulant (e.g. herbal supplement) composition [291–293] showed an increase on RMR lasting at least 4 hours (time in which the measurement finished). Taking all aforementioned evidence together, the RMR still elevated for a minimum of 4 hours after the stimulant ingestion. Therefore, the current recommendations are to assess the RMR at least 4 hours after a stimulant ingestion [21]; nonetheless, other authors recommend at least 10-12 hours [61].

Nicotine consumption prior to the RMR assessment: nicotine consumption through cigarette smoking has been tested to verify its effect on RMR assessments [21]. A non-randomized crossover study [294] compared the 'baseline' assessed RMR and the effect of low smoking (0.8 mg of nicotine ≈ 1 cigarette) and high smoking (1.74 mg of nicotine ≈2-3 cigarettes) on RMR. At 140 minutes after the low smoke condition, the RMR was increased about 5.2%, while at the same time, for the high smoke condition the RMR was increased about 9.3% [294]. Unfortunately, the time to recover baseline RMR values after smoking was not assessed. Thus, whether the RMR continues elevated more than 140 minutes remained unknown as no others studies have been found studying this issue [21]. Moreover, the effect of other devices

used to deliver nicotine (e.g. electronic cigarettes, patches, chewing, etc.) on RMR is also unknown [21]. Thus, the recommendations done by Fullmer et al. [21] is to avoid nicotine consumption for at least 2.5 hours before the RMR assessment.

Effects of exercise on the RMR assessment and related parameters: firstly it is important to mention that most of studies are focused on determine the increase of EE as excess post-exercise oxygen consumption (i.e. EPOC; which is defined as the increased VO_2 after the ending of exercise) rather than RMR [21]. The EPOC process may be divided into two different phases. The first (acute or short-term EPOC) is relatively rapid and is represented by the increase in VO_2 after finishing the exercise, which usually last for 10 to 90 minutes (depending on the intensity, duration, and type of exercise among others) [295]. The second is the long-term (or slow) EPOC, which can remain even up to 48 hours after the cessation of the exercise [296]. Thus, caution should be taken if moderate-vigorous exercise is performed prior the RMR assessment. In this regard, the recommendations are to avoid moderate intensity exercise (regardless if the exercise is focused on resistance or endurance exercise) 24 hours before the assessment, and 48 hours for the vigorous intensity exercise [297,298].

Effect of a 'post-calorimetric correction procedure' after the RMR assessment: although this post-calorimetric correction is not included in the aforementioned guidelines [21] nor is commonly used yet, it should be considered. In brief, this procedure proposed by Schadewaldt et al. [161], is based on the infusion of pure gases to improve the 'quality' of the RMR assessment. Concretely, using this procedure the accuracy and precision, as well as the comparability between metabolic carts is enhanced. Using high-precision mass-flow controllers (immediately after the subject's RMR assessment) a continuous infusion of pure N_2 and CO_2 gases is performed to simulating the subject's VO_2 and VCO_2 readouts. Therefore, the subject´s VO_2 and VCO_2 can then be corrected by 'using' the measured metabolic cart error (i.e. the difference between the infused gases [expected values] and the readouts of the metabolic cart [measured values]) [161,198,199]. This procedure has shown improvements in both, the RMR and RER assessed by two well-known metabolic carts– the Deltatrac and the Vmax Encore 29n [161]. However, the information available is scarce. Conversely, other authors [299] have proposed that this post-calorimetric correction procedure may not be as useful as suggested, arguments that are based on several reasons: firstly, using this procedure one is assuming that such post-test infusion is representative of the entire RMR assessment (or post-prandial assessment as in the study of Galgani et al. [199]) and test conditions. Although this fact might be true for short assessments (e.g. ≈30 minutes or 1 hour), may be not for assessments lasting a few hours (e.g. postprandial studies) [299]. Secondly, when infusing N_2 and CO_2 gases through the hose tube, the system is measuring almost steady-state amounts of VO_2 and VCO_2 [299], but during a 'real' RMR assessment (even with a canopy attached to a mixing chamber system) both FeO_2 and $FeCO_2$ will vary along with the respiratory cycle [299]. Lastly, even though all manufacturers supply information regarding the response times of their gas analyzers systems, the response times of O_2 and CO_2 analyzers not always are the same [113,299]. Thus, different response times of the gas analyzers may introduce a non-detectable error, as this infusion produced 'steady-state' simulations (i.e. non-variable gas exchange). Regardless the aforementioned limitations of the post-calorimetric correction procedure, it is much better to use it than have no objection, and strongly believe, that the instrument's data are the 'absolute truth'.

In summary, both, the referred guidelines [21] and the aforementioned evidence and studies presented are supposed to allow (or facilitate) researchers and/or clinicians to obtain accurate RMR assessments. Nonetheless, after obtaining the RMR data, another process is necessary to select those values that would be representative of the whole RMR assessment. The process, as well as the most common methods used in literature for gas exchange data selection are presented below (**Section 6.1**).

6.1. METHODS FOR GAS EXCHANGE DATA SELECTION

Once the RMR assessment has finished, the resulting (i.e. measured) VO_2 and VCO_2 data need to be processed and selected to estimate, for example, the RMR (i.e. resting EE), the RER (or RQ) and/or the substrate oxidation (i.e. FATox and CHOox). In this sense, from the entire VO_2 and VCO_2 record, it is broadly assumed that the first 5 minutes of assessment should be discarded [21,300], for avoiding possible technical and biological artifacts (e.g. non-metabolic variation in the gas exchange). Importantly, excluding from further analysis this period (i.e. the first 5 minutes) is a common practice [21] independently of the method for gas exchange data selection used. Therefore, the remaining VO_2 and VCO_2 data (e.g. 25 minutes if the RMR assessment lasted 30 minutes) will be further select depending on the 'approach' or method for gas exchange data selection used. For illustrative purposes, and because is supposed to be the normal practice [21] for the following explanations the duration of the entire assessment will be consider to be 30 minutes. Lastly, as the different metabolic carts allow to export the data at different time frequencies (e.g. every 5 seconds, every 10 seconds, 1 minute, etc.), in our examples presented below the data frequency will be establish at 30 seconds.

Regardless the type of assessment performed (e.g. BMR or RMR), three different approaches may be followed for most of IC assessments using metabolic carts. These approaches are: (a) the aforementioned SSt (i.e. *steady state time methods*), (b) the *time interval methods* (TI) and (c) the *filtering methods*. Importantly, both SSt and TI methods for gas exchange data selection may apply to different time lengths; while in the other hand, filtering methods may be divided regarding their 'intensity'. Examples of the aforementioned methods are presented below. But before it should be noted that the filtering methods are 'relatively new', and thus, they have not been widely used yet.

In general, although little differences can be found among studies [21,189,301–306], the aforementioned methods may be classified on the following '*sub-methods*' and/or conditions:

SSt: the SSt methods are often determined by achieving ≤10% CV for a specified interval of time in one or more of the following parameters [21,208]: VO_2, VCO_2, minute ventilation (VE), and/or RER (normally ≤5% CV). Thus, the obtained data that not achieve these criteria or thresholds are discarded from further steps and analysis [21,208]. Importantly, the SSt may vary by (a) the used time lengths (e.g. from 3-min to 10-min periods [3 min SSt and 10 min SSt respectively]), (b) the CV thresholds (ranging from ≤5% to 10%), and (c) the combination of the gas exchange variables (e.g. VO_2, VCO_2 and RER [301,302,304,306] vs. VO_2, VCO_2, RER and VE [189]).

Short TI: following this approach the data is averaged as a fixed intervals of 5 minutes, as for example: *6–10 min* (i.e. data is averaged from the 6th to the 10th minute), *11–15 min*, *16–20 min*, *21–25 min*, and *26–30 min* [189,301–304,306].

Long TI: the data is averaged including more minutes than in the Short TI approach. Examples of the Long TI approach are *6–20 min* (i.e. data is averaged from the 6th to the 20th minute), *6–15 min*, *6–25 min*, *6–30 min,* and other different combinations [189,301,302,304,306].

Filtering: following this approach certain thresholds are established depending on the mean RMR value (i.e. resting EE) estimated. In brief, the range of the threshold will determine the 'intensity' of the filter applied (e.g. discarding either those values <85% or >115% of the mean RMR). Example of the filtering approach are the *low filter*, the *medium filter* or the *strong filter*.

Below, a detailed description, as well as illustrative examples (using real data), of the different methods for gas exchange data selection (i.e. SSt, TIs and filtering) are presented.

Firstly, with reference to the SSt, after discarding the first 5 minutes of measurement [21,300], a period considered to be markedly stable is selected for estimating EE as was aforementioned [21,208]. To determine such stability the selection of the SSt is based on the variability (expressed as CV) of certain parameters which may vary between studies [21]. Moreover, while some authors have proposed that SSt should be represented by the period presenting the lowest CV (mean of the CV for VO_2, VCO_2, RER and VE) trying to ensure the 'best' gas exchange stability [189], others have defined the SSt as the first 5 minutes period in which the SSt criteria (CV for VO_2 and VCO_2 was <10%) is achieved [301–304,306]. The belief that SSt methods for gas exchange data selection provides an accurate estimation of RMR emerge from studies mostly performed in hospitalized and/or ventilated patients [301]. However, there is not strong evidence yet that the same assumption can be followed in healthy spontaneously breathing subjects [189].

For explanatory purposes, the criteria established in the Sanchez-Delgado et al. [189] work will be followed to define the SSt criteria. Thus, in first place, for each period of 3, 4, 5 and 10 consecutive minutes the CVs of VO_2, VCO_2 and RER were calculated as well as the *mean CVs* variable (i.e. an average of all the aforementioned CVs) for each period examined. For example, for the 4 min SSt, every consecutive period of 4 minutes is examined (the 6th to 9th, the 7th to 10th, etc.). Then, the period selected as '*the SSt*' (for each condition; i.e. 3, 4, 5 and 10 min SSt) is this presenting the lowest mean CVs (e.g. for the 4 min SSt, the selected one is the 6th to 9th period) [189]. Finally, with these processed data (i.e. VO_2 and VCO_2 data) the equations presented in the **Table 3** can be used and thus, EE, RER, and substrate oxidation estimated. An example of the aforementioned process for selecting representative gas exchange data using the SSt method is presented in **Figure 15**.

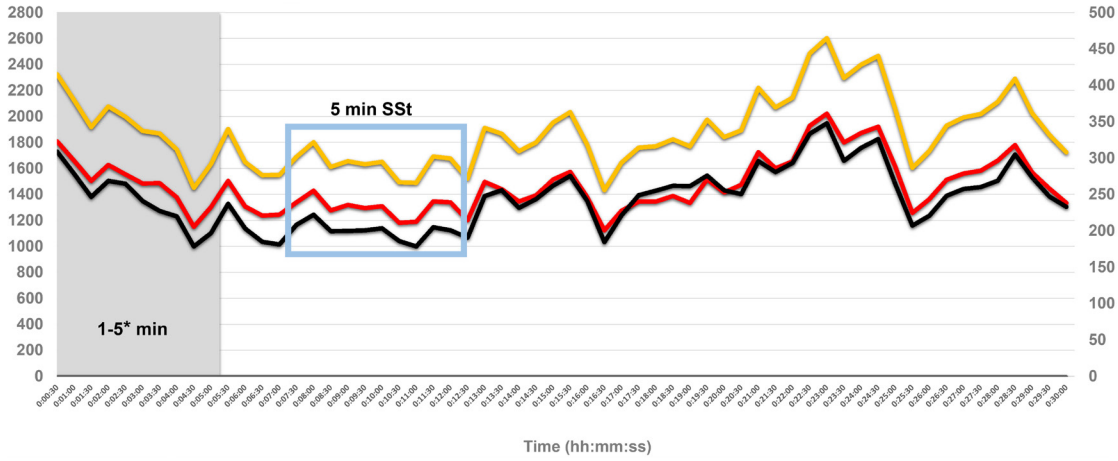


Figure 15. Example of gas exchange data selection using the Steady-State time (SSt) method defined as proposed by Sanchez-Delgado et al. [189]. *Only the 5 min SSt method has been illustrated, although the process is similar for the rest of SSt methods (i.e. 3, 4 and 10 minutes SSt methods). Continuous yellow line represents the 'raw' resting metabolic rate (RMR; in kcal/day), continuous red line represents the 'raw' oxygen consumption (in milliliters per minute) and black line represents the 'raw' carbon dioxide production (in milliliters per minute). The data is from a real subject (a healthy male, 22 years old, 173 centimeters, and 83.5 kilograms) assessed using the Omnical metabolic cart (Maastricht Instruments, Maastricht, The Netherlands). Light gray columns represents the first 5 minutes of gas exchange data which are normally excluded* [21]. *Blue box represents the selected 5 minutes of gas exchange data, which will be further processed (e.g. to calculate substrate oxidation) using the 5 minutes SSt method.*

In the TI methods, a pre-established fixed TI period is selected, and normally its stability (expressed as CV) is not considered [189,301–304,306]. As aforementioned, in BMR or RMR assessments, the TI methods may be divided into short TI (normally consecutive periods of 5 minutes) or long TI [189,301,302,304,306]. The duration of the long TI may vary across studies, for example long TI from *6–15 min* to *6–45 min* [302]. It should be noted that in certain works (e.g. [303]), although is not broadly extended, the stability of the measurement (expressed as CV) is tested after calculating a TI period, thus, determining if such TI achieved the SS criteria (in the aforementioned example, i.e. [303], defined as CV for VO_2 and VCO_2 ≤10% over 5 consecutive minutes). Finally, for explanatory purposes (as occurred previously) the different short TI and long TI proposed in the Sanchez-Delgado et al. [189] work will be used and are presented in **Figure 16**.

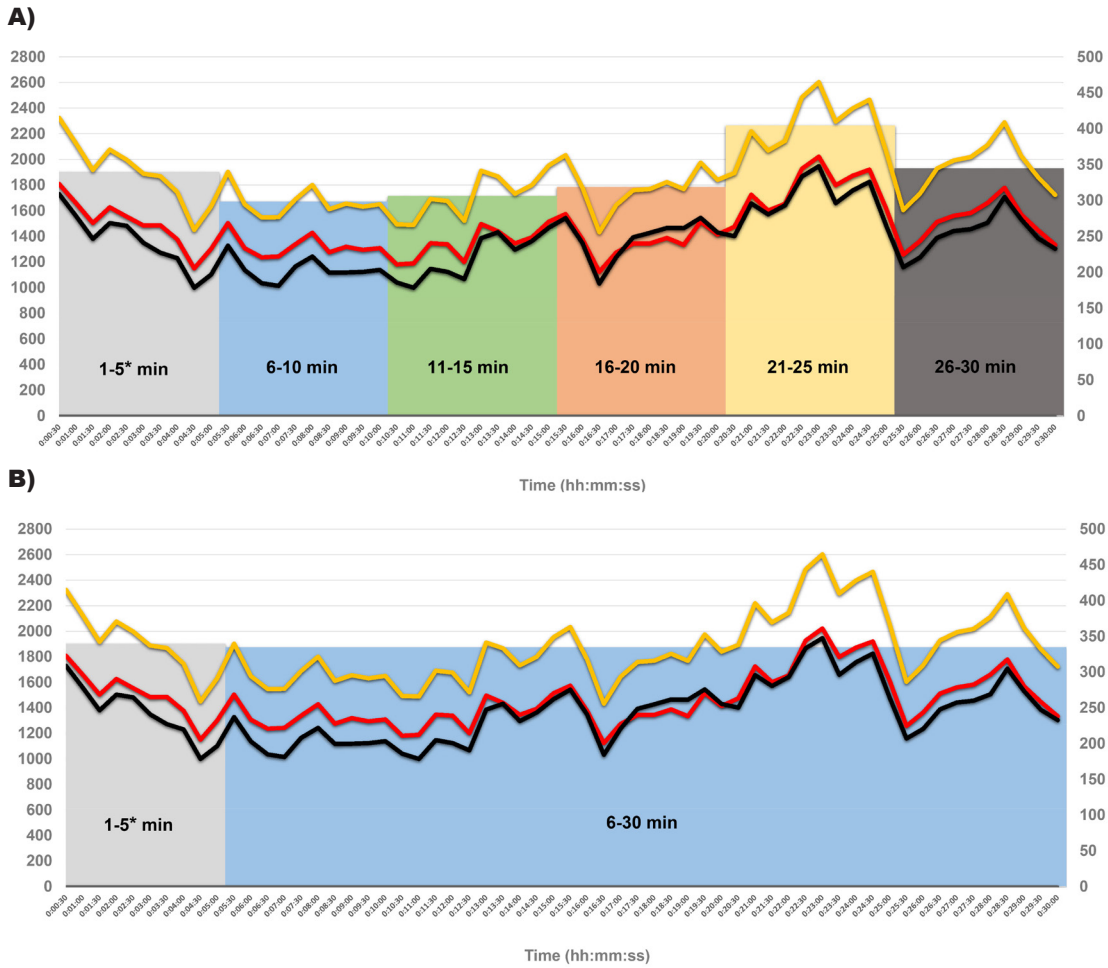


Figure 16. Examples of gas exchange data selection using both the short Time Interval (Short TIs; Panel A) and the long Time Interval (Long TIs; Panel B) methods as proposed by Sanchez-Delgado et al. [189]. *The short TIs of 6–10 min (i.e. data is averaged from the 6th to the 10th minute), 11–15 min, 16–20 min, 21–25 min, and 26–30 min have been represented in Panel A. Only the 6–30 min long TIs has been represented (i.e. the 6–25 min has not been included, although the rationale is the same) in Panel B. Continuous yellow line represents the 'raw' resting metabolic rate (RMR; in kcal/day), continuous red line represents the 'raw' oxygen consumption (in milliliters per minute) and black line represents the 'raw' carbon dioxide production (in milliliters per minute). The data is from a real subject (a healthy male, 22 years old, 173 centimeters, and 83.5 kilograms) assessed using the Omnical metabolic cart (Maastricht Instruments, Maastricht, The Netherlands). Light gray columns represents the first 5 minutes of gas exchange data which are normally excluded [21]. Blue column represents the 5 minutes of gas exchange data using the 6–10 min short TI method in Panel A and the long TI method (6-30 min) in Panel B. Green column represents the 11–15 min short TI method. Orange column represents the 16–20 min short TI method. Yellow column represents the 21–25 min short TI method. Dark gray column represents the 26–30 min short TI method.*

The filtering methods could be considered as a method between the SSt and the TI methods. When using the filtering methods, the data points exhibiting either higher or lower values than a given threshold are discarded. Such thresholds are normally established as the mean RMR value (i.e. resting EE) ± *X*% of the mean, where *X* is represented by the 'intensity' of the filter applied. In this regard, the thresholds for the *low filter* are <85% or >115% of the mean RMR, <90% or >110% of the mean RMR for the *medium filter*, and <95 or >105% for the *strong filter*. For exemplifying this method a hypothetical 30 minutes RMR assessment has been chosen [21]. The first step consists on discarding the first 5 minutes as mentioned [21,300], and then the remaining VO_2 and VCO_2 values (i.e. 25 minutes) are used to calculate the $mean_{25\text{-}min}$ RMR. Secondly, the resting EE for each time period (of the remaining 25 minutes) is averaged at 1 minute intervals and also estimated (e.g. RMR for the 6th minute, for the 7th minute, etc.; *per minute RMR*). Then, those *per minute RMR* data exhibiting resting EE values <85% or >115% than the $mean_{25\text{-}min}$ RMR are discarded (i.e. *low filter*); those *per minute RMR* values <90% or >110% than the $mean_{25\text{-}min}$ RMR (i.e. medium filter); or those *per minute RMR* values <95% or >105% than the $mean_{25\text{-}min}$ RMR (i.e. strong filter). Lastly, the mean EE is calculated only including the *per minute RMR* data that pass these filtering thresholds. An example of the process for selecting representative gas exchange data using the filtering methods is presented in **Figure 17**.

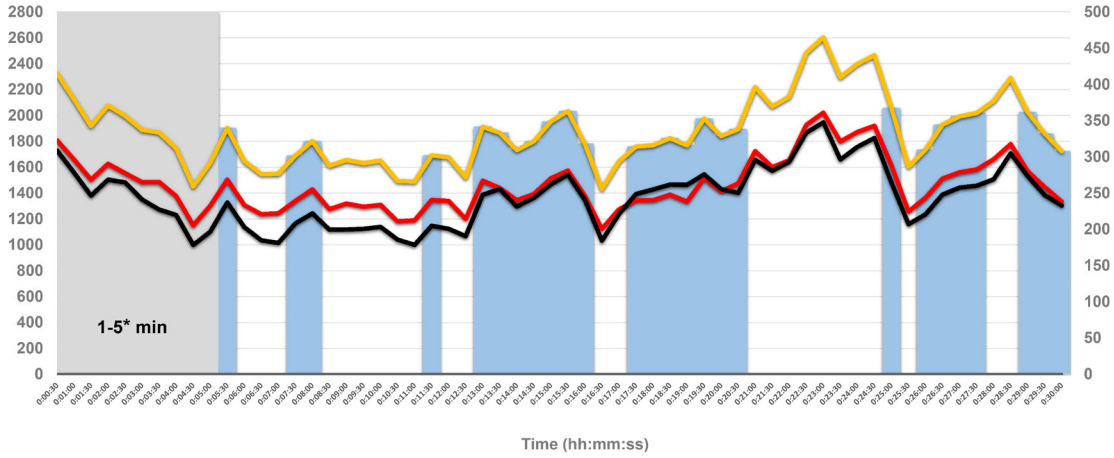


Figure 17. Example of gas exchange data selection using the filtering method. *Only the medium filter method has been illustrated, although the process is similar for the other filtering methods (i.e. low and strong filters). Continuous yellow line represents the 'raw' resting metabolic rate (RMR; in kcal/dayay), continuous red line represents the 'raw' oxygen consumption (in milliliters per minute) and black line represents the 'raw' carbon dioxide production (in milliliters per minute). The data is from a real subject (a healthy male, 22 years old, 173 centimeters, and 83.5 kilograms) assessed using the Omnical metabolic cart (Maastricht Instruments, Maastricht, The Netherlands). Light gray columns represents the first 5 minutes of gas exchange data which are normally excluded [21]. Medium filter thresholds used were those per minute RMR values <90% or >110% than the mean (of 25 minutes) RMR. Blue columns represents the periods of gas exchange data that pass these filtering thresholds and will be further processed (e.g. to calculate substrate oxidation).*

As stated, the SSt and TI methods can be used under different time lengths and periods, while the data yielded by the filtering methods may vary (e.g. from 1 minute of data to 25 minutes; assuming that the measurement last 30 minutes), as it directly depends on accomplish the aforementioned threshold criteria (criteria that may vary depending on the 'intensity' of the filter). The use of different methods may result in different resting EE (i.e. RMR) and RER estimations (as well as nutrient oxidation rates) being made [189,301,302]. In this regard, RMR estimates based on the SSt method are normally lower than those yielded by the TI method (regardless its duration) [189,302]; although, achieving a SSt is not always

feasible [21,301,302]. Given the definition of RMR (i.e. the necessary EE for maintaining vital functions and whole-body homeostasis on an awake person), it has been suggested that the lowest RMR estimates yielded by the SSt method may be more accurate [301,302] and reproducible [189] than those provided by the TI methods. However, the underestimation of the 'real' RMR cannot be dismissed [189]. Further, there is no consensus yet, neither for TI nor for SSt methods, about the time length for data selection [21].

Achieving a high day-to-day biological reproducibility (i.e. reducing inter-day differences) is fundamental to, for example, detect the relatively small changes in resting EE (i.e. RMR or BMR) after an intervention [159]. Furthermore, although resting EE estimation is mainly (but not entirely) determined by VO_2 [93], the RER is essential for inferring nutrient oxidation rates [99]. Therefore, accomplishing a high RER day-to-day biological reproducibility is also vital for a method for gas exchange data selection to be able to also detect changes in substrate oxidation while resting. However, just a few studies have examined the impact of different methods (using TI and SSt) for gas exchange data selection on both day-to-day RMR (or BMR) and RER biological reproducibility [189]. Importantly, whether the filtering methods yielded either better estimations or better day-to-day RMR (or BMR) and RER biological reproducibility remains unknown, as to best of the author's knowledge, no previous study regarding this issue has been published yet.

REFERENCES

1. Kenny, G.P.; Notley, S.R.; Gagnon, D. Direct calorimetry: a brief historical review of its use in the study of human metabolism and thermoregulation. *Eur. J. Appl. Physiol.* **2017**, *117*, 1765–1785.
2. McLean, J.; Tobin, G. *Animal and human calorimetry*; 1st editio.; Cambridge University Press: Cambridge, 1987;
3. Schoffelen, P.F.M. *Measurement of human energy expenditure: biological variability and technical validity*; Datawyse / Universitaire Pers Maastricht: Maastricht, 2017;
4. Lavoisier, A.; Laplace, P.S. Memoire sur la chaleur. In *Histoire de l'Academie Royale des Sciences (Paris), Anne 1780, avec les Memoires de Mathematique et Physique*; Paris, 1780; pp. 355–408.
5. Santorio, S. *Ars ... de statica medicina, aphorismorum sectionibus septem comprehensa.*; Apud Nicolaum Polum: Venetiis, 1614;
6. Lavoisier, M. Experiences sur la respiration des animaux, et sur les changemens qui arrivent a l'air en passant par leur poumon. In *Memories de l'Academie des Sciences de Paris*; Paris, 1777; pp. 185–194.
7. Zuntz, N.; Schumburg, W. *Studien zu einer Physiologie des Marsches*; Bibliothek von Coler Bd. 6: Berlin, 1901;
8. Atwater, W.O.; Benedict, F.G. A respiration calorimeter with appliances for the direct determination of oxygen. *Caregie Inst. Washington, Publ. No.* **1905**, *42*.
9. Hill, A.; Hill, A. A self-recording calorimeter for large animals. *J. Physiol.* **1914**, xiii–xiv.
10. Haldane, J. A new form of Apparatus for measuring the Respiratory Exchange of Animals. *J. Physiol.* **1892**, *13*, 419–430.
11. Zuntz, N.; Lehmann, C. Remarks on the Chemistry of respiration in the Horse during Rest and Work. *J. Physiol.* **1890**, *11*, 396–398.
12. Frayn, K. *Metabolic regulation: a human perspective*; Wiley-Blackwell: Oxford, 2010;
13. Westerterp, K. *Energy balance in motion*; Springer: New York, NY/Dordrecht/London, 2013;
14. Thaylor, B.N.; Thompson, A. *Nist Special Publication 330 2008 Edition*; National Institute of Standards and Technology: Gaithersburg, 2008;
15. FAO-WHO expert committee Energy and protein requirements: report of a joint FAO-WHO ad hoc expert committee. Rome, 22 March - 2 April 1971. *World Heal. Organ. - Tech. Rep. Ser.* **1973**, *522*, 1–118.
16. Haines, P.; Heal, G.; Laye, P.; Price, D.; Warrington, S.; Willson, R. *Principles of thermal analysis and calorimetry*; Haines, P.J., Ed.; The Royal Society of Chemistry: Cambridge, 2002;
17. Lam, Y.Y.; Ravussin, E. Indirect calorimetry: An indispensable tool to understand and predict obesity. *Eur. J. Clin. Nutr.* **2017**, *71*, 318–322.
18. Lam, Y.Y.; Ravussin, E. Analysis of energy metabolism in humans: A review of methodologies. *Mol. Metab.* **2016**, *5*, 1057–1071.
19. Swinburn, B.; Ravussin, E.; Saris, W.H.M.; Sauer, P. Energy balance or fat balance? In Proceedings of the American Journal of Clinical Nutrition; Am J Clin Nutr, 1993; Vol. 57.
20. Schwartz, M.W.; Seeley, R.J.; Zeltser, L.M.; Drewnowski, A.; Ravussin, E.; Redman, L.M.; Leibel, R.L. Obesity pathogenesis: An endocrine society scientific statement. *Endocr. Rev.* **2017**, *38*, 267–296.
21. Fullmer, S.; Benson-Davies, S.; Earthman, C.P.; Frankenfield, D.C.; Gradwell, E.; Lee, P.S.P.; Piemonte, T.; Trabulsi, J. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J. Acad. Nutr. Diet.* **2015**, *115*, 1417-1446. e2.
22. Simonson, D.C.; DeFronzo, R.A. Indirect calorimetry: methodological and interpretative problems. *Am. J. Physiol. Metab.* **1990**, *258*, E399–E412.
23. Raben, A.; Christensen, N.J.; Madsen, J.; Holst, J.J.; Astrup, A. Decreased postprandial thermogenesis and fat oxidation but increased fullness after a high-fiber meal compared with a low-fiber meal. *Am. J. Clin. Nutr.* **1994**, *59*, 1386–1394.
24. Jones, P.P.; Van Pelt, R.E.; Johnson, D.G.; Seals, D.R. Role of sympathetic neural activation in age- and habitual exercise-related differences in the thermic effect of food. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 5138–5144.
25. van Baak, M.A. Meal-induced activation of the sympathetic nervous system and its cardiovascular and thermogenic effects in man. *Physiol. Behav.* **2008**, *94*, 178–186.
26. Ruddick-Collins, L.C.; King, N.A.; Byrne, N.M.; Wood, R.E. Methodological considerations for meal-induced thermogenesis: measurement duration and reproducibility. *Br. J. Nutr.* **2013**, *110*, 1978–1986.
27. Scott, C.B.; Devore, R. Diet-induced thermogenesis: Variations among three isocaloric meal-replacement shakes. *Nutrition* **2005**, *21*, 874–877.
28. Donahoo, W.T.; Levine, J.A.; Melanson, E.L. Variability in energy expenditure and its components. *Curr. Opin. Clin. Nutr. Metab. Care* **2004**, *7*, 599–605.
29. Blondin, D.P.; Tingelstad, H.C.; Mantha, O.L.; Gosselin, C.; Haman, F. Maintaining thermogenesis in cold exposed humans: Relying on multiple metabolic pathways. *Compr. Physiol.* **2014**, *4*, 1383–1402.
30. van Marken Lichtenbelt, W.D.; Kingma, B.; van der Lans, A.; Schellen, L. Cold exposure - an approach to increasing energy expenditure in humans. *Trends Endocrinol. Metab.* 2014, *25*, 165–167.

31. Sanchez-Delgado, G.; Alcantara, J.M.A.; Acosta, F.M.; Martinez-Tellez, B.; Amaro-Gahete, F.J.; Merchan-Ramirez, E.; Löf, M.; Labayen, I.; Ravussin, E.; Ruiz, J.R. Energy Expenditure and Macronutrient Oxidation in Response to an Individualized Nonshivering Cooling Protocol. *Obesity* **2020**, *00*, 1–9.
32. Pontzer, H.; Durazo-arvizu, R.; Dugas, L.; Plange-rhule, J.; Forrester, T.E.; Lambert, E. V; Cooper, R.S.; Dale, A.; Luke, A. Constrained Total Energy Expenditure and Metabolic Adaptation to Physical Activity in Adult Humans. *Curr. Biol.* **2016**, *26*, 410–417.
33. Thurber, C.; Dugas, L.R.; Ocobock, C.; Carlson, B.; Speakman, J.R.; Pontzer, H. Extreme events reveal an alimentary limit on sustained maximal human energy expenditure. *Sci. Adv.* **2019**, *5*.
34. Aristotle, O.W. *Aristotle on youth & old age, life & death and respiration: translated, with introduction and notes*; Longman's, Green and Co.: London, 1897;
35. Priestley, J. *Experiments and observations on different kinds of air, by Joseph Priestley*; J. Johnson: London, 1774;
36. Grimaux, E. *Lavoisier, 1743-1794: d'après sa correspondance, ses manuscrits, ses papiers de famille et d'autres documents inédits*; F. Alcan: Paris, 1888;
37. Webb, P. *Human Calorimeters*; Greenwood Publishing Group, 1985;
38. West, J.B. The collaboration of Antoine and Marie-Anne Lavoisier and the first measurements of human oxygen consumption. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2013**, *305*.
39. Lavoisier, A. *Traite elementaire de chimie*; Paris, 1789;
40. Mendelsohn, E. *Heat and life*; Harvard University Press: Cambridge, 1964;
41. Blaxter, K.L. Adair Crawford and Calorimetry. *Proc. Nutr. Soc.* **1978**, *37*, 1–3.
42. Cypess, A.M.; Lehman, S.; Williams, G.; Tal, I.; Rodman, D.; Goldfine, A.B.; Kuo, F.C.; Palmer, E.L.; Tseng, Y.H.; Doria, A.; et al. Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* **2009**, *360*, 1509–1517.
43. Sturkenboom, M.G.G.; Franssen, E.J.F.; Berkhof, J.; Hoekstra, O.S. Physiological uptake of [f] fluorodeoxyglucose in the neck and upper chest region: are there predictive characteristics? *Nucl. Med. Commun.* **2004**, *25*, 1109–1111.
44. Wang, Q.; Zhang, M.; Xu, M.; Gu, W.; Xi, Y.; Qi, L.; Li, B.; Wang, W. Brown adipose tissue activation is inversely related to central obesity and metabolic parameters in adult human. *PLoS One* **2015**, *10*.
45. Yoneshiro, T.; Aita, S.; Matsushita, M.; Okamatsu-Ogura, Y.; Kameya, T.; Kawai, Y.; Miyagawa, M.; Tsujisaki, M.; Saito, M. Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity* **2011**, *19*, 1755–1760.
46. Martinez-Tellez, B.; Sanchez-Delgado, G.; Garcia-Rivero, Y.; Alcantara, J.M.A.; Martinez-Avila, W.D.; Muñoz-Hernandez, M. V.; Olza, J.; Boon, M.R.; Rensen, P.C.N.; Llamas-Elvira, J.M.; et al. A new personalized cooling protocol to activate brown adipose tissue in young adults. *Front. Physiol.* **2017**, *8*.
47. Bakker, L.E.H.; Boon, M.R.; van der Linden, R.A.D.; Arias-Bouda, L.P.; van Klinken, J.B.; Smit, F.; Verberne, H.J.; Jukema, J.W.; Tamsma, J.T.; Havekes, L.M.; et al. Brown adipose tissue volume in healthy lean south Asian adults compared with white Caucasians: A prospective, case-controlled observational study. *Lancet Diabetes Endocrinol.* **2014**, *2*, 210–217.
48. Saito, M.; Okamatsu-Ogura, Y.; Matsushita, M.; Watanabe, K.; Yoneshiro, T.; Nio-Kobayashi, J.; Iwanaga, T.; Miyagawa, M.; Kameya, T.; Nakada, K.; et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: Effects of cold exposure and adiposity. *Diabetes* **2009**, *58*, 1526–1531.
49. Cohade, C.; Mourtzikos, K.A.; Wahl, R.L. "USA-Fat": prevalence is related to ambient outdoor temperature-evaluation with 18F-FDG PET/CT. *J Nucl Med* **2003**, *44*, 1267–1270.
50. Despretz, C. La cause de la chaleur animale. *J. Physiol. Exp. Pathol.* **1824**, *4*, 143–159.
51. Dulong, P.R. Memoire sur la chaleur animale. *Ann. Chim. Physics, 3e série* **1841**, *1*, 440–455.
52. Lefèvre, J. *Chaleur Animal et Bioenergetique*; Masson et Cie.: Paris, 1911;
53. Regnault, V.; Reiset, J. Recherches chimiques sur la respiration des animaux des diverses classes. *Ann. Chim. Physics, 3e série* **1849**, *26*, 299–519.
54. Pettenkofer, M. Ueber die respiration. *Ann. der Chemie und Pharm.* **1862**, *123*, 1–52.
55. Pettenkofer, M.; Voit, C. Untersuchungen über den stoffverbrauch des normalen menchen. *Z. Biol.* **1866**, *2*, 478–573.
56. Rubner, M. Die quelle der thierschen warme. *Z. Biol.* **1894**, *30*, 73–142.
57. d'Arsonval, M.A. Recherches de calorimetrie. *J. l'Anatomie la Physiol.* **1886**, *22*, 113–161.
58. Atwater, W.O.; Rosa, E.B. Description of a new respiration calorimeter and experiments on the conservation of energy in the human body. *Unites States Dep. Agric. Off. Exp. Station. Bull.* **1899**, *63*.
59. Gorup-Besanez, E. *Lehrbuch der Chemie vol 3. Lehrbuch der Physiologischen Chemie*; Friedrich Vieweg und Sohn: Braunschweig, 1867;
60. Schoffelen, P.F.M.; Plasqui, G. Classical experiments in whole-body metabolism: open-circuit respirometry—diluted flow chamber, hood,

or facemask systems. *Eur. J. Appl. Physiol.* **2017**.
61. Chen, K.Y.; Smith, S.; Ravussin, E.; Krakoff, J.; Plasqui, G.; Tanaka, S.; Murgatroyd, P.; Brychta, R.; Bock, C.; Carnero, E.; et al. Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0): A Guide to Conducting and Reporting Human Whole-Room Calorimeter Studies. *Obesity* **2020**, *28*, 1613–1625.
62. Laulanié, F. De la marche des alterations de l'air dans l'asphyxie en vase clos. *Arch. Physiol. Norm. Pathol.* **1894**, *26*, 845–859.
63. Charlet-Lery, G.; Laplace, J.P. Utilization of a tracheal cannula in the pig. In *Energy Metabolism of Fram Animals. Proceedings of the Symposium on Energy Metabolism, Clermont-Ferrand, France (EAAP Publication No. 19)*; Vermorel, M., Ed.; G. de Bussac: Clemont-Ferrand, 1976; pp. 339–342.
64. Blaxter, K.L.; Brockway, J.M.; Boyne, A.W. A new method for estimating the heat production of animals. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **1972**, *57*, 60–72.
65. Aulic, L.H.; Arnhold, H.; Hauder, E.H.; Mason, A.D. A new open and closed circuit respiration chamber. *Q. J. Exp. Physiol.* **1983**, *68*, 351–357.
66. Armsby, H.P. The respiration calorimeter at the Pennsylvania Experiment Station. In *Annual Report of the Pennsylvania State College*; 1904; pp. 208–220.
67. Möllgaard, H.; Anderson, A.C. Respiration apparatus: its significance and use in experiments with dairy cattle. *Beret. fra Forsogslaboratoriet Kobenhaven* **1917**, *94*, 1–180.
68. Ritzman, E.G.; Benedict, F.G. Simplified techniques and apparatus for measuring energy requirements of cattle. *New Hampsh. Agric. Exp. Stn. Bull. 240* **1929**.
69. Mitchell, H.H.; Hamilton, T.S. The effect of the amount of feed consumed by cattle on the utilisation of its energy content. *J. Agric. Res.* **1932**, *45*, 163–191.
70. Kleiber, M. The California apparatus for respiration trials with large animals. *Hilgardia* **1935**, *9*, 1–70.
71. Marston, H.R. Energy transactions in the sheep. *Aust. J. Sci. Res. Ser. B (Biological Sci.* **1948**, *1*, 93–129.
72. Sonden, K.; Tigerstedt, R. Untersuchungen uber de respiration und dem gesamm-stoff weschel des menschen. *Arch. für Physiol.* **1895**, *6*, 1–224.
73. Tissot, J. Nouvelle méthode de mesure et d'inscription du débit et des mouvements respiratoires de l'homme et des animaux. *J. Physiol. Pathol. Générale* **1904**, *6*, 688–700.
74. Douglas, C.G. A method for determining the total respiratory exchange in man. *J. Physiol.* **1911**, *42*, xvii–xviii.
75. Macfarlane, D.J. Open-circuit respirometry: a historical review of portable gas analysis systems. *Eur. J. Appl. Physiol.* 2017, *117*, 2369–2386.
76. Zuntz, N.; Loewy, A.; Müller, F.; Caspari, W. *Höhenklima und bergwanderungen: in ihrer Wirkung auf den manschen*; Deutsches Verlagshaus Bong & Company: Berlin, 1906;
77. Benzinger, T.H.; Kitzinger, C. Direct calorimetry by means of the gradient principle. *Rev. Sci. Instrum.* **1949**, *20*, 849–860.
78. Wolff, H.S. The integrating motor pneumotachograph: a new instrument for the measurement of energy expenditure by indirect calorimetry. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **1958**, *43*, 270–283.
79. Webb, P.; Troutman, S.J. An instrument for continuous measurement of oxygen consumption. *J. Appl. Physiol.* **1970**, *28*, 867–871.
80. Sanchez-Delgado, G.; Ravussin, E. Assessment of energy expenditure: are calories measured differently for different diets? *Curr. Opin. Clin. Nutr. Metab. Care* **2020**, *23*, 312–318.
81. Westerterp, K.R. Doubly labelled water assessment of energy expenditure: principle, practice, and promise. *Eur. J. Appl. Physiol.* **2017**, *117*, 1277–1285.
82. Lifson, N.; Gordon, G.B.; Visscher, M.B.; Nier, A.O. The fate of utilized molecular oxygen and the source of the oxygen of respiratory carbon dioxide, studied with the aid of heavy oxygen. *J. Biol. Chem.* **1949**, *180*, 803–811.
83. Lifson, N.; Gordon, G.B.; McClintock, R. Measurement of total carbon dioxide production by means of D2O18. *J. Appl. Physiol.* **1955**, *7*, 704–710.
84. Lifson, N.; McClintock, R. Theory of use of the turnover rates of body water for measuring energy and material balance. *J. Theor. Biol.* **1966**, *12*, 46–74.
85. Schoeller, D.A.; Van Santen, E. Measurement of energy expenditure in humans by doubly labeled water method. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1982**, *53*, 955–959.
86. Speakman, J.R.; Roberts, S.B. Recent Advances in the Doubly Labeled Water Technique. *Obes. Res.* **1995**, *3*, 1–2.
87. Speakman, J.R.; Hambly, C. Using doubly-labelled water to measure free-living energy expenditure: Some old things to remember and some new things to consider. *Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol.* 2016, *202*, 3–9.
88. Westerterp, K.R.; Kayser, B.; Brouns, F.; Herry, J.P.; Saris, W.H.M. Energy expenditure climbing Mt. Everest. *J. Appl. Physiol.* **1992**, *73*, 1815–1819.
89. Speakman, J.R. Principles, problems and a paradox with the measurement of energy expenditure of free-living subjects using doubly-labelled water. *Stat. Med.* **1990**, *9*, 1365–1380.
90. Westerterp, K.R.; Brouns, F.; Saris, W.H.M.; Ten Hoor, F. Comparison of doubly labeled water with respirometry at low- and high-activity levels. *J. Appl. Physiol.* **1988**, *65*, 53–56.
91. Speakman, J.R. Calculation of CO2 production in doubly-labelled water studies. *J. Theor. Biol.* **1987**, *126*, 101–104.
92. Schoeller, D.A.; Taylor, P.B. Precision of the doubly labelled water method using the two-point calculation. *Hum. Nutr. Clin. Nutr.* **1987**, *41*, 215–223.
93. Weir, J.B. de V. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **1949**, *109*, 1–9.
94. Livesey, G.; Elia, M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *Am. J. Clin. Nutr.* **1988**, *47*, 608–628.
95. Consolazio, C.F.; Johnson, R.E.; Pecora, L.J. Physiological measurements of metabolic functions in man. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **1963**, *48*, 305–305.
96. Brouwer, E. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. *Acta Physiol Pharmacol Neerl.* **1957**, *6*, 795–802.
97. Lusk, G. Food values. *Science (80-.).* **1917**, *45*, 345–350.
98. Carpenter, T.M. *Tables, factors, and formulas for*

computing respiratory exchange and biological transformations of energy: prepared by Thorne M. Carpenter; Carnegie Institution of Washington: Washington DC, 1948;
99. Frayn, K.N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1983**, *55*, 628–634.
100. Cathcart, E.P.; Cuthbertson, D.P. The composition and distribution of the fatty substances of the human subject. *J. Physiol.* **1931**, *72*, 349–360.
101. Lusk, G. *The elements of the science of nutrition*; W.B. Saunders Co.: Philadelphia, 1928;
102. Webb, P.; Saris, W.H.M.; Schoffelen, P.F.M.; Schenau, G.J.V.I.; Hoor, F. Ten The work of walking: A calorimetric study. *Med. Sci. Sports Exerc.* **1988**, *20*, 331–337.
103. Dauncey, M.J.; Murgatroyd, P.R.; Cole, T.J. A human calorimeter for the direct and indirect measurement of 24 h energy expenditure. *Br. J. Nutr.* **1978**, *39*, 557–566.
104. Whipp, B.J.; Wasserman, K. Efficiency of muscular work. *J. Appl. Physiol.* **1969**, *26*, 644–648.
105. Jéquier, E.; Acheson, K.; Schutz, Y. Assessment Of Energy Expenditure And Fuel Utilization In Man. *Annu. Rev. Nutr.* **1987**, *7*, 187–208.
106. Reardon, F.D.; Leppik, K.E.; Wegmann, R.; Webb, P.; Ducharme, M.B.; Kenny, G.P. The Snellen human calorimeter revisited, re-engineered and upgraded: Design and performance characteristics. *Med. Biol. Eng. Comput.* **2006**, *44*, 721–728.
107. Webster, J.D.; Welsh, G.; Garrow, J.S. Description of a human direct calorimeter, with a note on the energy cost of clerical work. *Br. J. Nutr.* **1986**, *55*, 1–6.
108. Levine, J.A. Measurement of energy expenditure. *Public Health Nutr.* **2005**, *8*, 1123–1132.
109. Tschegg, E.; Sigmund, A.; Veitl, V.; Schmid, P.; Irsigler, K. An isothermic, gradient-free, whole-body calorimeter for long-term investigations of energy balance in man. *Metabolism* **1979**, *28*, 764–770.
110. Snellen, J.W. An improved estimation of mean body temperature using combined direct calorimetry and thermometry. *Eur. J. Appl. Physiol.* **2000**, *82*, 188–196.
111. Snellen, J.W.; Chang, K.S.; Smith, W. Technical description and performance characteristics of a human whole-body calorimeter. *Med. Biol. Eng. Comput.* **1983**, *21*, 9–20.
112. Spinnler, G.; Jequier, E.; Favre, R.; Dolivo, M.; Vannotti, A. Human calorimeter with a new type of gradient layer. *J. Appl. Physiol.* **1973**, *35*, 158–165.
113. Lighton, J.R.B. *Measuring metabolic rates: a manual for scientists*; Oxford University Press: Oxford, 2008;
114. Mtaweh, H.; Tuira, L.; Floh, A.A.; Parshuram, C.S. Indirect calorimetry: History, technology, and application. *Front. Pediatr.* 2018, *6*.
115. Ferrannini, E. The theoretical bases of indirect calorimetry: A review. *Metabolism* **1988**, *37*, 287–301.
116. Flatt, J.P.; Blackburn, G.L. The matabolic fuel regulatory system: implications for protein-sparing therapies during caloric deprivation and disease. *Am. J. Clin. Nutr.* 1974, *27*, 175–187.
117. Bistrian, B.R.; Blackburn, G.L.; Flatt, J.P.; Sizer, J.; Scrimshaw, N.S.; Sherman, M. Nitrogen metabolism and insulin requirements in obese diabetic adults on a protein sparing modified fast. *Diabetes* **1976**, *25*, 494–504.
118. Mariotti, F.; Tomé, D.; Mirand, P.P. Converting nitrogen into protein - Beyond 6.25 and Jones' factors. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 177–184.
119. Jones, D.B. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. *USDA Circ. No.* **1931**, *183*, 1–21.
120. Elia, M.; Livesey, G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. *World Rev. Nutr. Diet.* **1992**, *70*, 68–131.
121. Long, C.L.; Jeevanandam, M.; Kinney, J.M. Metabolism and recycling of urea in man. *Am. J. Clin. Nutr.* **1978**, *31*, 1367–1382.
122. Walser, M.; Bodenlos, L.J. Urea metabolism in man. *J. Clin. Invest.* **1959**, *38*, 1617–1626.
123. Jéquier, E. Direct and indirect calorimetry in man. In *Substrate and Energy Metabolism*; Garrow, J.S., Halliday, D., Eds.; Libbey: London, 1985.
124. Webb, P. The measurement of energy exchange in man: An analysis. *Am. J. Clin. Nutr.* **1980**, *33*, 1299–1310.
125. Webb, P.; Annis, J.F.; Troutman, S.J. Energy balance in man measured by direct and indirect calorimetry. *Am. J. Clin. Nutr.* **1980**, *33*, 1287–1298.
126. Kenny, G.P.; Webb, P.; Ducharme, M.B.; Reardon, F.D.; Jay, O. Calorimetric measurement of postexercise net heat loss and residual body heat storage. *Med. Sci. Sports Exerc.* **2008**, *40*, 1629–1636.
127. Kenny, G.P.; Dorman, L.E.; Webb, P.; Ducharme, M.B.; Gagnon, D.; Reardon, F.D.; Hardcastle, S.G.; Jay, O. Heat balance and cumulative heat storage during intermittent bouts of exercise. *Med. Sci. Sports Exerc.* **2009**, *41*, 588–596.
128. Blaxter, K. *Energy metabolism in animals and man*; Cambridge University Press: Cambridge, 1989;
129. Gerrits, W.J.J.; Labussière, E. *Indirect calorimetry: techniques, computations and applications*; Gerrits, W.J.J., Labussière, E., Eds.; Wageningen Academic Publishers: Wageningen, 2015;
130. Armour Forse, R. Comparison of Gas Exchange Measurements With a Mouthpiece, Face Mask, and Ventilated Canopy. *J. Parenter. Enter. Nutr.* **1993**, *17*, 388–391.
131. Darren M. Roffey; Nuala M. Byrne; and Andrew P. Hills Day-to-Day Variance in Measurement of Resting Metabolic Rate Using Ventilated-Hood and Mouthpiece & Nose-Clip Indirect Calorimetry Systems. *J. Parenter. Enter. Nutr.* **2006**, *30*, 426–432.
132. Jéquier, E. Long-term measurement of energy expenditure in man: direct or indirect calorimetry? In *Recent Advances in Obesity Research III*; Bjorntorp, P., Cairella, M., Howard, A.N., Eds.; Libbey: London, 1981; pp. 130–135.
133. Melanson, E.L.; Ingebrigtsen, J.P.; Bergouignan, A.; Ohkawara, K.; Kohrt, W.M.; Lighton, J.R.B. A new approach for flow-through respirometry measurements in humans. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **2010**, *298*, 1571–1579.
134. Matarese, L.E. Indirect calorimetry: Technical aspects. *J. Am. Diet. Assoc.* 1997, *97*, S154–S160.
135. Levine, J.; Morgan, M. Measurement of Energy Expenditure in Man: A Review of Available Methods. *J. Nutr. Med.* 1990, *1*, 325–343.
136. Weissman, C.; Damask, M.C.; Askanazi, J.; Rosenbaum, S.H.; Kinney, J.M. Evaluation of a non-invasive method for the measurement of metabolic rate in humans. *Clin. Sci.* **1985**, *69*, 135–141.
137. Scholander, P.F. Accurate analysis of respiratory

gases in 0.5 cubic centimeter samples Available online: https://pubmed.ncbi.nlm.nih.gov/20244250/ (accessed on Oct 20, 2020).
138. Haldane, J. Some Improved Methods of Gas Analysis. *J. Physiol.* **1898**, *22*, 465–480.
139. Schoffelen, P.F.M. Evaluation of an automatic indirect calorimeter for measurement of energy balance in man. In *van Es AJH (ed) European Nutrition Report No 5*; Koninklijke Bibliotheck: The Hague, 1985.
140. Nguyen, T.; de Longe, L.; Smith, S.R.; Bray, G.A. Chamber for indirect calorimetry with accurate measurement and time discrimination of metabolic plateaus of over 20 min. *Med. Biol. Eng. Comput.* **2003**, *41*, 572–578.
141. Schoffelen, P.F.M.; Westerterp, K.R.; Saris, W.H.M.; Ten Hoor, F. A dual-respiration chamber system with automated calibration. *J. Appl. Physiol.* **1997**, *83*, 2064–2072.
142. Seale, J.L.; Rumpler, W. V.; Moe, P.W. Description of a direct-indirect room-sized calorimeter. *Am. J. Physiol. - Endocrinol. Metab.* **1991**, *260*.
143. Minghelli, G.; Schutz, Y.; Charbonnier, A.; Whitehead, R.; Jéquier, E. Twenty-four-hour energy expenditure and basal metabolic rate measured in a whole-body indirect calorimeter in Gambian men. *Am. J. Clin. Nutr.* **1990**, *51*, 563–570.
144. Charbonnier, A.; Jones, C.D.; Schutz, Y.; Murgatroyd, P.R.; Whitehead, R.G.; Jéquier, E.; Spinnler, G. A whole body transportable indirect calorimeter for human use in the tropics Available online: https://pubmed.ncbi.nlm.nih.gov/2269251/ (accessed on Oct 20, 2020).
145. Ravussin, E.; Lillioja, S.; Anderson, T.E.; Christin, L.; Bogardus, C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J. Clin. Invest.* **1986**, *78*, 1568–1578.
146. Jequier, E.; Schutz, Y. Long-term measurements of energy expenditure in humans using a respiration chamber. *Am. J. Clin. Nutr.* **1983**, *38*, 989–998.
147. Dauncey, M.J.; Murgatroyd, P.R. A direct and indirect calorimeter for studies on energy expenditure in man over 24 h periods [proceedings] Available online: https://pubmed.ncbi.nlm.nih.gov/731578/ (accessed on Oct 20, 2020).
148. Henning, B.; Löfgren, R.; Sjöström, L. Chamber for indirect calorimetry with improved transient response. *Med. Biol. Eng. Comput.* **1996**, *34*, 207–212.
149. Tokuyama, K.; Ogata, H.; Katayose, Y.; Satoh, M. Algorithm for transient response of whole body indirect calorimeter: Deconvolution with a regularization parameter. *J. Appl. Physiol.* **2009**, *106*, 640–650.
150. Schutz, Y.; Ravussin, E.; Diethelms, R.; Jequier, E. Spontaneous physical activity measured by radar in obese and control subject studied in a respiration chamber - PubMed Available online: https://pubmed.ncbi.nlm.nih.gov/7068313/ (accessed on Oct 20, 2020).
151. Sun, M.; Hill, J.O. A method for measuring mechanical work and work efficiency during human activities. *J. Biomech.* **1993**, *26*, 229–241.
152. Moon, J.K.; Vohra, F.A.; Valerio Jimenez, O.S.; Puyau, M.R.; Butte, N.F. Closed-loop control of carbon dioxide concentration and pressure improves response of room respiration calorimeters. *J. Nutr.* **1995**, *125*, 220–228.
153. Sun, M.; Reed, G.W.; Hill, J.O. Modification of a whole room indirect calorimeter for measurement of rapid changes in energy expenditure. *J. Appl. Physiol.* **1994**, *76*, 2686–2691.
154. Cooper, B.G.; McLean, J.A.; Taylor, R. An evaluation of the Deltatrac indirect calorimeter by gravimetric injection and alcohol burning. *Clin. Phys. Physiol. Meas.* **1991**, *12*, 333–341.
155. Cooper, J.A.; Watras, A.C.; O'Brien, M.J.; Luke, A.; Dobratz, J.R.; Earthman, C.P.; Schoeller, D.A. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J. Am. Diet. Assoc.* **2009**, *109*, 128–32.
156. Graf, S.; Karsegard, V.L.; Viatte, V.; Maisonneuve, N.; Pichard, C.; Genton, L. *Comparison of three indirect calorimetry devices and three methods of gas collection: A prospective observational study*; Clin Nutr, 2013; Vol. 32;.
157. Delsoglio, M.; Dupertuis, Y.M.; Oshima, T.; van der Plas, M.; Pichard, C. Evaluation of the accuracy and precision of a new generation indirect calorimeter in canopy dilution mode. *Clin. Nutr.* **2019**, 1–8.
158. Oshima, T.; Dupertuis, Y.M.; Delsoglio, M.; Graf, S.; Heidegger, C.P.; Pichard, C. In vitro validation of indirect calorimetry device developed for the ICALIC project against mass spectrometry. *Clin. Nutr. ESPEN* **2019**, *32*, 50–55.
159. Kennedy, S.; Ryan, L.; Fraser, A.; Clegg, M.E. Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis. *J. Nutr. Sci.* **2014**, *3*.
160. Schoeller, D.A. Making Indirect Calorimetry a Gold Standard for Predicting Energy Requirements for Institutionalized Patients. *J. Am. Diet. Assoc.* **2007**, *107*, 390–392.
161. Schadewaldt, P.; Nowotny, B.; Strassburger, K.; Kotzka, J.; Roden, M. Indirect calorimetry in humans: a postcalorimetric evaluation procedure for correction of metabolic monitor variability. *Am. J. Clin. Nutr.* **2013**, *97*, 763–73.
162. Black, C.; Grocott, M.P.W.; Singer, M. Metabolic monitoring in the intensive care unit: A comparison of the Medgraphics Ultima, Deltatrac II, and Douglas bag collection methods. *Br. J. Anaesth.* **2015**, *114*, 261–268.
163. Rising, R.; Whyte, K.; Albu, J.; Pi-Sunyer, X. Evaluation of a new whole room indirect calorimeter specific for measurement of resting metabolic rate. *Nutr. Metab. (Lond).* **2015**, *12*, 46.
164. Haugen, H.A.; Melanson, E.L.; Tran, Z.V.; Kearney, J.T.; Hill, J.O. Variability of measured resting metabolic rate. *Am. J. Clin. Nutr.* **2003**, *78*, 1141–1144.
165. Bader, N.; Bosy-Westphal, A.; Dilba, B.; Müller, M.J. Intra- and interindividual variability of resting energy expenditure in healthy male subjects – biological and methodological variability of resting energy expenditure. *Br. J. Nutr.* **2005**, *94*, 843–849.
166. Weststrate, J.A. Resting metabolic rate and diet-induced thermogenesis: a methodological reappraisal. *Am J Clin Nutr.* **1993**, *58*, 592–601.
167. Kaviani, S.; Schoeller, D.A.; Ravussin, E.; Melanson, E.L.; Henes, S.T.; Dugas, L.R.; Dechert, R.E.; Mitri, G.; Schoffelen, P.F.M.; Gubbels, P.; et al. Determining the Accuracy and Reliability of Indirect Calorimeters Utilizing the Methanol Combustion Technique. *Nutr. Clin. Pract.* **2018**, *33*, 206–216.
168. Branson, R. The measurement of energy expenditure: Instrumentation, practical considerations, and clinical application. *Respir. Care* **1990**, *35*, 640–659.
169. Proctor, D.N.; Beck, K.C. Delay time adjustments to minimize errors in breath-by-breath

measurement of V˙O2 during exercise. *J. Appl. Physiol.* **1996**, *81*, 2495–2499.
170. Yamamoto, Y.; Takei, Y.; Mokushi, K.; Morita, H.; Mutoh, Y.; Miyashita, M. Breath-by-breath measurement of alveolar gas exchange with a slow-response gas analyser. *Med. Biol. Eng. Comput.* **1987**, *25*, 141–146.
171. Noguchi, H.; Ogushi, Y.; Yoshiya, I. Breath-by-breath V˙CO2 and V˙O2 require compensation for transport delay and dynamic response. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1982**, *52*, 79–84.
172. Wessel, H.U.; Stout, R.L.; Bastanier, C.K.; Paul, M.H. Breath-by-breath variation of FRC: Effect on Vo2 and Vco2 measured at the mouth. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1979**, *46*, 1122–1126.
173. Perret, C.; Mueller, C. Validation of a new portable ergospirometric device (Oxycon Mobile®) during exercise. *Int. J. Sports Med.* **2006**, *27*, 363–367.
174. Larsson, P.U.; Wadell, K.M.E.; Jakobsson, E.J.I.; Burlin, L.U.; Henriksson-Larsén, K.B. Validation of the MetaMax II Portable Metabolic Measurement System. *Int. J. Sports Med.* **2004**, *25*, 115–123.
175. Prieur, F.; Castells, J.; Denis, C. A methodology to assess the accuracy of a portable metabolic system (VmaxSTTM). *Med. Sci. Sports Exerc.* **2003**, *35*, 879–885.
176. Aliverti, A.; Kayser, B.; Macklem, P.T. Breath-by-breath assessment of alveolar gas stores and exchange. *J. Appl. Physiol.* **2004**, *96*, 1464–1469.
177. Potter, C.R.; Childs, D.J.; Houghton, W.; Armstrong, N. Breath-to-breath "noise" in the ventilatory and gas exchange responses of children to exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* **1999**, *80*, 118–124.
178. Grafe, E. Ein kopfrespirationsapparat. *Dtsch. Arch. Klin. Med.* **1909**, *95*, 529–542.
179. Benedict, F.G. Helmet for use in clinical studies of gaseous metabolism. *N. Engl. J. Med.* **1930**, *203*, 150–158.
180. Da Rocha, E.E.M.; Alves, V.G.F.; Da Fonseca, R.B. V Indirect calorimetry: Methodology, instruments and clinical application. *Curr. Opin. Clin. Nutr. Metab. Care* **2006**, *9*, 247–256.
181. Mullen, J.L. Indirect calorimetry in critical care. *Proc. Nutr. Soc.* **2005**, *50*, 239–244.
182. Weissman, C.; Sardar, A.; Kemper, M. In Vitro Evaluation of a Compact Metabolic Measurement Instrument. *J. Parenter. Enter. Nutr.* **1990**, *14*, 216–221.
183. Tissot, S.; Delafosse, B.; Bertrand, O.; Bouffard, Y.; Viale, J.P.; Annat, G. Clinical validation of the Deltatrac monitoring system in mechanically ventilated patients. *Intensive Care Med.* **1995**, *21*, 149–153.
184. Isbell, T.R.; Klesges, R.C.; Meyers, A.W.; Klesges, L.M. Measurement reliability and reactivity using repeated measurements of resting energy expenditure with a face mask, mouthpiece, and ventilated canopy. *J. Parenter. Enter. Nutr.* **1991**, *15*, 165–168.
185. Wang, X.; Wang, Y.; Ma, Z.; Xu, Y.; Wu, Q. Indirect calorimetry using a ventilated hood may be easier than using a facemask to achieve steady state when measuring resting energy expenditure. *Nutr. Res.* **2017**, *48*, 33–39.
186. Welle, S. Metabolic responses to a meal during rest and low-intensity exercise. *Am. J. Clin. Nutr.* **1984**, *40*, 990–994.
187. Siler, W.L. Is running style and economy affected by wearing respiratory apparatus? Available online: https://pubmed.ncbi.nlm.nih.gov/8450730/ (accessed on Oct 20, 2020).
188. Namiesnik, J.; Wardencki, W. Water vapour removal from gaseous samples used for analytical purposes. A review. *Int. J. Environ. Anal. Chem.* **1999**, *73*, 269–280.
189. Sanchez-Delgado, G.; Alcantara, J.; Ortiz-Alvarez, L.; Xu, H.; Martinez-Tellez, B.; Labayen, I.; Ruiz, J. Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. *Clin. Nutr.* **2018**, *37*, 1618–1624.
190. McAnena, O.J.; Harvey, L.P.; Katzeff, H.L.; Daly, J.M. Indirect calorimetry: comparison of hood and mask systems for measuring resting energy expenditure in healthy volunteers. *JPEN. J. Parenter. Enteral Nutr.* **1986**, *10*, 555–557.
191. Segal, K.R. Comparison of indirect calorimetric measurements of resting energy expenditure with a ventilated hood, face mask, and mouthpiece. *Am. J. Clin. Nutr.* **1987**, *45*, 1420–1423.
192. Leff, M.L.; Hill, J.O.; Yates, A.A.; Cotsonis, G.A.; Heymsfield, S.B. Resting metabolic rate: Measurement reliability. *J. Parenter. Enter. Nutr.* **1987**, *11*, 354–359.
193. Ravussin, E.; Burnand, B.; Schutz, Y.; Jequier, E. Twenty-four-hour energy expenditure and resting metabolic rate in obese, moderately obese, and control subjects. *Am. J. Clin. Nutr.* **1982**, *35*, 566–573.
194. JCGM *JCGM 200:2008 International vocabulary of metrology-Basic and general concepts and associated terms (VIM)*; BIPM: Sèvres, 2008;
195. Miodownik, S.; Melendez, J.; Carlon, V.A.; Burda, B.; Melendez, J.; Arslan, V.; Quantitative, B.B. Quantitative methanol-burning lung model for validating gas-exchange measurements over wide ranges of FIO2. *J. Appl. Physiol.* **1998**, *84*, 2177–2182.
196. Brychta, R.J.; Rothney, M.P.; Skarulis, M.C.; Chen, K.Y. Optimizing energy expenditure detection in human metabolic chambers. In Proceedings of the Proceedings of the 31st Annual International Conference of the IEEE Engineering in Medicine and Biology Society: Engineering the Future of Biomedicine, EMBC 2009; IEEE Computer Society, 2009; Vol. 2009, pp. 6864–6868.
197. Chakravarthy, M.; Parsons, S.; Lassman, M.E.; Butterfield, K.; Lee, A.Y.H.; Chen, Y.; Previs, S.; Spond, J.; Yang, S.; Bock, C.; et al. Effects of 13-hour hyperglucagonemia on energy expenditure and hepatic glucose production in humans. *Diabetes* **2017**, *66*, 36–44.
198. Galgani, J.E.; Castro-Sepulveda, M.; Pérez-Luco, C.; Fernańdez-Verdejo, R.; Fernández-Verdejo, R. Validity of predictive equations for resting metabolic rate in healthy humans. *Clin. Sci. (Lond).* **2018**, *132*, 1741–1751.
199. Galgani, J.E.; Castro-Sepulveda, M.A. Influence of a Gas Exchange Correction Procedure on Resting Metabolic Rate and Respiratory Quotient in Humans. *Obesity* **2017**, *25*, 1941–1947.
200. Schoffelen, P.F.M.; den Hoed, M.; van Breda, E.; Plasqui, G. Test-retest variability of VO 2max using total-capture indirect calorimetry reveals linear relationship of VO 2 and Power. *Scand. J. Med. Sci. Sport.* **2019**, *29*, 213–222.
201. Adriaens, M.P.E.; Schoffelen, P.F.M.; Westerterp, K.R. Intra-individual variation of basal metabolic rate and the influence of daily habitual physical activity before testing. *Br. J. Nutr.* **2003**, *90*, 419–423.
202. Schoffelen, P.F.M.; Westerterp, K.R. Intra-individual variability and adaptation of overnight- and

sleeping metabolic rate. *Physiol. Behav.* **2008**, *94*, 158–163.
203. Lin, C.C.; Chang, K.C.; Lee, K.S. Effects of treatment by laser-assisted uvuloplasty on sleep energy expenditure in obstructive sleep apnea patients. *Metabolism.* **2002**, *51*, 622–627.
204. Plasqui, G.; Kester, A.D.M.; Westerterp, K.R. Seasonal variation in sleeping metabolic rate, thyroid activity, and leptin. *Am. J. Physiol. - Endocrinol. Metab.* **2003**, *285*.
205. van Dale, D.; Schoffelen, P.F.; ten Hoor, F.; Saris, W.H. Effects of addition of exercise to energy restriction on 24-hour energy expenditure, sleeping metabolic rate and daily physical activity. *Eur. J. Clin. Nutr.* **1989**, *43*, 441–451.
206. Murgatroyd, P.R.; Davies, H.L.; Prentice, A.M. Intra-individual variability and measurement noise in estimates of energy expenditure by whole body indirect calorimetry. *Br. J. Nutr.* **1987**, *58*, 347–356.
207. Zitting, K.M.; Vujovic, N.; Yuan, R.K.; Isherwood, C.M.; Medina, J.E.; Wang, W.; Buxton, O.M.; Williams, J.S.; Czeisler, C.A.; Duffy, J.F. Human Resting Energy Expenditure Varies with Circadian Phase. *Curr. Biol.* **2018**, *28*, 3685-3690.e3.
208. Compher, C.; Frankenfield, D.; Keim, N.; Roth-Yousey, L.; Evidence Analysis Working Group Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *J. Am. Diet. Assoc.* **2006**, *106*, 881–903.
209. Kostyak, J.C.; Kris-Etherton, P.; Bagshaw, D.; Delany, J.P.; Farrell, P.A. Relative fat oxidation is higher in children than adults. *Nutr. J.* **2007**, *6*.
210. Melanson, E.L.; Gavin, K.M.; Shea, K.L.; Wolfe, P.; Wierman, M.E.; Schwartz, R.S.; Kohrt, W.M. Regulation of energy expenditure by estradiol in premenopausal women. *J. Appl. Physiol.* **2015**, *119*, 975–981.
211. Johnstone, A.M.; Murison, S.D.; Duncan, J.S.; Rance, K.A.; Speakman, J.R. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am. J. Clin. Nutr.* **2005**, *82*, 941–948.
212. Wong, W.W.; Butte, N.F.; Ellis, K.J.; Hergenroeder, A.C.; Hill, R.B.; Stuff, J.E.; Smith, E.O. Pubertal African-American Girls Expend Less Energy at Rest and During Physical Activity than Caucasian Girls 1 . *J. Clin. Endocrinol. Metab.* **1999**, *84*, 906–911.
213. Piaggi, P.; Masindova, I.; Muller, Y.L.; Mercader, J.; Wiessner, G.B.; Chen, P.; Kobes, S.; Hsueh, W.C.; Mongalo, M.; Knowler, W.C.; et al. A genome-wide association study using a custom genotyping array identifies variants in GPR158 associated with reduced energy expenditure in American Indians. In Proceedings of the Diabetes; American Diabetes Association Inc., 2017; Vol. 66, pp. 2284–2295.
214. Acosta, F.M.; Martinez-Tellez, B.; Sanchez-Delgado, G.; Alcantara, J.M.A.; Acosta-Manzano, P.; Morales-Artacho, A.J.; Ruiz, J.R. Physiological responses to acute cold exposure in young lean men. *PLoS One* **2018**, *13*.
215. Sanchez-Delgado, G.; Alcantara, J.; Acosta, F.M.; Martinez-Tellez, B.; Amaro-Gahete, F.J.; Ortiz-Alvarez, L.; Löf, M.; Labayen, I.; Ruiz, J.R. Estimation of non-shivering thermogenesis and cold-induced nutrient oxidation rates: Impact of method for data selection and analysis. *Clin. Nutr.* **2018**, *38*, 1–7.
216. Karst, H.; Steiniger, J.; Noack, R.; Steglich, H.-D. Diet-Induced Thermogenesis in Man: Thermic Effects of Single Proteins, Carbohydrates and Fats Depending on Their Energy Amount. *Ann. Nutr. Metab.* **1984**, *28*, 245–252.
217. Nguo, K.; Huggins, C.E.; Truby, H.; Brown, J.; Bonham, M.P. Effect of macronutrient composition on meal-induced thermogenesis in adolescents with obesity. *Eur. J. Nutr.* **2018**, *0*, 0.
218. Quatela, A.; Callister, R.; Patterson, A.; Macdonald-Wicks, L. The energy content and composition of meals consumed after an overnight fast and their effects on diet induced thermogenesis: A systematic review, meta-analyses and meta-regressions. *Nutrients* **2016**, *8*, 1–30.
219. Kinabo, J.L.; Durnin, J.V.G.A. Thermic effect of food in man: Effect of meal composition, and energy content. *Br. J. Nutr.* **1990**, *64*, 37–44.
220. Piaggi, P. Metabolic Determinants of Weight Gain in Humans. *Obesity* 2019, *27*, 691–699.
221. Hall, K.D.; Guo, J. Obesity Energetics: Body Weight Regulation and the Effects of Diet Composition. *Gastroenterology* **2017**, *152*, 1718-1727.e3.
222. Esparza, J.; Fox, C.; Harper, I.T.; Bennett, P.H.; Schulz, L.O.; Valencia, M.E.; Ravussin, E. Daily energy expenditure in Mexican and USA Pima Indians: Low physical activity as a possible cause of obesity. *Int. J. Obes.* **2000**, *24*, 55–59.
223. Henry, C.J.K. Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutr.* **2005**, *8*, 1133–52.
224. Mifflin, D.; Jeor, T.; Daugherty, A.; Hill, A.; Scott, J.; Daugherty, S.; Kho, Y. A new predictive equation in healthy individuals for resting energy. *Am. J. Clin. Nutr.* **1990**, *51*, 241–247.
225. Weyer, C.; Snitker, S.; Rising, R.; Bogardus, C.; Ravussin, E. Determinants of energy expenditure and fuel utilization in man: Effects of body composition, age, sex, ethnicity and glucose tolerance in 916 subjects. *Int. J. Obes.* **1999**, *23*, 715–722.
226. Fernández-Verdejo, R.; Aguirre, C.; Galgani, J.E. Issues in Measuring and Interpreting Energy Balance and Its Contribution to Obesity. *Curr. Obes. Rep.* **2019**, *8*, 88–97.
227. Ravussin, E.; Bogardus, C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am. J. Clin. Nutr.* 1989, *49*, 968–975.
228. Heymsfield, S.B.; Thomas, D.; Bosy-Westphal, A.; Shen, W.; Peterson, C.M.; Müller, M.J. Evolving concepts on adjusting human resting energy expenditure measurements for body size. *Obes. Rev.* **2012**, *13*, 1001–1014.
229. Watson, L.P.E.; Raymond-Barker, P.; Moran, C.; Schoenmakers, N.; Mitchell, C.; Bluck, L.; Chatterjee, V.K.; Savage, D.B.; Murgatroyd, P.R. An approach to quantifying abnormalities in energy expenditure and lean mass in metabolic disease. *Eur. J. Clin. Nutr.* **2014**, *68*, 234–240.
230. Thiebaud, D.; Schutz, Y.; Acheson, K. Energy cost of glucose storage in human subjects during glucose-insulin infusions. *Am. J. Physiol. - Endocrinol. Metab.* **1983**, *7*.
231. Trayhurn, P.; James, W.P.T. Thermogenesis: dietary and non-shivering aspects. In *The body weigth regulatory system: normal and disturbed mechanisms*; Cioff, L.A., James, W.P.T., Itallie, T.B., Eds.; Raven: New York, 1981.
232. Acheson, K.; Jequier, E.; Wahren, J. Influence of β-adrenergic blockade on glucose-induced thermogenesis in man. *J. Clin. Invest.* **1983**, *72*, 981–986.
233. Danforth, E.; Burger, A.G.; Goldman, R.F.; Sims, E.A.H. Thermogenesis during weight gain. In

Recent Advances in Obesity Research III; Bray, G.A., Ed.; Newman: London, 1978.
234. Riumallo, J.A.; Schoeller, D.; Barrera, G.; Gattas, V.; Uauy, R. Energy expenditure in underweight free-living adults: Impact of energy supplementation as determined by doubly labeled water and indirect calorimetry. *Am. J. Clin. Nutr.* **1989**, *49*, 239–246.
235. Sims, E.A.H.; Danforth, E. Expenditure and storage of energy in man. *J. Clin. Invest.* 1987, *79*, 1019–1025.
236. Sims, E.A.H.; Danforth, E.; Horton, E.S. Endocrine and metabolic effects of experimental obesity in man. *Recent Prog. Horm. Res.* **1973**, *29*, 457–496.
237. Melanson, K.J.; Saltzman, E.; Vinken, A.G.; Russell, R.; Roberts, S.B. The effects of age on postprandial thermogenesis at four graded energetic challenges: Findings in young and older women. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **1998**, *53*.
238. D'Alessio, D.A.; Kavle, E.C.; Mozzoli, M.A.; Smalley, K.J.; Polansky, M.; Kendrick, Z. V.; Owen, L.R.; Bushman, M.C.; Boden, G.; Owen, O.E. Thermic effect of food in lean and obese men. *J. Clin. Invest.* **1988**, *81*, 1781–1789.
239. Westerterp, K.R. Diet induced thermogenesis. *Nutr. Metab. (Lond).* **2004**, *1*, 5.
240. Ogata, H.; Kobayashi, F.; Hibi, M.; Tanaka, S.; Tokuyama, K. A novel approach to calculating the thermic effect of food in a metabolic chamber. *Physiol. Rep.* **2016**, *4*, 1–11.
241. Tataranni, P.A.; Larson, D.E.; Snitker, S.; Ravussin, E. Thermic effect of food in humans: methods and results from use of a respiratory chamber Available online: https://pubmed.ncbi.nlm.nih.gov/7733021/ (accessed on Oct 20, 2020).
242. Thearle, M.S.; Pannacciulli, N.; Bonfiglio, S.; Pacak, K.; Krakoff, J. Extent and determinants of thermogenic responses to 24 hours of fasting, energy balance, and five different overfeeding diets in humans. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 2791–2799.
243. Schutz, Y.; Bessard, T.; Jequier, E. Diet-induced thermogenesis measured over a whole day in obese and nonobese women. *Am. J. Clin. Nutr.* **1984**, *40*, 542–552.
244. Westerterp, K.R.; Wilson, S.A.J.; Rolland, V. Diet induced thermogenesis measured over 24 h in a respiration chamber: Effect of diet composition. *Int. J. Obes.* **1999**, *23*, 287–292.
245. Dabbech, M.; Aubert, R.; Apfelbaum, M.; Boulier, A. Reproducible measurement of postprandial energy expenditure in young healthy males. *Am. J. Clin. Nutr.* **1994**, *60*, 849–854.
246. Houde-Nadeau, M.; De Jonge, L.; Garrel, D.R. Thermogenic response to food: Intra-individual variability and measurement reliability. *J. Am. Coll. Nutr.* **1993**, *12*, 511–516.
247. Piers, L.S.; Soares, M.J.; Makan, T.; Shetty, P.S. Thermic effect of a meal. *Br. J. Nutr.* **1992**, *67*, 165–175.
248. Armellini, F.; Zamboni, M.; Mino, A.; Bissoli, L.; Micciolo, R.; Bosello, O. Postabsorptive resting metabolic rate and thermic effect of food in relation to body composition and adipose tissue distribution. *Metabolism.* **2000**, *49*, 6–10.
249. Laville, M.; Cornu, C.; Normand, S.; Mithieux, G.; Beylot, M.; Riou, J.P. Decreased glucose-induced thermogenesis at the onset of obesity. *Am. J. Clin. Nutr.* **1993**, *57*, 851–856.
250. Nelson, K.M.; Weinsier, R.L.; James, L.D.; Darnell, B.; Hunter, G.; Long, C.L. Effect of weight reduction on resting energy expenditure, substrate utilization, and the thermic effect of food in moderately obese women. *Am. J. Clin. Nutr.* **1992**, *55*, 924–933.
251. Katzeff, H.L.; Danforth, E. Decreased thermic effect of a mixed meal during overnutrition in human obesity. *Am. J. Clin. Nutr.* **1989**, *50*, 915–921.
252. Schutz, Y.; Golay, A.; Felber, J.P.; Jequier, E. Decreased glucose-induced thermogenesis after weight loss in obese subjects: A predisposing factor for relapse of obesity? *Am. J. Clin. Nutr.* **1984**, *39*, 380–387.
253. Tentolouris, N.; Pavlatos, S.; Kokkinos, A.; Perrea, D.; Pagoni, S.; Katsilambros, N. Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. *Metabolism.* **2008**, *57*, 313–320.
254. Welle, S.L.; Campbell, R.G. Normal thermic effect of glucose in obese women. *Am. J. Clin. Nutr.* **1983**, *37*, 87–92.
255. Segal, K.R.; Chun, A.; Coronel, P.; Cruz-Noori, A.; Santos, R. Reliability of the measurement of postprandial thermogenesis in men of three levels of body fatness. *Metabolism* **1992**, *41*, 754–762.
256. Janský, L. Non-shivering thermogenesis and its thermoregulatory significance. *Biol. Rev. Camb. Philos. Soc.* 1973, *48*, 85–132.
257. Blondin, D.P.; Labbé, S.M.; Phoenix, S.; Guérin, B.; Turcotte, É.E.; Richard, D.; Carpentier, A.C.; Haman, F. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. *J. Physiol.* **2015**, *593*, 701–714.
258. Blondin, D.P.; Daoud, A.; Taylor, T.; Tingelstad, H.C.; Bézaire, V.; Richard, D.; Carpentier, A.C.; Taylor, A.W.; Harper, M.E.; Aguer, C.; et al. Four-week cold acclimation in adult humans shifts uncoupling thermogenesis from skeletal muscles to brown adipose tissue. *J. Physiol.* **2017**, *595*, 2099–2113.
259. Blondin, D.P.; Frisch, F.; Phoenix, S.; Guérin, B.; Turcotte, É.E.; Haman, F.; Richard, D.; Carpentier, A.C. Inhibition of Intracellular Triglyceride Lipolysis Suppresses Cold-Induced Brown Adipose Tissue Metabolism and Increases Shivering in Humans. *Cell Metab.* **2017**, *25*, 438–447.
260. van der Lans, A.A.J.J.; Wierts, R.; Vosselman, M.J.; Schrauwen, P.; Brans, B.; van Marken Lichtenbelt, W.D. Cold-activated brown adipose tissue in human adults: Methodological issues. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 2014, *307*.
261. Chen, K.Y.; Cypess, A.M.; Laughlin, M.R.; Haft, C.R.; Hu, H.H.; Bredella, M.A.; Enerbäck, S.; Kinahan, P.E.; Lichtenbelt, W. van M.; Lin, F.I.; et al. Brown Adipose Reporting Criteria in Imaging STudies (BARCIST 1.0): Recommendations for Standardized FDG-PET/CT Experiments in Humans. *Cell Metab.* 2016, *24*, 210–222.
262. Blondin, D.P.; Tingelstad, H.C.; Noll, C.; Frisch, F.; Phoenix, S.; Guérin, B.; Turcotte, É.E.; Richard, D.; Haman, F.; Carpentier, A.C. Dietary fatty acid metabolism of brown adipose tissue in cold-acclimated men. *Nat. Commun.* **2017**, *8*.
263. Delsoglio, M.; Achamrah, N.; Berger, M.M.; Pichard, C. Indirect Calorimetry in Clinical Practice. *J. Clin. Med.* **2019**, *8*, 1387.
264. Achamrah, N.; Delsoglio, M.; De Waele, E.; Berger, M.M.; Pichard, C. Indirect calorimetry: The 6 main issues. *Clin. Nutr.* 2020.
265. Fredrix, E.W.H.M.; Soeters, P.B.; von Meyenfeldt, M.F.; Saris, W.H.M. Measurement of resting energy expenditure in a clinical setting. *Clin. Nutr.* **1990**, *9*, 299–304.
266. Turley, K.R.; McBride, P.J.; Wilmore, J.H. Resting

metabolic rate measured after subjects spent the night at home vs at a clinic. *Am. J. Clin. Nutr.* **1993**, *58*, 141–144.
267. Frankenfield, D.C.; Coleman, A. Recovery to Resting Metabolic State after Walking. *J. Am. Diet. Assoc.* **2009**, *109*, 1914–1916.
268. Kashiwazaki, H.; Dejima, Y.; Suzuki, T. Influence of upper and lower thermoneutral room temperatures (20 degrees C and 25 degrees C) on fasting and post-prandial resting metabolism under different outdoor temperatures. *Eur. J. Clin. Nutr.* **1990**, *44*, 405–413.
269. Schols, A.M.W.J.; Schoffelen, P.F.M.; Ceulemans, H.; Wouters, E.F.M.; Saris, W.H.M. Measurement of resting energy expenditure in patients with chronic obstructive pulmonary disease in a clinical setting. *J. Parenter. Enter. Nutr.* **1992**, *16*, 364–368.
270. Mellecker, R.R.; McManus, A.M. Measurement of resting energy expenditure in healthy children. *J. Parenter. Enter. Nutr.* **2009**, *33*, 640–645.
271. Snell, B.; Fullmer, S.; Eggett, D.L. Reading and Listening to Music Increase Resting Energy Expenditure during an Indirect Calorimetry Test. *J. Acad. Nutr. Diet.* **2014**, *114*, 1939–1942.
272. Buchowski, M.S.; Majchrzak, K.M.; Blomquist, K.; Chen, K.Y.; Byrne, D.W.; Bachorowski, J.A. Energy expenditure of genuine laughter. *Int. J. Obes.* **2007**, *31*, 131–137.
273. Saeidifard, F.; Medina-Inojosa, J.R.; Supervia, M.; Olson, T.P.; Somers, V.K.; Erwin, P.J.; Lopez-Jimenez, F. Differences of energy expenditure while sitting versus standing: A systematic review and meta-analysis. *Eur. J. Prev. Cardiol.* **2018**, *25*, 522–538.
274. Miles-Chan, J.L.; Sarafian, D.; Montani, J.P.; Schutz, Y.; Dulloo, A.G. Sitting comfortably versus lying down: Is there really a difference in energy expenditure? *Clin. Nutr.* **2014**, *33*, 175–178.
275. Amaro-Gahete, F.J.; Sanchez-Delgado, G.; Alcantara, J.M.A.; Martinez-Tellez, B.; Acosta, F.M.; Merchan-Ramirez, E.; Löf, M.; Labayen, I.; Ruiz, J.R. Energy expenditure differences across lying, sitting, and standing positions in young healthy adults. *PLoS One* **2019**, *14*.
276. Brandi, L.S.; Bertolini, R.; Janni, A.; Gioia, A.; Angeletti, C.A. Energy metabolism of thoracic surgical patients in the early postoperative period: Effect of posture. *Chest* **1996**, *109*, 630–637.
277. Levine, J.A.; Schleusner, S.J.; Jensen, M.D. Energy expenditure of nonexercise activity. *Am. J. Clin. Nutr.* **2000**, *72*, 1451–1454.
278. Sujatha, T.; Shatrugna, V.; Venkataramana, Y.; Begum, N. Energy expenditure on household, childcare and occupational activities of women from urban poor households. *Br. J. Nutr.* **2000**, *83*, 497–503.
279. Taguri, E.; Tanaka, S.; Ohkawara, K.; Ishikawa-Takata, K.; Hikihara, Y.; Miyake, R.; Yamamoto, S.; Tabata, I. Validity of physical activity indices for adjusting energy expenditure for body size: Do the indices depend on body size? *J. Physiol. Anthropol.* **2010**, *29*, 109–117.
280. Weststrate, J.A.; Weys, P.J.M.; Poortvliet, E.J.; Deurenberg, P.; Hautvast, J.G.A.J. Diurnal variation in postabsorptive resting metabolic rate and diet-induced thermogenesis. *Am. J. Clin. Nutr.* **1989**, *50*, 908–914.
281. Claessens-Van Ooijen, A.M.J.; Westerterp, K.R.; Wouters, L.; Schoffelen, P.F.M.; Van Steenhoven, A.A.; Van Marken Lichtenbelt, W.D. Heat production and body temperature during cooling and rewarming in overweight and lean men. *Obesity* **2006**, *14*, 1914–1920.
282. Van Ooijen, A.M.J.; Van Marken Lichtenbelt, W.D.; Van Steenhoven, A.A.; Westerterp, K.R. Seasonal changes in metabolic and temperature responses to cold air in humans. *Physiol. Behav.* **2004**, *82*, 545–553.
283. Belko, A.Z.; Barbieri, T.F. Effect of meal size and frequency on the thermic effect of food. *Nutr. Res.* **1987**, *7*, 237–242.
284. Levine; Harris; Morgan Energy expenditure in chronic alcohol abuse. *Eur. J. Clin. Invest.* **2000**, *30*, 779–786.
285. Segal, K.R.; Gutin, B. Thermic effects of food and exercise in lean and obese women. *Metabolism* **1983**, *32*, 581–589.
286. Weststrate, J.A.; Hautvast, J.G.A.J. The effects of short-term carbohydrate overfeeding and prior exercise on resting metabolic rate and diet-induced thermogenesis. *Metabolism* **1990**, *39*, 1232–1239.
287. Bielinski, R.; Schutz, Y.; Jequier, E. Energy metabolism during the postexercise recovery in man. *Am. J. Clin. Nutr.* **1985**, *42*, 69–82.
288. Arciero, P.J.; Bougopoulos, C.L.; Nindl, B.C.; Benowitz, N.L. Influence of age on the thermic response to caffeine in women. *Metabolism.* **2000**, *49*, 101–107.
289. Belza, A.; Toubro, S.; Astrup, A. The effect of caffeine, green tea and tyrosine on thermogenesis and energy intake. *Eur. J. Clin. Nutr.* **2009**, *63*, 57–64.
290. Yoshida, T.; Sakane, N.; Umekawa, T.; Kondo, M. Relationship between basal metabolic rate, thermogenic response to caffeine, and body weight loss following combined low calorie and exercise treatment in obese women. *Int J Obes Relat Metab Disord* **1994**, *18*, 345–350.
291. Komatsu, T.; Nakamori, M.; Komatsu, K.; Hosoda, K.; Okamura, M.; Toyama, K.; Ishikura, Y.; Sakai, T.; Kunii, D.; Yamamoto, S. Oolong tea increases energy metabolism in Japanese females. *undefined* **2003**.
292. Belza, A.; Frandsen, E.; Kondrup, J. Body fat loss achieved by stimulation of thermogenesis by a combination of bioactive food ingredients: A placebo-controlled, double-blind 8-week intervention in obese subjects. *Int. J. Obes.* **2007**, *31*, 121–130.
293. Greenway, F.L.; De Jonge, L.; Blanchard, D.; Frisard, M.; Smith, S.R. Effect of a dietary herbal supplement containing caffeine and ephedra on weight, metabolic rate, and body composition. *Obes. Res.* **2004**, *12*, 1152–1157.
294. Collins, L.C.; Walker, J.; Stamford, B.A. Smoking multiple high- versus low-nicotine cigarettes: Impact on resting energy expenditure. *Metabolism.* **1996**, *45*, 923–926.
295. Molé, P.A. Impact of Energy Intake and Exercise on Resting Metabolic Rate. *Sport. Med.* 1990, *10*, 72–87.
296. Speakman, J.R.; Selman, C. Physical activity and resting metabolic rate. *Proc. Nutr. Soc.* **2003**, *62*, 621–634.
297. Garber, C.E.; Blissmer, B.; Deschenes, M.R.; Franklin, B.A.; Lamonte, M.J.; Lee, I.M.; Nieman, D.C.; Swain, D.P. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. *Med. Sci. Sports Exerc.* **2011**, *43*, 1334–1359.
298. Williamson, D.L.; Kirwan, J.P. A single bout of concentric resistance exercise increases basal

metabolic rate 48 hours after exercise in healthy 59-77-year-old men. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **1997**, *52*.
299. Acheson, K.J. Indirect calorimetry: a case for improved standard operating procedures. *Eur. J. Clin. Nutr.* **2014**, *68*, 1–1.
300. Horner, N.K.; Lampe, J.W.; Patterson, R.E.; Neuhouser, M.L.; Beresford, S.A.; Prentice, R.L. Indirect Calorimetry Protocol Development for Measuring Resting Metabolic Rate as a Component of Total Energy Expenditure in Free-Living Postmenopausal Women. *J. Nutr.* **2001**, *131*, 2215–2218.
301. McClave, S.A.; Spain, D.A.; Skolnick, J.L.; Lowen, C.C.; Kleber, M.J.; Wickerham, P.S.; Vogt, J.R.; Looney, S.W. Achievement of steady state optimizes results when performing indirect calorimetry. *J. Parenter. Enter. Nutr.* **2003**, *27*, 16–20.
302. Irving, C.J.; Eggett, D.L.; Fullmer, S. Comparing Steady State to Time Interval and Non-Steady State Measurements of Resting Metabolic Rate. *Nutr. Clin. Pract.* **2017**, *32*, 77–83.
303. Borges, J.H.; Langer, R.D.; Cirolini, V.X.; Páscoa, M.A.; Guerra, G.; Gonçalves, E.M. Minimum Time to Achieve the Steady State and Optimum Abbreviated Period to Estimate the Resting Energy Expenditure by Indirect Calorimetry in Healthy Young Adults. *Nutr. Clin. Pract.* **2016**, *31*, 349–354.
304. Borges, J.H.; Guerra-Júnior, G.; Gonçalves, E.M. Methods for data analysis of resting energy expenditure measured using indirect calorimetry. *Nutrition* **2019**, *59*, 44–49.
305. Popp, C.J.; Tisch, J.J.; Sakarcan, K.E.; Bridges, W.C.; Jesch, E.D. Approximate Time to Steady-state Resting Energy Expenditure Using Indirect Calorimetry in Young, Healthy Adults. *Front. Nutr.* **2016**, *3*.
306. Popp, C.; Butler, M.; Curran, M.; Illiano, P.; Sevick, M.A.; St-Jules, D. Evaluating steady-state resting energy expenditure using indirect calorimetry in adults with overweight and obesity. *Clin. Nutr.* **2019**, *39*, 2220–2226.

AIMS

AIMS

The overall aim of the present Doctoral Thesis is to study the performance of diverse metabolic carts for assessing the resting metabolic rate in healthy humans, trying to find the metabolic cart which can replace the former gold standard. Further, as the method for gas exchange data selection may influence the metabolic cart performance and the day-to-day biological reproducibility, to determine the most suitable method is of vital importance. This overall aim is addressed in four different studies which are grouped on two sections.

Section 1 – Metabolic carts for assessing resting metabolic rate

- General objective 1: To determine the accuracy, precision, biological reproducibility and comparability of different metabolic carts to assess resting metabolic rate and respiratory exchange ratio.

    - Specific objective 1.1: To investigate the biological reproducibility and comparability of resting energy expenditure and nutrient oxidation rates assessments provided by two different and commercially available breath-by-breath metabolic carts (**Study I**).

    - Specific objective 1.2: To investigate the accuracy, precision, biological reproducibility and comparability of resting energy expenditure and nutrient oxidation rates assessments provided by four different, commercially available and recently manufactured, mixing chamber and breath-by-breath metabolic carts (**Study II**).

    - Specific objective 1.3: To investigate whether a post-calorimetric correction procedure improves the biological reproducibility and comparability of resting energy expenditure and nutrient oxidation rates assessments provided by four different, commercially available and recently manufactured, mixing chamber and breath-by-breath metabolic carts (**Study II**).

Section 2 – Methods for gas exchange data selection in resting metabolic rate assessments

- General objective 2: To examine the influence of different methods of gas exchange data selection for inferring the resting metabolic rate (i.e. resting energy expenditure) and nutrient oxidation rates, as supplied by different metabolic carts.

    - Specific objective 2.1: To analyze the influence of different methods for gas exchange data selection on resting metabolic rate and nutrient oxidation rates, using two different and commercially available breath-by-breath metabolic carts (**Study III**).

    - Specific objective 2.2: To analyze the influence of different methods for gas exchange data selection on the biological variability of resting metabolic rate and nutrient oxidation rates, using four different, commercially available and recently manufactured, mixing chamber and breath-by-breath metabolic carts (**Study IV**).

Table 4 shows the methodological overview of the four studies included in the present Doctoral Thesis.

Table 4. Methodological overview of the studies included in the Doctoral Thesis.

Study	Design	Study (reference)	Participants characteristics†	Metabolic carts used	Independent variable	Dependent variables	Statistical approach and analyses
Study I	Repeated measures	RMC study 1	17 (65%); 23.2±1.7 years; 168.0±9.0 cm; 63.2±11.5 kg; 22.4±2.6 kg/m^2	- CPX Ultima CardiO2[1] - CCM Express[1]	RMR	- RMR (i.e. REE) - RER	- B&A - Two-factor (MC x day) ANOVA - Paired *t*-tests - Linear regression
Study II	Repeated measures	RMC study 2	29 (38%); 25.0±4.3 years; 171.0±12.9 cm; 71.2±7.5 kg; 24.1±3.2 kg/m^2	- Q-NRG[2] - Vyntus CPX[3] - Omnical[4] - Ultima CardiO2[1]	- Simulated EE - RMR	- EE - RER - RMR (i.e. REE)	- One-factor ANOVA - Two-factor (MC × PCCP) ANOVA - Paired *t*-tests - B&A - Linear regression
Study III	Cross-sectional study	ACTIBATE study [1] FIT-AGEING study [2]	107 (67%); 22.2±2.2 years; 167.8±8.7 cm; 69.3±15.9 kg; 24.5±4.4 kg/m^2 74 (53); 53.5±5.3 years; 167.8±9.8 cm; 75.7±15.0 kg; 26.7±3.8 kg/m^2	- CPX Ultima CardiO2*[1] - CCM Express[1]	RMR	- RMR (i.e. REE) - RER - FATox - CHOox	- One-factor ANOVA - Linear regression
Study IV	Repeated measures	RMC study 2	17 (35%); 25.1±4.0 years; 171.1±8.8 cm; 68.6±12.9 kg; 23.3±2.9 kg/m^2	- Q-NRG[2] - Vyntus CPX[3] - Omnical[4] - Ultima CardiO2[1]	RMR	- RMR (i.e. REE) - RER - FATox - CHOox	- One-factor ANOVA - Linear regression - Paired *t*-tests

*RMC: acronym for the 'reproducibility of metabolic carts' study. ACTIBATE: acronym for the 'activating brown adipose tissue through exercise' study. FIT-AGEING: acronym for the 'physical fitness as klotho protein stimulator' study. † Presented as mean ± standard deviation otherwise stated, N (and percentage of women); age (years); height (centimeters); body weight (kilograms); and body mass index (BMI; in kilograms/meters^2). * Two different units of the same metabolic cart model were used. B&A: Bland and Altman analysis and/or plots [3]; RMR: resting metabolic rate; REE: resting energy expenditure (in kilocalories per day); RER: respiratory exchange ratio; EE: energy expenditure (in kilocalories per day); FATox: fat oxidation (in grams per minute); CHOox: carbohydrate oxidation (in grams per minute); MC: metabolic cart; ANOVA: repeated-measures analyses of variance; PCCC: post-calorimetric correction procedure. [1]Metabolic cart brand reference: Medgraphics Corporation, St. Paul, MN, USA; [2]Metabolic cart brand reference: Cosmed, Rome, Italy; [3]Metabolic cart brand reference: Jaeger-CareFusion, Höchberg, Germany; [4]Metabolic cart brand reference: Maastricht Instruments, Maastricht, The Netherlands. References are presented below.*

REFERENCES

[1] Sanchez-Delgado, G., et al., Activating brown adipose tissue through exercise (ACTIBATE) in young adults: Rationale, design and methodology. *Contemp. Clin. Trials* **2015**, 45, 416–425.

[2] Amaro-Gahete, F.J., et al., Exercise training as S-Klotho protein stimulator in sedentary healthy adults: Rationale, design, and methodology. *Contemp. Clin. Trials Commun.* **2018**, 11, 10–19.

[3] Bland, J.M.; Altman, D.G. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet (London, England)*, **1986**, 1, 307–10.

RESULTS AND DISCUSION

SECTION 1

STUDY I

BACKGROUND

Indirect calorimetry using a metabolic cart is a noninvasive reference method for the determination of human resting metabolic rate (RMR) in healthy, non-critically ill, and ventilated individuals [1–3], as well as in both, clinical and research settings [4]. It uses measured oxygen consumption (VO_2) and carbon dioxide production (VCO_2) obtained during a relatively short period of time (between 5 – 30 min) [1], from which a shorter period of the recorded data is analyzed (normally 5 or 10 min) [5,6]. Respiratory exchange ratio (RER) is determined as VCO_2/VO_2 and it is used to calculate the net rate of carbohydrates and fat oxidation rates [7].

The Deltatrac (DTC) Metabolic Monitor (VIASYS Health-care Inc, SensorMedics, Yorba Linda, CA) was considered the gold standard. Its day-to-day biological reproducibility was <4% (expressed as day-to-day coefficient of variation [$CV_{D\text{-}to\text{-}D}$]) in healthy individuals, which is thought to be comparable with the individuals' day-to-day physiological variation in RMR [8], while the biological reproducibility on RER values was low [9,10]. Nevertheless, the DTC is no longer manufactured [8,10–12] and, currently, there is no recognized gold standard metabolic cart [12], despite some suggest the Vmax Encore 2900 as the new gold standard [13]. Most of the commercially available metabolic carts have shown a day-to-day biological reproducibility above 10% in RMR assessments which could be clinically unacceptable [8]. Of note, there are no clear reasons for such high variability [8].

Achieving high day-to-day biological reproducibility is a key factor for being able to analyze the magnitude of change in RMR, for instance after an intervention [10]. It is also important to know the difference between metabolic carts (i.e. comparability) to be able to compare the RMR obtained with different equipment. Although some metabolic carts seem more reliable than others [12], studies analyzing this issue sometimes used different cohorts and metabolic carts. This could indicate that such RMR inter- and intra-day differences were attributable to biological differences of participants [3,8,14,15], instead of to the metabolic cart. Therefore, there is a need to better understand whether some metabolic carts are more reliable than others. If so, it is mandatory to identify the most commercially available reliable metabolic cart.

The aims of this study were: i) to determine the comparability of RMR and RER measurements with two commercially available breath-by-breath (B×B) metabolic carts, i.e. the CCM Express (CCM) and the Ultima CardiO2 (MGU) (Medgraphics Corp, Minnesota, USA) in young adults; and ii) to analyze the day-to-day biological reproducibility of RMR and RER measurements with the CCM and the MGU metabolic carts.

METHODS OVERVIEW

Subjects

Seventeen (11 women) healthy young adults aged 18-26 years participated in the study (see **Table 4**). All the participants met the following criteria: i) being non-physically active (less than 20 minutes of physical activity 3 days/week); ii) having a stable body weight (maximum changes of 3 kg) over the last 3 months; iii) not being enrolled in a weight loss program; iv) being non-smokers; v) not taking any medication; vi) not having acute or chronic illness; and vii) not being pregnant. All these criteria were confirmed by the participants. The study protocol and informed consent were performed in accordance with the Declaration of Helsinki (revision of 2013), and was approved by the Human Research Ethics Committee of both University of Granada (n°924) and Servicio Andaluz de Salud (Centro de Granada, CEI-Granada). Before their enrollment, all participants signed an informed consent.

Indirect Calorimetry assessment

The study was conducted between February and April 2016. A repeated-measures design was used over 2 consecutive days. RMR measurements were conducted between 7.30 AM and 11 AM, and the measurement hour for each participant was replicated. Participants arrived at the research center by car or by bus (they had to avoid any physical activity) and in fasting conditions (at least 8 hours). Also, they were advised to refrain from any physical activity both moderate (24 hours) and vigorous intensity (48 hours) before the RMR measurements.

On both days, RMR measurements were performed during two consecutive 30-minute periods with two different metabolic carts: the CCM and the MGU (Medgraphics Corp, Minnesota, USA). A neoprene face-mask equipped with a directconnect™ metabolic flow sensor (Medgraphics Corp, Minnesota, USA) was used for gases collection. The device order was counterbalanced between participants and the same order was replicated on both testing days. Both B×B metabolic carts use the same non-dispersive infrared analyzer for VCO_2 (resolution ±0.1%) and a galvanic fuel cell for VO_2 (accuracy <1%; resolution ±0.1%) [3,15], and both measures the percent concentration of VCO_2 and VO_2 along with the calculated flow rate. From these, the VO_2 and VCO_2 are calculated by the Breeze Software (MGCDiagnostic®, Breeze Suite 8.1.0.54 SP7). Flow calibration was performed using a 3-L calibration syringe at the beginning of every testing day, and gas analyzers were calibrated before each measurement using 2 standard gas concentrations (Calibration gas: CO_2, 5%±0.02% absolute; O_2, 12%±0.02% absolute; Nitrogen, balance. Reference gas: O_2, 21%±0.02% absolute; Nitrogen, balance) following the manufacturers' instructions.

The measurement of indirect calorimetry was performed following the recommended guidelines for RMR assessment [16]. Briefly, RMR was measured in the same quiet room with dim lighting, with controlled ambient temperature (22-24°C) and humidity (35-45%) conditions, and by the same trained researcher. On each testing day, before performing the measurements, participants had to confirm that they met the aforementioned study conditions, and then, they lay on a reclined bed in a supine position for a minimum of 20 minutes prior the RMR measurement as an adaptation period. Participants were covered by a sheet during the measurements. Furthermore, they were instructed to breathe normally, and not to fidget, talk, or sleep while measurements were being taken. The same instructions were followed during the two 30-minute RMR measurements. The time interval between the two measurements was 5 minutes, and during this time, participants stayed in bed.

On the first day, participants' weight and height were measured without shoes and with light clothing using a Seca scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany).

Obtained gases data were automatically averaged every minute by the Brezee (MGCDiagnostic®, Breeze Suite 8.1.0.54 SP7) software. For data selection, we calculated the coefficient of variance (CV) of VO_2, VCO_2, RER, and minute ventilation (VE) for every 5-minute period after discarding the first 5 minutes (i.e. from 6th to 10th, from 7th to 11th, etc.). Later, we selected the 5-minute period that met the most of the steady state criteria (i) CV <10% for VO_2, ii) CV<10% for VCO_2, iii) CV<10% for VE, and iv) CV<5% for RER) and that presented the lowest average between CVs of VO_2, VCO_2, VE, and RER. RMR average (calculated using the Weir equation [17]) and RER values of this period were included in further analysis. 17 RMR valid measurements were performed with the MGU metabolic cart and 16 with the CCM metabolic cart. The exclusion of the participant with the CCM metabolic cart was due to problems in the measurement protocol.

Statistical analyses

Analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 21.0, IBM SPSS Statistics, IBM Corporation), and the level of significance was set to <0.05. We used the Bland-Altmant method [18] to analyze comparability and day-to-day biological reproducibility of the CCM and MGU metabolic carts. To analyze comparability (aim 1), RMR and RER data obtained with the CCM were subtracted from those measured with the MGU. Positive differences would indicate that the MGU values were higher than those obtained with the CCM. To study day-to-day biological reproducibility (aim 2), day 1 measurements were subtracted from day 2; so, a positive difference would indicate that measurements on day 2 were higher than on day 1. In order to analyze whether the error changes as the magnitude of the measure changes, we studied heteroscedasticity. We conducted linear regression analysis by using the mean RMR or RER values and the absolute values of the RMR and RER differences.

A two-way (Metabolic cart × day) analysis of variance (ANOVA) was conducted to analyze systematic bias between metabolic carts (comparability) and between days (day-to-day biological reproducibility).

To study differences on day-to-day biological reproducibility between metabolic carts (aim 2), we compared the absolute value of inter-day differences in RMR and RER values (e.g. |RMR Day1 – RMR Day2|) obtained with the CCM and the MGU using 2-sided paired *t*-tests. Finally, we studied the association between the day-to-day biological reproducibility achieved with both metabolic carts. We conducted linear regression analysis using the absolute value of inter-day differences in RMR and RER values obtained with both metabolic carts.

RESULTS

Table 4 shows the descriptive characteristics of participants.

	Day 1		**Day 2**	
Age (years)	23.2	(1.7)		
Height (cm)	168	(9)		
Weight (kg)	63.2	(11.5)		
BMI (kg/m^2)	22.4	(2.6)		
CCM – RMR (kcal/day)	1239	(289)	1292	(195)
CCM – RER	0.83	(0.04)	0.85	(0.05)
MGU – RMR (kcal/day)	1297	(361)	1372	(320)
MGU – RER	0.85	(0.03)	0.84	(0.05)

Data are presented as mean and (standard deviation). BMI: Body mass index. RMR: Resting metabolic rate. RER: Respiratory exchange ratio.

Comparability

Figure 18 shows Bland and Altman plots comparing RMR (**panels A** and **B**) and RER (**panels C** and **D**) values obtained with the CCM and MGU metabolic carts on study day 1 and 2. RMR values were higher with the MGU than with the CCM (Day 1: 1303±372 kcal/day and 1238±289 kcal/day, respectively; mean difference: 65±161 kcal/day; 12±7.6%; Day 2: 1385±326 kcal/day and 1291±194 kcal/day, respectively, mean difference: 94±161 kcal/day; 10±6.3%; two-way ANOVA P=0.021). There were no differences on RER values obtained by both metabolic carts (two-way ANOVA P=0.642). We did not detect heteroscedasticity on day 1 in RMR (β=0.105; P=0.114) and RER (β=-0.225; P=0.30) nor on day 2 in RER (β=0.397; P=0.197). Nevertheless, we observed heteroscedasticity on day 2 RMR (β=0.362; P<0.001) measurements (**Figure 18B**).

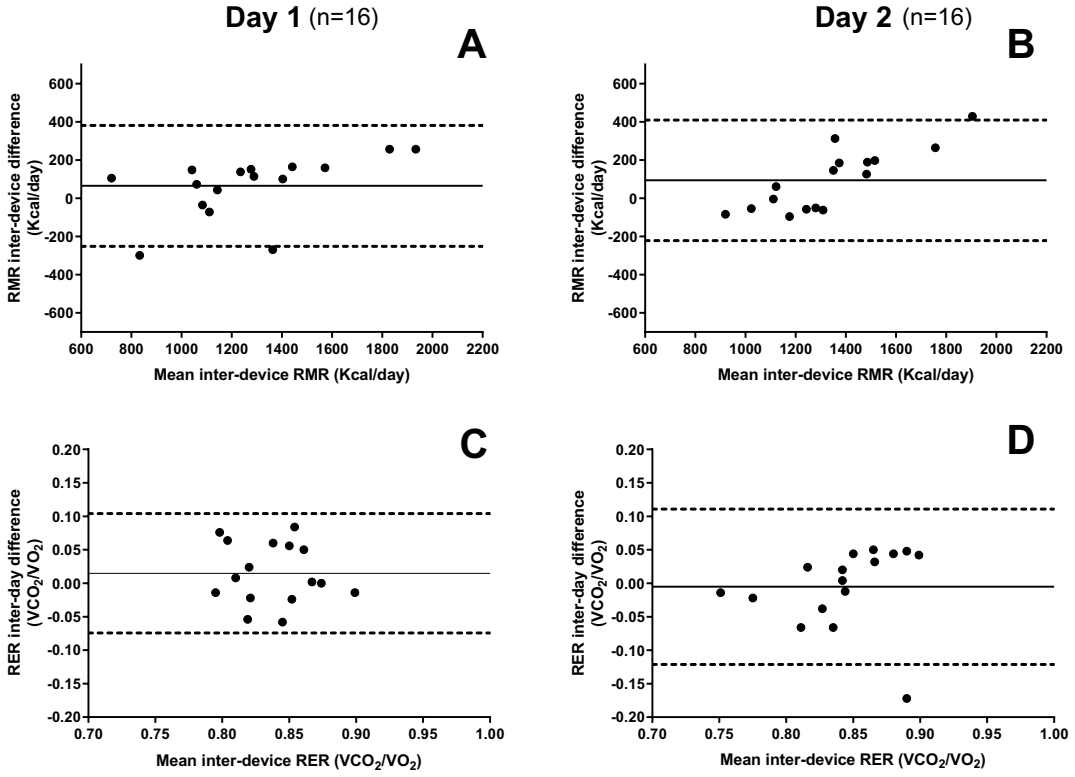


Figure 18. Bland-Altman plots for inter-devices (panel A and B) resting metabolic rate (RMR) and respiratory exchange ratio (RER) comparisons (panel C and D) on Day 1 and Day 2.

Day-to-day biological reproducibility

Figure 19 shows Bland and Altman plots comparing RMR and RER values obtained on day 1 and day 2 with both metabolic carts. There was no significant day effect on the two-way ANOVA, meaning there was no systematic bias when comparing RMR or RER Day 1 vs. Day 2 measurements (all P>0.25). There was no heteroscedasticity neither in the MGU RMR (β=-0.680; P=0.110, **Figure 19B**) and RER (β=-0.465; P=0.133, **Figure 19D**), nor in the CCM RMR (β=-0.488; P=0.198, **Figure 19A**). Nevertheless, we found heteroscedasticity on the CCM RER (β=0.889; P=0.004, **Figure 19C**) measurements. Absolute day-to-day RMR differences obtained with the MGU were higher (i.e. less reproducible or reliable) than those obtained with the CCM (219±185 vs. 158±154 kcal/day, respectively, P=0.002; and 18.3±17.2% vs. 13.5±15.3% respectively, P=0.046; **Figure 20A**). There were no differences on RER day-to-day differences between the metabolic carts (P=0.871; **Figure 20B**).

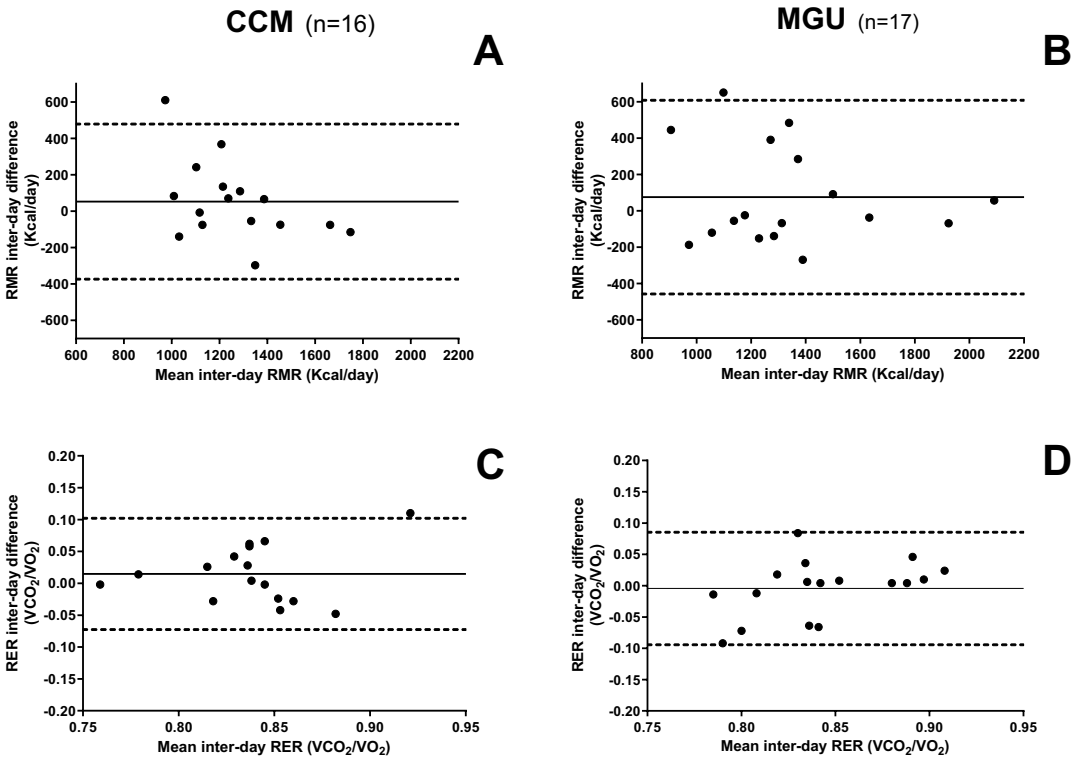


Figure 19. Bland-Altman plots for resting metabolic rate (RMR) and respiratory exchange ratio (RER) on Day 1 and Day 2 with the CCM (panel A and C) and MGU (panel B and D) metabolic carts.

There was a strong association between day-to-day biological reproducibility achieved with the CCM and MGU metabolic carts (β=0.717, R^2=0.743, P<0.001, **Figure 20C**), which persisted when using day-to-day percentages instead of absolute values (β=0.962, R^2=0.735, P<0.001). On the other hand, there was no association between absolute day-to-day differences in RER obtained with both metabolic carts (β=-0.002, R^2<0.001, P=0.992, **Figure 20D**).

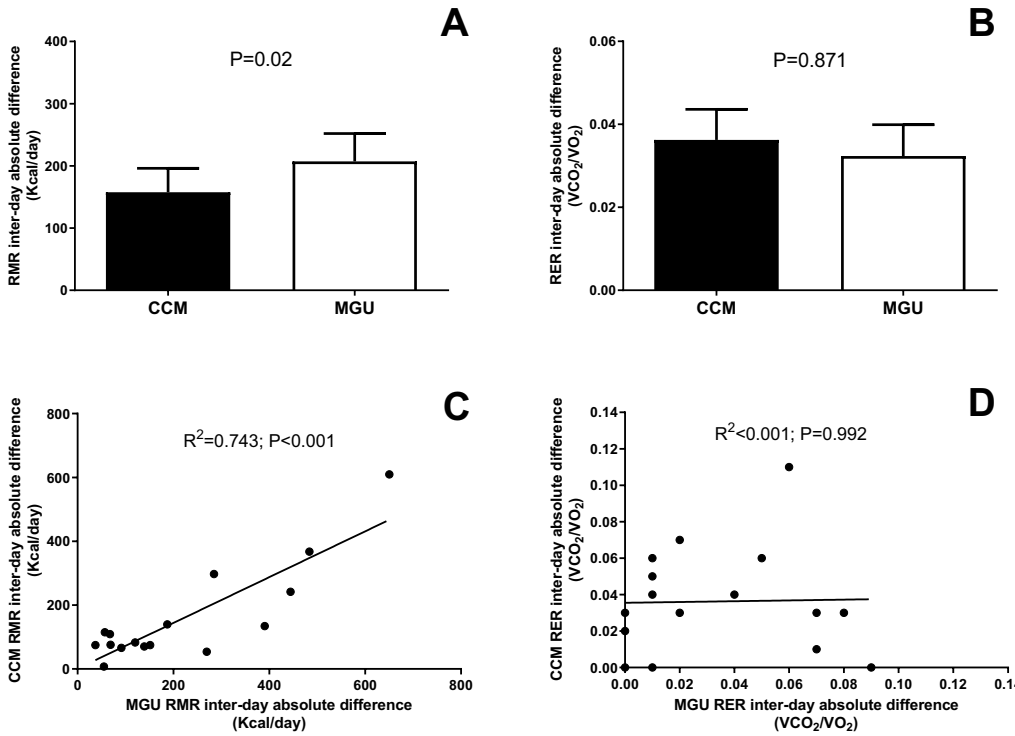


Figure 20. Comparisons (panel A and B) and associations (panel C and D) of inter-day differences between CCM and MGU metabolic carts.

DISCUSSION

The aims of this study were to analyze the comparability and day-to-day biological reproducibility of RMR and RER measurements with the CCM and MGU commercially available metabolic carts in young adults. Firstly, we observed that the MGU RMR values were higher than those obtained with the CCM metabolic cart, yet no differences in RER values were found. Secondly, there was no systematic bias when comparing RMR or RER Day 1 and Day 2 measurements with both metabolic carts, however, the results suggest that the CCM metabolic cart is more reliable than the MGU metabolic cart. We also observed a very significant association of day-to-day differences in RMR with both metabolic carts. Such association suggests that the individual's biological variability has a greater impact on RMR biological reproducibility than the metabolic cart used. Consequently, caution must be taken when different cohorts are studied or compared, or when the results from intervention studies are analyzed.

Comparability

RMR is defined as the energy needed for maintaining the vital signs, normal body functions, and homeostasis at resting state over a 24-hour period [16]. Therefore, RMR is virtually the lowest energy expenditure obtained in an individual awake during the resting assessment. Our results show that the RMR values obtained with the CCM metabolic cart were lower (10-12%) than those obtained with the MGU metabolic cart. We cannot discard however that the CCM underestimates RMR compared with the MGU. These differences are surprising taking into account that both metabolic carts use the same VO_2 galvanic fuel cell and the same VCO_2 non-dispersive infrared analyzer, and also, both are manufactured by the same

company (Medgraphics Corp). The observed differences in RMR, but not in RER, could be due to differences in the flow created by the constants from the calibration. There might be some inherent issues during the flow calibration whereby one of the metabolic carts has slightly different calibration constants for flow vs. the other. Unfortunately, we cannot know which metabolic cart is providing more accurate values as no gold standard was used.

Several studies analyzed the validity of the CCM and MGU metabolic carts against the DTC. Sundström et al. [15] compared the DTC, the Quark RMR (Cosmed, Italy), and the CCM metabolic cart, and showed that RMR measured with the CCM was 64% higher compared with the DTC (2876±656 vs. 1749±389 kcal/day for CCM and DTC respectively). In addition, they reported disagreements higher than 1000 kcal/day between the CCM and the DTC in some participants, which could be explained either by the overestimation of the CCM compared with the DTC or by a measurement error (i.e. air leaks). Graf et al. [11] used the same metabolic carts (DTC, Quark RMR, and CCM) and also found differences between the CCM and the DTC. They showed that the CCM overestimated the RMR compared with the DTC.

Cooper et al. [12] showed that the MGU (older model than the one used in our study) also overestimated RMR compared with the DTC metabolic cart. They found significant differences in the comparison between metabolic carts (P=0.02) and the values for within subject $CV_{D\text{-}to\text{-}D}$ was 11.1% and for within subject difference (kcal/day) was 107±180. In another study, Black et al. [3] compared the MGU vs. the DTC and the MGU vs. the Douglas bag. They found that when the MGU was compared with the DTC wider limits of agreement were obtained, as well as high random errors for VO_2 (41%), VCO_2 (31%), and RMR (37%). Moreover, they presented data about the precision for each previous parameter comparing the MGU vs. the DTC. The results obtained were -56 to 35 ml min-1, -0.6 to 68 ml min-1, and -249 to 305 kcal/day for VO_2, VCO_2, and RMR respectively, and they concluded that the MGU was not an accurate metabolic cart due to the differences obtained in their study.

It is important to note that the CCM and the MGU are B×B metabolic carts while the DTC uses a mixing chamber technique [3]. The B×B technique samples the concentrations of VO_2 and VCO_2 at each breath and, afterwards, the metabolic cart averages and calculates the data obtained over time [19]. This technique is supposed to be more effective in cases with problems of incomplete mixing of inspired gas (i.e. mechanically ventilated patients), unstable fraction of inspired oxygen, effects of water vapor, and dead space (no exchange of gases) [19]. On the other hand, with the mixing chamber technique the expired gas is directed into the mixing chamber, and the metabolic cart samples the gas collection at a factory-selected intervals (normally ranged from 1 to 5 minutes) [20]. This system is supposed to work better for steady-state RMR assessments (i.e. non-critically ill patients) [20]. Therefore, these differences in data sampling and processing could partially explain the reported B×B vs. mixing chamber differences.

Day-to-day biological reproducibility

We observed no systematic bias on RMR and RER day-to-day biological reproducibility with both CCM and MGU metabolic carts (**Figure 19**), yet the absolute inter-day RMR differences were lower in the CCM. Cooper et al. [12], in the previously mentioned study, reported a day-to-day biological reproducibility MGU $CV_{D\text{-}to\text{-}D}$ ranging from 4.8 to 10.9%, whereas the difference was higher in our study (18.3±17.2%). Differences between studies could be explained by the participants' characteristics in Cooper et al. [12] (n=12, 7 women; age: 24±11 years old; BMI: 21.8±2.1 kg/m^2) and by the biological variability instead of the metabolic cart. Black et al. [3] also showed similar results to those reported by Cooper et al. [12] in terms of agreement for RMR assessed with the MGU metabolic cart. However, this study was conducted in mechanically lung ventilated patients and day-to-day biological reproducibility (i.e. $CV_{D\text{-}to\text{-}D}$) for RMR measurement were not reported. They also found that 3 of 39 measurements (7%) were unusable due to unstable VO_2 [3]. Conversely, in our study, no measurements were

discarded. However, as previously mentioned, caution must be taken comparing results between different cohorts.

Sundström et al. [15] analyzed the day-to-day biological reproducibility of the DTC, the Quark RMR, and the CCM metabolic carts in mechanically ventilated patients (n=24, 9 women; age: 36 to 79 years; BMI: 18.3 to 43.1 kg/m^2). According to their study, the $CV_{D\text{-}to\text{-}D}$ in RMR for the CCM was 7.9±8.6%, which was lower than the one obtained in our study ($CV_{D\text{-}to\text{-}D}$ of 13.5±15.3% in RMR). These differences could be partially explained by the participants' characteristics, as our participants were healthy, younger (23.2±1.7 years), and with lower BMI (22.4±2.6 kg/m^2). As previously mentioned, caution is needed when comparing different cohorts. Furthermore, Graf et al. [11] compared the CCM (in canopy, face-tent and face-mask gases collection systems) with the previously mentioned metabolic carts (Quark and DTC) in adults (n=24, 15 women; age: 53±15 years; BMI: 25.5±7.1 kg/m^2). They found that RMR assessed with the face-mask (1626±336 kcal) or face-tent (1666±315 kcal) gas collection systems were lower than RMR measured with the canopy (1741±360 kcal). It was argued that such differences may have been related to air leaks inherent to the gases collection system, and unfortunately, no inter-gas collection system day-to-day variability was reported. In our study, we used face-mask gas collection system and results were similar on both days (1238±289; and 1291±194 kcal/day for Day 1 and 2, respectively).

Caution must be taken when comparing studies or cohorts due to the intra and inter-individual biological variability in RMR. Intra-individual variability in RMR refers mainly to the individual's biological variance, despite the fact that methodological variance cannot be excluded [21]. Inter-individual variability might be explained by age, sex, fat-free mass, or fat mass [22]. Therefore, whereas some studies argue that devices are not reliable (i.e. showed low day-to-day biological reproducibility) or that the results are in disagreement with others, it is plausible that such RMR inter-day differences are attributable to individuals [3,8,14,15] rather than to the metabolic carts. We observed a strong association of absolute inter-day differences in RMR measured with the CCM and the MGU (R^2=0.743, P<0.001, **Figure 20C**), which suggest that the observed inter-day variability might be greatly explained (74%) by the individual's biological variability. We observed a RMR $CV_{D\text{-}to\text{-}D}$ of 13% and 18% for the CCM and the MGU respectively, whereas others reported a range of 2-10% [23,24] in adults. Probably, these differences are due to the previously mentioned biological variability [9] (because different cohorts were studied), plus the protocol followed in each study for data collection (methodological variability). Taken together, these findings suggest that studies analyzing inter-day variability or biological reproducibility of RMR measurement should be conducted in the same cohort.

The results of the present study should be considered with caution, as there are some limitations. Our study was carried out in healthy young adults, and we do not know if our results apply to older or unhealthy individuals, or to other models of the CCM and MGU metabolic carts [6]. Also, we did not record the menstrual cycle and therefore its effect on RMR of our sample is unknown [25,26]. However, due the intra-individual design and the 24 hours test-retest it is likely that the potential effect of menstrual cycle on RMR day-to-day differences is negligible. We did not conduct a priory sample size calculations, yet despite having a relatively small sample size, it is similar to other studies with similar research questions, and it allowed us to observed statistical significant differences between devices.

CONCLUSION

The CCM metabolic cart provides lower RMR values and better day-to-day biological reproducibility than the MGU in our study population of young adults. Our findings also suggest that the individual's biological variability could had a greater impact on RMR than the metabolic cart used in the measurement.

REFERENCES

1. da Rocha, E.E.M.; Alves, V.G.F.; da Fonseca, R.B. V Indirect calorimetry: methodology, instruments and clinical application. *Curr. Opin. Clin. Nutr. Metab. Care* **2006**, *9*, 247–256.

2. Schadewaldt, P.; Nowotny, B.; Strassburger, K.; Kotzka, J.; Roden, M. Indirect calorimetry in humans: a postcalorimetric evaluation procedure for correction of metabolic monitor variability. *Am. J. Clin. Nutr.* **2013**, *97*, 763–73.

3. Black, C.; Grocott, M.P.W.; Singer, M. Metabolic monitoring in the intensive care unit: A comparison of the Medgraphics Ultima, Deltatrac II, and Douglas bag collection methods. *Br. J. Anaesth.* **2015**, *114*, 261–268.

4. Lam, Y.Y.; Ravussin, E. Indirect calorimetry: An indispensable tool to understand and predict obesity. *Eur. J. Clin. Nutr.* **2017**, *71*, 318–322.

5. Irving, C.J.; Eggett, D.L.; Fullmer, S. Comparing Steady State to Time Interval and Non-Steady State Measurements of Resting Metabolic Rate. *Nutr. Clin. Pract.* **2017**, *32*, 77–83.

6. Darren M. Roffey; Nuala M. Byrne; and Andrew P. Hills Day-to-Day Variance in Measurement of Resting Metabolic Rate Using Ventilated-Hood and Mouthpiece & Nose-Clip Indirect Calorimetry Systems. *J. Parenter. Enter. Nutr.* **2006**, *30*, 426–432.

7. Simonson, D.C.; DeFronzo, R.A. Indirect calorimetry: methodological and interpretative problems. *Am. J. Physiol.* **1990**, *258*, E399-412.

8. Schoeller, D.A. Making Indirect Calorimetry a Gold Standard for Predicting Energy Requirements for Institutionalized Patients. *J. Am. Diet. Assoc.* **2007**, *107*, 390–392.

9. Acheson, K.J. Indirect calorimetry: a case for improved standard operating procedures. *Eur. J. Clin. Nutr.* **2014**, *68*, 1–1.

10. Kennedy, S.; Ryan, L.; Fraser, A.; Clegg, M.E. Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis. *J. Nutr. Sci.* **2014**, *3*.

11. Graf, S.; Karsegard, V.L.; Viatte, V.; Maisonneuve, N.; Pichard, C.; Genton, L. *Comparison of three indirect calorimetry devices and three methods of gas collection: A prospective observational study*; **2013**; Vol. 32;.

12. Cooper, J.A.; Watras, A.C.; O'Brien, M.J.; Luke, A.; Dobratz, J.R.; Earthman, C.P.; Schoeller, D.A. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J. Am. Diet. Assoc.* **2009**, *109*, 128–32.

13. Rising, R.; Whyte, K.; Albu, J.; Pi-Sunyer, X. Evaluation of a new whole room indirect calorimeter specific for measurement of resting metabolic rate. *Nutr. Metab. (Lond).* **2015**, *12*, 46.

14. Bader, N.; Bosy-Westphal, A.; Dilba, B.; Müller, M.J. Intra- and interindividual variability of resting energy expenditure in healthy male subjects – biological and methodological variability of resting energy expenditure. *Br. J. Nutr.* **2005**, *94*, 843–849.

15. Sundström, M.; Tjäder, I.; Rooyackers, O.; Wernerman, J. Indirect calorimetry in mechanically ventilated patients. A systematic comparison of three instruments. *Clin. Nutr.* **2013**, *32*, 118–121.

16. Fullmer, S.; Benson-Davies, S.; Earthman, C.P.; Frankenfield, D.C.; Gradwell, E.; Lee, P.S.P.; Piemonte, T.; Trabulsi, J. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J. Acad. Nutr. Diet.* **2015**, *115*, 1417-1446.e2.

17. Weir, J. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **1949**, *109*, 1–9.

18. Bland, J.M.; Altman, D.G. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet (London, England)* **1986**, *1*, 307–10.

19. Branson, R. The measurement of energy expenditure: Instrumentation, practical considerations, and clinical application. *Respir. Care* **1990**, *35*, 640–659.

20. Matarese, L.E. Indirect calorimetry: Technical aspects. *J. Am. Diet. Assoc.* **1997**, *97*, S154–S160.

21. Müller, M.J.; Bosy-Westphal, A.; Klaus, S.; Kreymann, G.; Lührmann, P.M.; Neuhäuser-Berthold, M.; Noack, R.; Pirke, K.M.; Platte, P.; Selberg, O.; et al. World Health Organization equations have shortcomings for predicting resting energy expenditure in persons from a modern, affluent population: generation of a new reference standard from a retrospective analysis of a German database of resting energy expe. *Am. J. Clin. Nutr.* **2004**, *80*, 1379–90.

22. Ravussin, E.; Lillioja, S.; Anderson, T.E.; Christin, L.; Bogardus, C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J. Clin. Invest.* **1986**, *78*, 1568–1578.

23. Soares, M.J.; Shetty, P.S. Intra-individual variations in resting metabolic rates of human subjects. *Hum. Nutr. Clin. Nutr.* **1986**, *40*, 365–9.

24. Weststrate, J.A. Resting metabolic rate and diet-induced thermogenesis: a methodological reappraisal. *Am. J. Clin. Nutr.* **1993**, *58*, 592–601.

25. Melanson, K.J.; Saltzman, E.; Russell, R.; Roberts, S.B. Postabsorptive and postprandial energy expenditure and substrate oxidation do not change during the menstrual cycle in young women. *J. Nutr.* **1996**, *126*, 2531–8.

26. Ferraro, R.; Lillioja, S.; Fontvieille, A.M.; Rising, R.; Bogardus, C.; Ravussin, E. Lower sedentary metabolic rate in women compared with men. *J. Clin. Invest.* **1992**, *90*, 780–784.

RESULTS AND DISCUSION

SECTION 1

STUDY II

BACKGROUND

Resting metabolic rate (RMR) is defined as the energy expenditure needed for maintaining normal body functions and homeostasis while an awake person is resting in thermoneutrality [1], and usually accounts for 60-70% of the total daily energy expenditure [2]. Indirect calorimetry is the reference method for assessing human RMR [3–5] via measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). In addition to RMR, indirect calorimetry also allows to determine the respiratory exchange ratio (RER), i.e. the VCO_2-to-VO_2 ratio, which gives valuable information about the type of energy substrates (fat vs. carbohydrate) being metabolized [6–8]. Metabolic carts are the most used indirect calorimeters for assessing RMR and RER. The Deltatrac metabolic cart (DTC; Datex Instrumentarium Corp, Helsinki, Finland) has been for long considered the gold standard for assessing RMR and RER. However, the DTC is no longer manufactured [3,9–13], and no other metabolic cart has been recognized yet as the new gold standard [12]. Consequently, there is a need for identifying a valid metabolic cart that can be considered as the new gold standard by the scientific community.

To identify a new reference metabolic cart, three approaches can be used [14]: (i) assessing its accuracy (i.e. the proximity of measurements to traceable standards [15]) and precision (i.e. the variability in repeated measures of the same quantity [15]) by alcohol burning tests, (ii) assessing its accuracy and precision by controlled pure gas (N_2 and CO_2) infusions and (iii) assessing its biological reproducibility (i.e. the similarity between in vivo measurements performed under the same conditions in different moments). Many of the currently commercially available metabolic carts have shown unacceptable accuracy, precision and/or biological reproducibility for RMR and/or RER assessment [9,16]. In contrast, the DTC typically presented high accuracy, precision and biological reproducibility (day-to-day coefficient of variation [$CV_{D\text{-}to\text{-}D}$] of < 4% [4,9–12,17,18], thought to be close to the RMR physiological variability) [19–22]. Of note, most previous studies examining the accuracy and/or precision of different metabolic carts have not compared them within the same settings and conditions (e.g. [12]) or have not used recently manufactured metabolic carts (e.g. [16]), which might bias the results due to the deterioration of the systems. On the other hand, comparing the biological reproducibility achieved by different metabolic carts must be done within the same cohort and conditions, as the individuals' characteristics have considerable influence on the RMR biological reproducibility [13].

Importantly, to improve RMR and RER measurements, overcoming some of the limitations of metabolic carts, a post-calorimetric correction procedure was proposed by Schadewaldt et al. [3]. In brief, this post-calorimetric correction procedure consists of simulating the subject's VO_2 and VCO_2 by infusing pure gases (N_2, for diluting ambient O_2, and CO_2), using high-precision mass-flow controllers, immediately after the subject's indirect calorimetry testing [3]. The subject´s VO_2 and VCO_2 can then be 'corrected' by the measured metabolic cart error (i.e. the difference between the infused gases and the readouts of the metabolic cart) [3]. Crucially, the application of this post-calorimetric correction procedure significantly improved the biological reproducibility and the comparability (i.e. how similar are the measurements obtained by different devices) of RMR and RER measured by two metabolic carts (DTC and Vmax Encore 29n [SensorMedics, Yorba Linda, CA, USA]; another no longer manufactured metabolic cart) [3]. This procedure also improved the post-prandial RER measured by the Vmax Encore 29n [23]. However, whether the application of this post-calorimetric correction procedure improves the biological reproducibility and comparability of RMR and RER using diverse commercially available metabolic carts remains to be determined.

The present study was designed to determine the accuracy and precision (by alcohol burning and pure gas infusions), and the biological reproducibility and comparability (in young healthy adults) of RMR and RER assessments provided by four commercially available, and recently manufactured, metabolic carts [the Q-NRG (Cosmed, Rome, Italy); the Vyntus

CPX (Jaeger-CareFusion, Höchberg, Germany; thereinafter called *Vyntus*); the Omnical (Maastricht Instruments, Maastricht, The Netherlands); and the Ultima CardiO2 (Medgraphics Corporation, St. Paul, MN, USA; thereinafter called *Ultima*)]. Further, we assessed whether the post-calorimetric correction improves the biological reproducibility and comparability of RMR and RER assessments yield by the four metabolic carts.

METHODS OVERVIEW

Metabolic carts and procedures

Detailed information and characteristics of the metabolic carts are presented in **Table 5**.

Table 5. Metabolic carts information and characteristics.

Metabolic cart	Calibration gases concentration		Gas analyzers		Flow sensor	Flow rate calibration method	Calibration frequency	Subjects' gases collection system	Minimum data frequency
	O_2	CO_2	O_2	CO_2					
Q-NRG	16%	5%	Galvanic fuel cell	Non-dispersive infrared	Bidirectional digital turbine	3 L syringe push and pull motion calibration	Monthly	Ventilated plastic canopy equipped with an anti-bacterial filter	30 seconds
Vyntus CPX	16%	5%	Fully digital, high speed analyzer, based on electrochemical principle	Fully digital, high speed analyzer based on principle of infrared absorption	Digital Volume Transducer flow sensor technology	Automated volume and flow calibration (rates of 0.2 and 2.0 L/s)	Every testing day	Ventilated disposable plastic canopy equipped with an anti-bacterial filter	10 seconds
Omnical	18%	0.8%	ABB H&B MAGNOS® dumbbell type paramagnetic[1]	ABB H&B URAS® infrared[1]	Unidirectional dry bellows flowmeter with digital counter	Automated and periodical flow and volume calibration	Every testing day	Ventilated plastic canopy	5 seconds
Ultima CardiO2	12%	5%	Galvanic fuel cell	Non-dispersive infrared	Bidirectional Pitot tube flow sensor	3 L syringe push and pull motion calibration	Every testing day	Face-tent equipped with an anti-bacterial filter and a PreVent® metabolic flow sensor[2]	10 seconds

Calibration frequency refers to the gas analyzers and flow rate calibrations; [1]ABB H&B MAGNOS® and ABB H&B URAS® are registered models/brands of the gas analyzers; [2]PreVent® is a registered metabolic flow sensor model (Medgraphics Corp, Minnesota, USA).

All the metabolic carts were calibrated (flow and gas analyzers) by the same researchers, strictly following the manufacturers' instructions. We conducted three experiments (see **Figure 21**): (i) methanol burning tests (hereinafter *alcohol burning*); (ii) controlled pure gas infusions (hereinafter *gas infusions*); and, (iii) in vivo assessment in young healthy adults (hereinafter *human study*).

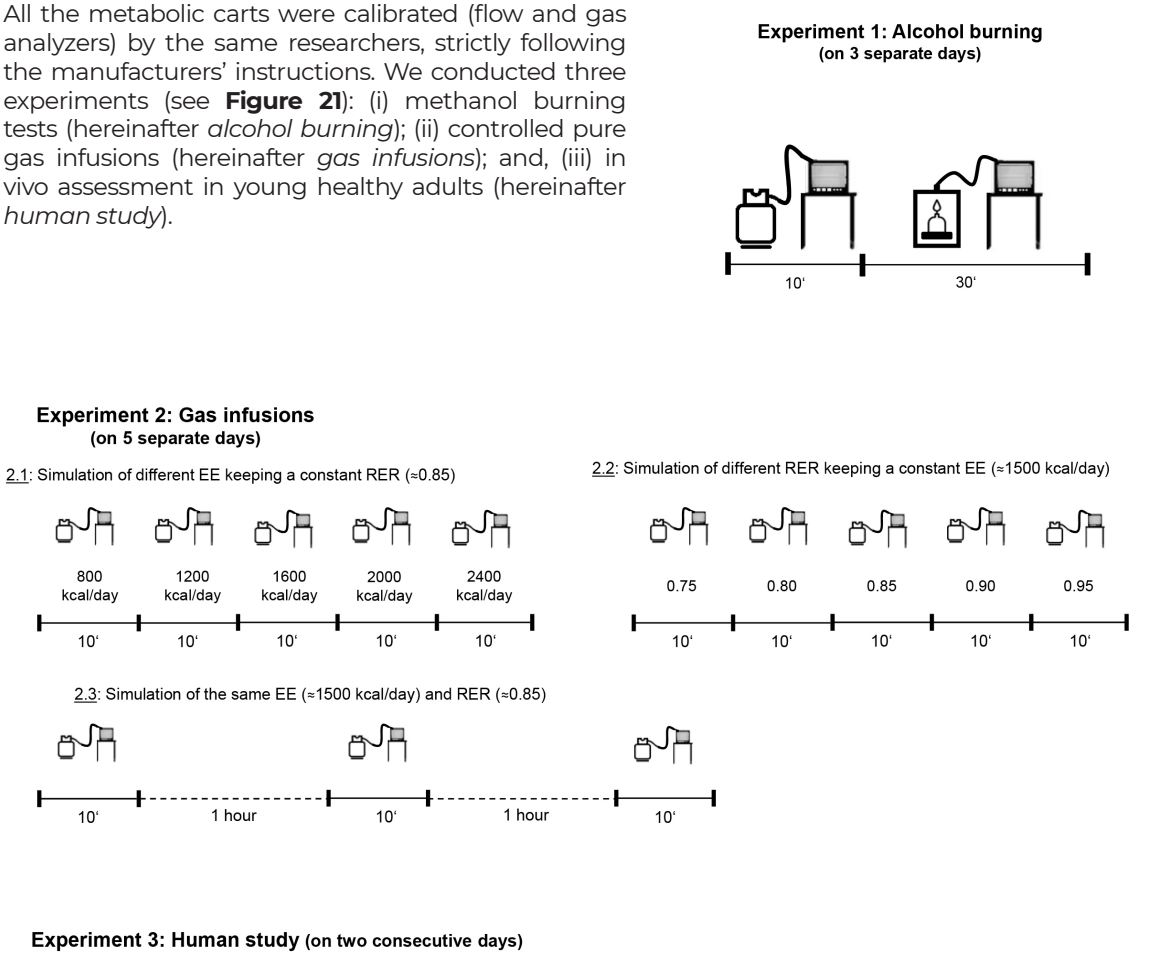


Figure 21. Study design. *Experiment 1 refers to the assessment of accuracy and precision using methanol burning tests; experiment 2 refers to the assessment of accuracy and precision using controlled gas infusions; experiment 3 refers to the assessment of biological reproducibility and comparability (with and without using the post-calorimetric correction) in young healthy adults. EE: energy expenditure; RER: respiratory exchange ratio.*

Experiment 1: Alcohol burning

On 3 separate days, we conducted methanol [purity ≥ 99.9% and water ≤ 0.05 % (EMSURE® ACS, ISO, Reag. Ph Eur, Merck, Darmstadt, Germany)] burning tests. We lighted the flame of the wick burning kit inside a methanol burning glass cage (Maastricht Instruments, Maastricht, The Netherlands) and let the methanol burn for 30 minutes, while the produced gases were directed to the metabolic carts' hose tube. The methanol weight was dynamically recorded using a calibrated scale (model MS 1602TS/00 precision scale, precision 0.01 g; Mettler Toledo, Giessen, Germany). The *expected value* considered for both VO_2 and VCO_2 recoveries were 100%, while the *expected value* for RER was 0.667 based on the following reaction [16,24]:

$$2CH_3OH + 3O_2 \rightarrow 2CO_2 + 4H_2O$$

The first 5 minutes of the burning were discarded, and the remaining data were averaged for further analysis. Then, the *measurement error* and the *percentage measurement error* for VO_2 and VCO_2 were calculated as follow:

(i) Measurement error = measured value - expected value

$$\textit{(ii) Percentage measurement error} = \left(\frac{\textit{measurement error}}{\textit{expected value}} \right) x\ 100$$

In order to compare the methanol burning and gas infusions (**Figure 21**), the methanol burning were immediately preceded by 10-minute N_2 and CO_2 infusions (see extended methodology below) simulating VO_2 and VCO_2 achieved during previous methanol burning tests.

Experiment 2: Gas infusions

We employed 3 different approaches to test the accuracy and precision of the metabolic carts using the controlled pure gas infusion method [14] (**Figure 21**): *a)* we simulated energy expenditure (EE) of 800, 1200, 1600, 2000 and 2400 kcal/day while keeping RER constant (≈0.85); *b)* we simulated RER of 0.75, 0.80, 0.85, 0.90 and 0.95 while keeping EE constant (≈1500 kcal/day); *c)* we studied the intra-day precision at the same simulated EE (≈1500 kcal/day) and RER (≈0.85) in three occasions, 1-hour apart. In both *a* and *b*, the metabolic cart recording was not stopped between simulations.

The controlled pure gas infusions were performed using two high-precision mass-flow controllers (358 Series, Analyt-MTC, Müllheim, Germany; 0-2 l/min). One controller was used for infusing pure N_2 (purity ≥ 99.9997%; Carburos Metálicos/Air Products and Chemicals, Inc., Barcelona, Spain) and the other for infusing pure CO_2 (purity ≥ 99.995%; Carburos Metálicos/Air Products and Chemicals, Inc., Barcelona, Spain) directly into the hose tube of the metabolic cart [3]. N_2 infusion is used to dilute ambient O_2, and therefore, the simulated VO_2 can be calculated by using the following equation [25]:

$$VO_2\ (ml/min) = infused\ N_2\ (ml/min) \times 0.2646$$

The three approaches (**Figure 21**) were repeated in 5 different days. All controlled pure gas infusions lasted 10 minutes each. The first 5 minutes were discarded, and the remaining data were averaged and considered for further analysis. The *measurement error* and the *percentage measurement error* for VO_2 and VCO_2 were calculated as previously described in experiment 1's description.

Experiment 3: Human study

Subjects

Twenty-nine young healthy adults participated in the study (Table 6). The inclusion criteria were: (i) being older than 18 years old; (ii) having a body mass index between 18.5 and 40 kg/m^2; (iii) having a stable body weight over the last 3 months (changes ≤3 kg) and not being enrolled in a weight loss program; (iv) non-smokers; (v) under no medication that could directly affect energy metabolism; (vi) not suffering from chronic or acute illness; and (vii) not being pregnant. All these criteria were verbally confirmed by the participants. Both the study protocol and written informed consent followed the 2013 revised Declaration of Helsinki and were approved by the Human Research Ethics Committee of the University of Granada (#836).

Table 6. Subjects' characteristics.

	All (n=29)					Men (n=18)					Women (n=11)				
	Mean	±	SD	Min	Max	Mean	±	SD	Min	Max	Mean	±	SD	Min	Max
Age (years)	25	±	4.3	18	36	24.9	±	4.2	18	34	25.8	±	5.0	20	36
Body weight (kg)	71.2	±	7.5	45.6	99.2	77.1	±	10.6	63.3	99.2	60.1	±	8.0	45.6	72.4
Height (cm)	171.0	±	12.9	154.6	184.5	174.9	±	5.5	160.5	184.5	164.7	±	5.6	154.6	174.3
BMI (kg/m^2)	24.1	±	3.2	19.1	31.9	25.2	±	3.4	21.6	31.9	22.1	±	2.3	19.1	25.9
Waist circumference (cm)	77.3	±	9.2	59.8	97.6	81.2	±	8.5	70.0	97.2	70.7	±	6.0	59.8	85.0
Lean mass (kg)	48.9	±	10.8	30.5	66.6	56.1	±	5.9	42.2	66.6	36.9	±	4.0	30.5	41.8
Fat mass (kg)	17.9	±	7.0	8.6	36.7	16.7	±	7.6	8.6	36.7	19.9	±	5.3	10.8	28.5
Fat mass (%)	26.0	±	8.6	13.5	41.1	21.5	±	7.2	13.5	40.4	33.4	±	5.6	24.2	41.1

SD: Standard deviation; Min: Minimum; Max: Maximum; BMI: body mass index.

Anthropometric and body composition assessment

On the first visit, subjects' height and body weight were measured using a stadiometer and scale (Seca model 799, Electronic Column Scale, Hamburg, Germany) without shoes and with light clothing. Waist circumference was measured twice using a plastic tape while the subjects were in a standing position, and the average of both assessments was used. Body composition was assessed by whole-body dual-energy X-ray absorptiometry (Discovery Wi, Hologic, Inc., Bedford, MA, USA).

Indirect calorimetry assessment

The participants arrived at the research center by public transportation or motorized vehicle (avoiding any moderate or intense physical activity since they woke up) and confirmed having consumed the standardized *ad-libitum* meal plan (**Table S1**) during the preceding 24 h, which included consuming the standardized dinner 12 h before the start of the first indirect calorimetry assessment. Further, they refrained from both moderate (previous 24 h) and vigorous intensity (previous 48 h) physical activity. RMR and RER were assessed with each cart on two consecutive days in the morning between 9 am and Noon. The assessment lasted 30 minutes on each cart, with a 20-minute period between measurements. The order of the carts was randomly assigned and replicated on the second day (**Figure 21**).

The assessments were performed in agreement with current methodological recommendations [1]. Subjects stayed motionless on a reclined bed in the supine position covered by a bed sheet for a minimum of 20 minutes before the first indirect calorimetry assessment. Moreover, the subjects were asked to lay on the bed during the last 15 minutes of every period between measurements (**Figure 21**). Subjects were instructed not to sleep, talk, or fidget, and to breathe normally during the assessments.

Urine collection and analysis

Twelve-hour urine samples were collected before arriving to the research center. For that purpose, subjects were provided with two airtight 2 L polyethylene containers. They were instructed to collect their urine from dinner (9 pm) to the indirect calorimetry assessment start (9 am). Total urine volume and urea concentration (Spinreact, UREA-37_R1, Girona, Spain) were measured, and nitrogen urine levels were estimated using a regression equation (see below) previously computed in our laboratory in a separate sample of 19 young adults [26], where nitrogen urine levels were determined by the Kjeldahl method [27].

N (g/l) = 0.0065 x urea (mg/dl) + 1.2598

Post-calorimetric correction procedure

During the subject's RMR assessments, both VO_2 and VCO_2 metabolic carts readouts were averaged for ten minutes (from the 11th to the 20th minute, *subject's readout*). Immediately after the RMR assessment (without stopping the metabolic cart recording), N_2 and CO_2 were infused during 10 minutes into the metabolic cart hose tube in volumes mimicking the subject's VO_2 and VCO_2 averaged readouts (*expected values*). The VO_2 and the VCO_2 readouts during the last 5 minutes of infusion were also averaged (*measured values*). Then, the VO_2 and VCO_2 *corrected values* were calculated as follow.

Corrected values = subject´s readout x expected value / measured value

RMR and RER calculations

The VO_2 and the VCO_2 data from the indirect calorimetry assessment were downloaded from all metabolic carts at their minimum data frequency (**Table 5**). Later, the first and last 5 minutes data were discarded, and the remaining 20 minutes data were averaged using an Excel 2013® spreadsheet (Microsoft Corp, Redmond, WA, USA) and considered for further analyses. The RER was calculated as VCO_2/VO_2 for both the *uncorrected* and *corrected* VO_2 and VCO_2 data. Lastly, with both the *uncorrected* and *corrected* VO_2 and VCO_2 values, the RMR (i.e. *uncorrected* and *corrected* RMR) was calculated using the Weir abbreviated equation [28], where N is urinary nitrogen excretion (N was considered to be 0 in experiments' 1 and 2 calculations):

RMR (kcal/day) = (3.941 x VO_2 (L/min) + 1.106 x VCO_2 (L/min) - 2.17 x N (g/min)) x 1440

Statistical analysis

Results are presented as mean ± standard deviation, unless otherwise stated. Analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 22.0, IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA) and the level of significance was set at $P<0.050$. Figures were created using Graph Pad Prism (GraphPad Software, v. 8.4.1, CA, USA). All gas values are provided under standard temperature, pressure, and dry (STPD) conditions.

Alcohol burning

The absolute value of measurement errors in VO_2, VCO_2, EE and RER obtained by the methanol burning test was calculated (e.g. |*measured* VO_2 – *expected* VO_2|) for each metabolic cart and burn, and was later expressed as a percentage of the expected values (i.e. *percentage of absolute measurement error*). We compared the *percentage of absolute measurement error* using a one-factor repeated-measures analyses of variance (ANOVA; assuming sphericity). We considered ±2% as an acceptable accuracy criterion [16]. We also compared the *percentage measurement errors* in VO_2, VCO_2, EE and the *measurement error* in RER obtained by the methanol burning test and by the gas infusions using paired *t*-test analyses.

Gas infusions

Both, the *measurement error* and the *percentage measurement error* in EE and the measurement error in RER were compared using one-factor ANOVAs with *post-hoc* LSD Tukey comparisons.

Human study

For every participant and cart, the $CV_{D\text{-}to\text{-}D}$ (e.g. [standard deviation *uncorrected* VO_2 / mean *uncorrected* VO_2] × 100) were calculated for both *uncorrected* and *corrected* VO_2, VCO_2, RMR and RER values. We also calculated the absolute value of inter-day differences (e.g. |*uncorrected* VO_2 Day 1 – *uncorrected* VO_2 Day 2|). Then, two-factor (metabolic cart × correction) ANOVA with *post-hoc* LSD Tukey comparisons were used to test differences across metabolic carts in VO_2, VCO_2, RMR and RER $CV_{D\text{-}to\text{-}D}$ and the absolute value of inter-day differences (biological reproducibility). We also conducted ANOVAs to compare the VO_2, VCO_2, RMR and RER among the metabolic carts (comparability). Bland-Altman analyses [29] of both *uncorrected* and *corrected* VO_2, VCO_2, RMR and RER were also used to test biological reproducibility and comparability.

Lastly, we studied the associations of both *uncorrected* and *corrected* RMR with their classical predictors including body weight, lean and fat masses, and sex [30]. We conducted simple linear regression analysis to study the association between RMR and body weight (Model 1), and multiple linear regression to study the associations between RMR and lean mass, fat mass and sex (Model 2).

RESULTS

Alcohol burning

Despite of the observed trends (**Figure 22**), no statistically significant differences were found in the *percentage of absolute measurement errors* in VO_2, VCO_2, EE and RER (all P≥0.083; n=3). The mean *percentage of absolute measurement errors* in VO_2 (**Figure 22A**) were 1.7±0.5% for the Omnical (range: 1.2 to 2.2%), 3.3±1.8% for the Q-NRG (range: 1.3 to 4.8%), 15.2±12.9% for the Ultima (range: 3.7 to 29.2%) and 16.1±7.1% for the Vyntus (range: 8.0 to 21.2%). The mean *percentage of absolute measurement errors* in VCO_2 (**Figure 22B**) were 1.8±1.0% for the Omnical (range: 0.8 to 2.8%), 6.7±3.3% for the Q-NRG (range: 3.1 to 9.6%), 7.3±8.4% for the Ultima (range: 0.1 to 16.4%) and 13.3±3.0% for the Vyntus (range: 10.3 to 16.2%). The mean *percentage of absolute measurement errors* in EE (**Figure 22C**) were 1.6±0.4% for the Omnical (range: 1.2 to 2.0%), 3.5±1.2% for the Q-NRG (range: 2.3 to 4.7%), 14.0±11.9% for the Ultima (range: 4.0 to 27.2%) and 15.7±6.1% for the Vyntus (range: 8.9 to 20.4%). The mean *percentage of absolute measurement errors* in RER (**Figure 22D**) were 2.2±1.1% for the Omnical (range: 1.5 to 3.5%), 5.9±1.7% for the Vyntus (range: 4.2 to 7.4%), 6.6±0.8% for the Q-NRG (range: 6.1 to 7.5%), and 7.7±5.2% for the Ultima (range: 1.8 to 11.4%). Lastly, no differences were observed between the *measurement error* estimated by the methanol burning vs. the gas infusions (**Figure S1** and **Table S2**).

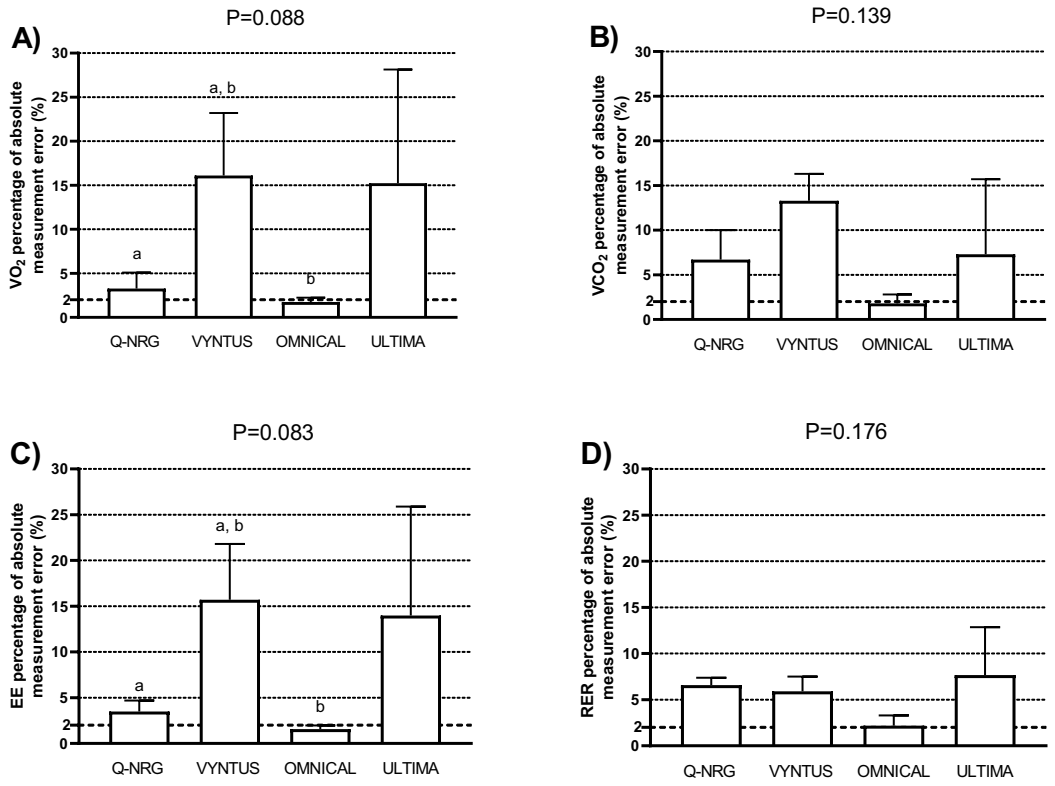


Figure 22. Percentage of absolute measurement error of oxygen consumption (VO_2; Panel A), carbon dioxide production (VCO_2; Panel B), energy expenditure (EE; Panel C) and respiratory exchange ratio (RER; Panel D) across metabolic carts, as determined by alcohol burning (i.e. methanol combustion). *The absolute value of measurement errors in VO_2, VCO_2, EE and RER obtained by the methanol combustion was calculated (e.g. |measured VO_2 – expected VO_2|) for each metabolic cart and burn, and was later expressed as a percentage of the expected values (percentage of absolute measurement error). P values from repeated measures analysis of variance (ANOVA, n=3). Identical indicatory letters represent significant differences as determined by post-hoc LSD Tukey analysis. Results are presented as mean and standard deviation.*

Gas infusions

The mean *percentage of absolute measurement errors* during the experiment 2 (i.e. mean of 50 gas infusions; combining approaches *a* and *b*) in VO_2 were 1.5±0.6% for the Omnical (range: 0.4 to 3.3%), 2.0±1.5% for the Q-NRG (range: 0.1 to 6.8%), 8.4±13.4% for the Ultima (range: 0.1 to 78.9%) and 12.4±4.4% for the Vyntus (range: 0.1 to 21.0%). The mean *percentage of absolute measurement errors* in VCO_2 were 1.2±1.2% for the Omnical (range: 0.1 to 4.8%), 5.0±4.1% for the Ultima (range: 0.1 to 26.8%), 6.2±1.8% for the Q-NRG (range: 3.5 to 10.6%) and 10.1±3.0% for the Vyntus (range: 1.2 to 14.6%). The mean *percentage of absolute measurement errors* in EE were 1.4±0.6% for the Omnical (range: 0.2 to 3.4%), 1.6±1.4% for the Q-NRG (range: 0.1 to 5.3 %), 7.3±10.0% for the Ultima (range: 0.4 to 57.9%) and 11.9±3.9% for the Vyntus (range: 1.4 to 18.6%). The mean *percentage of absolute measurement errors* in RER were 1.2±0.8% for the Omnical (range: 0.1 to 3.3%), 3.1±2.4% for the Vyntus (range: 0.2 to 11.3%), 5.9±7.7% for the Ultima (range: 0.2 to 43.0%) and 6.6±3.0% for the Q-NRG (range: 0.8 to 16.0%). The *measurement error* gradually increased in parallel to the simulated EE for the Q-NRG, the Vyntus and the Omnical (all P≤0.014, **Figure 23A-C**) metabolic carts, but not for the Ultima (P=0.134, **Figure 23D**). On the other hand, the *percentage measurement error* was not different (all P≥0.303) across simulated EEs for the Vyntus (**Figure 23F**), the Omnical (**Figure 23G**) and the Ultima (**Figure 23H**), but it was for the Q-NRG (P=0.005, **Figure 23E**) metabolic cart.

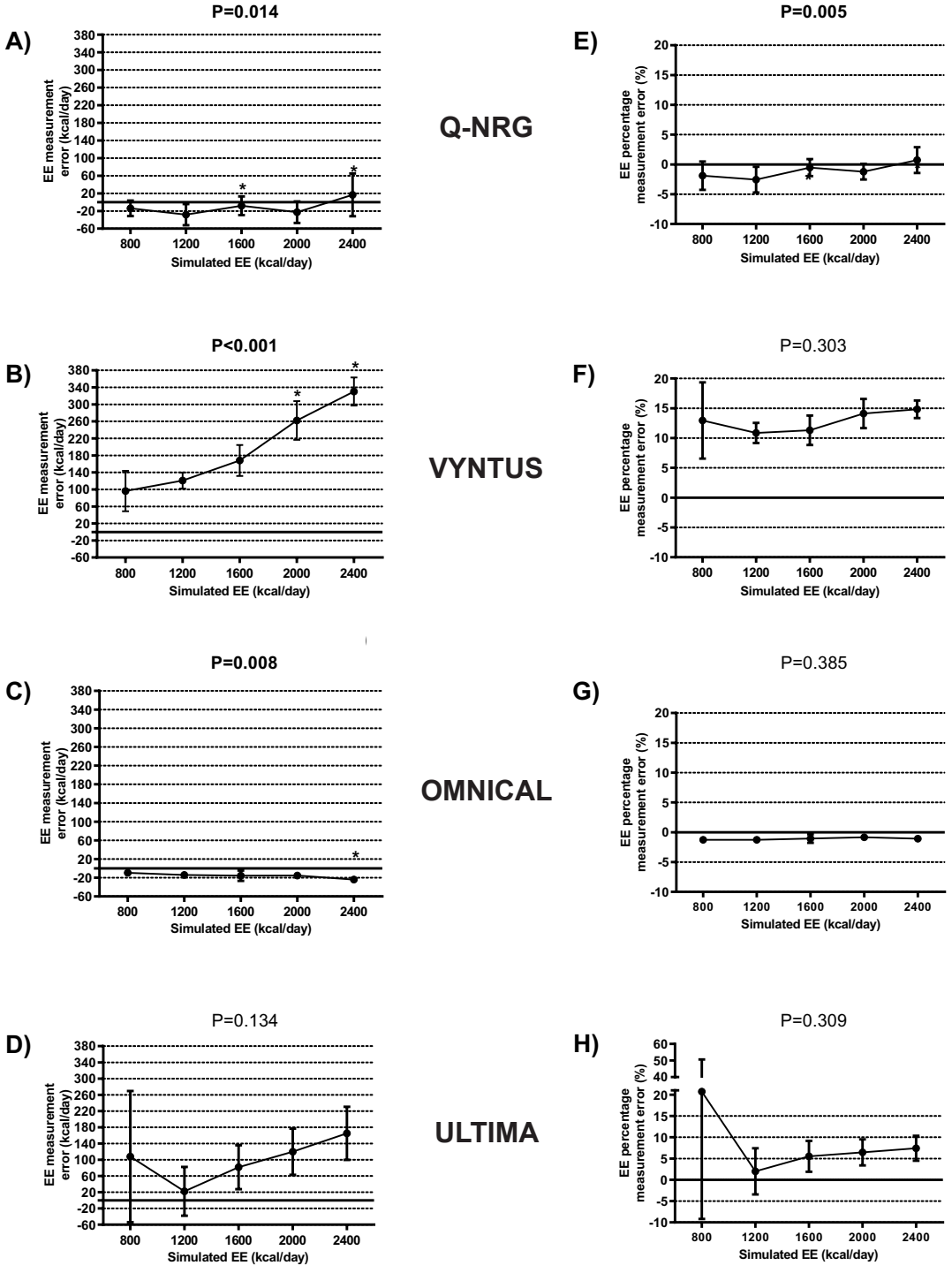


Figure 23. Measurement error of the four metabolic carts at different simulated energy expenditure (EE) by gas infusions. *Results are presented as measurement error (measured value – expected value) for Panels A-D, and as percentage measurement error ([measured value – expected value] / expected value) × 100 for Panels E-H. P values from repeated measures analysis of variance (ANOVA, n=5). * represent significant differences vs. the immediately lower simulated EE (LSD Tukey post-hoc test). Results are presented as mean and standard deviation.*

We observed that the *measurement error* was not different at different simulated RER for the Vyntus (**Figure 24B**) and the Omnical (**Figure 24C**) metabolic carts (both P≥0.136). In contrast, it was different for the Q-NRG (**Figure 24A**) and the Ultima (**Figure 24D**) metabolic carts (both P≤0.040).

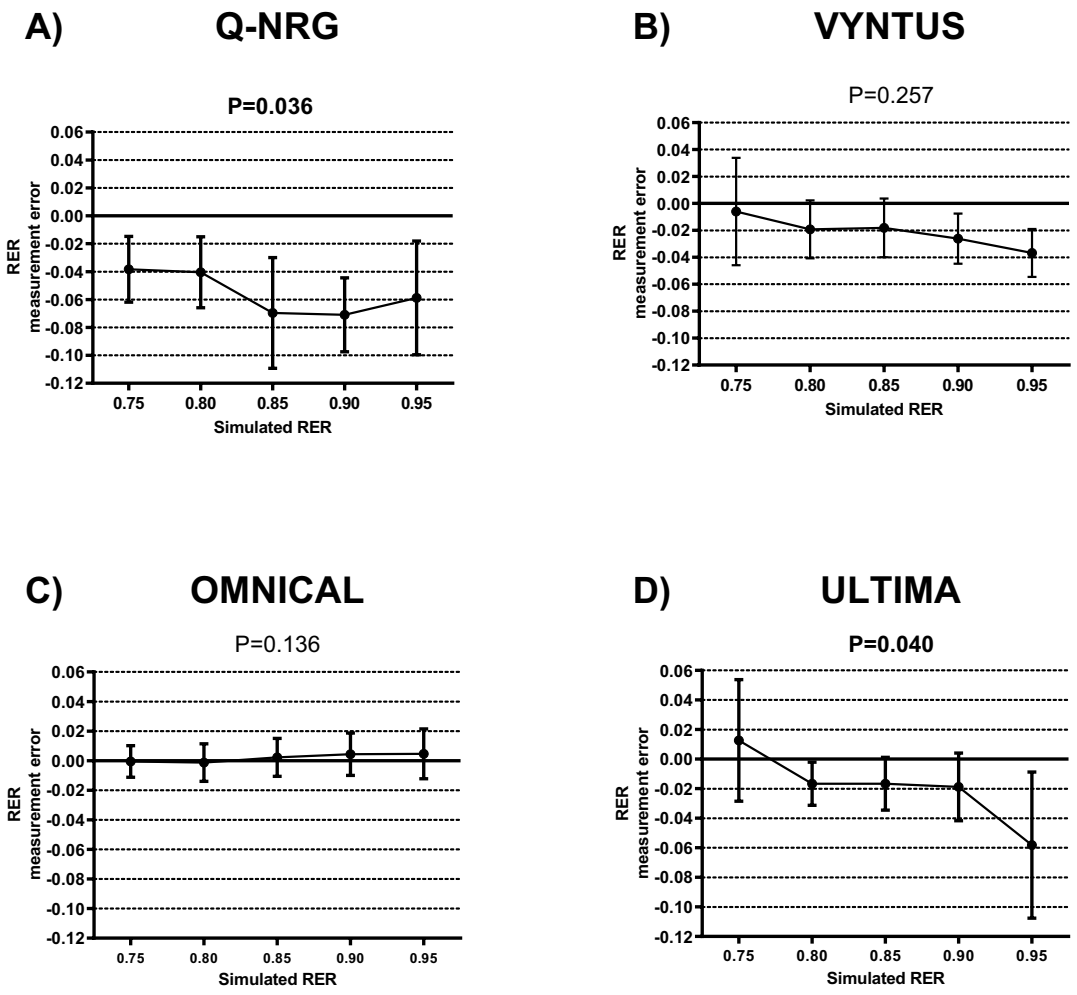


Figure 24. Measurement error of the four metabolic carts at different simulated respiratory exchange ratios (RER) by gas infusions. *Measurement error was calculated as measured value – expected value. P values from repeated measures analysis of variance (ANOVA, n=5). Results are presented as mean and standard deviation.*

Finally, we observed that the intra-day error (i.e. intra-day precision - approach *c*) did not vary in any of the metabolic carts (all P≥0.114, **Figure 25**).

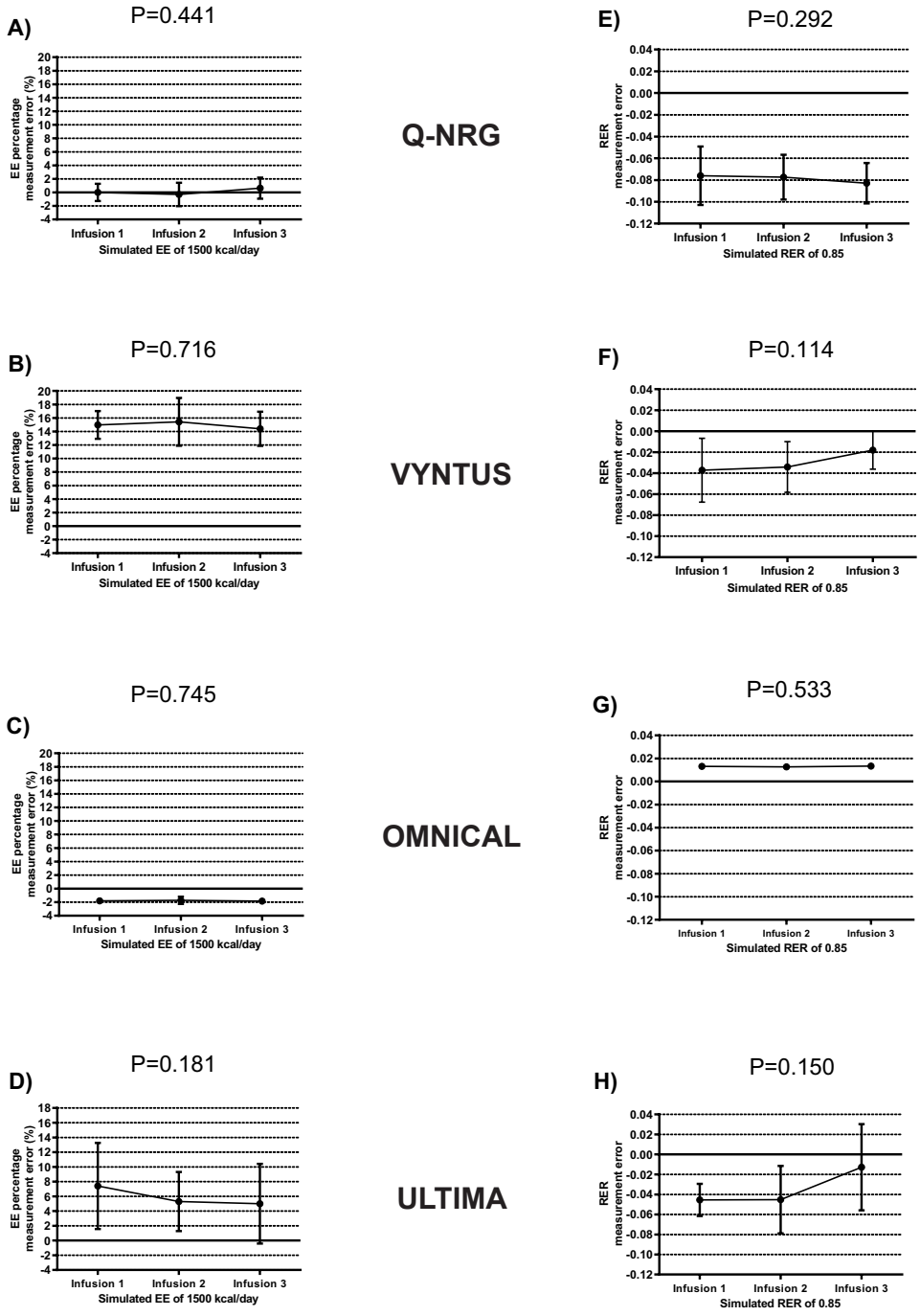


Figure 25. Intra-day precision at the same simulated energy expenditure and respiratory exchange ratio (RER) by three gas infusions one-hour apart. *Results are presented as percentage measurement error ([measured value – expected value] / expected value) × 100 for Panels A-D, and as measurement error (measured value – expected value) for Panels E-H. P values from repeated measures analysis of variance (ANOVA, n=5). Results are presented as mean and standard deviation.*

Human study

When expressed as $CV_{D\text{-}to\text{-}D}$, no differences were found in RMR biological reproducibility across metabolic carts (P=0.058, **Figure 26A**), nor between *uncorrected* and *corrected* RMR values (P=0.656, **Figure 26A**), and there was no metabolic cart × correction interaction effect on RMR biological reproducibility (P=0.496, **Figure 26A**). When RMR biological reproducibility was analyzed as absolute values of inter-day differences, we detected a metabolic cart main effect (P=0.026, **Figure 26C**), and *post-hoc* comparisons revealed that the Q-NRG RMR inter-day differences were lower than the Ultima RMR inter-day differences. We did not find a significant correction effect (P=0.729) nor a metabolic cart × correction interaction effect (P=0.415).

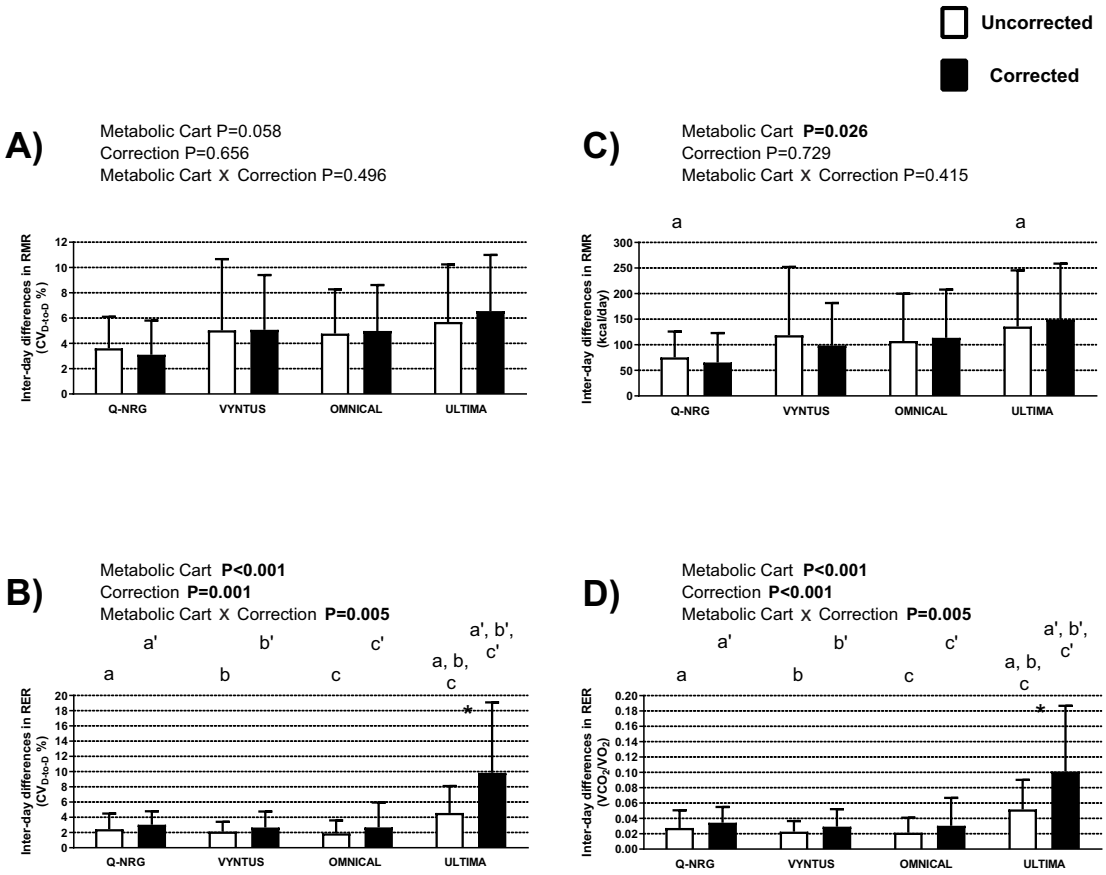


Figure 26. Inter-day precision of resting metabolic rate (RMR) and respiratory exchange ratio (RER) across metabolic carts, with and without using the post-calorimetric correction procedure. Panels A and B are expressed as day-to-day coefficient of variation ($CV_{D\text{-}to\text{-}D}$ %), while Panels C and D are expressed as absolute value of the differences (i.e. |Day 1 – Day 2|). *P values from a two-factor (Metabolic Cart × Correction) repeated measures analysis of variance (ANOVA, n=29). Identical indicatory letters represent significant differences as determined by post-hoc LSD Tukey analysis for uncorrected values. Identical prime indicatory letters represent significant differences as determined by post-hoc LSD Tukey analysis for corrected values. * represent significant differences between the uncorrected and the corrected values. Results are presented as mean and standard deviation.*

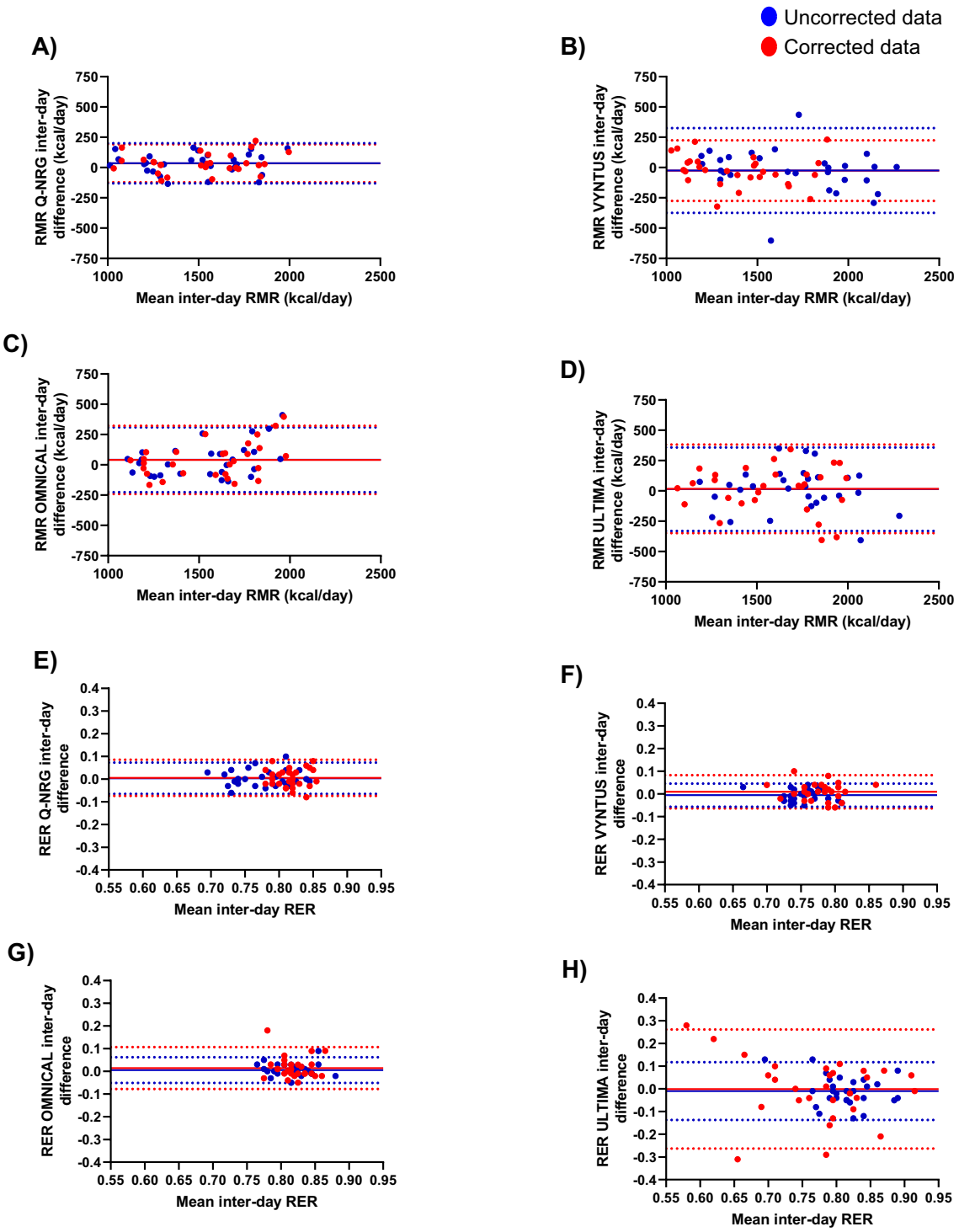


Figure 27. Bland-Altman plots for inter-day precision on resting metabolic rate (RMR; Panels A-D) and respiratory exchange ratio (RER; Panels E-H) across metabolic carts, with and without correcting using the post-calorimetric correction procedure (n=29). *Solid line represents the systematic error between day 1 and day 2. Dashed lines represent the upper and lower limits of agreement (Mean±1.96 standard deviation).*

The RER biological reproducibility was different across metabolic carts either when expressed as $CV_{D\text{-}to\text{-}D}$ or as absolute values of inter-day differences (both P<0.001, **Figures 26B** and **26D**). *Post-hoc* analyses revealed that the Ultima metabolic cart presented higher mean RER inter-day differences than the other 3 metabolic carts. We detected a significant effect of the post-calorimetric correction procedure (both P≤0.001, **Figure 26B** and **26D**), and *post-hoc* analyses showed that the application of the post-calorimetric correction procedure reduced RER inter-day biological reproducibility in the Ultima metabolic cart. **Figure 27** shows the Bland-Altman plots for biological reproducibility of RMR and RER, while VO_2 and VCO_2 data are shown in **Figures S2** and **S3**.

When testing comparability, we found that the RMR and RER values were different across metabolic carts in both days 1 and 2 (all P<0.001, **Figure 28**). Moreover, the correction main effect and the metabolic cart × correction interaction were significant (all P<0.035), except the correction effect on RER on day 2 (P=0.702, **Figure 28D**). *Corrected* RMR and RER values were different than *uncorrected* values for the Q-NRG and the Vyntus, while only the RMR was different for the Omnical and Ultima metabolic carts. Using the post-calorimetric correction procedure increased the RMR estimations yielded by the Q-NRG and the Omnical, while it reduced the RMR estimation yielded by the Vyntus metabolic cart (**Figure 28A** and **28B**). Bland and Altman plots comparing the *uncorrected* and *corrected* RMR and RER across metabolic carts are in **Figures S4** and **S5** respectively.

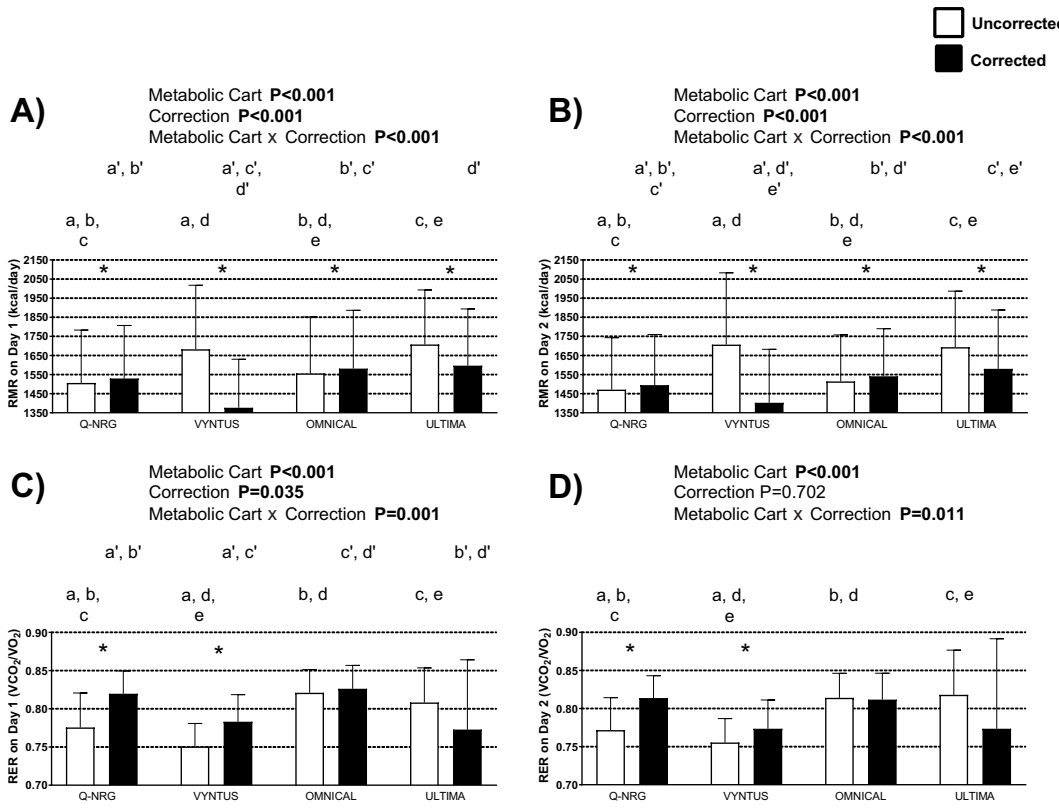


Figure 28. Resting metabolic rate (RMR) and respiratory exchange ratio (RER) across metabolic carts on day 1 (Panels A and C) and day 2 (Panels B and D), with and without correcting using the post-calorimetric correction procedure. *P values from two-factor (Metabolic Cart × Correction) repeated measures analysis of variance (n=29). Identical indicatory letters represent significant differences as determined by post-hoc LSD Tukey analysis for uncorrected values. Identical prime indicatory letters represent significant differences as determined by post-hoc LSD Tukey analysis for corrected values. * represent significant differences between the uncorrected vs. the corrected values. Results are presented as mean and standard deviation.*

The variance in RMR explained by body weight was 73% for the Q-NRG, 72% for the Omnical, 65% for the Ultima and 61% for the Vyntus (**Figure 29A**) whereas the variance in RMR explained by lean mass, fat mass and sex was 93% for the Omnical, 90% for the Q-NRG, 73% for the Ultima and 71% for the Vyntus (**Figure 29B**). The variance in RMR explained by the aforementioned models was higher when using the *uncorrected values* than when using the *corrected values* in all cases (i.e. using the RMR from the day 1, 2 or mean of both days) except in the Vyntus (**Figure 29** and **Table S3**).

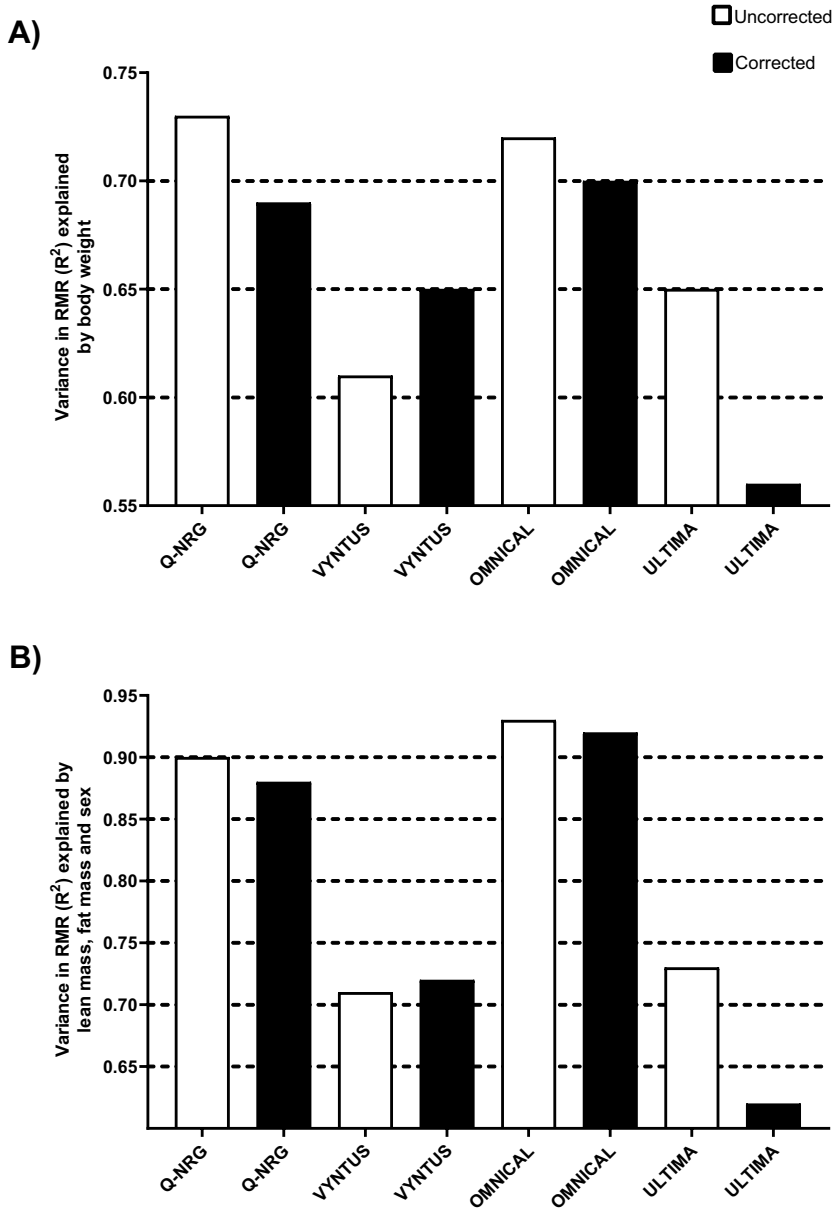


Figure 29. Explained resting metabolic rate (RMR; mean of both testing days) variance by body weight (Panel A), lean mass, fat mass and sex (Panel B) across metabolic carts, with and without correcting using the post-calorimetric correction procedure. *Results are presented as adjusted R^2 from simple (Panel A) and multiple (Panel B) linear regression models.*

DISCUSSION

The assessment of RMR and RER is considered of relevance in both clinical and research contexts [2,31]. Here we analyzed the accuracy and precision (by alcohol burning and pure gas infusions) and the biological reproducibility and comparability (in healthy young adults) of four commercially available - and recently manufactured - metabolic carts (the Q-NRG, the Vyntus, the Omnical and the Ultima) for assessing RMR and RER. The Omnical metabolic cart showed the most accurate and precise results for RMR and RER. The RMR and RER biological reproducibility was similar for all the metabolic carts examined except for the Ultima, which presented a higher RER variability. The application of a post-calorimetric correction procedure does not improve the RMR and RER biological reproducibility nor the comparability across the metabolic carts used in the present study.

Validity of the four metabolic carts for assessing RMR and RER

Accuracy is defined as the proximity of measurements to traceable standards. In indirect calorimetry, these traceable standards are commonly the gas production during alcohol/alkane combustion and/or controlled infusion of pure gases [15]. Previous studies [16] stated ±2% as an acceptable measurement error, although this criterion might vary across laboratories [15]. In our study, the Omnical was the only metabolic cart presenting an acceptable accuracy (error lower than 2%) in all variables except RER (2.2±1.1% when determined by alcohol burning, 1.2±0.8% when determined by pure gas infusions; see Figure 22). The Omnical also presented the best precision as indicated by the lower standard deviations and ranges for both methanol burning and pure gas infusions and the most stable measurement error in the within day repeated measurement experiment (Figure 25). The Omnical results are in agreement with those of Kaviani et al. [16] and Schoffelen et al. [32]. However, it should be noted that Kaviani et al. [16] used 2 different Omnical units, and only one of them showed an accuracy higher than ±2%. Noteworthy, in our study, the Q-NRG showed similar accuracy than the Omnical for the assessment of VO_2 and EE, but worse accuracy for assessing VCO_2 and RER. In a previous study [33], the accuracy of 3 Q-NRG units was tested by alcohol burning showing that the RER *measurement error* (-0.001, -0.012 and 0.008 for each unit) was lower than the mean RER *measurement error* observed in our study (-0.044). These differences could be partially explained because Delsoglio et al. [33] used ethanol (purity=96%), while we used methanol (purity≥99.9%). Finally, the lack of accuracy showed by the Vyntus in our study (RER *measurement error* determined by alcohol burning: -0.050, -0.036 and -0.028 obtained in the three different tests) is similar than the *measurement error* reported by a previous study [34] using butane burning at different volumes (RER *measurement error*: -0.043, -0.059 and -0.047 simulating low, medium and high exercise intensities respectively).

Previous studies have reported that the RMR biological reproducibility achieved by several metabolic carts is unacceptably high (>10%) [4,10-13,35,36]. The DTC metabolic cart, for long considered the gold standard, presented a RMR biological reproducibility, expressed as $CV_{D\text{-}to\text{-}D}$, below 4% [4,9-12,17,18]. Of note, in our study, the Q-NRG achieved a similar RMR biological reproducibility (3.6±0.5%). Nonetheless, all the metabolic carts included in this study achieved an RMR biological reproducibility below 6%, with no differences between metabolic carts. Another study has previously determined the Omnical's RMR biological reproducibility [32], showing an RMR inter-day precision of 2.9±1.0% (expressed as $CV_{D\text{-}to\text{-}D}$; n=10), while we observed a inter-day precision of 4.8±0.7%. Nevertheless, caution must be taken when comparing biological reproducibility between different cohorts, as the individuals' characteristics and study procedures could had a greater influence on the RMR biological reproducibility than the metabolic cart itself [13,15].

Despite of the RMR biological reproducibility was not different across metabolic carts, the RMR estimates yield by the Q-NRG and the Omnical were better predicted by body weight, and by body composition and sex, than the RMR estimates yield by the Vyntus or the Ultima. This might suggest that the Q-NRG and the Omnical provided more valid RMR estimates than the Vyntus and the Ultima, which is in line with the results obtained by the alcohol burning and the gas infusions tests. Unlike in RMR, we detected differences in RER biological reproducibility across metabolic carts. The Ultima RER biological reproducibility was worse than the obtained by the 3 others metabolic carts, while similar RER biological reproducibility was observed for the Q-NRG, the Vyntus, and the Omnical.

Overall, our results suggest that, considering the accuracy and precision (by alcohol burning and gas infusions tests) and the biological reproducibility in young healthy adults, the Omnical is the most valid metabolic cart for assessing RMR and RER (and therefore nutrient oxidation rates). Of note, despite the Q-NRG performance was considerably worse than the Omnical for RER measurements, it was much similar for assessing RMR. Although our results suggest that the Omnical should be the option of choice for assessing RMR and RER, the Q-NRG might be also considered a valid option for RMR assessment. Commonly, other characteristics beyond performance, such as affordability and difficulty of use, are considered for selecting a metabolic cart in a large variety of clinical/research settings [31]. Importantly, the Omnical can be upgraded to exercise testing and even to a basic-room indirect-calorimeter, while the Q-NRG cannot. On the other hand, the Omnical is the most expensive while the Q-NRG is the cheapest metabolic cart among the included in this study. The Omnical is considerably bigger and more difficult to move/transport than the Q-NRG, which is a compact, lightweight and battery-powered metabolic cart. Moreover, the Q-NRG only requires a monthly calibration, while the Omnical need a daily calibration. It should be noted however that the Q-NRG requires a manual adjustment of the fan speed during the RMR assessment, while the Omnical modifies it automatically. Finally, both metabolic carts run with a user-friendly and intuitive software.

Post-calorimetric correction procedure

Schadelwaldt et al. [3] proposed a post-calorimetric correction procedure in an attempt to correct the *measurement error* of metabolic carts, and showed that this procedure improves the comparability of two metabolic carts (DTC and Vmax Encore 29n) [3]. We therefore hypothesized that the application of this procedure would improve the biological reproducibility and comparability of the RMR and RER estimations yielded by the four metabolic carts included in this study. However, the results clearly showed that the application of the post-calorimetric correction procedure does not improve neither the biological reproducibility nor the comparability across metabolic carts of the RMR and RER estimations. In fact, this procedure even impaired the RER biological reproducibility in the Ultima metabolic cart. Further, the variance in *corrected* RMR *values* explained by body weight or by body composition and sex was lower than the variance in *uncorrected* RMR *values* in all metabolic carts except the Vyntus. Although one may expect that those metabolic carts presenting higher measurement error would benefit from this procedure, we observed that this was not the case in our study (e.g. Vyntus and Ultima).

Overall, our results suggest that the application of the post-calorimetric correction procedure proposed by Schadelwaldt et al. [3] is not advisable when using any of the four metabolic carts included in this study. Nevertheless, the gas infusions test seems to be a valid way of periodically validating the metabolic carts (i.e. determining the metabolic cart *measurement error*) [15]. In fact, our results show that the *measurement error* determined by the gas infusions test was comparable to the *measurement error* determined by the alcohol burning test, which is commonly considered the reference method to validate indirect calorimeters [14,16,17,24]. It should be considered however that, even if both options seem valid

for determining the *measurement error* of a cart, none of them produces similar humidity than the present in expired human gases [15].

The present study results should be considered with caution as some limitations exists. Firstly, our study was performed in young healthy adults, and thus, whether the present results apply to other population remains unknown. Secondly, previous studies suggest that the performance of a single unit might not represent the performance of all manufactured units [16,37], and therefore, our study need to be replicated before drawing firm conclusions. Moreover, our study did not include the DTC (former gold-standard) or other commercially available metabolic carts which have been reported to provide accurate results (e.g. ParvoMedics TrueOne 2400) [16]. Moreover, we used the methanol burning glass cage manufactured by Maastricht Instruments, and thus, it might have favored the Omnical, although the agreement between methanol burning and gas infusions invite to discard this possibility. Lastly, we did not control the menstrual cycle in female participants [38,39], although considering the within subject design of the human study and that both assessment were performed within 24 h its potential impact on RMR or RER biological reproducibility is likely negligible.

CONCLUSION

Our study showed that four commercially available, and recently manufactured, metabolic carts (the Q-NRG, the Vyntus CPX, the Omnical and the Ultima CardiO2) provide similar RMR and RER biological reproducibility in young healthy adults, although yield non-comparable RMR and RER estimations. Alcohol burning and gas infusions tests showed however that the Omnical provide more accurate and precise estimations of RMR and RER. Finally, our study showed that using the post-calorimetric procedure previously proposed by Schadelwaldt et al. [3] in these metabolic carts does not improve the RMR and RER biological reproducibility and worsen the association of RMR with its classical predictors.

SUPPLEMENTARY MATERIAL

Table S1. Standardized meal plan options for the previous day of each testing day.

Option 1		**Option 2**		**Option 3**	
Lunch		***Lunch***		***Lunch***	
Tomato sauce pasta	Spaghetti or macaroni Tomato sauce Tuna Olive oil	*Tomato sauce pasta*	Spaghetti or macaroni Tomato sauce Tuna Olive oil	*Tomato sauce boiled rice*	Boiled rice Tomato sauce Minced pork Olive oil
Snack		***Snack***		***Snack***	
	Banana Nuts		Apple Sweetened natural yogurt		Banana Sweetened natural yogurt
Dinner		***Dinner***		***Dinner***	
Mixed salad *Baked chicken and potato*	Salad Tomato Cheese Olive oil Chicken Potato Olive oil	*Mixed salad* *Baked chicken and potato*	Salad Tomato Cheese Olive oil Chicken Potato Olive oil	*Mixed salad* *Spanish omelette*	Salad Tomato Cheese Olive oil Eggs Potato Olive oil

Subjects selected one out of the three menu options and consumed it in both days.

Table S2. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), energy expenditure (EE) and respiratory exchange ratio (RER) yielded by different metabolic carts during an alcohol burning test (methanol combustion) and gas infusions performed in three different days.

	Alcohol Burning							
	Expected VO_2	**Expected VCO_2**	**Expected EE**	**Expected RER**	**Measured VO_2**	**Measured VCO_2**	**Measured EE**	**Measured RER**
Q-NRG								
Day 1	350	233	2352	0.667	345	216	2295	0.63
Day 2	349	232	2345	0.667	336	210	2235	0.63
Day 3	370	246	2486	0.667	387	239	2569	0.62
Vyntus								
Day 1	514	342	3454	0.667	612	378	4063	0.62
Day 2	386	257	2594	0.667	490	309	3264	0.63
Day 3	365	243	2453	0.667	442	283	2951	0.64
Omnical								
Day 1	505	336	3394	0.667	498	327	3339	0.66
Day 2	317	211	2131	0.667	310	209	2088	0.67
Day 3	361	240	2426	0.667	367	236	2452	0.64
Ultima								
Day 1	506	338	3403	0.667	654	393	4323	0.60
Day 2	402	268	2703	0.667	417	283	2812	0.68
Day 3	369	246	2481	0.667	416	246	2743	0.59
	Gas infusions							
	Expected VO_2	**Expected VCO_2**	**Expected EE**	**Expected RER**	**Measured VO_2**	**Measured VCO_2**	**Expected VO_2**	**Expected VCO_2**
Q-NRG								
Day 1	348	216	2322	0.62	347	201	2289	0.58
Day 2	348	216	2316	0.62	340	204	2255	0.60
Day 3	368	229	2456	0.62	373	211	2451	0.56
Vyntus								
Day 1	471	314	3414	0.67	517	352	3492	0.68
Day 2	219	136	1461	0.62	267	155	1764	0.58
Day 3	364	226	2604	0.62	425	261	2829	0.61
Omnical								
Day 1	471	314	3174	0.67	469	316	3166	0.67
Day 2	325	202	2165	0.62	319	200	2129	0.63
Day 3	359	223	2394	0.62	360	215	2383	0.60
Ultima								
Day 1	471	314	3174	0.67	482	344	3282	0.71
Day 2	400	249	2664	0.62	392	262	2643	0.67
Day 3	368	228	2451	0.62	357	229	2394	0.64

VO_2 and VCO_2 are presented in ml/min; EE is presented in kcal/day; RER was calculated as VCO_2/ VO_2.

Table S3. Associations between the resting metabolic rate (RMR) estimations yielded by different metabolic carts and classical determinants of RMR.

	Uncorrected RMR (kcal/day)			Corrected RMR (kcal/day)		
	R^2	β	P	R^2	β	P
			Q-NRG			
Model 1						
Day 1						
Body weight (kg)	0.69	18.2 ± 2.3	**<0.001**	0.67	18.0 ± 2.4	**<0.001**
Constant		219.5 ± 162.9	0.189		256.5 ± 168.6	0.14
Day 2						
Body weight (kg)	0.73	18.4 ± 2.1	**<0.001**	0.68	17.2 ± 2.2	**<0.001**
Constant		174.2 ± 150.2	0.256		275.5 ± 160.0	0.096
Mean (Day 1 and Day 2)						
Body weight (kg)	0.73	18.3 ± 2.1	**<0.001**	0.69	17.6 ± 2.2	**<0.001**
Constant		196.9 ± 149.8	0.2		266.0 ± 158.6	0.105
Model 2						
Day 1						
Lean Mass (kg)	0.89	16.1 ± 3.6	**<0.001**	0.87	13.2 ± 3.8	**0.002**
Fat Mass (kg)		6.8 ± 2.7	**0.017**		7.8 ± 2.9	**0.012**
Sex		-200.6 ± 79.3	**0.018**		-263.4 ± 85.0	**0.005**
Constant		873.9 ± 267.9	**0.003**		1107.1 ± 287.1	**0.001**
Day 2						
Lean Mass (kg)	0.87	19.8 ± 3.7	**<0.001**	0.86	15.3 ± 3.8	**<0.001**
Fat Mass (kg)		7.3 ± 2.8	**0.016**		6.6 ± 2.9	**0.03**
Sex		-14.2	0.253		-187.3 ± 84.4	**0.036**
Constant		507.0 ± 281.5	0.084		887.3 ± 285.2	**0.005**
Mean (Day 1 and Day 2)						
Lean Mass (kg)	0.90	18.0 ± 3.3	**<0.001**	0.88	14.3 ± 3.5	**<0.001**
Fat Mass (kg)		7.1 ± 2.5	**0.008**		7.2 ± 2.6	**0.011**
Sex		-149.1 ± 72.9	0.051		-225.3 ± 77.2	**0.007**
Constant		690.5 ± 246.2	**0.01**		997.2 ± 260.8	**0.001**
	R^2	β	P	R^2	β	P
			Vyntus			
Model 1						
Day 1						
Body weight (kg)	0.56	19.9 ± 3.3	**<0.001**	0.60	15.5 ± 2.4	**<0.001**
Constant		278.1 ± 237.3	0.251		278.9 ± 171.6	**0.116**
Day 2						

Body weight (kg)	0.58	22.8 ± 3.6	**<0.001**	0.62	17.5 ± 2.6	**<0.001**
Constant		95.6 ± 257.6	0.713		167.0 ± 183.1	0.37
Mean (Day 1 and Day 2)						
Body weight (kg)	0.61	21.3 ± 3.2	**<0.001**	0.65	16.5 ± 2.3	**<0.001**
Constant		186.9 ± 228.9	0.422		223.0 ± 164.0	0.185
Model 2						
Day 1						
Lean Mass (kg)	0.62	16.3 ± 7.9	**0.049**	0.62	15.1 ± 5.9	**0.017**
Fat Mass (kg)		12.5 ± 6.0	**0.046**		10.6 ± 4.5	**0.025**
Sex		-209.1 ± 176.0	0.246		-89.7 ± 131.7	0.502
Constant		951.8 ± 594.4	0.122		571.0 ± 445.0	0.211
Day 2						
Lean Mass (kg)	0.72	25.8 ± 7.5	**0.002**	0.72	13.5 ± 5.6	**0.023**
Fat Mass (kg)		6.9 ± 5.7	0.235		10.0 ± 4.2	**0.026**
Sex		-113.1 ± 167.6	0.506		-214.0 ± 124.8	0.099
Constant		480.6 ± 566.2	0.404		858.4 ± 421.4	0.052
Mean (Day 1 and Day 2)						
Lean Mass (kg)	0.71	21.0 ± 7.0	**0.006**	0.72	14.3 ± 5.2	**0.011**
Fat Mass (kg)		9.7 ± 5.3	0.078		10.3 ± 4.0	**0.015**
Sex		-161.1 ± 155.9	0.311		-151.8 ± 117.0	0.206
Constant		716.2 ± 526.6	0.186		714.7 ± 394.7	0.082
	R^2	**β**	**P**	**R^2**	**β**	**P**
			Omnical			
Model 1						
Day 1						
Body weight (kg)	0.67	19.1 ± 2.5	**<0.001**	0.63	19.2 ± 2.8	**<0.001**
Constant		209.1 ± 181.2	0.258		226.3 ± 197.8	0.263
Day 2						
Body weight (kg)	0.70	16.1 ± 2.0	**<0.001**	0.68	16.3 ± 2.1	**<0.001**
Constant		379.2 ± 142.3	**0.013**		390.5 ± 149.0	**0.014**
Mean (Day 1 and Day 2)						
Body weight (kg)	0.72	17.6 ± 2.0	**<0.001**	0.70	17.7 ± 2.2	**<0.001**
Constant		294.2 ± 146.6	0.055		308.4 ± 158.2	**0.062**
Model 2						
Day 1						
Lean Mass (kg)	0.84	12.0 ± 4.5	**0.013**	0.83	10.1 ± 4.7	**0.042**
Fat Mass (kg)		10.3 ± 3.4	**0.006**		10.0 ± 3.6	**0.01**
Sex		-314.8 ± 100.1	**0.004**		-375.1 ± 105.7	**0.002**
Constant		1219.1 ± 338.1	**0.001**		1424.6 ± 357.0	**0.001**

Day 2						
Lean Mass (kg)	0.92	16.6 ± 2.6	**<0.001**	0.92	15.6 ± 2.7	**<0.001**
Fat Mass (kg)		4.3 ± 2.0	**0.041**		4.5 ± 2.1	**0.04**
Sex		-127.2 ± 59.1	**0.041**		-163.9 ± 61.2	**0.013**
Constant		800.6 ± 199.5	**<0.001**		927.1 ± 206.7	**<0.001**
Mean (Day 1 and Day 2)						
Lean Mass (kg)	0.93	14.3 ± 2.7	**<0.001**	0.92	12.8 ± 2.9	**<0.001**
Fat Mass (kg)		7.3 ± 2.1	**0.002**		7.2 ± 2.2	**0.003**
Sex		-221.0 ± 60.6	**0.001**		-269.5 ± 63.8	**<0.001**
Constant		1010.0 ± 204.7	**<0.001**		1175.9 ± 215.4	**<0.001**
	R^2	**β**	**P**	**R^2**	**β**	**P**
			Ultima			
Model 1						
Day 1						
Body weight (kg)	0.68	18.5 ± 2.4	**<0.001**	0.51	16.9 ± 3.1	**<0.001**
Constant		397.0 ± 173.2	**0.03**		402.0 ± 221.0	0.08
Day 2						
Body weight (kg)	0.51	16.6 ± 3.1	**<0.001**	0.50	17.3 ± 3.2	**<0.001**
Constant		517.0 ± 218.6	**0.026**		353.8 ± 232.2	0.139
Mean (Day 1 and Day 2)						
Body weight (kg)	0.65	17.6 ± 2.4	**<0.001**	0.56	17.1 ± 2.8	**<0.001**
Constant		457.0 ± 173.3	**0.014**		377.9 ± 203.0	0.074
Model 2						
Day 1						
Lean Mass (kg)	0.76	15.2 ± 5.3	**0.008**	0.55	11.5 ± 7.6	0.14
Fat Mass (kg)		10.5 ± 4.0	**0.014**		11.3 ± 5.7	0.059
Sex		-198.3 ± 118.0	0.105		-227.8 ± 168.7	0.189
Constant		1052.0 ± 399.0	**0.014**		1147.3 ± 569.8	0.055
Day 2						
Lean Mass (kg)	0.56	20.0 ± 7.4	**0.012**	0.55	15.6 ± 7.9	0.058
Fat Mass (kg)		6.1 ± 5.6	0.288		9.1 ± 5.9	0.139
Sex		-29.2 ± 164.8	0.861		-155.0 ± 175.7	0.386
Constant		647.3 ± 556.6	0.256		868.6 ± 593.3	0.156
Mean (Day 1 and Day 2)						
Lean Mass (kg)	0.73	17.6 ± 5.4	**0.003**	0.62	13.6 ± 6.8	**0.056**
Fat Mass (kg)		8.3 ± 4.1	0.055		10.2 ± 5.1	0.057
Sex		-113.8 ± 121.6	0.358		-191.4 ± 151.0	0.217
Constant		849.6 ± 410.6	**0.049**		1008.0 ± 510.1	0.059

Results are presented as adjusted R^2, unstandardized β ± standard deviation and P value from a simple (Model 1)/ multiple (Model 2) regression models. Sex: 1=men; 2=women. Significant P values (P<0.05) are presented in bold numbers.

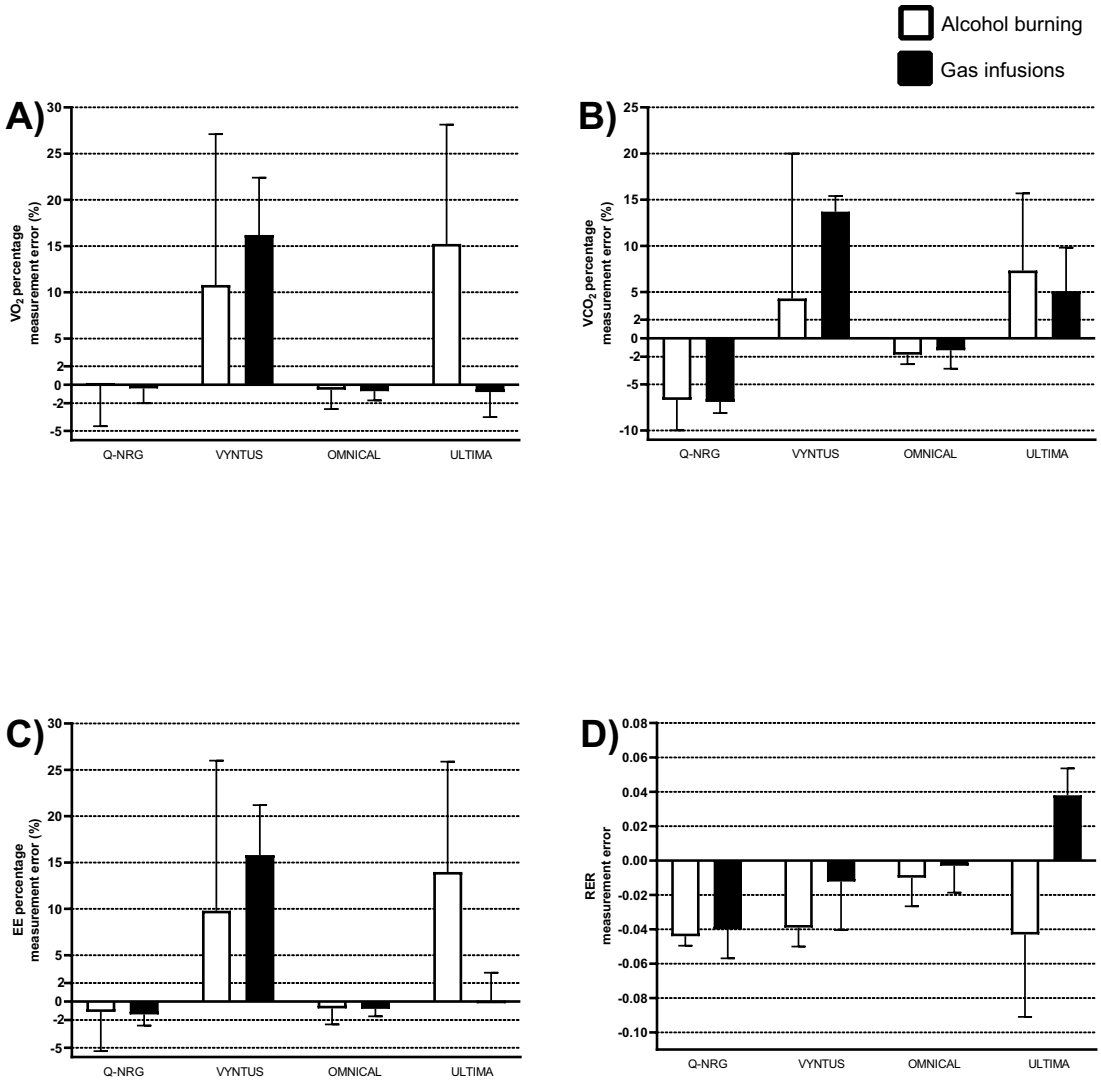


Figure S1. Percentage measurement error of oxygen consumption (VO_2; Panel A), carbon dioxide production (VCO_2; Panel B), energy expenditure (EE; Panel C) and measurement error of respiratory exchange ratio (RER; Panel D) across metabolic carts, as determined by alcohol burning (i.e. methanol combustion; white bars) and gas infusions (black bars). *The percentage measurement error was calculated as ([measured value – expected value] / expected value) × 100 for and measurement error as (measured value – expected value). * represent significant differences (paired t-test) between the methanol combustion vs. the gas infusions values (n=3). Results are presented as mean and standard deviation.*

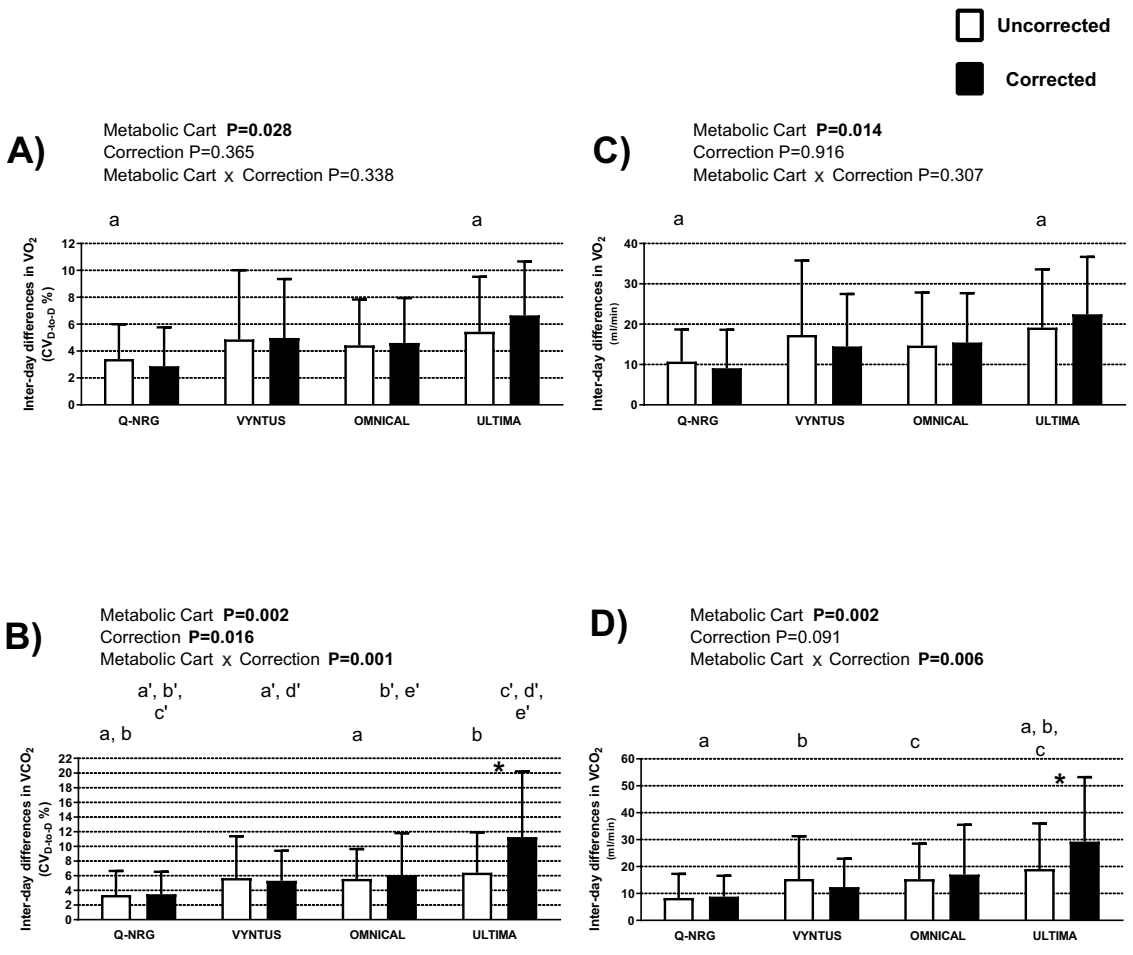


Figure S2. Inter-day precision of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) across metabolic carts, with and without using the post-calorimetric correction procedure. *Panels A and B are expressed as day-to-day coefficient of variation (CVD-to-D %), while Panels C and D are expressed as absolute value of the differences (i.e. |Day 1 – Day 2|). P values from a two-factor (Metabolic Cart × Correction) repeated measures analysis of variance (ANOVA, n=29). Identical indicatory letters represent significant differences as determined by post-hoc LSD Tukey analysis for uncorrected values. Identical prime indicatory letters represent significant differences as determined by post-hoc LSD Tukey analysis for corrected values. * represent significant differences between the uncorrected and the corrected values. Results are presented as mean and standard deviation.*

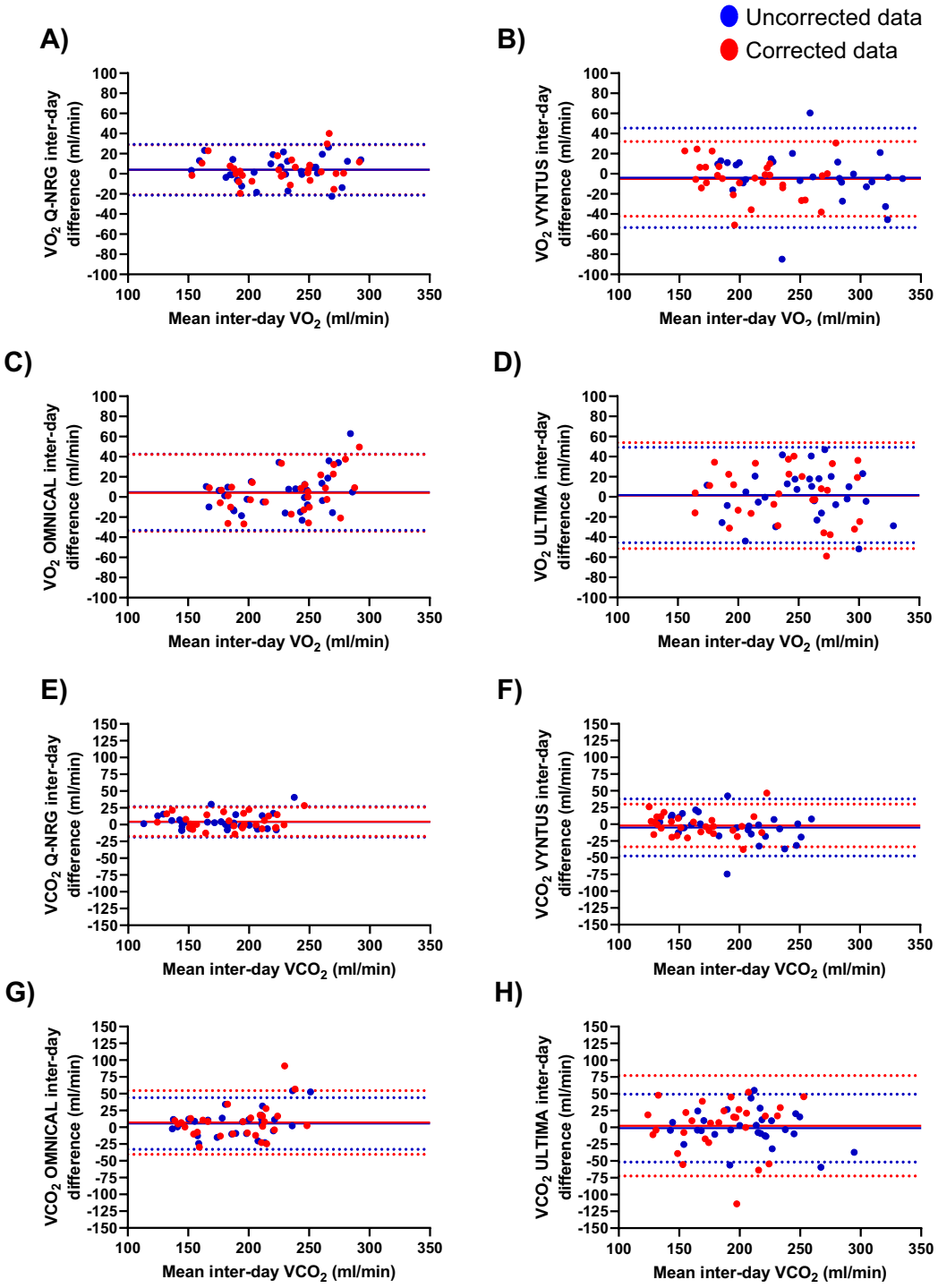


Figure S3. Bland-Altman plots for inter-day precision on oxygen consumption (VO_2; Panels A-D) and carbon oxide production (VCO_2; Panels E-H) across metabolic carts, with and without correcting using the post-calorimetric correction procedure (n=29). *Solid line represents the systematic error between day 1 and day 2. Dashed lines represent the upper and lower limits of agreement (Mean±1.96 standard deviation).*

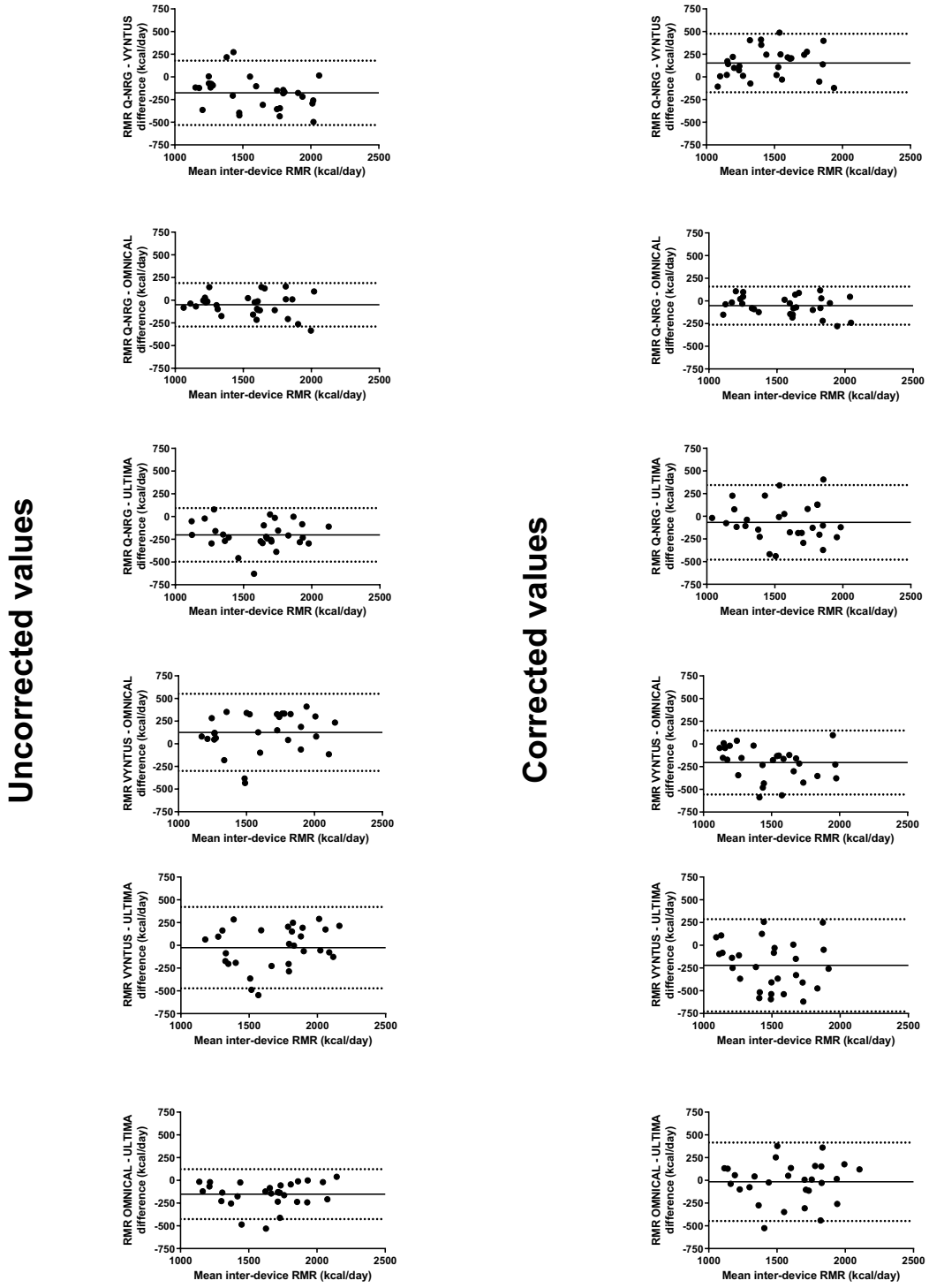


Figure S4. Bland-Altman plots for inter-devices differences (i.e. comparability) on resting metabolic rate (RMR) across metabolic carts, with and without correcting using the post-calorimetric correction procedure (n=29). *Solid line represents the systematic error between metabolic carts. Dashed lines represent the upper and lower limits of agreement (Mean±1.96 standard deviation). Represented results from the day 1.*

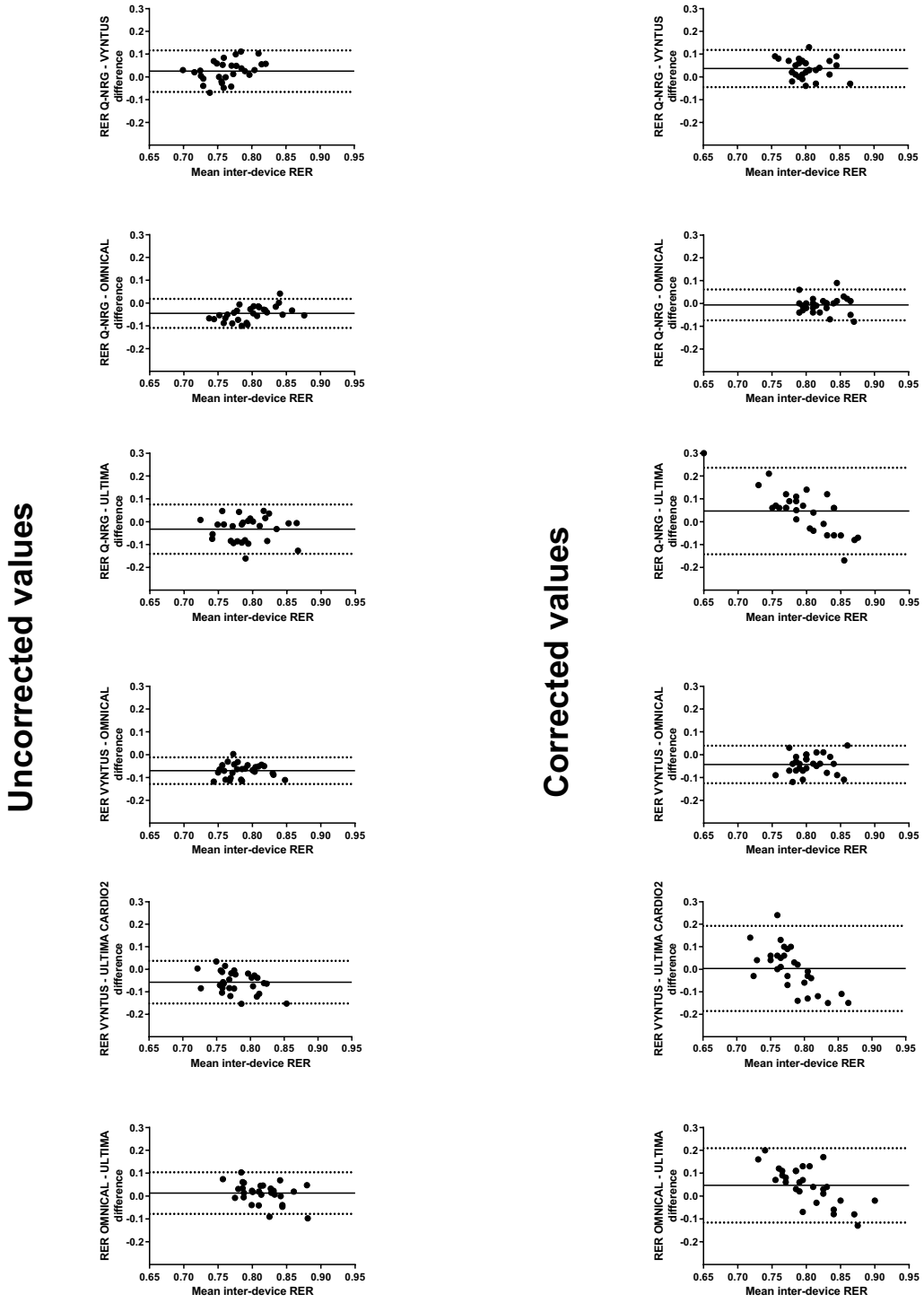


Figure S5. Bland-Altman plots for inter-devices differences (i.e. comparability) on respiratory exchange ratio (RER) across metabolic carts, with and without correcting using the post-calorimetric correction procedure (n=29). *Solid line represents the systematic error between metabolic carts. Dashed lines represent the upper and lower limits of agreement (Mean±1.96 standard deviation). Represented results from the day 1.*

REFERENCES

1. Fullmer, S.; Benson-Davies, S.; Earthman, C.P.; Frankenfield, D.C.; Gradwell, E.; Lee, P.S.P.; Piemonte, T.; Trabulsi, J. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J. Acad. Nutr. Diet.* **2015**, *115*, 1417-1446.e2.
2. Lam, Y.Y.; Ravussin, E. Indirect calorimetry: An indispensable tool to understand and predict obesity. *Eur. J. Clin. Nutr.* **2017**, *71*, 318–322.
3. Schadewaldt, P.; Nowotny, B.; Strassburger, K.; Kotzka, J.; Roden, M. Indirect calorimetry in humans: a postcalorimetric evaluation procedure for correction of metabolic monitor variability. *Am. J. Clin. Nutr.* **2013**, *97*, 763–73.
4. Black, C.; Grocott, M.P.W.; Singer, M. Metabolic monitoring in the intensive care unit: A comparison of the Medgraphics Ultima, Deltatrac II, and Douglas bag collection methods. *Br. J. Anaesth.* **2015**, *114*, 261–268.
5. Da Rocha, E.E.M.; Alves, V.G.F.; Da Fonseca, R.B. V Indirect calorimetry: Methodology, instruments and clinical application. *Curr. Opin. Clin. Nutr. Metab. Care* **2006**, *9*, 247–256.
6. Ferrannini, E. The theoretical bases of indirect calorimetry: A review. *Metabolism* **1988**, *37*, 287–301.
7. Livesey, G.; Elia, M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *Am. J. Clin. Nutr.* **1988**, *47*, 608–628.
8. Frayn, K.N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* **1983**, *121*, 628–634.
9. Schoeller, D.A. Making Indirect Calorimetry a Gold Standard for Predicting Energy Requirements for Institutionalized Patients. *J. Am. Diet. Assoc.* **2007**, *107*, 390–392.
10. Kennedy, S.; Ryan, L.; Fraser, A.; Clegg, M.E. Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis. *J. Nutr. Sci.* **2014**, *3*.
11. Graf, S.; Karsegard, V.L.; Viatte, V.; Maisonneuve, N.; Pichard, C.; Genton, L. *Comparison of three indirect calorimetry devices and three methods of gas collection: A prospective observational study*; 2013; Vol. 32;.
12. Cooper, J.A.; Watras, A.C.; O'Brien, M.J.; Luke, A.; Dobratz, J.R.; Earthman, C.P.; Schoeller, D.A. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J. Am. Diet. Assoc.* **2009**, *109*, 128–32.
13. Alcantara, J.M.A.; Sanchez-Delgado, G.; Martinez-Tellez, B.; Merchan-Ramirez, E.; Labayen, I.; Ruiz, J.R. Congruent validity and inter-day reliability of two breath by breath metabolic carts to measure resting metabolic rate in young adults. *Nutr. Metab. Cardiovasc. Dis.* **2018**, *28*, 929–936.
14. Schoffelen, P.F.M.; Plasqui, G. Classical experiments in whole-body metabolism: open-circuit respirometry—diluted flow chamber, hood, or facemask systems. *Eur. J. Appl. Physiol.* **2017**.
15. Chen, K.Y.; Smith, S.; Ravussin, E.; Krakoff, J.; Plasqui, G.; Tanaka, S.; Murgatroyd, P.; Brychta, R.; Bock, C.; Carnero, E.; et al. Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0): A Guide to Conducting and Reporting Human Whole-Room Calorimeter Studies. *Obesity* **2020**, *28*, 1613–1625.
16. Kaviani, S.; Schoeller, D.A.; Ravussin, E.; Melanson, E.L.; Henes, S.T.; Dugas, L.R.; Dechert, R.E.; Mitri, G.; Schoffelen, P.F.M.; Gubbels, P.; et al. Determining the Accuracy and Reliability of Indirect Calorimeters Utilizing the Methanol Combustion Technique. *Nutr. Clin. Pract.* **2018**, *33*, 206–216.
17. Cooper, B.G.; McLean, J.A.; Taylor, R. An evaluation of the Deltatrac indirect calorimeter by gravimetric injection and alcohol burning. *Clin. Phys. Physiol. Meas.* **1991**, *12*, 333–341.
18. Tissot, S.; Delafosse, B.; Bertrand, O.; Bouffard, Y.; Viale, J.P.; Annat, G. Clinical validation of the Deltatrac monitoring system in mechanically ventilated patients. *Intensive Care Med.* **1995**, *21*, 149–153.
19. Weststrate, J.A. Resting metabolic rate and diet-induced thermogenesis: a methodological reappraisal. *Am J Clin Nutr.* **1993**, *58*, 592–601.
20. Bader, N.; Bosy-Westphal, A.; Dilba, B.; Müller, M.J. Intra- and interindividual variability of resting energy expenditure in healthy male subjects – biological

and methodological variability of resting energy expenditure. *Br. J. Nutr.* **2005**, *94*, 843–849.
21. Haugen, H.A.; Melanson, E.L.; Tran, Z.V.; Kearney, J.T.; Hill, J.O. Variability of measured resting metabolic rate. *Am. J. Clin. Nutr.* **2003**, *78*, 1141–1144.
22. Donahoo, W.T.; Levine, J.A.; Melanson, E.L. Variability in energy expenditure and its components. *Curr. Opin. Clin. Nutr. Metab. Care* **2004**, *7*, 599–605.
23. Galgani, J.E.; Castro-Sepulveda, M.A. Influence of a Gas Exchange Correction Procedure on Resting Metabolic Rate and Respiratory Quotient in Humans. *Obesity* **2017**, *25*, 1941–1947.
24. Miodownik, S.; Melendez, J.; Carlon, V.A.; Burda, B.; Melendez, J.; Arslan, V.; Quantitative, B.B. Quantitative methanol-burning lung model for validating gas-exchange measurements over wide ranges of FIO2. *J. Appl. Physiol.* **1998**, *84*, 2177–2182.
25. Murgatroyd, P.R.; Davies, H.L.; Prentice, A.M. Intra-individual variability and measurement noise in estimates of energy expenditure by whole body indirect calorimetry. *Br. J. Nutr.* **1987**, *58*, 347–356.
26. Sanchez-Delgado, G.; Alcantara, J.M.A.; Acosta, F.M.; Martinez-Tellez, B.; Amaro-Gahete, F.J.; Merchan-Ramirez, E.; Löf, M.; Labayen, I.; Ravussin, E.; Ruiz, J.R. Energy Expenditure and Macronutrient Oxidation in Response to an Individualized Nonshivering Cooling Protocol. *Obesity* **2020**, *00*, 1–9.
27. Kjeldahl, J. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. *Fresenius' Zeitschrift für Anal. Chemie* **1883**, *22*, 366–382.
28. Weir, J. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **1949**, *109*, 1–9.
29. Bland, J.; Altman, D. Statistical Methods for Assessing Agreement Between Two Methods of Clinical Measurement. *Lancet* **1986**, *327*, 307–310.
30. Johnstone, A.M.; Murison, S.D.; Duncan, J.S.; Rance, K.A.; Speakman, J.R. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am. J. Clin. Nutr.* **2005**, *82*, 941–948.
31. Oshima, T.; Berger, M.M.; De Waele, E.; Guttormsen, A.B.; Heidegger, C.P.; Hiesmayr, M.; Singer, P.; Wernerman, J.; Pichard, C. Indirect calorimetry in nutritional therapy. A position paper by the ICALIC study group. *Clin. Nutr.* **2017**, *36*, 651–662.
32. Schoffelen, P.F.M.; den Hoed, M.; van Breda, E.; Plasqui, G. Test-retest variability of VO 2max using total-capture indirect calorimetry reveals linear relationship of VO 2 and Power. *Scand. J. Med. Sci. Sport.* **2019**, *29*, 213–222.
33. Delsoglio, M.; Dupertuis, Y.M.; Oshima, T.; van der Plas, M.; Pichard, C. Evaluation of the accuracy and precision of a new generation indirect calorimeter in canopy dilution mode. *Clin. Nutr.* **2019**, 1–8.
34. Perez-Suarez, I.; Martin-Rincon, M.; Gonzalez-Henriquez, J.J.; Fezzardi, C.; Perez-Regalado, S.; Galvan-Alvarez, V.; Juan-Habib, J.W.; Morales-Alamo, D.; Calbet, J.A.L. Accuracy and Precision of the COSMED K5 Portable Analyser. *Front. Physiol.* **2018**, *9*, 1–12.
35. Poulsen, M.K.; Thomsen, L.P.; Kjærgaard, S.; Rees, S.E.; Karbing, D.S. Reliability of, and Agreement Between, two Breath-by-Breath Indirect Calorimeters at Varying Levels of Inspiratory Oxygen. *Nutr. Clin. Pract.* **2019**, *00*, 1–8.
36. Sundström, M.; Tjäder, I.; Rooyackers, O.; Wernerman, J. Indirect calorimetry in mechanically ventilated patients. A systematic comparison of three instruments. *Clin. Nutr.* **2013**, *32*, 118–121.
37. Darren M. Roffey; Nuala M. Byrne; and Andrew P. Hills Day-to-Day Variance in Measurement of Resting Metabolic Rate Using Ventilated-Hood and Mouthpiece & Nose-Clip Indirect Calorimetry Systems. *J. Parenter. Enter. Nutr.* **2006**, *30*, 426–432.
38. Melanson, K.J.; Saltzman, E.; Russell, R.; Roberts, S.B. Postabsorptive and Postprandial Energy Expenditure and Substrate Oxidation Do Not Change during the Menstrual Cycle in Young Women. *J. Nutr.* **1996**, *126*, 2531–2538.
39. Ferraro, R.; Lillioja, S.; Fontvieille, A.M.; Rising, R.; Bogardus, C.; Ravussin, E. Lower sedentary metabolic rate in women compared with men. *J. Clin. Invest.* **1992**, *90*, 780–784.

RESULTS AND DISCUSION

SECTION 2

STUDY III

BACKGROUND

The resting metabolic rate (RMR) is the lowest energy expenditure of a person who is awake [1], after at least 12 h of fasting, being in physical rest, and in a state of mental relaxation in an ambient environmental temperature; it accounts for some 60%–70% of the total daily energy expenditure [2]. The assessment of RMR is important when studying human energy balance, both in clinical and research settings [2,3]. Indirect calorimetry is the reference method for assessing RMR [2,4–6], which is estimated from the consumption of oxygen (VO_2) and the production of carbon dioxide (VCO_2) [6]. The measurement of VO_2 and VCO_2, together with urinary nitrogen excretion, also allows for the estimation of the nutrient (carbohydrate and fat) oxidation rate [7]. Indeed, the VCO_2/VO_2 ratio, i.e., the respiratory exchange ratio (RER), is an indicator of the relative predominance of fat (FATox) and carbohydrate (CHOox) oxidation.

The assessment of RMR using indirect calorimetry is normally performed over a 10–30 min period. It is widely assumed that the first 5 min of data recorded should be discarded [8,9]. A short (e.g., 5 min) steady respiratory state period, i.e., a period in which the indirect calorimetry record is markedly stable, then has to be selected from the remaining dataset for estimating the RMR [6,7,10]. The assumption that steady state (SSt) methods for gas exchange data selection provide a better estimate of RMR than the other methods available arose from studies performed in ventilated patients [10]. However, there is no strong evidence that the same can be assumed in healthy, non-ventilated subjects—and indeed different methods have been used. These methods can be grouped into three categories: (i) the selection of an SSt (defined as that providing a coefficient of variance [CV] of <10% for VO_2, VCO_2, and minute ventilation [VE], and of <5% for RER [11]), (ii) the selection of a pre-defined time interval (TI), without taking the stability of the results obtained into consideration [11], and (iii) "filtering", in which data above or below a given RMR threshold are discarded. Both the SSt and TI methods can be used under different time conditions [1]. Unfortunately, the use of different methods for gas exchange data selection could result in different estimates of RMR and nutrient oxidation rates being made [1,11,12]. For instance, in a study involving healthy subjects, Irving et al. [1] reported RMR estimates made by the SSt and TI methods to differ by some −101 to +121 kcal/day.

Certainly, SSt-based RMR estimates are usually lower than those provided by the TI method [1,11]. Given the above definition of RMR [1], it has been proposed that the lowest estimates obtained by the SSt method should be deemed more accurate than those provided by TI. However, the under-estimation of the homeostatic RMR cannot be ruled out in the SSt method, nor has any study checked whether the filtering methods for data selection provide lower RMR estimates than either SSt or TI.

The present work examines whether the SSt, TI, or filtering method yields the lowest RMR value in healthy, non-ventilated subjects, and determines in which method the greatest variance in RMR is explained by the classical determinants of this variable (i.e., body weight, body composition, and sex) [13].

METHODS OVERVIEW

Subjects

The participants of this retrospective study were 107 young adults (72 women) enrolled in the ACTIBATE study [14] and 74 middle-aged adults (39 women) enrolled in the FIT-AGEING study [15]. Detailed information about the methodology of the aforementioned studies can

be found elsewhere [14,15]. Briefly, the inclusion criteria were: (i) being physically inactive (<20 min of moderate–vigorous physical activity on <3 days/week), (ii) having a stable body weight (change <3 kg over the last 3 months [ACTIBATE] or <5 kg over the last 5 months [FIT-AGEING]), (iii) not being enrolled in a weight loss program, (iv) not being a smoker, (v) not suffering from an acute or chronic illness, and (vi) not being pregnant. The ACTIBATE study protocol was approved by the Committee for Research Involving Human Subjects at the University of Granada (Reference #924) and the Servicio Andaluz de Salud (Centro de Granada, CEI-Granada), while the FIT-AGEING was approved by the Human Research Ethics Committee of the Junta de Andalucía (0838-N-2017). Both studies were performed in accordance with the Declaration of Helsinki (2013 revision) and registered on the clinicaltrials.gov platform (IDs: NCT02365129 for the ACTIBATE study and NCT03334357 for the FIT-AGEING study). Oral and written informed consent was obtained from all the subjects before their enrolment.

Procedures

In both the above studies, subjects underwent a 30 min indirect calorimetry assessment of RMR at rest, early in the morning, following an overnight fast. All subjects were instructed to refrain from moderate (24 h) and vigorous physical activity (48 h) before the test day. On the previous evening, all subjects consumed a standardized meal of an egg omelet, boiled rice, and tomato sauce (ad libitum amounts). They were also instructed to avoid physical activity after they woke up, and to come to the research center by car or bus early in the morning, having had no breakfast (ensuring a ~12 h fast). Upon arrival, and after confirming their compliance with these above conditions, body weight and height were measured using a Seca model 799 electronic column scale (Seca, Hamburg, Germany) with the subjects barefoot and wearing light clothing. Thereafter, the subjects laid on a bed in the supine position for 20–30 min. Gas exchange data were then recorded by indirect calorimetry for 30 min in a quiet room with dim lighting, controlled at 22–24 °C and 35%–45% relative humidity [8]. During this time the subjects were covered with a bed sheet and instructed to remain silent, stay awake, avoid fidgeting, and to breathe normally.

Gas Exchange Assessments

Gas exchange was recorded using either a CCM Express or a CPX Ultima CardiO2 (two different devices were used only in the middle-aged adults cohort) breath-by-breath metabolic cart (Medical Graphics Corp, St. Paul, MN, USA). Both instruments require the use of a face mask equipped with a Directconnect™ flow sensor (Medical Graphics Corp, St. Paul, MN, USA). Both determine VCO_2 using a non-dispersive infrared analyzer, and both determine VO_2 using a galvanic fuel cell [16]. The flow rate was calibrated using a 3 L syringe at the beginning of every test. The gas analyzers were calibrated before each measurement using standard gases according to the manufacturers' instructions [16].

Methods for Gas Exchange Data Selection

The collected gas exchange data were processed using MGCDiagnostic® Breeze Suite 8.1.0.54 SP7 software (Medical Graphics Corp., St. Paul, MN, USA) to yield a data point for each variable for every minute (i.e., the means of all ventilation data (per minute ventilation data—pMVD) for each particular minute). The first 5 min of data were discarded [8,9]; the remaining 25 min period dataset was processed using three different methods to select representative gas exchange data for determining the RMR and nutrient oxidation rate.

Time Interval Method

Short TIs of 6–10 min, 11–15 min, 16–20 min, 21–25 min, and 26–30 min, and long TIs of 6–25 min and 6–30 min were established [11], and the means of the pMVD values for all variables available for these time periods calculated. These processed data were used to calculate the RMR and nutrient oxidation rate (see below for details).

Steady-State Time Method

The CVs of VO_2, VCO_2, VE, and RER were calculated for every period of 3, 4, 5, and 10 min (e.g., for the 3 min SSt we processed all the 25 min period datasets and we examined the 6th to 8th min period, the 7th to 9th period, etc.) and the mean CVs for each variable calculated for each time period. The periods selected for the final analyses were those with the lowest mean CV for each (e.g., from the 3 min SSt examined periods, we selected the 7th to 9th) [11]. The means of the available pMVD values for these time periods were then calculated. These processed data were used to calculate the RMR and nutrient oxidation rate (see below for details).

Filtering Method

The pMVD values for VO_2 and VCO_2 for the entire 25 min data collection period—i.e., with no division into SSt or TI periods—were used to calculate the $mean_{25\ min}$ RMR (see below for details). Furthermore, pMVD RMR values were also calculated, discarding either (i) those values <85% or >115% of the $mean_{25\ min}$ RMR (low filter), (ii) <90% or >110% of the $mean_{25\ min}$ RMR (medium filter), or (iii) <95% or >105% of the $mean_{25\ min}$ RMR (strong filter). For the minutes that passed these filters, the means were calculated for all pMVD values available.

Calculating the Resting Metabolic and Nutrient Oxidation Rates

Weir's equation (assuming zero urinary nitrogen excretion) [17] was used to calculate RMR values from the mean pMVD for VO_2 and VCO_2 obtained with each gas exchange data selection method. The FATox and CHOox rates were calculated using Frayn's stoichiometric equations [18], also assuming zero urinary nitrogen excretion. Finally, the mean RMR, RER, VO_2, VCO_2, FATox, and CHOox were calculated for all gas exchange data selection methods under each different condition.

Body Composition assessment

Body composition was determined by dual energy X-ray absorptiometry using a Discovery Wi device (Hologic Inc., Bedford, MA, USA). Quality controls, the positioning of participants and analysis of the results were performed according to the manufacturer's recommendations.

Statistical analysis

Results are presented as mean±SD unless otherwise stated. All analyses were conducted using the Statistical Package for the Social Sciences v.22.0 (IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA). Significance was set at $p < 0.05$. Repeated-measures analysis of variance (ANOVA) with a *post-hoc* Bonferroni test was used to detect differences in RMR, RER, VO_2, VCO_2, FATox, and CHOox estimates across the methods for gas exchange data selection. The CVs for VO_2, VCO_2, RER, and VE obtained via the different methods were also compared. Two different ANOVA models were used: one with four levels of fixed factors (i.e., short TI, long TI,

SSt, and filtering), and one with 14 levels (all methods and their different conditions).

The differences between the methods in terms of the variance in RMR explained by its classical determinants (i.e., body weight, body composition [lean and fat masses], and sex) [13] were examined by either simple linear regression (associations between RMR and body weight) or multiple linear regression (associations between RMR and sex and body weight; RMR and sex and lean and fat masses).

RESULTS

Table 7. Subject descriptive characteristics.

	Young Adults (n = 107)				Middle-Aged Adults (n = 74)			
		Min	Max	Percentile 10 - 90		Min	Max	Percentile 10 - 90
Age (years)	22.2 ± 2.2	18.2	26.6	19.1–25.2	53.5 ± 5.3	45.0	66.0	47.0–61.7
Sex (n, %)								
Women	72 , 67				39 , 53			
Men	35 , 33				35 , 47			
Metabolic cart used (n, %)								
CCM	46 , 43				0 , 0			
Ultima	61 , 57				74 , 100			
Body weight (kg)	69.3 ± 15.9	45.0	118.5	52.2–90.6	75.7 ± 15.0	50.6	110.7	57.8–94.6
Height (cm)	167.8 ± 8.7	148.5	195.1	157.2–180	167.8 ± 9.8	148.3	189.8	155.8–181.6
BMI (kg/m^2)	24.5 ± 4.4	17.2	38.4	19.4–30.9	26.7 ± 3.8	19.0	38.0	22.0–31.7
WC (cm)	79.8 ± 13.2	58.0	125.6	65.0–97.8	95.1 ± 11.7	68.6	118.7	79.2–107.8
LM (kg)	41.4 ± 9.5	28.1	66.8	31.0–55.2	43.5 ± 11.7	22.7	63.6	30.5–59.6
FM (kg)	24.0 ± 8.9	9.9	51.7	14.6–36.4	30.0 ± 8.4	14.5	55.8	20.6–40.7
FM (%)	35.0 ± 7.8	15.3	51.9	26.2–44.4	39.9 ± 9.1	23.0	59.4	26.7–51.1

Data are presented as mean±SD unless otherwise stated. CCM: CCM Express metabolic cart; Ultima: CPX Ultima CardiO2 metabolic cart; BMI: Body mass index; WC: waist circumference; LM: lean mass; FM: fat mass.

Influence of the gas exchange data selection method on estimates of RMR, RER and nutrient oxidation

Figure 30 shows the RMR and RER estimates yielded by the gas exchange data selection methods for both the young and middle-aged adults. In the young adults, the short and long TI methods provided higher mean RMR estimates than either the SSt or filtering methods (taking all conditions together; *post-hoc* Bonferroni $p < 0.001$; **Figure 30A**). In the middle-aged adults they also provided higher mean RMR estimates than the filtering method (taking all conditions together; *post-hoc* Bonferroni $p < 0.001$; **Figure 30B**). No differences were seen between the RMR estimates yielded by the short and long TIs, nor between the SSt and the filtering methods (taking all conditions together) in either the young or the middle-aged adults (all *post-hoc* Bonferroni $p = 1.000$; **Figure 30A,B**). For the young adults, the lowest mean RMR values were obtained with the SSt 4 min method (1440 kcal/day; **Figure 30E**); however, the SSt 4 min method was only statistically different from the TI 6–10 min and the TI 11–15 min. In the middle-aged adults the lowest mean RMR values were provided by the strong-filter method (1493 kcal/day; **Figure 30F**); the strong-filter method was statistically different from all the different methods, with the exception of the TI 11–15 min, and the SSt 3, 4, and 5 min conditions. **Table S4** shows the comparisons (i.e., *post-hoc* Bonferroni) between the different methods. Similar patterns were observed when analyzing the influence of gas exchange data selection method on VO_2 and VCO_2 estimates (**Figure S6**).

Lastly, the periods in which the SSt were achieved (with the different SSt methods applied) in the young and the middle-aged adults are presented in **Figure S7**. We observed that ~50% of young and middle-aged adults achieved their SSts (i.e., the one presenting lower mean CV) during the first half of the 30 min indirect calorimetry assessment. On the other hand, we found differences between the first steady state achieved (i.e., the first period in which the CVs of $VO_2 < 10$, $VCO_2 < 10$, $VE < 10$, and $RER < 5$) and the "best" SSt achieved (i.e., the period with the lowest mean CVs) in RMR estimation in young adults (**Figure S8**).

The RER estimates yielded by the short TI method were significantly higher than all others in the young adult cohort (all *post-hoc* Bonferroni $p < 0.002$; **Figure 30C**) and that filtering method (taking all conditions together) in the middle-aged adults cohort (*post-hoc* Bonferroni $p = 0.038$; **Figure 30D**). Moreover, the long TI method provided higher RER estimates than the SSt and filtering methods (taking all conditions together) in the young adult (both Bonferroni *post-hoc* $p < 0.013$; **Figure 30C**). No differences in RER estimates were seen when comparing the SSt and filtering methods (taking all conditions together) in either the young or the middle-aged adults (*post-hoc* Bonferroni $p = 1.000$; **Figure 30C,D**). Furthermore, the long TI and the SSt were not significantly different than either the short TI method or the filtering methods (taking all conditions together) in the middle-aged adults (**Figure 30D**). The lowest mean RER values were obtained when using the strong-filter method (0.84) in the young adults (**Figure 30G**). However, the strong-filter method was only statistically different from the TI 26–30 min and the TI 6–30 min. The lowest mean RER values were obtained when using the SSt 3 min method (0.80) in the middle-aged adults (**Figure 30H**). However, no statistical differences were observed. As expected, the data selection methods yielding higher RER estimates also provided higher CHOox and lower FATox estimates, and vice versa (**Figure 31**).

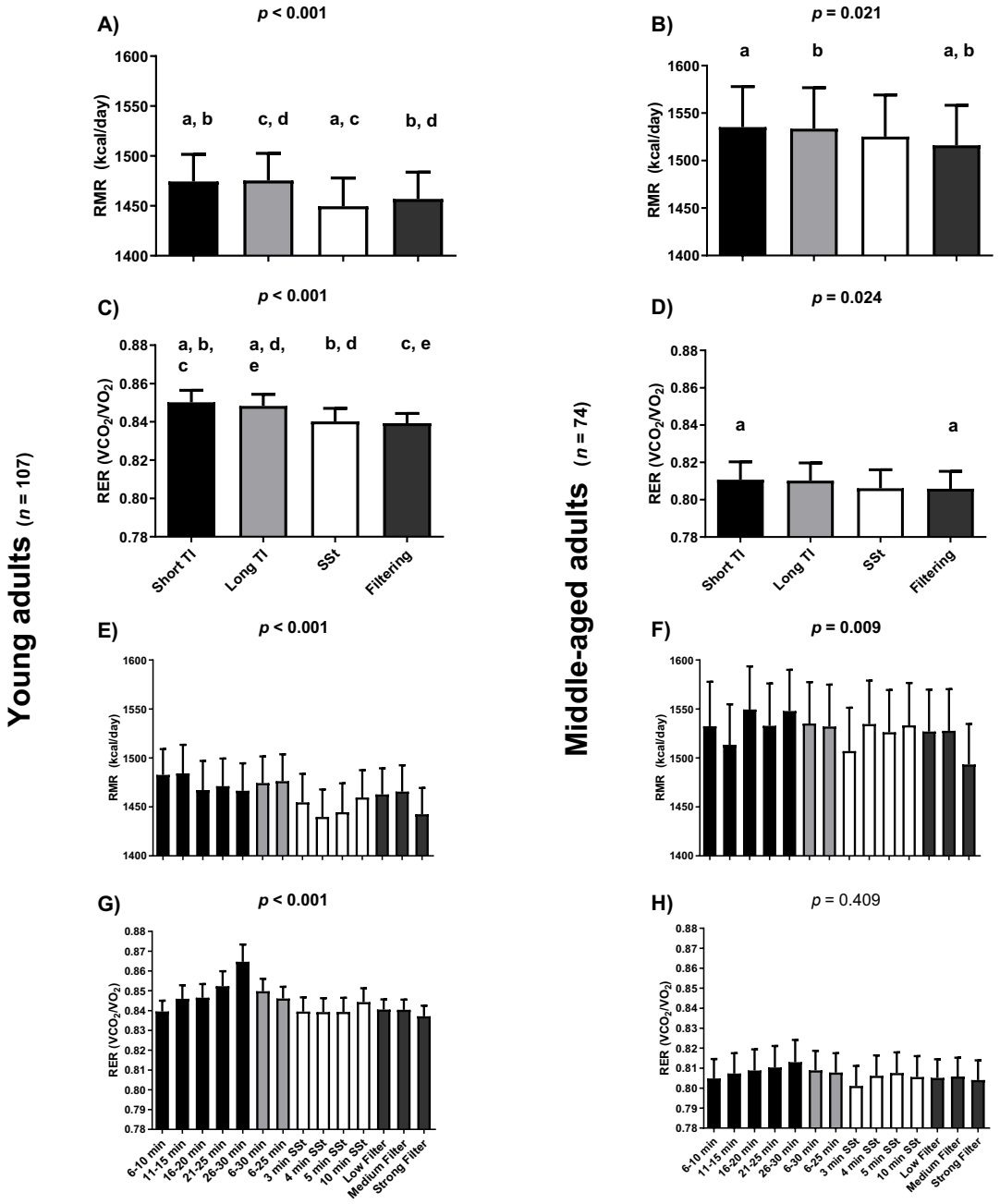


Figure 30. Differences among gas exchange data selection methods with respect to resting metabolic rate (RMR) and respiratory exchange ratio (RER) estimates. *Black columns represent short time interval (TI) periods (i.e., the means of the per minute ventilation data (pMVD]) values for all variables available for these time periods, panels A–D; the pMVD values for each short TI period, panels E,F). Light grey columns represent long TI periods (i.e., the means of the pMVD values for all variables available for these time periods, panels A–D; the means of the pMVD values for each long TI period, panels E,F). White columns represent steady state (SSt) periods (i.e., the means of the pMVD values for all variables available for these SSt periods, panels A-D; the means of the pMVD values for each SSt period, panels E,F). Dark grey columns represent filtering methods (i.e., the means of the pMVD values for all variables available for these filtering periods, panels A–D; the means of the pMVD values for each filtering period, panels E,F). p-values come from repeated-measures analysis of variance (ANOVA). Identical indicatory letters highlight differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM). Min: minutes; VCO_2: carbon dioxide production; VO_2: oxygen consumption.*

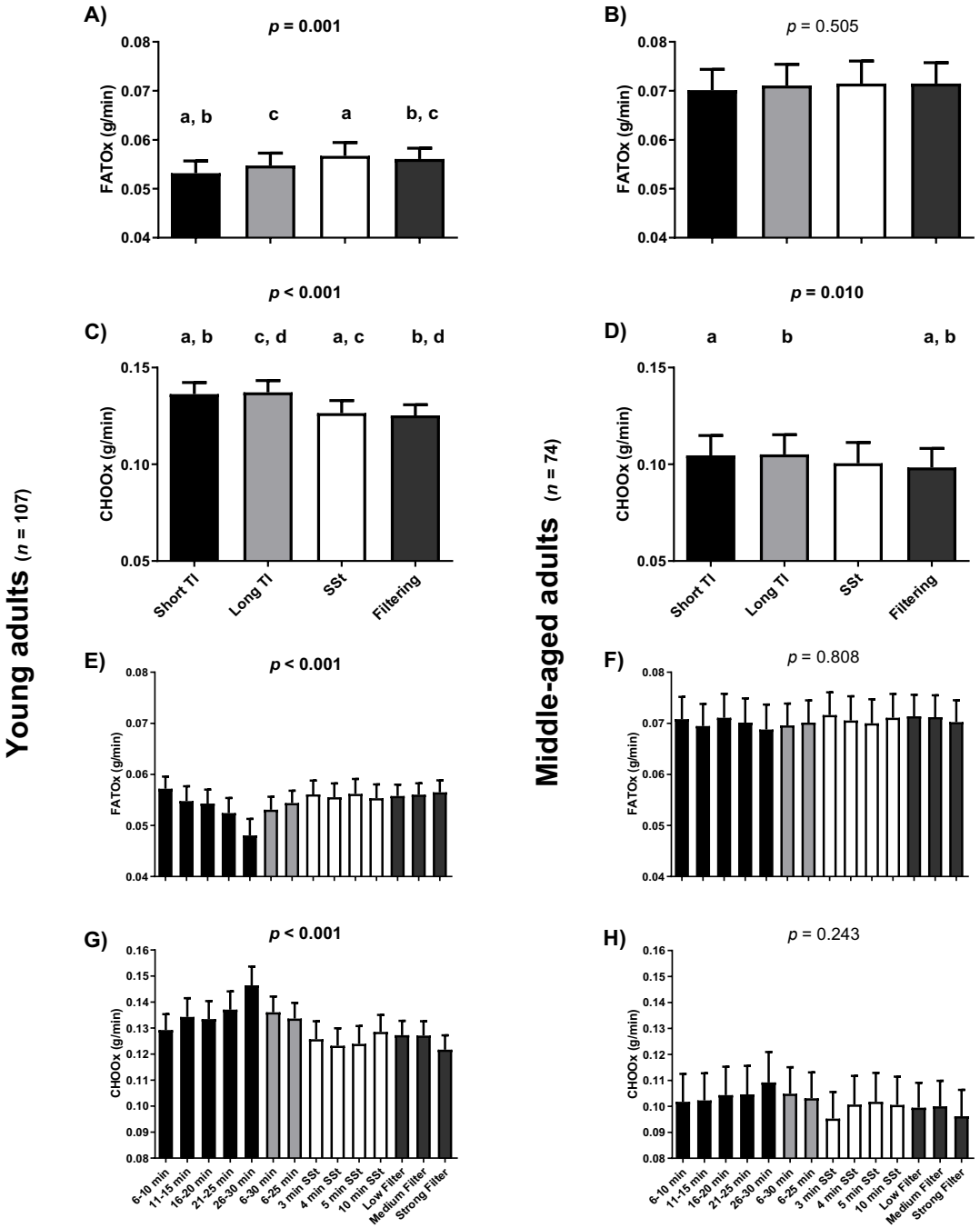


Figure 31. Differences among gas exchange data selection methods with respect to fat oxidation (FATox) and carbohydrate oxidation (CHOox) rates. *Black columns represent short time interval (TI) periods (i.e., the means of the per minute ventilation data [pMVD] values for all variables available for these time periods, panels A–D; the pMVD values for each short TI period, panels E,F). Light grey columns represent long TI periods (i.e., the means of the pMVD values for all variables available for these time periods, panels A–D; the means of the pMVD values for each long TI period, panels E,F). White columns represent steady state (SSt) periods (i.e., the means of the pMVD values for all variables available for these SSt periods, panels A–D; the means of the pMVD values for each SSt period, panels E,F). Dark grey columns represent filtering methods (i.e., the means of the pMVD values for all variables available for these filtering periods, panels A–D; the means of the pMVD values for each filtering period, panels E,F). p-values come from repeated-measures analysis of variance (ANOVA). Identical indicatory letters highlight differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM). Min: minutes.*

Differences between the methods in terms of the variance in RMR explained by its classical determinants

The variance in RMR explained by body weight (taking all conditions together) was 36%, 36%, 34%, and 38% for the short TI, long TI, SSt, and filtering methods respectively in young adults, and 50%, 51%, 52%, and 51% respectively in the middle-aged adults. The most explained variance was obtained using the low-filter method (40%) in young adults and the TI 6–10 min method (54%) in the middle-aged adults.

The variance explained increased to 34%–45% and 54%–68% in the young and middle-aged adults respectively after including subject sex in the model (**Table 8**). The most explained variance was obtained with the low-filter method in young adults, and both the TI 21–25 min and the medium-filter methods in the middle-aged adults. However, little difference was seen among the methods in terms of the variance explained by the classical determinants of RMR (**Table 8**).

A further model including subject sex, lean, and fat masses increased the variance in RMR explained by ~5% in the young adults, but not in the middle-aged adults (Table 3). The low-filter method explained the greatest variance in RMR in the young adults, and the TI 21–25 min method did so in the middle-aged adults. However, once again, little difference was seen among the methods in terms of the variance explained by the classical determinants of RMR (**Table 9**).

Table 8. Variance in resting metabolic rate (RMR) explained by sex and body weight in each of the gas exchange data selection methods.

	Young Adults (n = 107)						Middle-Aged Adults (n = 74)					
			Sex		Weight (kg)				Sex		Weight (kg)	
Method of data selection	**Model R^2**	**Constant**	**β**	**p**	**β**	**p**	**Model R^2**	**Constant**	**β**	**p**	**β**	**p**
TI 6–10 min	0.44	1232	−171.6	**0.001**	7.8	**<0.001**	0.67	1503	−424.0	**0.001**	8.9	**0.001**
TI 11–15 min	0.40	1340	−214.4	**<0.001**	7.3	**<0.001**	0.67	1616	−418.5	**0.001**	7.1	**0.004**
TI 16–20 min	0.40	1300	−214.5	**<0.001**	7.6	**<0.001**	0.60	1758	−445.2	**0.001**	6.2	**0.031**
TI 21–25 min	0.36	1342	−199.5	**0.001**	6.7	**<0.001**	0.68	1935	−507.5	**0.001**	4.9	0.051
TI 26–30 min	0.34	1208	−155.7	**0.009**	7.5	**<0.001**	0.54	1754	−409.8	**0.001**	5.5	0.058
TI 6–30 min	0.42	1284	−191.2	**0.001**	7.4	**<0.001**	0.66	1713	−441.0	**0.001**	6.5	**0.010**
TI 6–25 min	0.43	1303	−200.0	**<0.001**	7.3	**<0.001**	0.67	1703	−448.8	**0.001**	6.8	**0.008**
SSt 3 min	0.39	1245	−194.9	**0.001**	7.8	**<0.001**	0.66	1669	−456.7	**0.001**	7.1	**0.008**
SSt 4 min	0.39	1169	−167.5	**0.004**	7.9	**<0.001**	0.67	1786	−481.1	**0.001**	6.4	**0.016**
SSt 5 min	0.36	1301	−207.0	**0.001**	7.1	**<0.001**	0.66	1763	−464.1	**0.001**	6.2	**0.016**
SSt 10 min	0.41	1232	−182.8	**0.001**	7.7	**<0.001**	0.66	1681	−443.8	**0.001**	7.0	**0.008**
Low-filter	0.45	1188	−170.6	**0.001**	8.1	**<0.001**	0.67	1701	−442.9	**0.001**	6.6	**0.009**
Medium-filter	0.43	1209	−170.8	**0.001**	7.8	**<0.001**	0.68	1725	−447.5	**0.001**	6.4	**0.010**
Strong-filter	0.44	1218	−182.0	**0.001**	7.7	**<0.001**	0.67	1684	−431.7	**0.001**	6.2	**0.012**

Unstandardized beta and p-values (significant values in bold) from multiple regression analyses, in which sex and body weight were included as independent variables, and the RMR estimates yielded by the different methods of gas exchange data selection were included as dependent variables. Sex: 1 = men; 2 = women.

Table 9. Variance in resting metabolic rate (RMR) explained by sex, lean mass (LM) and fat mass (FM) in each of the gas exchange data selection methods.

	Young adults (n = 107)								Middle-aged adults (n = 74)							
			Sex		LM (kg)		FM (kg)				Sex		LM (kg)		FM (kg)	
Method of data selection	Model R^2	Constant	β	p	β	p	β	p	Model R^2	Constant	β	p	β	p	β	p
TI 6–10 min	0.47	668	−3.5	0.967	18.7	**<0.001**	2.1	0.450	0.66	1436	−400.4	**0.001**	10.5	**0.020**	8.4	**0.011**
TI 11–15 min	0.45	483	39.2	0.683	23.4	**<0.001**	−1.2	0.697	0.67	1583	−407.0	**0.001**	8.0	**0.050**	6.8	**0.022**
TI 16–20 min	0.44	497	23.2	0.814	22.8	**<0.001**	−0.3	0.925	0.60	1533	−370.0	**0.001**	10.4	**0.029**	4.3	0.210
TI 21–25 min	0.41	542	37.0	0.702	21.7	**<0.001**	−1.2	0.697	0.68	1713	−433.1	**0.001**	9.0	**0.030**	3.0	0.320
TI 26–30 min	0.38	433	73.8	0.451	22.1	**<0.001**	−0.2	0.957	0.55	1500	−324.4	**0.005**	10.2	**0.034**	3.3	0.346
TI 6–30 min	0.47	525	34.0	0.701	21.7	**<0.001**	−0.2	0.955	0.66	1553	−381.0	**0.001**	9.6	**0.022**	5.2	0.089
TI 6–25 min	0.47	548	24.0	0.787	21.6	**<0.001**	−0.2	0.956	0.67	1566	−402.5	**0.001**	9.5	**0.024**	5.6	0.065
SSt 3 min	0.44	417	49.5	0.610	23.3	**<0.001**	−0.3	0.919	0.66	1657	−452.0	**0.001**	7.6	0.085	7.0	**0.029**
SSt 4 min	0.45	306	87.7	0.344	24.2	**<0.001**	−0.5	0.868	0.67	1695	−451.0	**0.001**	8.2	0.060	5.7	0.073
SSt 5 min	0.42	379	65.7	0.510	24.3	**<0.001**	−2.0	0.530	0.66	1694	−441.0	**0.001**	7.7	0.073	5.7	0.066
SSt 10 min	0.45	464	44.8	0.628	22.2	**<0.001**	0.1	0.965	0.66	1534	−394.1	**0.001**	9.9	**0.023**	5.7	0.069
Low filter	0.50	451	47.8	0.571	22.1	**<0.001**	0.8	0.770	0.67	1577	−400.9	**0.001**	9.1	**0.030**	5.6	0.066
Medium filter	0.48	424	62.0	0.471	22.7	**<0.001**	0.1	0.989	0.67	1597	−404.1	**0.001**	8.9	**0.029**	5.3	0.073
Strong filter	0.49	416	55.4	0.516	22.8	**<0.001**	−0.3	0.918	0.67	1534	−381.2	**0.001**	9.1	**0.025**	4.9	0.094

Unstandardized beta and p-values (significant values in bold) from multiple regression analyses, in which sex, LM and FM were included as independent variables, and the RMR estimates yielded by the different methods of gas exchange data selection were included as dependent variables. Sex: 1 = men; 2 = women.

DISCUSSION

The present results show that when using breath-by-breath metabolic carts, RMR, and RER estimates yielded by the SSt and filtering methods are lower than those yielded by the TI method, while no differences were seen between the SSt and filtering methods. The variance in the RMR explained by its classical determinants (i.e., weight, body composition, and sex) was similar in all methods. These findings largely concurred in the cohorts of young adults and middle-aged adults examined, which further reinforced the consistency of the results.

Influence of the gas exchange data selection method on RMR, RER and nutrient Oxidation

Given that RMR is defined as the lowest energy expenditure of a person who is awake [1], the present results suggest that the SSt and filtering methods provide better RMR estimates. These results are in line with those reported in the literature [1,12] and concur with those reported in our previous study [11], in which lower RMR and RER estimates were yielded by the SSt method than the TI method. As expected, the SSt method showed less variability in the results returned than the TI method (Figures S9 and S10). This supports the notion that the assessment of stable state respiratory gas exchange variables provides the best results [8,19].

The SSt method purportedly reflects the baseline physiological state [10], thus reflecting the homeostatic RMR and nutrient oxidation rates [20,21]. Reeves et al. [19] reported that SSt measurements for assessing RMR were more accurate when taking data over short periods (e.g., 30 min). In line with this, McClave et al. [10] reported that the RMR obtained with the SSt 5 min method provides an accurate representation of the 24 h total energy expenditure in bedridden hospitalized patients owing to their low level of physical activity. However, in healthy individuals, the RMR does not represent the 24 h total energy expenditure [6]. McClave et al. [10] recommended that: (i) a steady respiratory state should be considered reached when changes in the CV of VO2 and VCO2 are <10% over a period of 5 consecutive min, (ii) RMR assessment should end when a steady respiratory state is achieved, and (iii) when a steady respiratory state cannot be reached, a more prolonged test (≥60 min) becomes necessary. Reeves et al. [19] showed that reducing the steady respiratory state time period for data collection from 5 to 4 min resulted in acceptable RMR values. It has been suggested that reducing it to 3 min might underestimate the RMR [19], but in our previous study [11] no differences were seen between the RMRs provided by the SSt 3, 4, or 5 min conditions. The present results agree with our previous findings in a smaller sample of young adults (17 vs. the present 107) and middle-aged adults [11]. In addition, the present results for both independent cohorts were similar (i.e., replicated). As we mentioned previously, the assessment of RMR using indirect calorimetry is normally performed over a 30 min period, and our results suggest that the SSt becomes more stable (i.e., less variable) as the RMR measurement progresses (Figure S8). On the other hand, we observed that after ~15 min of measurement, ~50% of the young and the middle-aged adults achieved the SSt (Figure S7). Thus, based on our results we would recommend that when RMR is assessed using indirect calorimetry the measurement should last at least 30 min, to obtain the most stable results as possible reducing the variability (Figure S8). Moreover, it could be interesting to test whether the stability increases (or not) if the RMR measurement lasts more than 30 min.

To our knowledge, no other study has compared the TI, SSt, and filtering methods, rendering further comparisons impossible. However, the present results suggest that those provided by the filtering method are similar to those provided by the SSt method. It might therefore be used as an alternative in subjects in whom a steady respiratory state is not achieved (although the present subjects all reached a steady respiratory state, which might bias this suggestion).

Mean FATox was higher and CHOox lower, when determined via the SSt and filtering methods compared to the TI method, with no differences seen among the different SSt periods or among the different filtering conditions. In contrast, differences were observed among the TI periods in terms of both the RER and nutrient oxidation rates, with an increase in the RER seen over each test period (more pronounced in the young adult cohort—Figure 31). This gradual increase in the RER, which might influence the nutrient oxidation rates estimates, may be related to the metabolic cart rather than subject factors or the method of gas exchange data selection used, since this increase was also observed in a previous study involving different subjects but using the same metabolic carts [11].

Differences between the methods in terms of the variance in RMR explained by its classical determinants

For the middle-aged adults, the variance in RMR explained by its classical determinants across the gas exchange data selection methods was in line with the results of previous studies (36%–56% explained by body weight [3,22–24]), although in the young adult cohort the variance explained was less than in the majority of the aforementioned studies. When comparing with previous studies in which body composition was included in the regression model, the variance in RMR explained across methods in the present study was also lower. In fact, Müller et al. [25] reported 72% of the variance in RMR to be explained by sex, lean mass, and fat mass. Korth et al. [22] reported 75% of the variance to be explained by lean mass alone, while Mifflin et al. [23] reported a value of 64%. Galgani and Castro-Sepulveda [3] reported 75% of the variance in RMR to be explained by fat-free mass, fat mass, and age. In the present study, the variance explained for the middle-aged adults was in line with the aforementioned studies (55%–68% depending on the gas exchange data selection method used—Table 9). The differences across studies might be related, to a greater or lesser extent, to the accuracy of the metabolic cart [16]. In fact, in that study [16] we compared the inter-day reliability and the congruent validity of the CCM Express and the CPX Ultima CardiO2. Firstly, we observed that the CCM Express metabolic cart is more reliable than the CPX Ultima CardiO2 (i.e., less RMR inter-day differences: 158 ± 154 kcal/day vs. 219 ± 185 kcal/day for the CCM Express and the CPX Ultima CardiO2 respectively) [16]. Secondly, we observed that the RMR values obtained using the CPX Ultima CardiO2 were higher than the values obtained using the CCM Express metabolic cart (mean difference between metabolic carts of 65 ± 161 kcal/day on study day 1 and 94 ± 161 kcal/day on study day 2) [16]. Thus, as mentioned, the differences across studies could be related to the accuracy of the metabolic cart, and it may be that neither of the breath-by-breath carts used (i.e., the CCM Express and CPX Ultima CardiO2) is sufficiently accurate for measuring RMR if the ability to explain the variance in RMR is taken as an indirect indicator of accuracy. However, the variance explained was quite similar for all the gas exchange data selection methods used; it may not, therefore, be "method-dependent".

The present results should be considered with some caution. The assessment of RMR was performed using two different breath-by-breath metabolic carts indiscriminately, both of which were equipped with a face-mask. The use of other metabolic carts or other gas collection systems (e.g., canopy collection) might influence the RMR results obtained [26,27]. Further studies involving different metabolic carts and gas collection systems, as well as with different subject populations, are needed to confirm the results.

CONCLUSION

The present findings suggest that when using CCM Express and CPX Ultima CardiO2 breath-by-breath metabolic carts, both the SSt and filtering methods yield the lowest RMR and RER estimates with the lowest amount of variability (i.e., lowest intra-measurement coefficients of variation). Moreover, the filtering method might be a valid alternative for use with subjects who do not achieve a steady respiratory state.

SUPPLEMENTARY MATERIAL

Table S4. Differences in resting metabolic rate (RMR), respiratory exchange ratio (RER), fat oxidation (FATox), carbohydrate oxidation (CHOox), oxygen consumption (VO_2) and carbon dioxide production (VCO_2) across methods for gas exchange data analysis, as revealed by *post-hoc* Bonferroni analysis.

Middle-aged adults (n=74)

Young adults (n=107)	TI-6-10 min	TI-11-15 min	TI-16-20 min	TI-21-25 min	TI-26-30 min	TI-6-30 min	TI-6-25 min	SSt-3 min	SSt-4 min	SSt-5 min	SSt-10 min	Low filter	Medium filter	Strong filter
TI-6-10 min														0.019
TI-11-15 min														
TI-16-20 min														<0.001
TI-21-25 min														0.004
TI-26-30 min														0.009
TI-6-30 min														<0.001
TI-6-25 min														<0.001
SSt-3 min														
SSt-4 min	0.023	0.01				0.001	0.001							
SSt-5 min		<0.001				0.005	0.001							
SSt-10 min							0.021							<0.001
Low filter														<0.001
Medium filter									0.019					<0.001
Strong filter	0.001	<0.001				<0.001	<0.001					<0.001	<0.001	

Middle-aged adults (n=74)

Young adults (n=107)	TI-6-10 min	TI-11-15 min	TI-16-20 min	TI-21-25 min	TI-26-30 min	TI-6-30 min	TI-6-25 min	SSt-3 min	SSt-4 min	SSt-5 min	SSt-10 min	Low filter	Medium filter	Strong filter
TI-6-10 min														
TI-11-15 min														
TI-16-20 min														
TI-21-25 min														
TI-26-30 min														
TI-6-30 min					0.046									
TI-6-25 min					0.030	0.019								
SSt-3 min					<0.001									
SSt-4 min					<0.001									
SSt-5 min					0.001									
SSt-10 min					0.003									
Low filter					0.002	0.006								
Medium filter					0.004	0.030								
Strong filter					0.001	0.007								

Middle-aged adults (n=74)

Young adults (n=107)	TI-6-10 min	TI-11-15 min	TI-16-20 min	TI-21-25 min	TI-26-30 min	TI-6-30 min	TI-6-25 min	SSt-3 min	SSt-4 min	SSt-5 min	SSt-10 min	Low filter	Medium filter	Strong filter
TI-6-10 min														
TI-11-15 min														
TI-16-20 min														
TI-21-25 min														
TI-26-30 min														
TI-6-30 min														
TI-6-25 min														
SSt-3 min					0.007									
SSt-4 min					0.005									
SSt-5 min					0.019									
SSt-10 min					0.005									
Low filter					0.006									
Medium filter					0.008									
Strong filter					0.002									

Middle-aged adults (n=74)

Young adults (n=107)

CHOox (g/min)	TI-6-10 min	TI-11-15 min	TI-16-20 min	TI-21-25 min	TI-26-30 min	TI-6-30 min	TI-6-25 min	SSt-3 min	SSt-4 min	SSt-5 min	SSt-10 min	Low filter	Medium filter	Strong filter
TI-6-10 min														
TI-11-15 min														
TI-16-20 min														
TI-21-25 min														
TI-26-30 min														
TI-6-30 min														
TI-6-25 min														
SSt-3 min					0.001									
SSt-4 min					<0.001	0.003								
SSt-5 min					0.001	0.025								
SSt-10 min					0.002	0.036								
Low filter					<0.001	<0.001	0.032							
Medium filter					0.001	0.001								
Strong filter				0.005	<0.001	<0.001	0.001							

Middle-aged adults (n=74)

Young adults (n=107)

VO_2 (ml/min)	TI-6-10 min	TI-11-15 min	TI-16-20 min	TI-21-25 min	TI-26-30 min	TI-6-30 min	TI-6-25 min	SSt-3 min	SSt-4 min	SSt-5 min	SSt-10 min	Low filter	Medium filter	Strong filter
TI-6-10 min														0.02
TI-11-15 min														
TI-16-20 min														<0.001
TI-21-25 min														0.012
TI-26-30 min														0.026
TI-6-30 min														<0.001
TI-6-25 min														<0.001
SSt-3 min														
SSt-4 min	0.038	0.044				0.006	0.004							
SSt-5 min		<0.001				0.044	0.004							
SSt-10 min							0.042							<0.001
Low filter														<0.001
Medium filter									0.041					<0.001
Strong filter	0.003	<0.001				<0.001	<0.001					<0.001	<0.001	

Middle-aged adults (n=74)

Young adults (n=107)

VCO_2 (ml/min)	TI-6-10 min	TI-11-15 min	TI-16-20 min	TI-21-25 min	TI-26-30 min	TI-6-30 min	TI-6-25 min	SSt-3 min	SSt-4 min	SSt-5 min	SSt-10 min	Low filter	Medium filter	Strong filter
TI-6-10 min														
TI-11-15 min														
TI-16-20 min														0.003
TI-21-25 min														0.02
TI-26-30 min														0.002
TI-6-30 min														<0.001
TI-6-25 min														<0.001
SSt-3 min														
SSt-4 min	0.046	0.001		0.005	0.001	<0.001	<0.001							
SSt-5 min		0.002			0.019	<0.001	<0.001							
SSt-10 min						0.008	0.036							<0.001
Low filter						<0.001	0.004		0.043					<0.001
Medium filter						<0.001	0.01		0.02					<0.001
Strong filter	<0.001	<0.001	0.015	<0.001	<0.001	<0.001	<0.001				0.005	<0.001	<0.001	

Significant P values returned by the post-hoc Bonferroni test are presented. P values for young adults are presented shaded in white while P values for middle-aged adults are presented shaded in light grey.

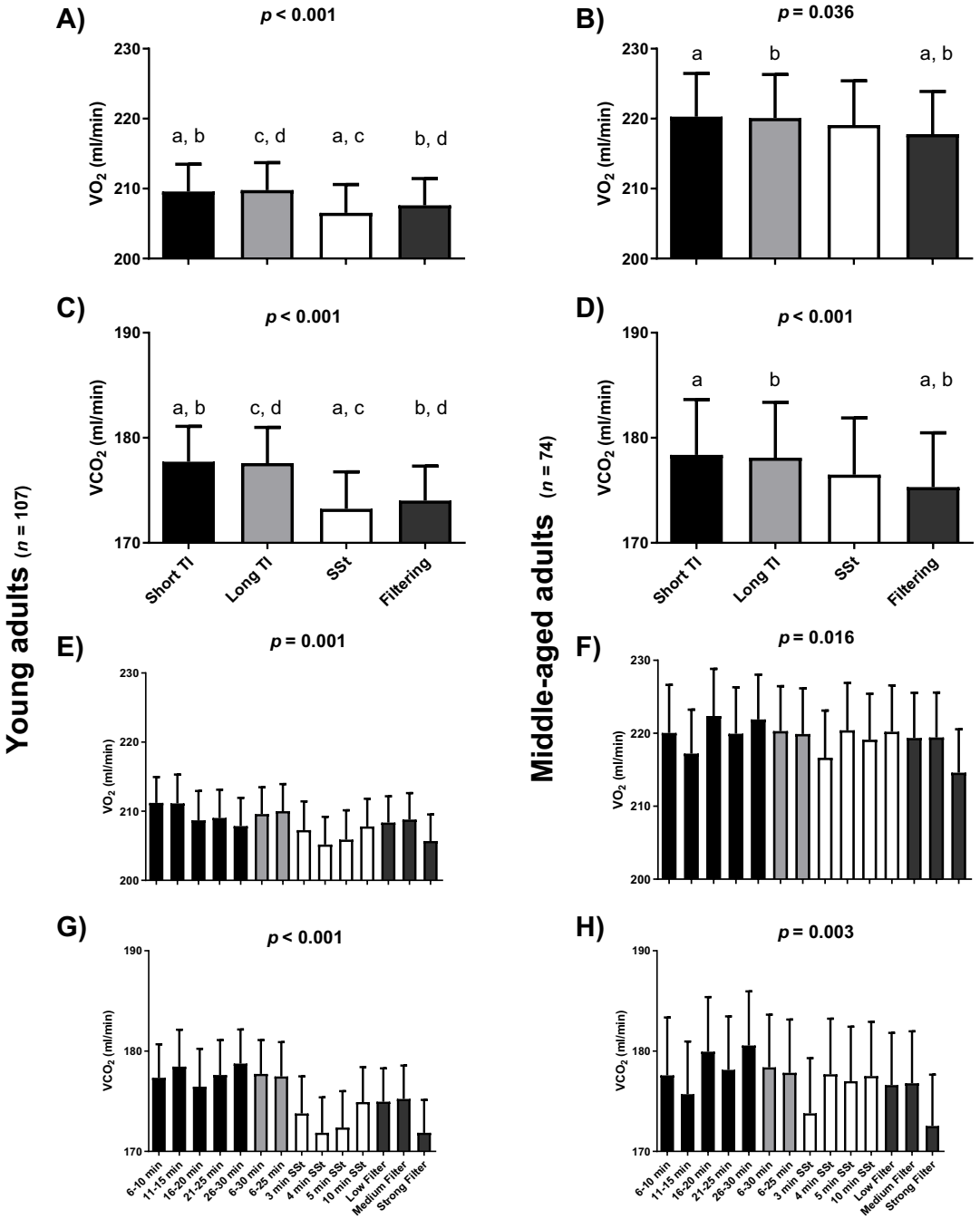


Figure S6. Differences among methods for gas exchange data selection with respect to oxygen consumption (VO_2) and carbon dioxide production (VCO_2). *Black columns represent short time interval (TI) periods (i.e. the means of the per minute ventilation data [pMVD] values for all variables available for these time periods, Panels A-D; the pMVD values for each short TI period, Panels E-F); light grey columns represent long TI periods (i.e. the means of the pMVD values for all variables available for these time periods, Panels A-D; the means of the pMVD values for each long TI period, Panels E-F); white columns represent steady state (SSt) periods (i.e. the means of the pMVD values for all variables available for these SSt periods, Panels A-D; the means of the pMVD values for each SSt period, Panels E-F); dark grey columns represent filtering methods (i.e. the means of the pMVD values for all variables available for these filtering periods, Panels A-D; the means of the pMVD values for each filtering period, Panels E-F). P values come from repeated-measures analysis of variance (ANOVA). Identical indicatory letters highlight differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM). Mil: millilitres; Min: minutes.*

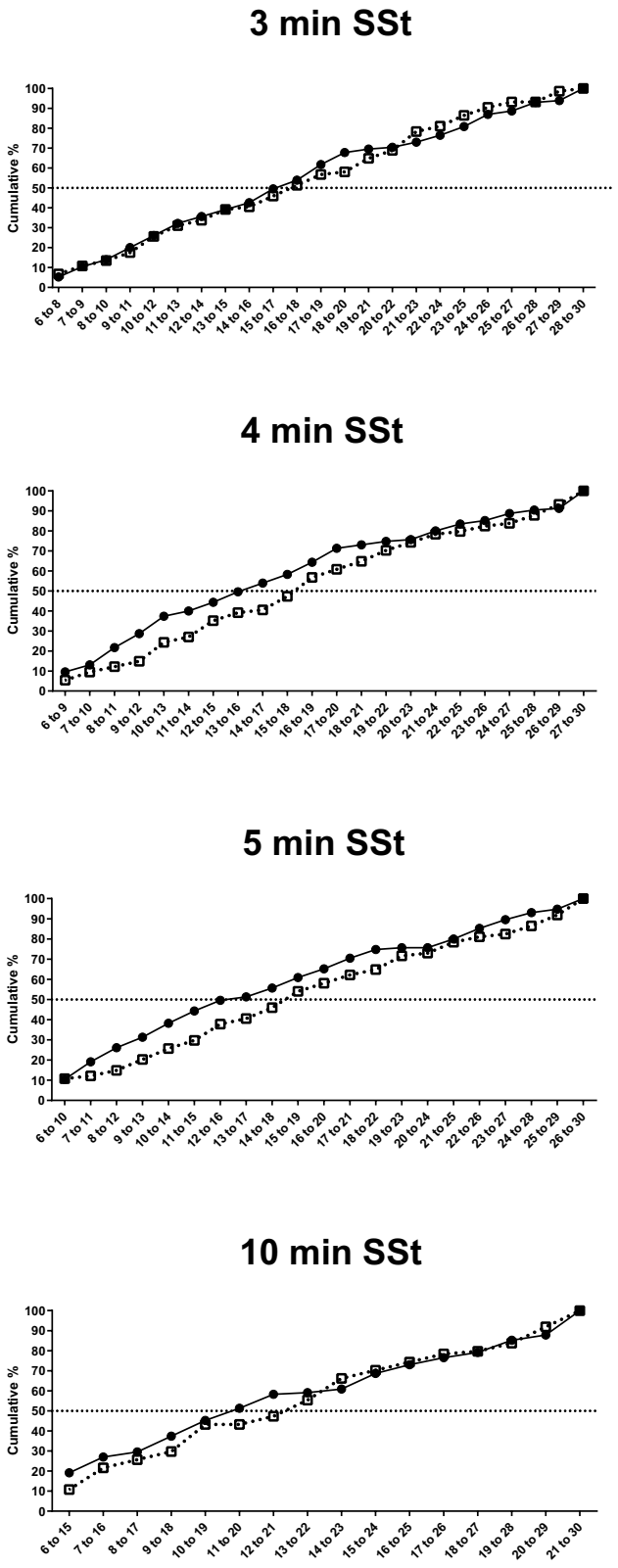


Figure S7. Period from the 30 min resting metabolic rate measurement in which the steady state (SSt) is achieved with respect to the SSt methods applied in each cohort. *Data are presented as a cumulative percentage of subjects whom achieved the SSt. Solid line represent the percentage for the young-adults' cohort; dashed line represent the percentage for the middle-aged adults' cohort.*

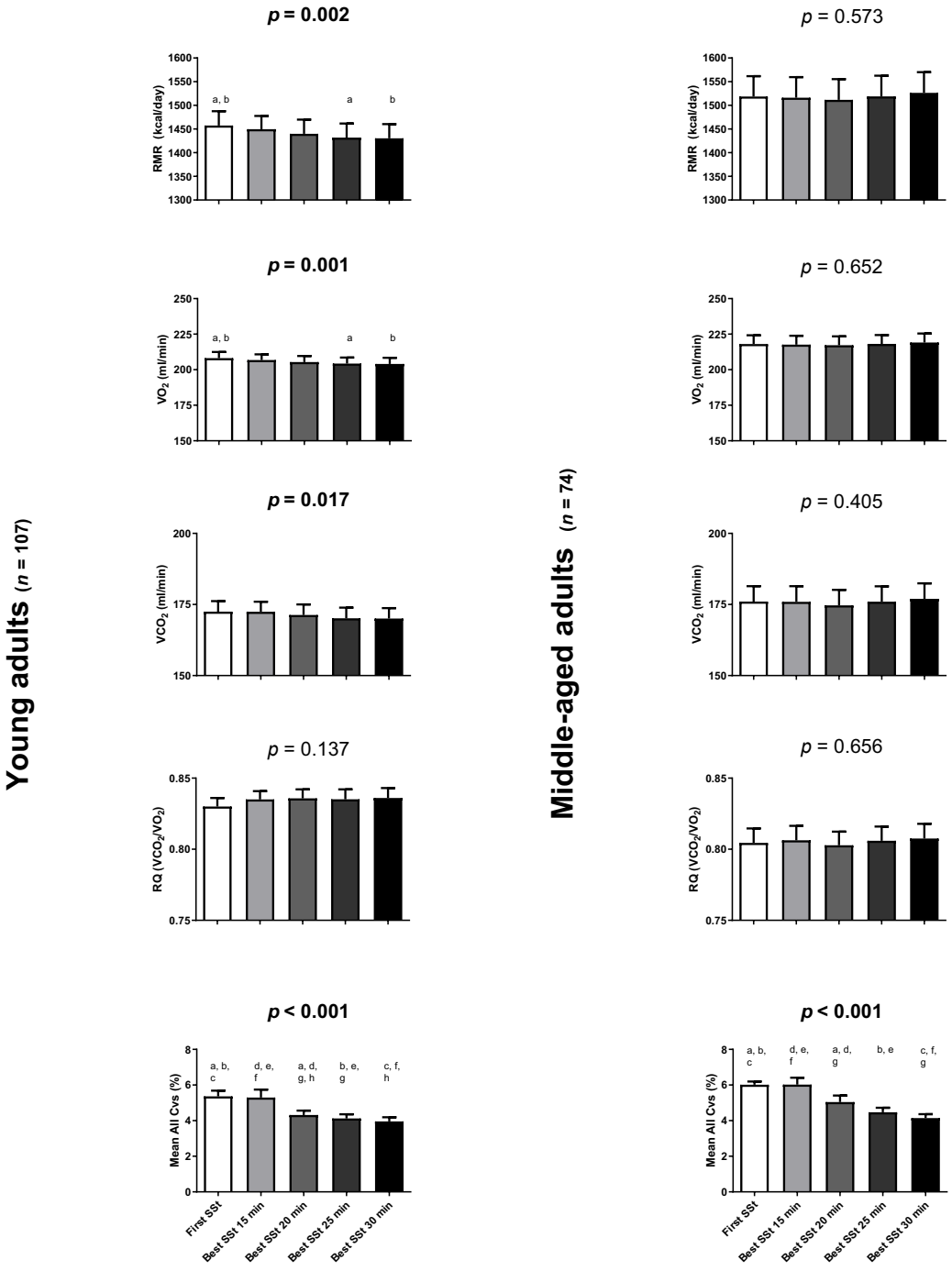


Figure S8. Differences among 5 min Steady State time (SSt) method achieved at different time lengths gas exchange data selection with respect to resting metabolic rate (RMR), oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER) and the mean of all coefficients of variation (Mean All CVs; i.e., coefficient of variation of VO_2, VCO_2, RER and minute ventilation). *First SSt represent the first SSt-5 min period in which the coefficient of variation of VO_2, VCO_2 and minute ventilation were lower than 10% and the coefficient of variation of RER was lower than 5%. Best SSt represent the SSt-5 min period with the lowest mean of all coefficients of variation aforementioned (i.e., Mean All CVs). P values come from repeated-measures analysis of variance (ANOVA). Identical indicatory letters highlight differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM). Mil: millilitres; Min: minutes.*

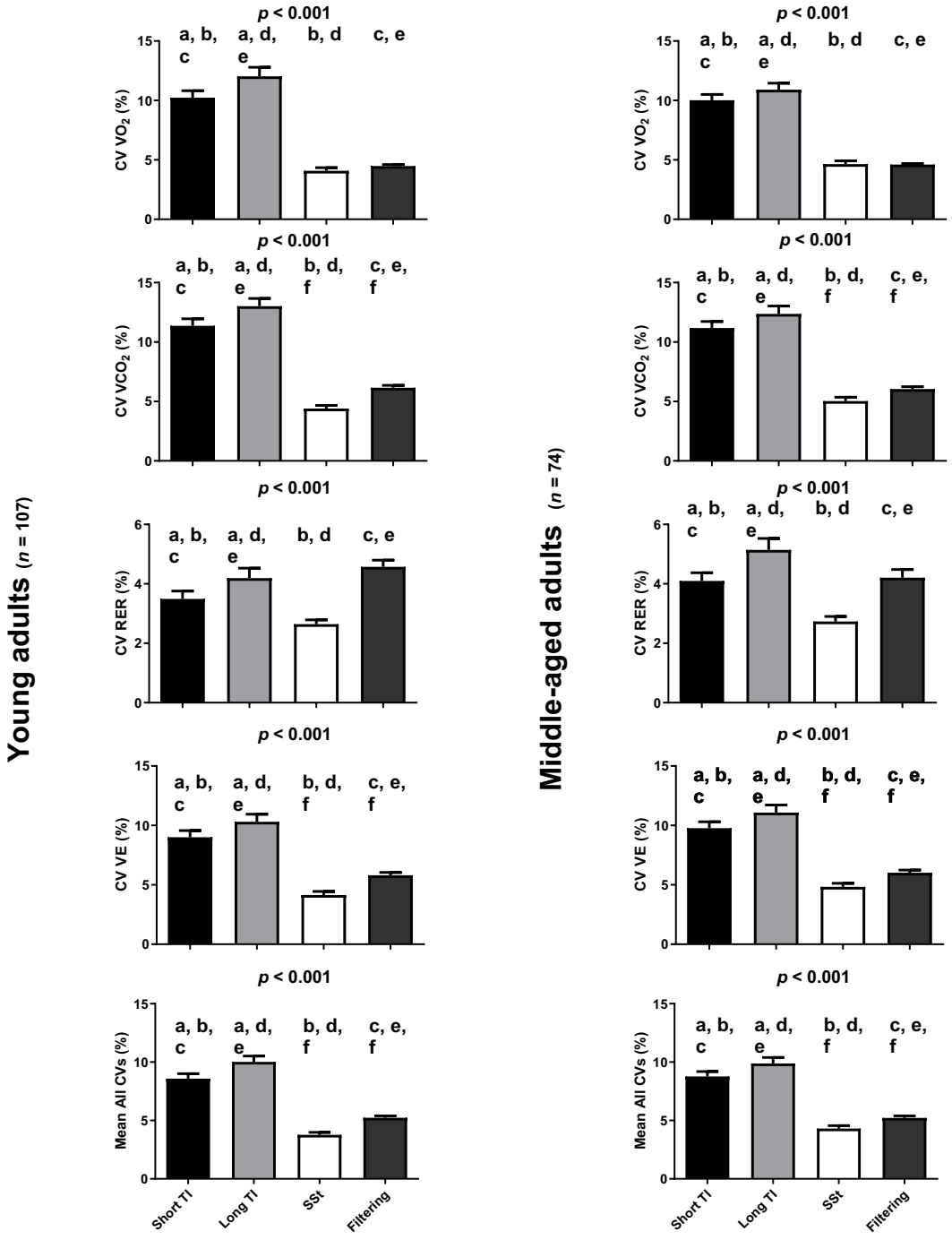


Figure S9. Differences among gas exchange data selection with respect to the coefficient of variation of oxygen consumption (CV VO_2), carbon dioxide production (CV VCO_2), respiratory exchange ratio (CV RER), ventilation (CV VE) and the mean of all coefficients of variation aforementioned (Mean All CVs). *Black columns represent short time interval (TI) periods; light grey columns represent Whole-measurement periods; white columns represent steady state (SSt) periods; and dark grey columns represent filtering methods. P values come from repeated-measures analysis of variance (ANOVA). Identical indicatory letters highlight differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM).*

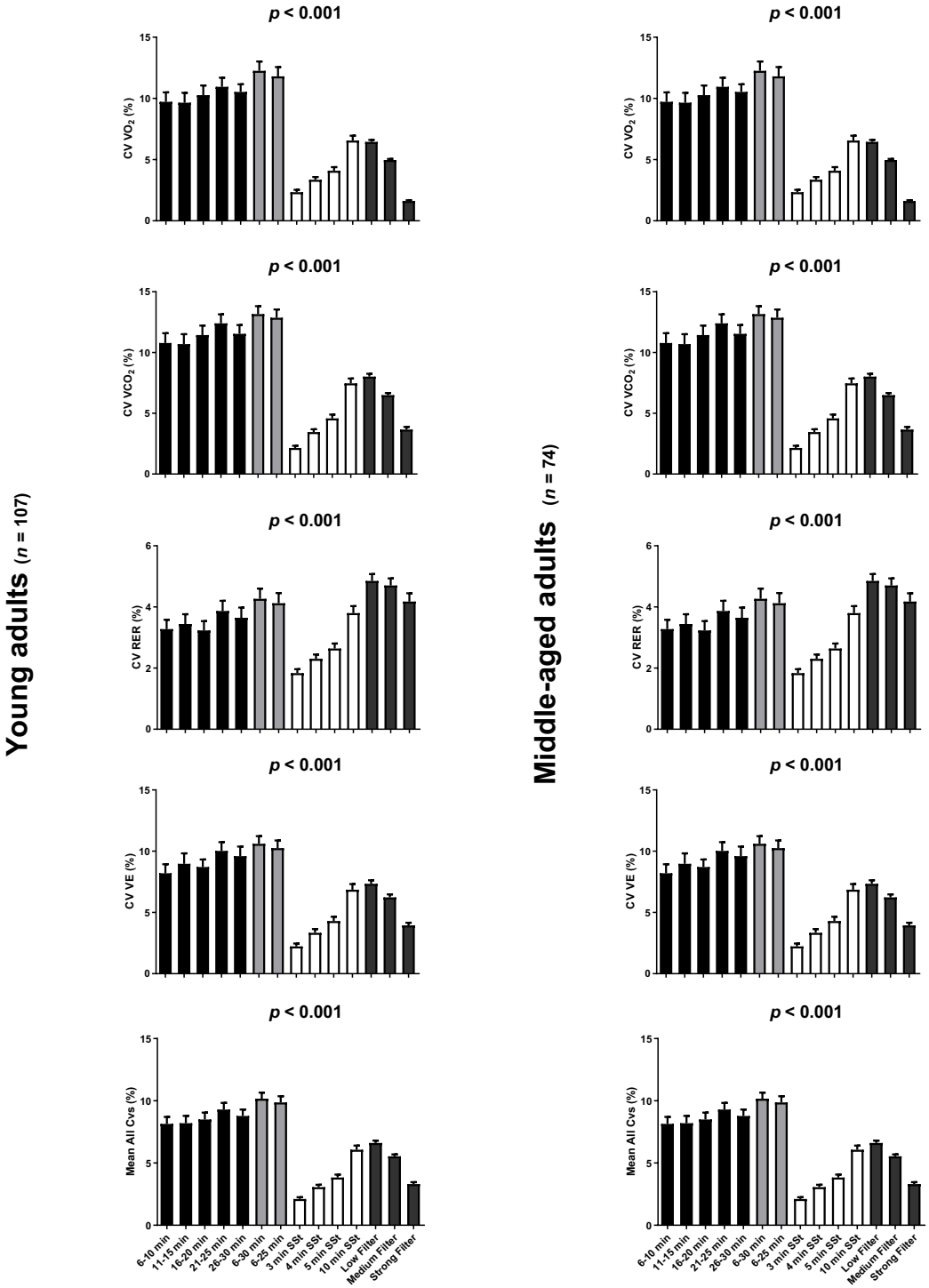


Figure S10. Differences among gas exchange data selection methods with respect to coefficient of variation of oxygen consumption (CV VO_2), carbon dioxide production (CV VCO_2), respiratory exchange ratio (CV RER), ventilation (CV VE), and the mean of all the aforementioned coefficients of variation (Mean All CVs). *Black columns represent short time interval (TI) periods; light grey columns represent long TI periods; white columns represent steady state (SSt) periods; dark grey columns represent filtering methods. P values come from repeated-measures analysis of variance (ANOVA). Identical indicatory letters highlight differences as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM).*

REFERENCES

1. Irving, C.J.; Eggett, D.L.; Fullmer, S. Comparing Steady State to Time Interval and Non-Steady State Measurements of Resting Metabolic Rate. *Nutr. Clin. Pract.* **2017**, *32*, 77–83.
2. Lam, Y.Y.; Ravussin, E. Indirect calorimetry: An indispensable tool to understand and predict obesity. *Eur. J. Clin. Nutr.* **2017**, *71*, 318–322.
3. Galgani, J.E.; Castro-Sepulveda, M.A. Influence of a Gas Exchange Correction Procedure on Resting Metabolic Rate and Respiratory Quotient in Humans. *Obesity* **2017**, *25*, 1941–1947.
4. Black, C.; Grocott, M.P.W.; Singer, M. Metabolic monitoring in the intensive care unit: A comparison of the Medgraphics Ultima, Deltatrac II, and Douglas bag collection methods. *Br. J. Anaesth.* **2015**, *114*, 261–268.
5. Cooper, J.A.; Watras, A.C.; O'Brien, M.J.; Luke, A.; Dobratz, J.R.; Earthman, C.P.; Schoeller, D.A. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J. Am. Diet. Assoc.* **2009**, *109*, 128–132.
6. Da Rocha, E.E.M.; Alves, V.G.F.; da Fonseca, R.B.V. Indirect calorimetry: Methodology, instruments and clinical application. *Curr. Opin. Clin. Nutr. Metab. Care* **2006**, *9*, 247–256.
7. Simonson, D.C.; DeFronzo, R.A. Indirect calorimetry: Methodological and interpretative problems. *Am. J. Physiol. Metab.* **2017**, *258*, E399–E412.
8. Fullmer, S.; Benson-Davies, S.; Earthman, C.P.; Frankenfield, D.C.; Gradwell, E.; Lee, P.S.P.; Piemonte, T.; Trabulsi, J. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J. Acad. Nutr. Diet.* **2015**, *115*, 1417–1446.
9. Horner, N.K.; Lampe, J.W.; Patterson, R.E.; Neuhouser, M.L.; Beresford, S.A.; Prentice, R.L. Indirect Calorimetry Protocol Development for Measuring Resting Metabolic Rate as a Component of Total Energy Expenditure in Free-Living Postmenopausal Women. *J. Nutr.* **2001**, *131*, 2215–2218.
10. McClave, S.A.; Spain, D.A.; Skolnick, J.L.; Lowen, C.C.; Kleber, M.J.; Wickerham, P.S.; Vogt, J.R.; Looney, S.W. Achievement of steady state optimizes results when performing indirect calorimetry. *J. Parenter. Enter. Nutr.* **2003**, *27*, 16–20.
11. Sanchez-Delgado, G.; Alcantara, J.M.A.; Ortiz-Alvarez, L.; Xu, H.; Martinez-Tellez, B.; Labayen, I.; Ruiz, J.R. Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. *Clin. Nutr.* **2018**, *37*, 1618–1624.
12. Borges, J.H.; Guerra-Júnior, G.; Gonçalves, E.M. Methods for data analysis of resting energy expenditure measured using indirect calorimetry. *Nutrition* **2019**, *59*, 44–49.
13. Johnstone, A.M.; Murison, S.D.; Duncan, J.S.; Rance, K.A.; Speakman, J.R. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am. J. Clin. Nutr.* **2005**, *82*, 941–948.
14. Sanchez-Delgado, G.; Martinez-Tellez, B.; Olza, J.; Aguilera, C.M.; Labayen, I.; Ortega, F.B.; Chillon, P.; Fernandez-Reguera, C.; Alcantara, J.M.A.; Martinez-Avila, W.D.; et al. Activating brown adipose tissue through exercise (ACTIBATE) in young adults: Rationale, design and methodology. *Contemp. Clin. Trials* **2015**, *45*, 416–425.

15. Amaro-Gahete, F.J.; Jurado-Fasoli, L.; Espuch-Oliver, A.; Robles-Gonzalez, L.; Navarro-Lomas, G.; de Haro, T.; Femia, P.; Castillo, M.J.; Gutierrez, A. Exercise training as S-Klotho protein stimulator in sedentary healthy adults: Rationale, design, and methodology. *Contemp. Clin. Trials Commun.* **2018**, *11*, 10–19.

16. Alcantara, J.M.A.; Sanchez-Delgado, G.; Martinez-Tellez, B.; Merchan-Ramirez, E.; Labayen, I.; Ruiz, J.R. Congruent validity and inter-day reliability of two breath by breath metabolic carts to measure resting metabolic rate in young adults. *Nutr. Metab. Cardiovasc. Dis.* **2018**, *28*, 929–936.

17. Weir, J. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **1949**, *109*, 1–9.

18. Frayn, K. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* **1983**, *55*, 628–634.

19. Reeves, M.M.; Davies, P.S.W.; Bauer, J.; Battistutta, D. Reducing the time period of steady state does not affect the accuracy of energy expenditure measurements by indirect calorimetry. *J. Appl. Physiol.* **2004**, *97*, 130–134.

20. Matarese, L.E. Indirect calorimetry: Technical aspects. *J. Am. Diet. Assoc.* **1997**, 97, S154–S160.

21. Mullen, J.L. Indirect calorimetry in critical care. *Proc. Nutr. Soc.* **2005**, *50*, 239–244.

22. Korth, O.; Bosy-Westphal, A.; Zschoche, P.; Glüer, C.C.; Heller, M.; Müller, M.J. Influence of methods used in body composition analysis on the prediction of resting energy expenditure. *Eur. J. Clin. Nutr.* **2007**, *61*, 582–589.

23. Mifflin, D.; Jeor, T.; Daugherty, A.; Hill, A.; Scott, J.; Daugherty, S.; Kho, Y. A new predictive equation in healthy individuals for resting energy. *Am. J. Clin. Nutr.* **1990**, *51*, 241–247.

24. Livingston, E.H.; Kohlstadt, I. Simplified resting metabolic rate-predicting formulas for normal-sized and obese individuals. *Obes. Res.* **2005**, *13*, 1255–1262.

25. Müller, M.J.; Bosy-Westphal, A.; Klaus, S.; Kreymann, G.; Lührmann, P.M.; Neuhäuser-Berthold, M.; Noack, R.; Pirke, K.M.; Platte, P.; Selberg, O.; et al. World Health Organization equations have shortcomings for predicting resting energy expenditure in persons from a modern, affluent population: Generation of a new reference standard from a retrospective analysis of a German database of resting energy expenditure. *Am. J. Clin. Nutr.* **2004**, *80*, 1379–1390.

26. Graf, S.; Karsegard, V.L.; Viatte, V.; Maisonneuve, N.; Pichard, C.; Genton, L. Comparison of three indirect calorimetry devices and three methods of gas collection: A prospective observational study. *Clin. Nutr.* **2013**, *32*, 1067–1072.

27. Forse, R.A. Comparison of Gas Exchange Measurements with a Mouthpiece, Face Mask, and Ventilated Canopy. *J. Parenter. Enter. Nutr.* **1993**, *17*, 388–391.

RESULTS AND DISCUSION

SECTION 2

STUDY IV

BACKGROUND

Assessing human resting metabolic rate (RMR) is of vital importance in clinical setting as well as in research [1,2]. Metabolic carts are the most used devices to assess RMR regardless the subject's age and health status [1–5]. Using diverse equations RMR, respiratory exchange ratio (RER), and substrate oxidation (i.e. fat and carbohydrates oxidation) can be assessed [6,7]. A few years ago, guidelines for performing RMR assessments in healthy and non-critically ill subjects were published [2]. Nevertheless, some issues remained unclear and new studies are needed to clarify them. One example is the necessity of identifying the method for gas exchange data selection providing the best day-to-day biological reproducibility (i.e. the similarity between in vivo measurements performed under the same conditions in different moments) [2].

When measuring oxygen consumption (VO_2) and carbon dioxide production (VCO_2) using metabolic carts, the subject gas exchange is normally measured during a period of time ranging from 10 to 30 minutes [2]. From the total gas exchange measurement, a common practice is to discard the first 5-minute data [2] and select a short period from the remaining data [3,8–14]. It has been broadly assumed that the steady state (SSt) gas exchange data selection method increase the validity of the RMR assessment [9]. This SSt method is commonly defined as selecting the period in which the coefficient of variation (CV) for VO_2 and VCO_2 is lower than a pre-determined threshold (usually 10%) [9], although others authors have proposed the inclusion of the CV for RER (usually 5%) [2] and minute ventilation (usually 10%, only when using breath-by-breath metabolic carts) [15]. Furthermore, the achievement of a period accomplishing the SSt criteria is not always feasible, and thus, others methods (e.g. time interval [TI] and/or filtering methods) have been proposed [10]. When using the TI methods a pre-defined time interval is selected (although the duration may vary; e.g. short TI [commonly periods of 5 minutes] or long TI [commonly periods ≥10 minutes]), without taking the stability of the obtained results into consideration. On the other hand, using the filtering methods, data above or below a given RMR threshold (defined as the mean RMR plus and minus a certain percentage of RMR) are discarded.

Achieving a high day-to-day biological reproducibility (i.e. reducing inter-day differences) is fundamental to, for example, detect the relatively small changes in RMR after an intervention [15–17]. Further, the RER is also vital for an accurate estimation of substrate oxidation rates [18], and thus, accomplishing a high RER biological reproducibility is also important for a method. Unfortunately, to the best of the author's knowledge, only few studies have determined the influence of different methods for gas exchange data selection on RMR and RER biological reproducibility (e.g. [15]), and there are no studies determining their biological reproducibility using the filtering methods. Moreover, whether the conclusions obtained using one metabolic cart can be extrapolated to others is still to be elucidated. Therefore, the influence of method of gas exchange data selection on RMR and RER biological reproducibility needs to be studied in diverse metabolic carts.

Of interest, to improve the RMR and RER assessments, a post-calorimetric correction procedure was developed [19]. Briefly, the subject's VO_2 and VCO_2 are simulated through the infusion of pure gases (N_2 and CO_2), employing high-precision mass-flow controllers, immediately after the subject's assessment. Therefore, the subject´s VO_2 and VCO_2 can then be 'corrected' using the determined metabolic cart error (i.e. the difference between the infused gases and the readouts of the metabolic cart) [19]. This procedure has shown to improve the RMR and RER biological reproducibility [19] and the post-prandial RER estimations [20] (with the Deltatrac [Datex Instrumentarium Corp, Helsinki, Finland] and the Vmax Encore 29n [SensorMedics, Yorba Linda, CA, USA] metabolic carts). However, whether the application of this procedure affects the biological reproducibility of RMR and RER using diverse methods for gas exchange data selection need to be determined.

The aim of the present study was to analyze the influence of methods for gas exchange data selection (TIs, SSt and filtering) on RMR and RER estimations and on their day-to-day biological reproducibility in young healthy adults. Further, the present study also investigated which method for gas exchange data selection resulted in greatest RMR variance explained by its classical determinants (i.e., body weight, sex and body composition [fat and fat free masses]) [21]. Lastly, we determined whether using a post-calorimetric correction procedure influences the RMR and RER estimates across methods (TIs, SSt and filtering).

METHODS OVERVIEW

Metabolic carts and procedures

RMR and RER were assessed using four different metabolic carts: the Q-NRG (Cosmed, Rome, Italy), the Vyntus CPX (Jaeger-CareFusion, Höchberg, Germany; thereinafter called *Vyntus*), the Omnical (Maastricht Instruments, Maastricht, The Netherlands), and the Ultima CardiO2 (Medgraphics Corporation, St. Paul, MN, USA; thereinafter called *Ultima*). Detailed information and characteristics are presented elsewhere (see Table 5, Chapter 2). Lastly, all the metabolic carts were calibrated (flow and gas analyzers) by the same researchers, strictly following the manufacturers' instructions.

Subjects

A repeated-measures design was used over 2 consecutive mornings, and the measurement hour for each participant was replicated (see below - Indirect calorimetry assessment section). A total of 29 healthy young adults (n=11 women) participated in the study. Of them, 17 subjects (n=6 women) had valid data on each method in both testing days, and thus only them were included in further analyses (Table S5). The inclusion criteria were: (i) being older than 18 years old; (ii) having a body mass index between 18.5 and 40 kg/m^2; (iii) having a stable body weight over the last 3 months (changes ≤3 kg) and not being enrolled in a weight loss program; (iv) non-smokers; (v) under no medication that could directly affect energy metabolism; (vi) not suffering from chronic or acute illness; and (vii) not being pregnant. All these criteria were verbally confirmed by the subjects. Both the study protocol and written informed consent followed the 2013 revised Declaration of Helsinki and were approved by the Human Research Ethics Committee of the University of Granada (#836).

Anthropometric and body composition assessment

On the first visit, subjects' height and body weight were measured using a stadiometer and scale (Seca model 799, Electronic Column Scale, Hamburg, Germany) without shoes and with light clothing. Waist circumference (at the midpoint between the costal margin and iliac crest in the mid-axillary line) was measured twice using a plastic tape while the subjects were in a standing position, and the average of both assessments was used. Body composition was assessed by whole-body dual-energy X-ray absorptiometry (Discovery Wi, Hologic, Inc., Bedford, MA, USA).

Indirect calorimetry assessment

The subjects arrived at the research center by public transportation or motorized vehicle (avoiding any moderate or intense physical activity since they woke up) and confirmed having consumed the standardized ad-libitum meal plan (see Table S1, Study II) during the preceding 24 hours, which included consuming the standardized dinner 12 hours before the start of the first IC assessment. Further, they refrained from both moderate (previous 24 hours) and vigorous intensity (previous 48 hours) physical activity. RMR and RER were assessed with each cart on two consecutive days in the morning between 9 am and Noon. The assessment lasted 30 minutes on each cart, with a 20-minute period between measurements. The order of the carts was randomly assigned and replicated on the second day (see Figure 21, Experiment 3, Study II).

The assessments were performed in agreement with current methodological recommendations [2]. Subjects stayed motionless on a reclined bed in the supine position covered by a bed sheet for a minimum of 20 minutes before the first IC assessment. Moreover, the subjects were asked to lay on the bed during the last 15 minutes of every period between measurements (see Figure 21, Experiment 3, Study II). Subjects were instructed not to sleep, talk, or fidget, and to breathe normally during the assessments.

The VO_2 and the VCO_2 data from the IC assessment were downloaded from all metabolic carts. Later, those data points presenting non-physiological RER (i.e. below 0.7 and above 1) as well as the first 5 minutes data were discarded [2], and the remaining 25 minutes data were averaged every 1 minute using an Excel 2013® spreadsheet (Microsoft Corp, Redmond, WA, USA).

Urine collection and analysis

Twelve-hour urine samples were collected before arriving to the research center. For that purpose, subjects were provided with two airtight 2 L polyethylene containers. They were instructed to collect their urine from dinner (9 pm) to the IC assessment start (9 am). Total urine volume and urea concentration (Spinreact, UREA-37_R1, Girona, Spain) were measured, and N urine levels were estimated using a regression equation (see below) previously computed in our laboratory in a separate sample of 19 young adults [22], where N urine levels were determined by the Kjeldahl method [23].

N (g/l) = 0.0065 x urea (mg/dl) + 1.2598

Post-calorimetric correction procedure

During the subject's RMR assessments, both VO_2 and VCO_2 metabolic carts readouts were averaged for ten minutes (from the 11th to the 20th minute, *subject's readout*). Immediately after the RMR assessment (without stopping the metabolic cart recording), N_2 and CO_2 were infused during 10 minutes into the metabolic cart hose tube in volumes mimicking the subject's VO_2 and VCO_2 averaged readouts (*expected values*). The VO_2 and the VCO_2 readouts during the last 5 minutes of infusion were also averaged (*measured values*). Then, the VO_2 and VCO_2 corrected values were calculated as follow.

Corrected values = subject´s readout x expected value / measured value

Gas exchange data selection methods

Time Interval Method

Short TIs of 6-10 min, 11-15 min, 16-20 min, 21-25 min, and 26-30 min, and long TIs of 6-25 min and 6-30 min were established [15], and the means of all variables calculated and used to estimate the RMR and RER.

Steady-State Time Method

The CVs of VO_2, VCO_2 and RER were calculated for every period of 3, 4, 5, and 10 min SSt (e.g. 6th to 8th, 7th to 9th period, etc.). Later, VO_2, VCO_2 and RER CVs were averaged to obtain a mean CV for each time period. The periods selected for the final analyses were those with the lowest mean CV [15]. The mean VO_2, VCO_2 for these periods were then used to estimate the RMR and RER.

Filtering Method

The mean VO_2 and VCO_2 for the entire 25 min data collection period (i.e. having discarded the first 5 minutes) were used to calculate the $mean_{25\ min}$ RMR (see below for details). Later, we identified and discarded those minute data being (i) <85% or >115% of the $mean_{25\ min}$ RMR (low filter), (ii) <90% or >110% of the $mean_{25\ min}$ RMR (medium filter), or (iii) <95% or >105% of the $mean_{25\ min}$ RMR (strong filter). For the minutes that passed these filters, the average VO_2 and VCO_2 were computed and used to estimate the RMR and RER.

RMR and RER estimations

For each type of gas exchange data selection method, the RER was calculated as VCO_2/VO_2 for both the uncorrected and corrected VO_2 and VCO_2 data. Lastly, with both the uncorrected and corrected VO_2 and VCO_2 values, the RMR (i.e. uncorrected and corrected RMR) was estimated using the Weir abbreviated equation [6], where N is urinary nitrogen excretion:

$$RMR\ (kcal/day) = (3.941 \times VO_2\ (L/min) + 1.106 \times VCO_2\ (L/min) - 2.17 \times N\ (g/min)) \times 1440$$

Statistical analysis

Results are presented as mean ± standard deviation, unless otherwise stated. Analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 22.0, IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA) and the level of significance was set at P<0.050. Figures were created using Graph Pad Prism (GraphPad Software, v. 8.4.1, CA, USA). Gas values are provided under standard temperature, pressure, and dry (STPD) conditions. All the analyses were performed separately in the Q-NRG, the Vyntus, the Omnical and the Ultima metabolic carts, and with both uncorrected and corrected values, unless otherwise stated.

Influence of methods for gas exchange data selection on Indirect Calorimetry assessments

A repeated-measures analysis of variance (ANOVA) was used to test differences in RMR, RER, VO_2 and VCO_2 across methods for gas exchange data selection on both testing days. Bonferroni correction were used to perform *post-hoc* comparisons. We also studied the associations of RMR (an average of both testing days) with their classical predictors including body weight, body composition (lean mass and fat mass), and sex [21]. We conducted simple linear regression analyses to study the association between RMR and body weight (i.e. Model 1), and multiple linear regressions to study the associations between RMR and lean mass, fat mass and sex (i.e. Model 2).

Influence of methods for gas exchange data selection on the day-to-day biological reproducibility

For every subject and metabolic cart, the absolute value of inter-day differences (e.g. |*uncorrected* RMR Day 1 - *uncorrected* RMR Day 2|) were calculated for RMR, RER VO_2 and VCO_2 (corrected and uncorrected values). We also calculated the day-to-day CV (i.e. $CV_{D\text{-}to\text{-}D}$ = [standard deviation *uncorrected* RMR / mean *uncorrected* RMR] × 100) of the RMR for both, uncorrected and corrected values. Then, ANOVA, with *post-hoc* Bonferroni comparisons, were used to test differences across metabolic carts in RMR, RER, VO_2 and VCO_2 (as absolute values) and the $CV_{D\text{-}to\text{-}D}$ of the RMR (as $CV_{D\text{-}to\text{-}D}$; percentage).

RESULTS

A total of 12 out of 29 subjects were retrospectively excluded from the analyses for not presenting data in at least one of the methods for data analyses (**Table S5**). The characteristics of the study subjects included (n=17) are presented in **Table 10**. Therefore, these 17 subjects had valid data for every metabolic cart (both testing days) and for every method for gas exchange data selection.

Table 10. Subject's characteristics.

	Men (n=11)					**Women (n=6)**				
	Mean	**±**	**SD**	**Min**	**Max**	**Mean**	**±**	**SD**	**Min**	**Max**
Age (years)	25.10	±	3.4	20.0	31.0	25.20	±	5.2	20.0	34.0
Body weight (kg)	74.5	±	10.8	63.3	99.2	57.8	±	8.7	45.6	72.4
Height (cm)	175.0	±	6.8	160.5	184.5	163.9	±	7.6	154.6	174.3
BMI (kg/m^2)	24.3	±	2.8	21.6	30.5	21.4	±	2.3	19.1	24.7
WC (cm)	79.7	±	8.6	70.0	96.6	68.3	±	4.8	59.8	72.9
Lean mass (kg)	54.7	±	5.9	42.2	63.1	35.3	±	3.8	30.5	40.7
Fat mass (kg)	15.5	±	6.7	9.3	31.3	19.7	±	5.9	10.8	28.5
Fat mass (%)	20.7	±	6.3	14.0	31.9	34.0	±	6.3	24.2	41.1

SD: Standard deviation; Min: Minimum; Max: Maximum; BMI: body mass index; WC: waist circumference.

Influence of methods for gas exchange data selection on Indirect Calorimetry assessments

Figure 32 shows uncorrected RMR (**panels A-D**) and RER estimations (**panels E-H**) across methods for gas exchange data selection obtained in day 1, while **Figure S11** shows the same parameters for the corrected RMR. Differences in uncorrected RMR for the Vyntus (P=0.031; **Figure 32B**) and in uncorrected RER for the Q-NRG (P=0.004; **Figure 32E**) were observed. Significant post-hoc differences were detected after applying the Bonferroni correction for the Vyntus (the strong filter vs. 21-25 min, 6-25 min, 6-30 min, and low and medium filters, all P≤0.032) and for the Q-NRG (the 6-10 min vs. 26-30 min, 6-25 min, 6-30 min, and low, medium and strong filters, all P≤0.031). These differences remained when the corrected values were used (**Figure S11B** and **Figure S11E** respectively). Further, significant differences in uncorrected VO_2 for the Vyntus (P=0.022; **Figure S14B**) were detected, while no significant differences were observed in uncorrected VCO_2. The results for the corrected VO_2 and VCO_2 were similar than these observed for the uncorrected values (see **Figure S15**). All the results observed in day 2 were similar to these observed in day 1 (data not shown).

The lowest uncorrected RMR value was obtained when following the strong filter method for every metabolic cart (mean RMR: 1445, 1631, 1476 and 1601 kcal/day for the Q-NRG, the Vyntus, the Omnical and the Ultima metabolic carts respectively; **Figure 32A-D**). On the other hand, the lowest mean corrected RMR value was obtained when following the low filter method for the Q-NRG (1333 kcal/day; **Figure S11A**), the 21-25 min for the Vyntus (1524 kcal/day; **Figure S11B**), the 26-30 min for the Omnical (1329 kcal/day; **Figure S11C**), and the 16-20 min for the Ultima (1511 kcal/day; **Figure S11D**).

Table 11 shows the variance in uncorrected RMR values (an average of both testing days) explained by body weight (Model 1) and by the subject sex, lean mass and fat mass (Model 2). Merging the gas exchange methods in each type - e.g. averaging 3, 4, 5 and 10 min SSt - the explained variance was 67% (Q-NRG), 62% (Vyntus), 63% (Omnical) and 50% (Ultima) for the short TI; 68%, 62%, 64% and 50% for the long TI; 64%, 60%, 46% and 52% for the SSt; and 66%, 61%, 61% and 50% for filtering methods using the Q-NRG, the Vyntus, the Omnical and the Ultima metabolic carts respectively (**Table 11**).

The highest explained variance was obtained using the 16-20 min method (72%) for the Q-NRG, the 21-25 min method (64%) for the Vyntus, the 6-10 min method (70%) for the Omnical, and the 6-10 min method (57%) for the Ultima (**Table 11**, Model 1). In the second model (**Table 11**, Model 2), the variance in uncorrected RMR vales explained increased compared to the Model 1. In this regard, in Model 2 the most explained variance was obtained using the 11-15 min method (92%) for the Q-NRG, the 4 min SSt method (85%) for the Vyntus, the 6-25 min and 6-30 min methods (both 95%) for the Omnical, and the 21-25 min method (86%) for the Ultima (**Table 11**, Model 2). Finally, **Table S6** shows the variance in corrected RMR values (an average of both testing days). The variance in RMR explained by its classical determinants was lower when using the corrected values instead of the uncorrected values (**Table S6**).

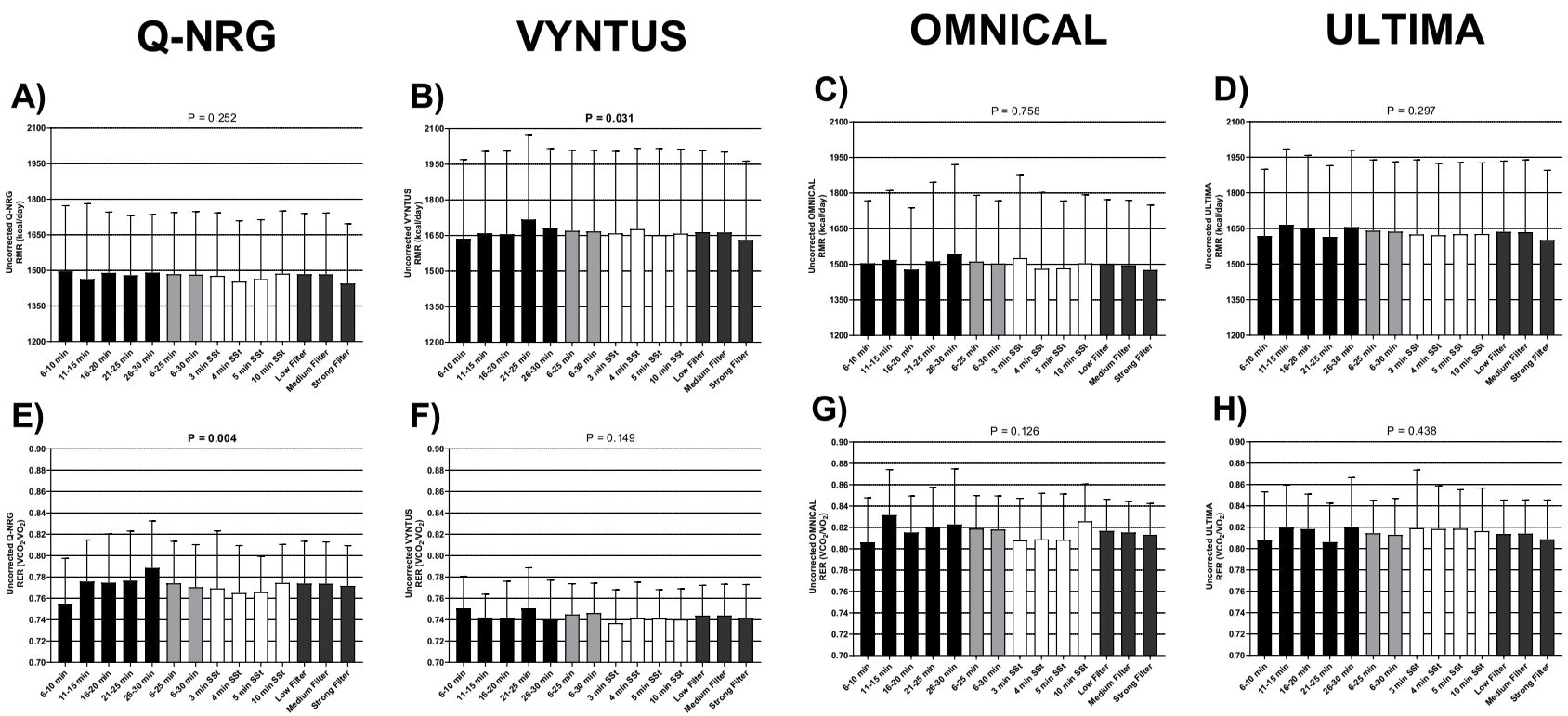


Figure 32. Differences among methods for gas exchange data selection in uncorrected resting metabolic rate (RMR; panels A-D) and uncorrected respiratory exchange ratio (RER; panels E-H) by metabolic cart. *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

Table 11. Variance in uncorrected resting metabolic rate (RMR) explained by its classical predictors in each of the gas exchange data selection methods and metabolic carts.

	Q-NRG	Vyntus	Omnical	Ultima
	R^2	R^2	R^2	R^2
Model 1				
Short TI	0.67	0.62	0.63	0.50
Long TI	0.68	0.62	0.64	0.50
SSt	0.64	0.60	0.46	0.52
Filtering	0.66	0.61	0.61	0.50
6-10 min	0.59	0.62	0.70	0.57
11-15 min	0.70	0.57	0.60	0.37
16-20 min	0.72	0.58	0.41	0.48
21-25 min	0.57	0.64	0.60	0.51
26-30 min	0.60	0.63	0.51	0.52
6-25 min	0.67	0.62	0.63	0.50
6-30 min	0.68	0.61	0.65	0.50
3 min SSt	0.60	0.60	0.35	0.49
4 min SSt	0.62	0.58	0.29	0.52
5 min SSt	0.61	0.60	0.51	0.53
10 min SSt	0.67	0.58	0.55	0.51
Low Filter	0.66	0.61	0.60	0.51
Medium Filter	0.66	0.61	0.61	0.49
Strong Filter	0.66	0.62	0.63	0.50
Model 2				
Short TI	0.90	0.80	0.95	0.82
Long TI	0.90	0.80	0.95	0.83
SSt	0.86	0.81	0.78	0.83
Filtering	0.90	0.80	0.94	0.83
6-10 min	0.75	0.80	0.80	0.81
11-15 min	0.92	0.74	0.88	0.78
16-20 min	0.90	0.75	0.81	0.78
21-25 min	0.79	0.83	0.90	0.86
26-30 min	0.89	0.83	0.85	0.77
6-25 min	0.90	0.80	0.95	0.82
6-30 min	0.90	0.79	0.95	0.83
3 min SSt	0.80	0.77	0.55	0.83
4 min SSt	0.91	0.85	0.68	0.81
5 min SSt	0.87	0.80	0.78	0.83
10 min SSt	0.85	0.80	0.89	0.83
Low Filter	0.90	0.79	0.93	0.85
Medium Filter	0.89	0.80	0.94	0.83
Strong Filter	0.90	0.80	0.95	0.82

Data are presented as adjusted R squared (R^2) from simple regression analyses (Model 1) and from multiple regression analyses (Model 2). In Model 1 body weight (BW; in kg) was included as independent variable, and the RMR estimates yielded by the different methods for gas exchange data selection were included as dependent variables. In Model 2 sex, lean mass and fat mass (both variables in kg) were included as independent variables, and the RMR estimates (in kilocalories per day) yielded by the different methods for gas exchange data selection were included as dependent variables.

Influence of methods for gas exchange data selection on the day-to-day biological reproducibility

A significant effect of the method for gas exchange data selection in the absolute value of inter-day uncorrected RMR differences was observed for the Q-NRG (P=0.041, **Figure 33A**) and for the uncorrected RMR and RER differences for the Omnical (P=0.009, Figure **33C**; P=0.013, Figure **33G**). These significant differences, in addition to the absolute value on inter-day corrected RER differences for the Vyntus (P=0.026, **Figure S12F**), were also observed when the corrected values were used instead (**Figure S12A, C** and **G**). Further, post-hoc analyses revealed significant differences between the SSt and the 6-25 min and 6-30 min methods in the Q-NRG and the Omnical, after Bonferroni comparisons. Similar results were observed when the inter-day RMR differences were expressed as $CV_{D\text{-}to\text{-}D}$ instead as the absolute value on inter-day differences (**Figure S13**). Interestingly, the lowest $CV_{D\text{-}to\text{-}D}$ was observed when employing long TI methods (i.e. 6-25 min and 6-30 min methods; **Figure S13**) in all metabolic carts for both uncorrected and corrected data, except for the uncorrected RMR values in the Ultima (**Figure S13D**).

Lastly, the same analyses were performed for uncorrected (**Figure S16**) and corrected (**Figure S17**) VO_2 and VCO_2 parameters. As occurred previously with the RMR and RER, significant effect of the method for gas exchange data selection in the absolute value of inter-day uncorrected VO_2 and VCO_2 differences was observed for the Q-NRG (P=0.027 and P=0.028, **Figure S16A** and **E** respectively) and for the Omnical (P=0.008 and P=0.005, **Figure S16C** and **G** respectively). These statistically significant differences remained when the corrected values were used instead (**Figure S17**).

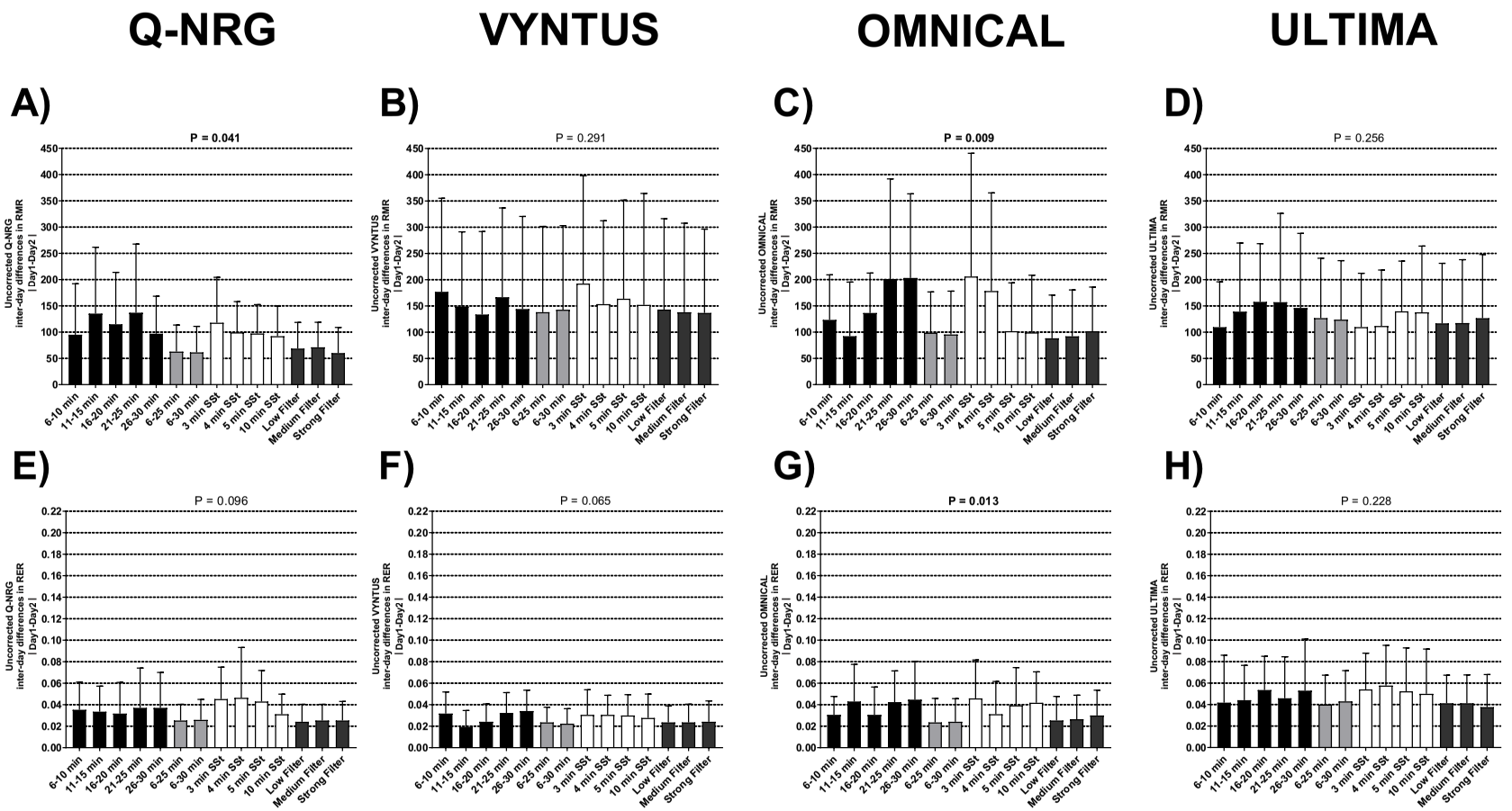


Figure 33. Differences among methods for gas exchange data selection with respect to uncorrected resting metabolic rate (RMR; panels A-D) and uncorrected respiratory exchange ratio (RER; panels E-H) absolute value of inter-day differences by metabolic cart. *Y axis represents absolute values of the inter-day differences (e.g. |RMR Day1 – RMR Day2|). Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

DISCUSSION

The present study aimed to analyze the influence of the TI, SSt and filtering methods for gas exchange data selection on the RMR and RER estimations and its day-to-day biological reproducibility, using four commercially available metabolic carts. The main findings of the present study show that the highest biological reproducibility was observed when applying the long TI (i.e. 6-25 and 6-30 min methods) especially in the Q-NRG and Omnical metabolic carts. Regarding the variance in RMR explained by its classical predictors, generally the uncorrected RMR values consistently showed the highest explained variance values.

On the one hand, our results showed that the method for gas exchange data selection may not influence the results in cross-sectional analyses, except the RER for the Q-NRG (**Figure 32E**), and the RMR for the Vyntus (**Figure 32B**). Nevertheless, we observed that the strong filter was the method presenting the lowest RMR value in all the metabolic carts when using the uncorrected values. Taking into account the RMR definition - i.e. the minimum energy needed in an awake subject for maintaining normal body homeostasis, while resting, and in thermoneutrality conditions [2] -, the present results suggest that the strong filter method may provide better RMR estimations for cross-sectional analyses/studies. However, the under-estimation of the homeostatic RMR cannot be ruled out in the strong filter method. To the best of the author's knowledge no previous study has compared the filtering vs. the TI and/or the SSt methods for gas exchange data selection which hamper further comparisons. The results obtained by the strong filter method may suggest that it is a real alternative for those subjects in whom a SSt is not possible or is not achieved [2]. Although it should be noted that in our study, all subjects accomplished the SSt criteria (for each different condition, i.e. 3, 4, 5 y 10 min SSt) while the strong filter criteria were not (see **Table S5**), fact that should be considered.

On the other hand, achieving a high RMR day-to-day reproducibility is of vital importance in order to be able to detect changes induced by interventions [16,17]. Factors that may influence the RMR day-to-day biological reproducibility have been studied previously [24,25], while the RER day-to-day biological reproducibility has received much less attention [24]. In our study the uncorrected RMR day-to-day reproducibility was higher (i.e. lower inter-day differences) when using the 6-25 min or the 6-30 min TI methods than using the others methods for gas exchange data selection, in the Q-NRG and Omnical (**Figure 35** and **Figure S13**). Interestingly these results disagree with those observed in our previous study [15]. In fact, in the aforementioned study we observed that RMR and RER day-to-day biological reproducibility was not influenced by the use of TI nor by the SSt methods [15], which indeed concur with our present findings in the Vyntus and Ultima metabolic carts. Consequently, future studies are needed to test whether this results also apply to other metabolic carts, or to other populations as non-critically or critically ill hospitalized patients. It is important to acknowledge that RER depends on both, VO_2 and VCO_2, while RMR depends mostly on VO_2. Therefore, using different methods for gas exchange data selection may influence the RMR and RER day-to-day biological reproducibility in a different manner. The results of the Q-NRG, the Vyntus and the Ultima are in agreement with those results by Sanchez-Delgado et al. [15], as in the present study we did not observe a considerable influence of the method for gas exchange data selection on the RER day-to-day biological reproducibility. Contrary to those results, and using the Omnical metabolic cart, the RER day-to-day biological reproducibility was significantly influenced by the method for gas exchange data selection used (**Figure 33G**). Moreover, it should be noted that the results for VO_2 and VCO_2 were similar to those observed for RMR and RER (regardless if were uncorrected or corrected values). Considering all together, our findings may suggest that the influence of the methods for gas exchange data selection on RMR and RER day-to-day biological reproducibility is 'metabolic cart dependent'. Therefore, we might recommend to use the long TI methods (i.e. the 6-25 min TI and 6-30 min TI) when the influence of the method on the day-to-day biological reproducibility cannot be assessed, although this fact should be tested for each metabolic cart.

The variance in RMR explained by its classical determinants [21] may also be considered as an indirect indicator of accuracy [8]. In the present study, the variance in RMR explained by its classical determinants (across the different methods for gas exchange data selection) was in agreement with the results observed in previous studies (reporting results ranging from 36% to 56%) explained by body weight [8,20,26–28], and even slightly higher (up to 72% for uncorrected RMR values and up to 66% for corrected RMR values; Model 1, **Table 12** and **Table S6** respectively). Moreover, including body composition in the regression model (i.e. Model 2) increased the aforementioned explained variance (ranging from 55% to 95% for uncorrected RMR values; **Table 12**). Nonetheless, the variance in RMR explained by its classical determinants was mostly similar for all the gas exchange data selection methods used in the present study, which therefore may suggest that such explained variance is not, in a greater or lesser extent, 'method-dependent'. Nevertheless, it should be mentioned that for both, the Q-NRG and the Omnical metabolic carts, the RMR explained variance by its classical determinants were lower in Model 1 (**Table 12**) and in Model 2 (**Table S6**) compared to the others methods. Regarding the corrected RMR values, as occurred with most of the previous parameters, we did not observe a significant influence of the post-calorimetric correction procedure in terms of variance in RMR explained by its classical determinants.

The results of this study should be considered with caution, as there are some limitations that should be acknowledged. The subjects were healthy young adults, and we do not know if these findings can be extended to older people and to ventilated or hospitalized patients. Whereas our results are different when using the Q-NRG or the Omnical vs. the Vyntus or the Ultima metabolic carts, we do not know if our findings apply to other metabolic carts, or even to other gases collection system which have been proved to result in different RMR estimation (e.g. face-mask) [29]. Lastly, we did not control the menstrual cycle in female participants [30,31], although considering the within subject design and that both assessment were performed within 24 hours its potential influence on day-to-day biological reproducibility is likely negligible. Nevertheless, we controlled the fasting time (12 hours) prior to the indirect calorimetry assessments, which is considered a mandatory condition to assess both RMR and RER [32], as well as previous meals composition (which could bias RER measurements) [33].

CONCLUSION

In summary, our findings suggest that despite RMR and RER estimations are not generally affected by the methods for gas exchange data selection, day-to-day RMR and RER biological reproducibility is influenced by the use of different methods for data selection when using some, but not all, metabolic carts. Consequently, whenever possible, RMR and RER day-to-day biological reproducibility should be tested for each method and metabolic cart. Nevertheless, our data suggest that the use of either the 6-25 min TI or the 6-30 min TI methods might be the recommended option in any metabolic cart.

SUPPLEMENTARY MATERIAL

Table S5. List of the subjects who were included in the study (had valid data, for every testing day and metabolic cart) or were retrospectively excluded (did not have data in every of the method for gas exchange data selection, testing day and/or metabolic cart).

ID	Sex	Included/Excluded	Reason for exclusion
3000	2	Retrospectively excluded	Day 2; Q-NRG; Strong filter
3001	2	Retrospectively excluded	Day 1; Q-NRG; Strong filter
3004	1	Retrospectively excluded	Day 1; Vyntus; Strong filter
3005	2	Included	
3007	2	Retrospectively excluded	Day 1; Q-NRG; Strong filter
3008	1	Included	
3012	1	Retrospectively excluded	Day 2; Omnical; 16-20 min
3013	1	Included	
3014	1	Included	
3015	2	Included	
3016	1	Retrospectively excluded	Day 2; Omnical; Strong filter
3019	1	Retrospectively excluded	Day 2; Ultima; Strong filter
3023	2	Included	
3024	1	Included	
3027	1	Included	
3028	1	Included	
3031	1	Retrospectively excluded	Day 2; Omnical; Strong filter and Day 2; Ultima; Strong filter
3032	2	Included	
3033	1	Included	
3035	2	Included	
3037	1	Included	
3043	2	Retrospectively excluded	Day 2; Omnical; Strong filter
3044	1	Included	
3046	2	Retrospectively excluded	Day 2; Omnical; Strong filter
3047	1	Retrospectively excluded	Day 1; Omnical; Medium and Strong filters
3050	1	Included	
3053	1	Included	
3054	1	Retrospectively excluded	Day 1; Ultima; Strong filter and Day 2; Omnical; Strong filter
3058	2	Included	

Sex: 1=men and 2=women. Reasons for exclude the subjects are presented as the day of assessment, the metabolic cart/s and the method for gas exchange data selection in which the criterion/criteria was/were not met.

Table S6. Variance in corrected resting metabolic rate (RMR) explained by its classical predictors in each of the gas exchange data selection methods and metabolic carts.

	Q-NRG	Vyntus	Omnical	Ultima
	R^2	R^2	R^2	R^2
Model 1				
Short TI	0.60	0.61	0.60	0.43
Long TI	0.61	0.61	0.61	0.42
SSt	0.58	0.60	0.43	0.43
Filtering	0.60	0.60	0.58	0.42
6-10 min	0.53	0.59	0.66	0.47
11-15 min	0.64	0.55	0.58	0.31
16-20 min	0.64	0.57	0.38	0.42
21-25 min	0.52	0.66	0.59	0.43
26-30 min	0.52	0.63	0.48	0.47
6-25 min	0.60	0.61	0.60	0.43
6-30 min	0.62	0.60	0.62	0.41
3 min SSt	0.55	0.60	0.32	0.40
4 min SSt	0.57	0.60	0.28	0.44
5 min SSt	0.56	0.60	0.49	0.45
10 min SSt	0.61	0.57	0.52	0.44
Low Filter	0.60	0.60	0.57	0.43
Medium Filter	0.60	0.60	0.57	0.41
Strong Filter	0.59	0.61	0.60	0.42
Model 2				
Short TI	0.86	0.74	0.93	0.64
Long TI	0.86	0.73	0.94	0.63
SSt	0.84	0.76	0.77	0.63
Filtering	0.86	0.73	0.91	0.64
6-10 min	0.73	0.69	0.77	0.63
11-15 min	0.87	0.66	0.88	0.58
16-20 min	0.84	0.68	0.79	0.61
21-25 min	0.78	0.82	0.91	0.65
26-30 min	0.83	0.78	0.82	0.65
6-25 min	0.86	0.74	0.93	0.64
6-30 min	0.86	0.73	0.94	0.63
3 min SSt	0.78	0.73	0.55	0.61
4 min SSt	0.89	0.82	0.71	0.61
5 min SSt	0.84	0.74	0.77	0.65
10 min SSt	0.82	0.74	0.88	0.65
Low Filter	0.86	0.73	0.90	0.65
Medium Filter	0.86	0.73	0.91	0.64
Strong Filter	0.86	0.74	0.92	0.64

Data are presented as adjusted R squared (R^2) from simple regression analyses (Model 1) and from multiple regression analyses (Model 2). In Model 1 body weight (BW; in kg) was included as independent variable, and the RMR estimates yielded by the different methods for gas exchange data selection were included as dependent variables. In Model 2 sex, lean mass and fat mass (both variables in kg) were included as independent variables, and the RMR estimates (in kilocalories per day) yielded by the different methods for gas exchange data selection were included as dependent variables.

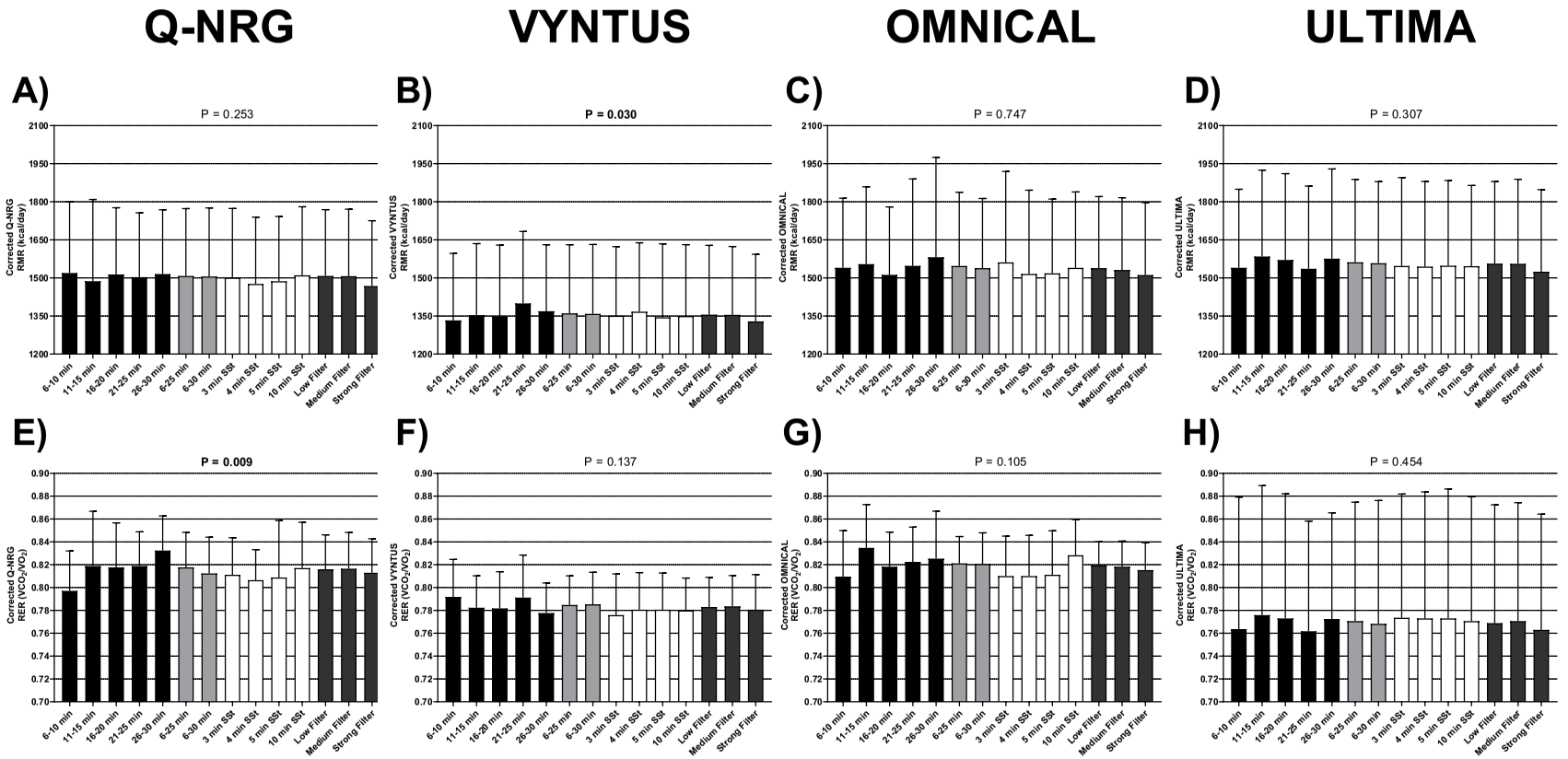


Figure S11. Differences among methods for gas exchange data selection with respect to corrected resting metabolic rate (RMR; panels A-D) and corrected respiratory exchange ratio (RER; panels E-H) estimates by metabolic cart. *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

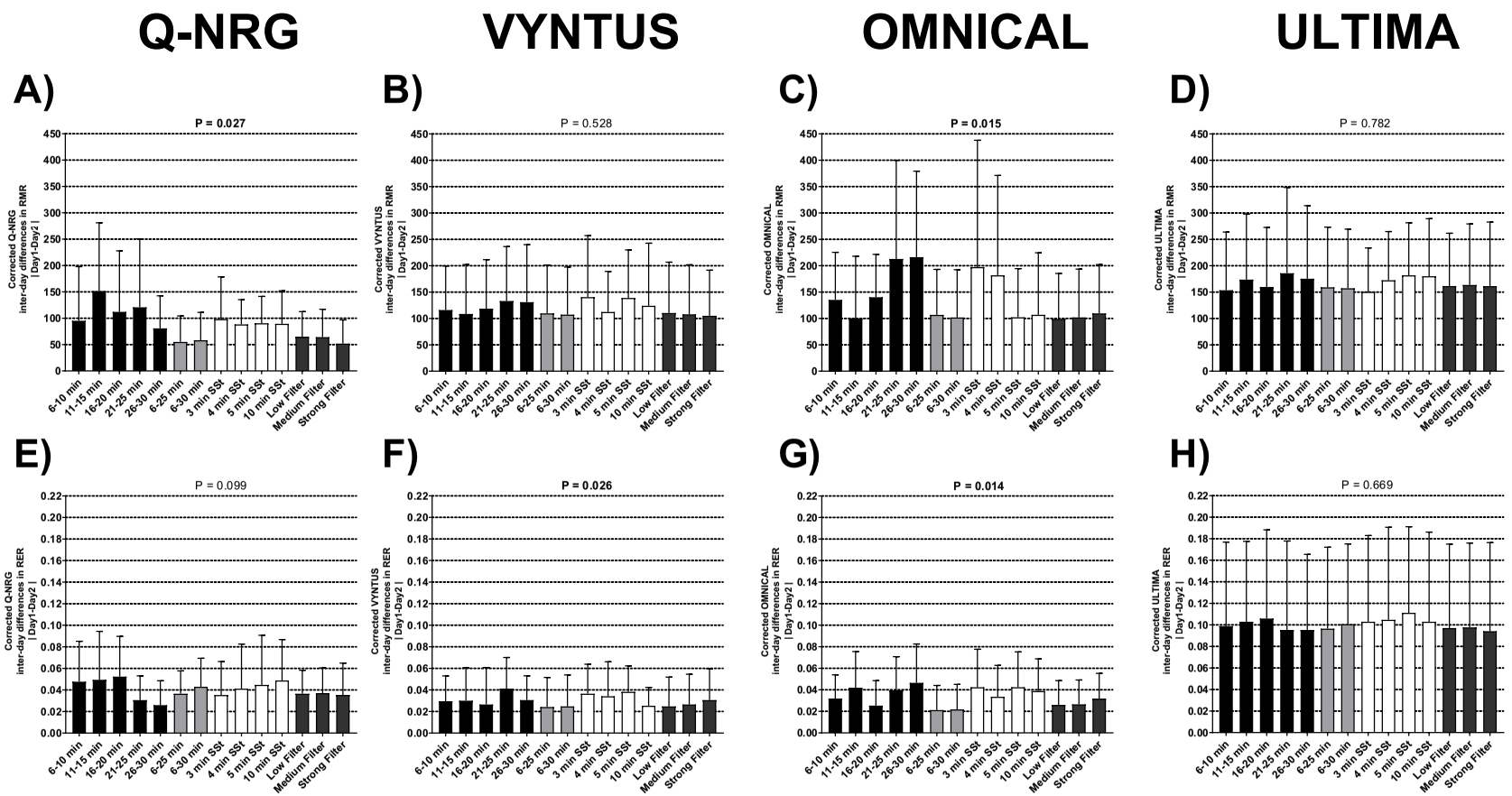


Figure S12. Differences among methods for gas exchange data selection with respect to corrected resting metabolic rate (RMR; panels A-D) and corrected respiratory exchange ratio (RER; panels E-H) absolute value of inter-day differences by metabolic cart. Y axis represents absolute values of the inter-day differences (e.g. |RMR Day1 – RMR Day2|). *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

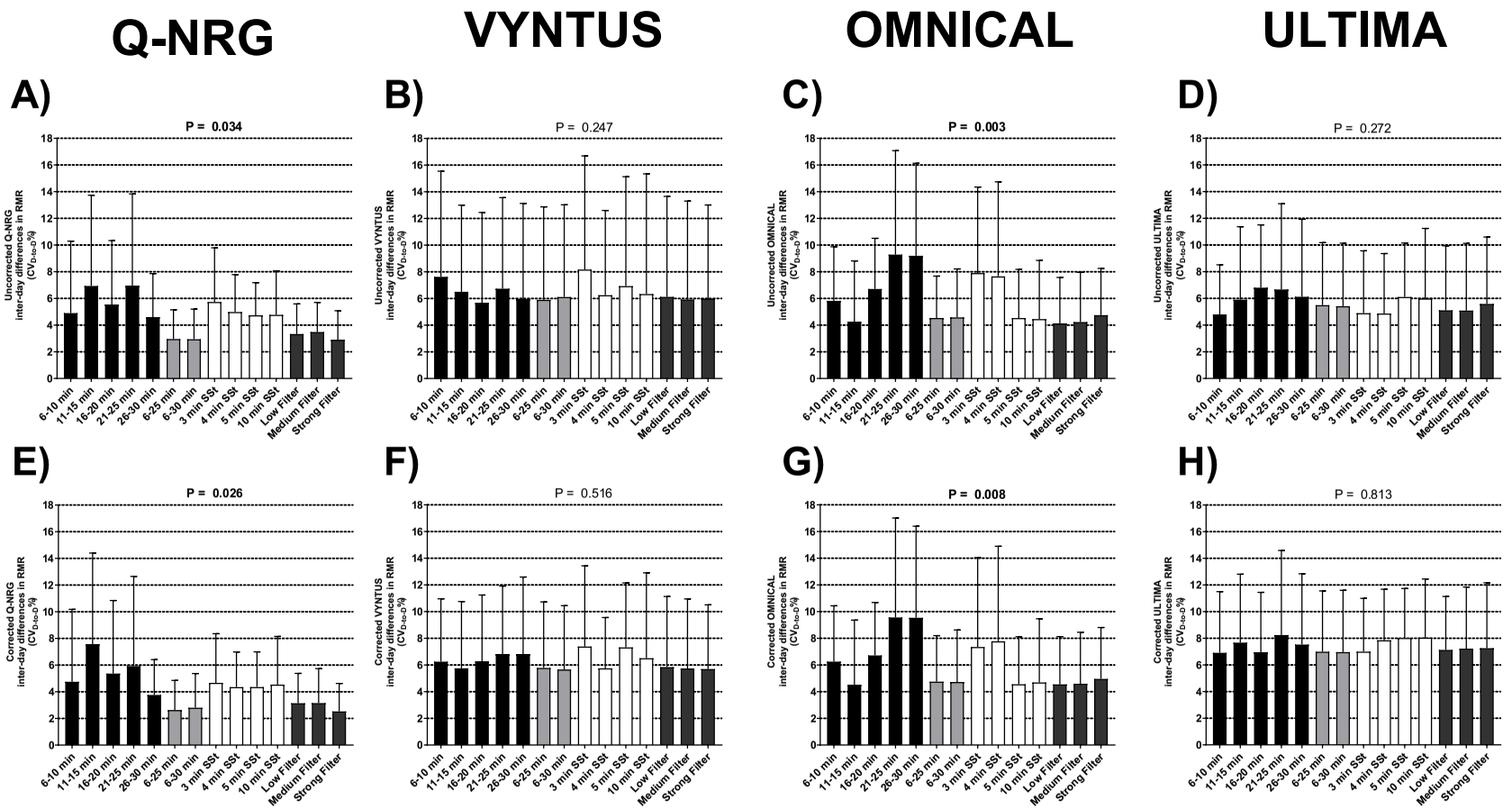


Figure S13. Differences among methods for gas exchange data selection with respect to uncorrected resting metabolic rate (RMR; panels A-D) and corrected RMR (panels E-H) value of inter-day differences both expressed as coefficient of variation ($CV_{D\text{-}to\text{-}D}$) by metabolic cart. Y axis represents the coefficient of variation of the inter-day differences (e.g. [standard deviation uncorrected RMR / mean uncorrected RMR] × 100). *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

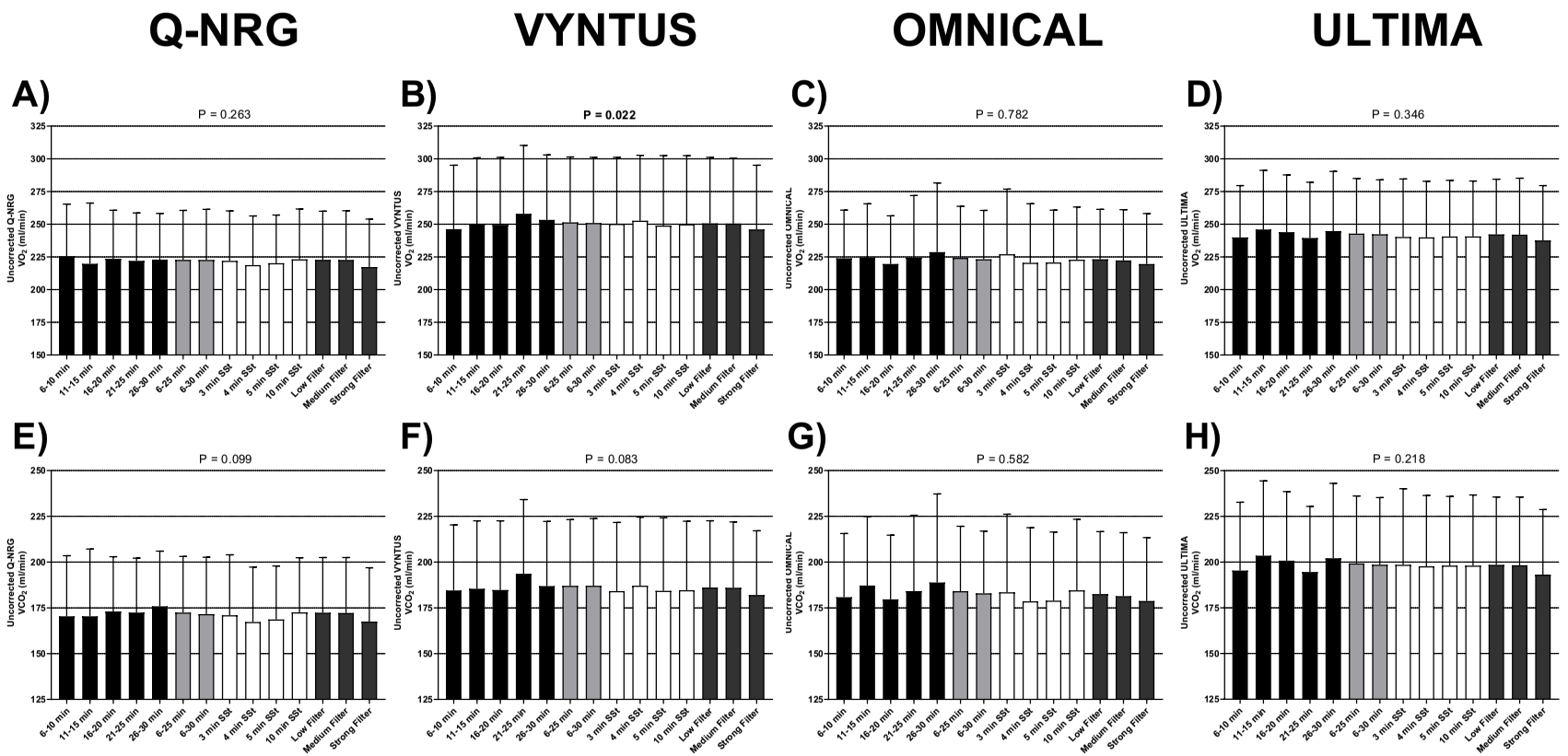


Figure S14. Differences among methods for gas exchange data selection with respect to uncorrected oxygen consumption (VO_2; panels A-D) and uncorrected carbon dioxide production (VCO_2; panels E-H) estimates by metabolic cart. *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

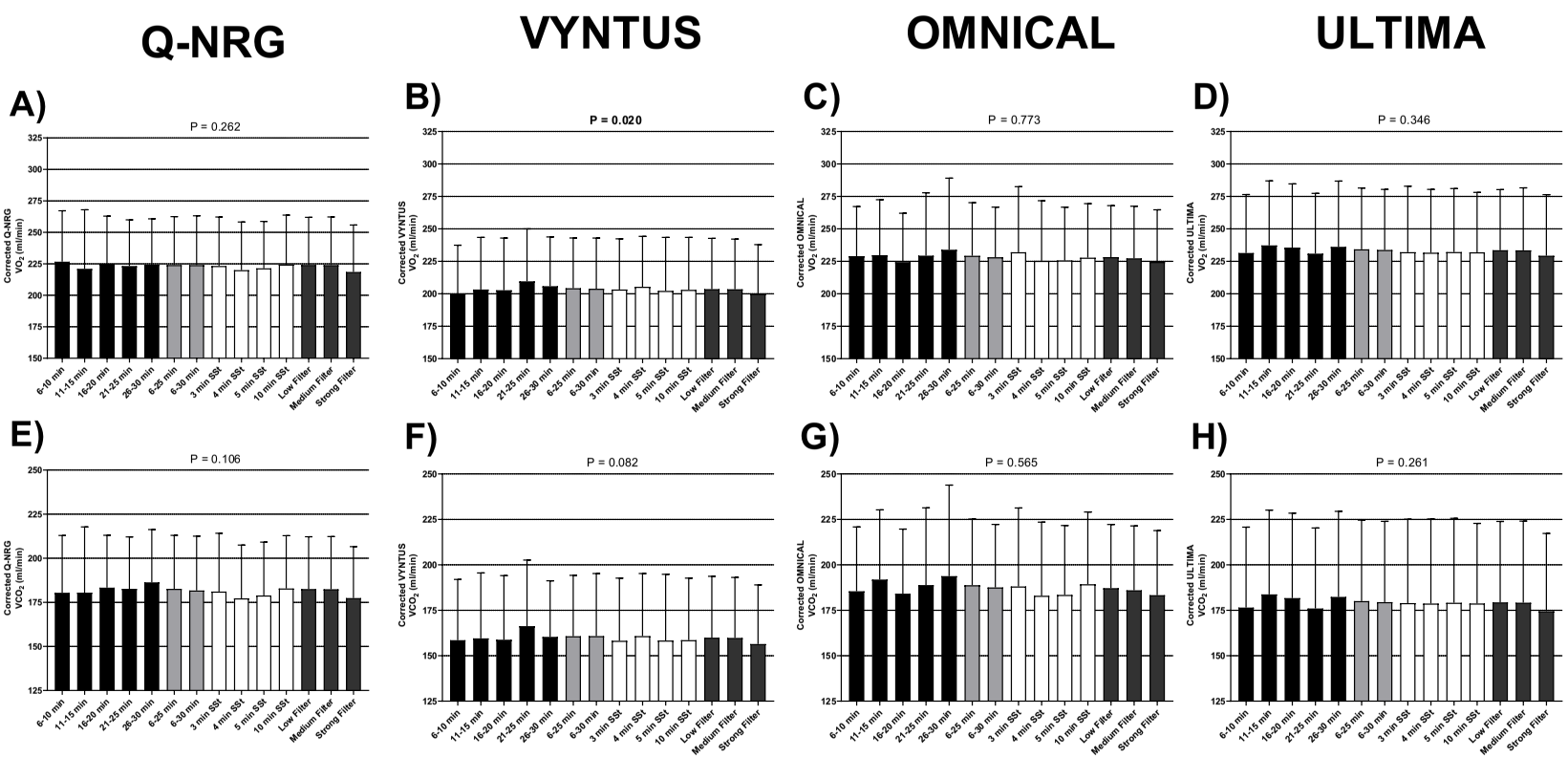


Figure S15. Differences among methods for gas exchange data selection with respect to corrected oxygen consumption (VO_2; panels A-D) and corrected carbon dioxide production (VCO_2; panels E-H) estimates by metabolic cart. *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

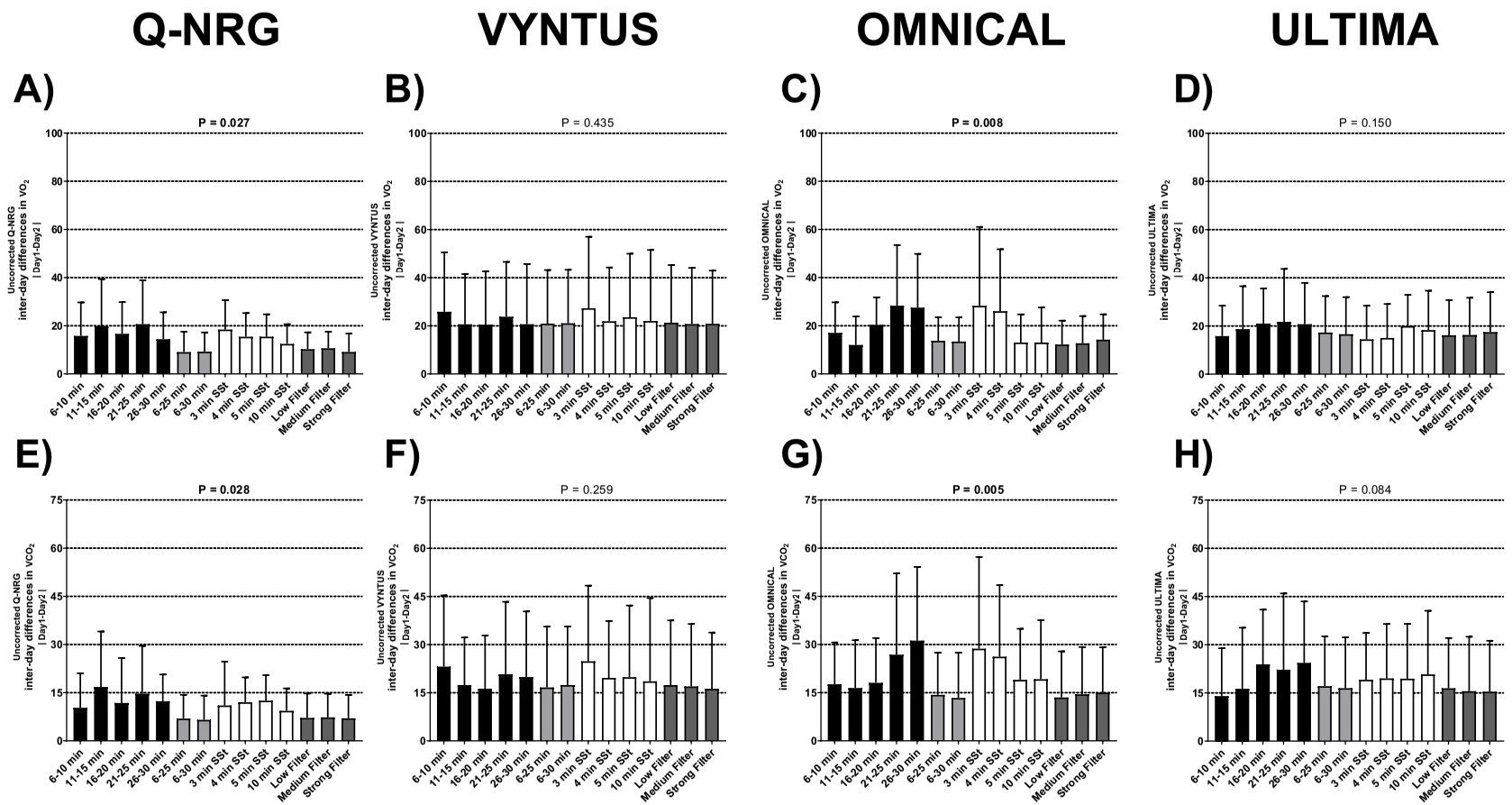


Figure S16. Differences among methods for gas exchange data selection with respect to uncorrected oxygen consumption (VO_2; panels A-D) and uncorrected carbon dioxide production (VCO_2; panels E-H) absolute value of inter-day differences by metabolic cart. Y axis represents absolute values of the inter-day differences (e.g. |uncorrected VO_2 Day1 – uncorrected VO_2 Day2|). *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

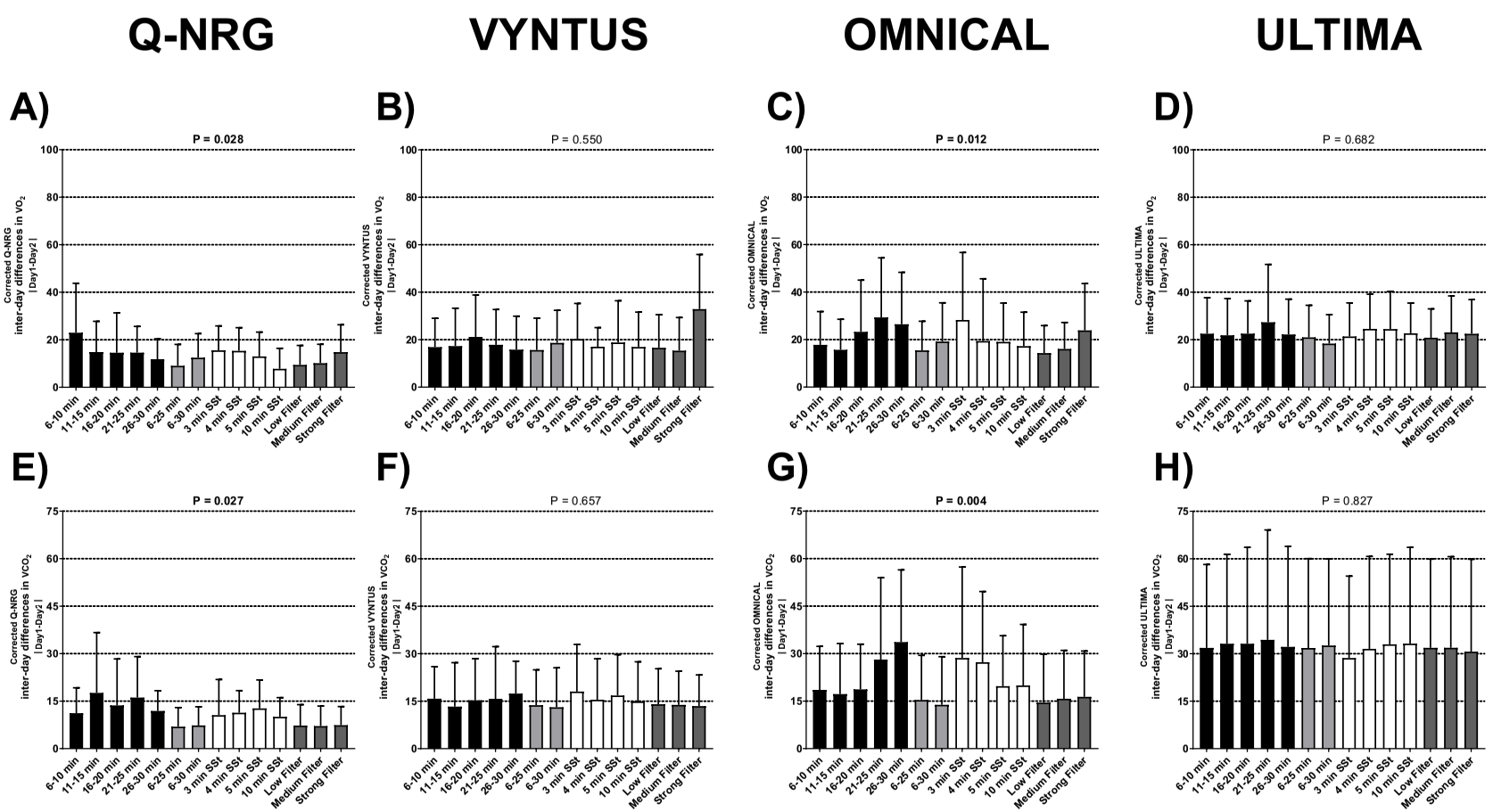


Figure S17. Differences among methods for gas exchange data selection with respect to corrected oxygen consumption (VO_2; panels A-D) and corrected carbon dioxide production (VCO_2; panels E-H) absolute value of inter-day differences by metabolic cart. Y axis represents absolute values of the inter-day differences (e.g. |corrected VO_2 Day1 – corrected VO_2 Day2|). *Black columns represent the values for each short TI period. Light grey columns represent the values for each long TI period. White columns represent the values for each steady state (SSt) period. Dark grey columns represent the values for each filtering period. P-values from repeated-measures analysis of variance (ANOVA). Data are presented as mean and standard deviation (SD). Min: minutes.*

REFERENCES

1. Lam, Y.Y.; Ravussin, E. Indirect calorimetry: An indispensable tool to understand and predict obesity. *Eur. J. Clin. Nutr.* **2017**, *71*, 318–322.

2. Fullmer, S.; Benson-Davies, S.; Earthman, C.P.; Frankenfield, D.C.; Gradwell, E.; Lee, P.S.P.; Piemonte, T.; Trabulsi, J. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J. Acad. Nutr. Diet.* **2015**, *115*, 1417-1446.e2.

3. Horner, N.K.; Lampe, J.W.; Patterson, R.E.; Neuhouser, M.L.; Beresford, S.A.; Prentice, R.L. Indirect Calorimetry Protocol Development for Measuring Resting Metabolic Rate as a Component of Total Energy Expenditure in Free-Living Postmenopausal Women. *J. Nutr.* **2001**, *131*, 2215–2218.

4. Ferrannini, E. The theoretical bases of indirect calorimetry: A review. *Metabolism* **1988**, *37*, 287–301.

5. Simonson, D.C.; DeFronzo, R.A. Indirect calorimetry: methodological and interpretative problems. *Am. J. Physiol. Metab.* **1990**, *258*, E399–E412.

6. Weir, J.B. de V. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **1949**, *109*, 1–9.

7. Frayn, K.N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1983**, *55*, 628–634.

8. Alcantara, J.M.A.; Delgado, G.S.; Gahete, F.J.A.; Galgani, J.E.; Ruiz, J.R. Impact of the Method Used to Select Gas Exchange Data for Estimating the Resting Metabolic Rate , as Supplied by Breath - by - Breath Metabolic Carts. *Nutrients* **2020**, *12*, 1–14.

9. Reeves, M.M.; Davies, P.S.W.; Bauer, J.; Battistutta, D. Reducing the time period of steady state does not affect the accuracy of energy expenditure measurements by indirect calorimetry. *J. Appl. Physiol.* **2004**, *97*, 130–134.

10. Irving, C.J.; Eggett, D.L.; Fullmer, S. Comparing Steady State to Time Interval and Non-Steady State Measurements of Resting Metabolic Rate. *Nutr. Clin. Pract.* **2017**, *32*, 77–83.

11. Borges, J.H.; Langer, R.D.; Cirolini, V.X.; Páscoa, M.A.; Guerra, G.; Gonçalves, E.M. Minimum Time to Achieve the Steady State and Optimum Abbreviated Period to Estimate the Resting Energy Expenditure by Indirect Calorimetry in Healthy Young Adults. *Nutr. Clin. Pract.* **2016**, *31*, 349–354.

12. Borges, J.H.; Guerra-Júnior, G.; Gonçalves, E.M. Methods for data analysis of resting energy expenditure measured using indirect calorimetry. *Nutrition* **2019**, *59*, 44–49.

13. Popp, C.J.; Tisch, J.J.; Sakarcan, K.E.; Bridges, W.C.; Jesch, E.D. Approximate Time to Steady-state Resting Energy Expenditure Using Indirect Calorimetry in Young, Healthy Adults. *Front. Nutr.* **2016**, *3*.

14. Popp, C.; Butler, M.; Curran, M.; Illiano, P.; Sevick, M.A.; St-Jules, D. Evaluating steady-state resting energy expenditure using indirect calorimetry in adults with overweight and obesity. *Clin. Nutr.* **2019**, *39*, 2220–2226.

15. Sanchez-Delgado, G.; Alcantara, J.; Ortiz-Alvarez, L.; Xu, H.; Martinez-Tellez, B.; Labayen, I.; Ruiz, J. Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. *Clin. Nutr.* **2018**, *37*, 1618–1624.

16. Darren M. Roffey; Nuala M. Byrne; and Andrew P. Hills Day-to-Day Variance in Measurement of Resting Metabolic Rate Using Ventilated-Hood and Mouthpiece & Nose-Clip Indirect Calorimetry Systems. *J. Parenter. Enter. Nutr.* **2006**, *30*, 426–432.

17. Kennedy, S.; Ryan, L.; Fraser, A.; Clegg, M.E. Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis. *J. Nutr. Sci.* **2014**, *3*.

18. Jéquier, E.; Acheson, K.; Schutz, Y.

Assessment Of Energy Expenditure And Fuel Utilization In Man. *Annu. Rev. Nutr.* **1987**, *7*, 187–208.

19. Schadewaldt, P.; Nowotny, B.; Strassburger, K.; Kotzka, J.; Roden, M. Indirect calorimetry in humans: a postcalorimetric evaluation procedure for correction of metabolic monitor variability. *Am. J. Clin. Nutr.* **2013**, *97*, 763–73.

20. Galgani, J.E.; Castro-Sepulveda, M.A. Influence of a Gas Exchange Correction Procedure on Resting Metabolic Rate and Respiratory Quotient in Humans. *Obesity* **2017**, *25*, 1941–1947.

21. Johnstone, A.M.; Murison, S.D.; Duncan, J.S.; Rance, K.A.; Speakman, J.R. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am. J. Clin. Nutr.* **2005**, *82*, 941–948.

22. Sanchez-Delgado, G.; Alcantara, J.M.A.; Acosta, F.M.; Martinez-Tellez, B.; Amaro-Gahete, F.J.; Merchan-Ramirez, E.; Löf, M.; Labayen, I.; Ravussin, E.; Ruiz, J.R. Energy Expenditure and Macronutrient Oxidation in Response to an Individualized Nonshivering Cooling Protocol. *Obesity* **2020**, *00*, 1–9.

23. Kjeldahl, J. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. *Fresenius' Zeitschrift für Anal. Chemie* **1883**, *22*, 366–382.

24. Haugen, H.A.; Melanson, E.L.; Tran, Z.V.; Kearney, J.T.; Hill, J.O. Variability of measured resting metabolic rate. *Am. J. Clin. Nutr.* **2003**, *78*, 1141–1144.

25. Cooper, J.A.; Watras, A.C.; O'Brien, M.J.; Luke, A.; Dobratz, J.R.; Earthman, C.P.; Schoeller, D.A. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J. Am. Diet. Assoc.* **2009**, *109*, 128–32.

26. Korth, O.; Bosy-Westphal, A.; Zschoche, P.; Glüer, C.C.; Heller, M.; Müller, M.J. Influence of methods used in body composition analysis on the prediction of resting energy expenditure. *Eur. J. Clin. Nutr.* **2007**, *61*, 582–589.

27. Mifflin, D.; Jeor, T.; Daugherty, A.; Hill, A.; Scott, J.; Daugherty, S.; Kho, Y. A new predictive equation in healthy individuals for resting energy. *Am. J. Clin. Nutr.* **1990**, *51*, 241–247.

28. Livingston, E.H.; Kohlstadt, I. Simplified resting metabolic rate-predicting formulas for normal-sized and obese individuals. *Obes. Res.* **2005**, *13*, 1255–1262.

29. Armour Forse, R. Comparison of Gas Exchange Measurements With a Mouthpiece, Face Mask, and Ventilated Canopy. *J. Parenter. Enter. Nutr.* **1993**, *17*, 388–391.

30. Melanson, K.J.; Saltzman, E.; Russell, R.; Roberts, S.B. Postabsorptive and Postprandial Energy Expenditure and Substrate Oxidation Do Not Change during the Menstrual Cycle in Young Women. *J. Nutr.* **1996**, *126*, 2531–2538.

31. Ferraro, R.; Lillioja, S.; Fontvieille, A.M.; Rising, R.; Bogardus, C.; Ravussin, E. Lower sedentary metabolic rate in women compared with men. *J. Clin. Invest.* **1992**, *90*, 780–784.

32. Shook, R.P.; Hand, G.A.; Paluch, A.E.; Wang, X.; Moran, R.; Hébert, J.R.; Jakicic, J.M.; Blair, S.N. High respiratory quotient is associated with increases in body weight and fat mass in young adults. *Eur. J. Clin. Nutr.* **2016**, *70*, 1197–1202.

33. Labayen, I.; Forga, L.; Martínez, J.A. Nutrient oxidation and metabolic rate as affected by meals containing different proportions of carbohydrate and fat, in healthy young women. *Eur. J. Nutr.* **1999**, *38*, 158–166.Sepulveda, M.A. Influence of a Gas Exchange Correction Procedure on Resting Metabolic Rate and Respiratory Quotient in Humans. *Obesity* **2017**, *25*, 1941–1947.

GENERAL DISCUSSION

GENERAL DISCUSSION

Obesity is one of the most important risk factors for developing cardiovascular disease, type 2 diabetes, as well as diverse types of cancer [1,2]. In simple terms, inducing a negative energy balance by increasing EE or by reducing EI is one of the main aims in the obesity treatment [2]. Consequently, numerous research groups around the world are studying human energy balance regulation. To this end, the use of accurate and precise calorimetry systems (i.e. capable to assess the day-to-day biological reproducibility) is mandatory to adequately estimate EE, which in turn is necessary for determining EI requirements [2]. IC is the reference method to estimate EE in humans, regardless their health status [2–7]. IC can be employed to assess 24hEE and its different components: SMR, BMR, TEF, CIT, and EAT [1]. When using IC, EE is estimated from the VO_2 and VCO_2 measurements [3,8–12], furthermore, IC also allows the estimation of the RQ and substrate oxidation. This, together with the technical advances achieved during the last decades, have made IC the preferred method to study the human metabolism in diverse situations and/or conditions [12]. To assess resting EE, the VO_2 and the VCO_2 are usually measured during a relatively short period of time (commonly 10-30 minutes) [4,5], from which even a shorter period of time is selected (using TI, SSt and/or filtering methods) and then analyzed (to estimate EE, RQ and/or substrate oxidation rates) [13,14].

Metabolic carts are the most extended IC system. A few years ago, the broadly considered gold standard metabolic cart for assessing human EE and RER, the DTC metabolic cart, was no longer manufactured and commercialized [15–23]. Importantly, to identify a reference metabolic cart three approaches can be used [24]: (i) assessing its accuracy and precision by alcohol burning tests, (ii) assessing its accuracy and precision by controlled pure gas infusions and (iii) assessing its biological reproducibility. The DTC was an accurate and precise metabolic cart [25]. Moreover, the day-to-day biological reproducibility observed using the DTC metabolic cart was suggested to be representative of the inherent human RMR biological variability [26]. Unfortunately, most of the current commercially available metabolic carts have shown an unacceptable low ($CV_{D\text{-}to\text{-}D}$≥10%) day-to-day biological reproducibility [21]. Therefore, there is an urgent need of characterize the accuracy, precision and biological reproducibility of commercially available metabolic carts to inform the scientific community about the best metabolic carts to study human energy metabolism nowadays.

Aiming to obtain better results when using metabolic carts, a novel technique called as 'post-calorimetric correction procedure' has been proposed by Schadewaldt et al. [22]. However, this post-calorimetric correction procedure has been only tested on two different metabolic carts (the DTC and the Vmax Encore 29n) [22,27]. Thus, the influence of this procedure on others metabolic carts, and the possible effect on their day-to-day biological reproducibility had not been tested until now. Another debated issue in IC methodology is the selection of gas exchange data from the whole measurement to compute EE, RER and nutrient oxidation rates. In fact, the method for gas exchange data selection is a key factor that may influence the VO_2 and VCO_2 values, and may also impact its day-to-day biological reproducibility [28].

This Doctoral Thesis aimed (i) to determine the accuracy, precision, biological reproducibility and comparability of different commercially available metabolic carts to assess RMR and RER in spontaneously breathing adults (**Study I** and **Study II**), and (ii) to examine the influence of different methods for gas exchange data selection on the RMR and RER estimates, as supplied by different metabolic carts (**Study III** and **Study IV**).

Alcohol burnings and continuous gas infusion tests, together with the assessment of the biological reproducibility are the most commonly accepted method for validating metabolic carts [24,29]. However, not all the metabolic carts allow the application of all these approaches. For example, the metabolic carts used in the **Study I** do not allow neither the gas infusion nor the burning test. Both the CCM and the MGU metabolic carts operates using the B×B technique. Generally, systems that are based on the B×B technique are not capable to detect the continuous and unidirectional air flow produced by these two validation approaches. The breathing has two well-differentiated phases, the inspiration and the expiration, and the B×B metabolic carts are specifically designed to detect such breathing pattern (i.e. air flowing in and out). On the other hand, in the other four metabolic carts used in

the **Study II** (i.e. the Q-NRG, the Vyntus, the Omnical and the Ultima), all the above-mentioned validation approaches are possible.

Accuracy may be defined as the proximity of measurements to traceable standards (i.e. the difference between the measured vs. the expected value) [29]. Previous studies have proposed a ±2% as an acceptable error in metabolic carts [16]. In this regard, in the **Study II**, the Omnical metabolic cart was the only metabolic cart presenting an error lower than 2% in VO_2, VCO_2 and EE and an error of 2.2% in RER when using the burning test validation methods, and even lower errors when using the pure gas infusions validation method. On the other hand, the Q-NRG metabolic cart showed similar accuracy than the Omnical for the assessment of VO_2 and EE, but worse accuracy for assessing VCO_2 and RER. The errors observed with the Vyntus and the Ultima metabolic cart were larger than those observed in the Omnical and the Q-NRG metabolic carts. It should be noted that the four metabolic carts included in the **Study II** were recently manufactured, thus preventing use-associated deterioration which may negatively influence the accuracy of the devices.

In the **Study II** we have also shown that the Q-NRG metabolic cart achieved a similar day-to-day RMR biological reproducibility than the one reported for the DTC ($CV_{D\text{-}to\text{-}D}$=3.6±0.5%). Nevertheless, all the metabolic carts included in this study achieved a day-to-day RMR biological reproducibility below 6% (and no statistical differences were observed among them). This contrast with the much higher day-to-day RMR biological reproducibility observed in the **Study I** with the CCM and MGU metabolic carts. Nevertheless, caution must be taken when comparing biological reproducibility results across different cohorts, as was showed in the **Study I.** These results suggest that the individual's characteristics could had a greater influence on the RMR biological reproducibility than the metabolic cart itself.

Nevertheless, the poorer biological reproducibility observed in **Study I** with the Medicalgraphics metabolic carts somehow aligns with observation of worse RER biological reproducibility obtain by the Ultima in the **Study II**, as compared to the others metabolic carts. It should be noted that the MGU metabolic cart (included in **Study I**) is an older model of the Ultima metabolic cart (included in **Study II**). As it can be observed the day-to-day RMR biological reproducibility observed in the **Study I** was almost three times higher than the observed in the **Study II** (i.e. 18.3±17.2% vs. 5.7±4.6% respectively). These differences may be explained (at least in part) by the individuals' characteristics and by the gases collection systems used in the **Study I** (i.e. neoprene face-mask) and in the **Study II** (face-tent), as it has been suggested by previous studies [30–33]. Nevertheless, other differences exist between the metabolic carts used in **Study I** and **Study II**. The flow-mass sensor used was different (directconnect™ metabolic flow sensor in **Study I** vs. PreVent™ metabolic flow sensor in **Study II**) and the MGU system (**Study I**) was older (≈3 years) compared to the Ultima metabolic cart (<1 year; **Study II**). Therefore, the possible influence of the deterioration of the different sub-systems and/or parts of the metabolic carts should not be discarded. Of note, the CCM metabolic cart (**Study I**) showed better RMR biological reproducibility than the observed by the MGU metabolic cart. This difference is somehow surprisingly, as both metabolic carts are manufactured by the same company (i.e. Medgraphics Corp.) and use the same VO_2 galvanic fuel cell and the same VCO_2 non-dispersive infrared analyzer. Despite the observed differences in RMR, but not in RER, might be attributable to differences in the calibration constants, and the fact that the CCM was newer than the MGU metabolic cart. Considering all together this might suggest that different metabolic carts within a same model/company might provide non comparable performance. This is in agreement with the observations of Kaviani et al. [16] and reinforce the need to replicate **Study II** results before concluding that the Omnical is the best metabolic cart among the ones analyzed in the present Doctoral Thesis.

Importantly, results from both **Study I** and **Study II** consistently show that the 6 metabolic carts analyzed in this Doctoral Thesis provide non-comparable RMR and RER estimations. In **Study I**, the RMR assessed using the CCM was 10-12% lower than the assessed by the MGU. In **Study II**, the RMR assessed by the Q-NRG was the lowest, followed by the Omnical, the Vyntus and the Ultima. This suggest that at least 5 out of 6 metabolic carts did not provide accurate RMR and RER. This reinforce the necessity to not only validate all metabolic carts before being used in research, but the need to periodically check their validity by using the aforementioned validation approaches [29]. Of note, the two metabolic carts showing better RMR accuracy as determined by burns and infusions (i.e. Q-NRG and Omnical), provide results

more similar to each other than to the rest of metabolic carts. This might suggest that, despite the **Study II** results showed no improvement of RMR or RER biological reproducibility by the application of Schadelwaldt' post-calorimetric correction, it might be useful for increasing the accuracy of the human measurement. Although it might be true to some extent, **Study II** results showed that corrected RMR and RER values are not comparable among metabolic carts, which is discouraging. Whether this post-calorimetric correction procedure provide more accurate results in different metabolic carts remained to be elucidated. Nevertheless, the gas infusions test seems to be a valid way for validating the metabolic carts as our results showed that the measurement error determined by the gas infusions test was comparable to the measurement error determined by the burning test, which is broadly considered as the reference method to validate indirect calorimeters [16,24,34,35].

The assessment of RMR using IC is normally performed over a 10–30 min period [4,5]. As aforementioned, it is widely assumed that the first 5 min of data recorded should be discarded [4,36]. Then, a shorter period is commonly selected from the remaining dataset for estimating the RMR, the RER and substrate oxidation [37–40]. It was assumed than SSt methods for gas exchange data selection provide better estimates of RMR than the other methods available (i.e. TI and filtering), after seminal studies performed in ventilated patients [4,39]. Noteworthy, the use of different methods for gas exchange data selection might result in different estimates of RMR, RER and substrate oxidation rates [28,41,42]. Indeed, the SSt-based RMR estimations are usually lower than those provided by the TI methods [28,41]. Therefore, considering that RMR is defined as the lowest EE in an awake person, it has been proposed that the lowest estimates obtained by the SSt method should be deemed more accurate than those provided by other methods. However, the under-estimation of the homeostatic RMR cannot be ruled out.

In the **Study III** we observed that the SSt and the filtering methods obtained lower RMR values than the TI methods, which was replicated in two different cohorts. In fact, these results were in line with previous literature [28,41,42]. Moreover, in the **Study IV**, we also observed that the filtering methods achieved lower RMR estimates in the four different metabolic carts used. But, as it was mentioned, the under-estimation of the homeostatic RMR cannot be dismissed. Interestingly, in the **Study IV**, we observed that the different methods for gas exchange data selection provided different day-to-day biological reproducibility (absolute inter-day differences and $CV_{D\text{-}to\text{-}D}$). In this regard, the methods that yielded the best day-to-day reproducibility across metabolic carts were the 6-25 min and the 6-30 min TI methods. Importantly, as the 'best' method for gas exchange data selection may depend on the metabolic cart, we strongly recommend to test which method for gas exchange data selection yield the best RMR and RER estimates.

Lastly, the variance in RMR explained by its classical determinants (sex, body weight and/or body composition) [43] may be considered also as an indirect approach for exploring the accuracy of the methods for gas exchange data selection. However, the variance in RMR explained was almost equal for all the methods for gas exchange data selection used in the **Study III** and **Study IV**, thus it may not be 'method-dependent'. Nevertheless, when the variance in RMR explained by its classical determinants is determined in the Q-NRG, the Vyntus, the Omnical and/or the Ultima metabolic carts (**Study IV**), the variance in RMR explained was almost two- to three-times (**Study IV**) the variance in RMR explained in the **Study III**. This fact could be explained, at least in part, by the fact that these metabolic carts are more accurate and precise than these included in the **Study III** (i.e. CCM and MGU).

GENERAL LIMITATIONS

The results presented in this Doctoral Thesis should be considered with caution since there are some limitations that should be acknowledged:

- All the studies included in this Doctoral Thesis included RMR data. Given that the RMR and the BMR are slightly different, we do not know whether our findings apply to BMR. However, all the RMR assessment followed the current recommendations [4], and therefore are likely similar to the BMR.

- The menstrual cycle was not recorded (**Study I** and **Study III**) or controlled (**Study II** and **Study IV**), and therefore its effect on the results is unknown [44,45]. However due to the intra-individual design and the 24 hours test-retest (except **Study III**) it is likely that the potential effect of menstrual cycle on RMR day-to-day differences is negligible.

- In **Studies I** and **III** we used a neoprene face-mask for the gas exchange collection while in the other two studies (**Study II** and **Study IV**) a ventilated hood canopy and a face-tent gas exchange collection systems were used. Therefore, the results may be influenced by the gas exchange collection system [30–33].

- All the studies included the assessment of human RMR using different metabolic carts. However, the DTC metabolic cart (the former gold standard) was not included as the reference metabolic cart.

- Previous studies suggest that the performance of a single unit may not represent the performance of all manufactured units [15,16]. Based on these studies, our results should be replicated before drawing firm conclusions.

- All the studies (except one in which a sub-sample of healthy middle-aged adults was included, **Study III**) were carried out in young healthy adults. Therefore, both the young age of most participant and their healthy state does not allow to apply our findings to other populations (e.g. critically ill ventilated patients, etc.). Therefore, it is necessary to replicate the findings of the present Doctoral Thesis on other populations (i.e. different age and/or health status).

REFERENCES

1. Lam, Y.Y.; Ravussin, E. Analysis of energy metabolism in humans: A review of methodologies. *Mol. Metab.* **2016**, *5*, 1057–1071.
2. Lam, Y.Y.; Ravussin, E. Indirect calorimetry: An indispensable tool to understand and predict obesity. *Eur. J. Clin. Nutr.* **2017**, *71*, 318–322.
3. McLean, J.; Tobin, G. *Animal and human calorimetry*; 1st editio.; Cambridge University Press: Cambridge, **1987**;
4. Fullmer, S.; Benson-Davies, S.; Earthman, C.P.; Frankenfield, D.C.; Gradwell, E.; Lee, P.S.P.; Piemonte, T.; Trabulsi, J. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J. Acad. Nutr. Diet.* **2015**, *115*, 1417-1446.e2.
5. Compher, C.; Frankenfield, D.; Keim, N.; Roth-Yousey, L.; Evidence Analysis Working Group Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *J. Am. Diet. Assoc.* **2006**, *106*, 881–903.
6. Achamrah, N.; Delsoglio, M.; De Waele, E.; Berger, M.M.; Pichard, C. Indirect calorimetry: The 6 main issues. *Clin. Nutr.* **2020**.
7. Oshima, T.; Berger, M.M.; De Waele, E.; Guttormsen, A.B.; Heidegger, C.P.; Hiesmayr, M.; Singer, P.; Wernerman, J.; Pichard, C. Indirect calorimetry in nutritional therapy. A position paper by the ICALIC study group. *Clin. Nutr.* **2017**, *36*, 651–662.
8. Lighton, J.R.B. *Measuring metabolic rates: a manual for scientists*; Oxford University Press: Oxford, **2008**;
9. Mtaweh, H.; Tuira, L.; Floh, A.A.; Parshuram, C.S. Indirect calorimetry: History, technology, and application. *Front. Pediatr.* **2018**, *6*.
10. Blaxter, K. *Energy metabolism in animals and man*; Cambridge University Press: Cambridge, **1989**;
11. Schoffelen, P.F.M. *Measurement of human energy expenditure: biological variability and technical validity*; Datawyse / Universitaire Pers Maastricht: Maastricht, **2017**;
12. Sanchez-Delgado, G.; Ravussin, E. Assessment of energy expenditure: are calories measured differently for different diets? *Curr. Opin. Clin. Nutr. Metab. Care* **2020**, *23*, 312–318.
13. Frayn, K.N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **1983**, *55*, 628–634.
14. Weir, J.B. de V. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **1949**, *109*, 1–9.
15. Cooper, J.A.; Watras, A.C.; O'Brien, M.J.; Luke, A.; Dobratz, J.R.; Earthman, C.P.; Schoeller, D.A. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *J. Am. Diet. Assoc.* **2009**, *109*, 128–32.
16. Kaviani, S.; Schoeller, D.A.; Ravussin, E.; Melanson, E.L.; Henes, S.T.; Dugas, L.R.; Dechert, R.E.; Mitri, G.; Schoffelen, P.F.M.; Gubbels, P.; et al. Determining the Accuracy and Reliability of Indirect Calorimeters Utilizing the Methanol Combustion Technique. *Nutr. Clin. Pract.* **2018**, *33*, 206–216.
17. Graf, S.; Karsegard, V.L.; Viatte, V.; Maisonneuve, N.; Pichard, C.; Genton, L. Comparison of three indirect calorimetry devices and three methods of gas collection: A prospective observational study; *Clin Nutr*, **2013**; Vol. 32;.
18. Delsoglio, M.; Dupertuis, Y.M.; Oshima, T.; van der Plas, M.; Pichard, C. Evaluation of the accuracy and precision of a new generation indirect calorimeter in canopy dilution mode. *Clin. Nutr.* **2019**, 1–8.
19. Oshima, T.; Dupertuis, Y.M.; Delsoglio, M.; Graf, S.; Heidegger, C.P.; Pichard, C. In vitro validation of indirect calorimetry device developed for the ICALIC project against mass spectrometry. *Clin. Nutr. ESPEN* **2019**, *32*, 50–55.
20. Kennedy, S.; Ryan, L.; Fraser, A.; Clegg, M.E. Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis. *J. Nutr. Sci.* **2014**, *3*.
21. Schoeller, D.A. Making Indirect Calorimetry a Gold Standard for Predicting Energy Requirements for Institutionalized Patients. *J. Am. Diet. Assoc.* **2007**, *107*, 390–392.
22. Schadewaldt, P.; Nowotny, B.; Strassburger, K.; Kotzka, J.; Roden, M. Indirect calorimetry in humans: a postcalorimetric evaluation procedure for correction of metabolic monitor variability. *Am. J. Clin. Nutr.* **2013**, *97*, 763–73.
23. Black, C.; Grocott, M.P.W.; Singer, M. Metabolic monitoring in the intensive care unit: A comparison of the Medgraphics Ultima, Deltatrac II, and Douglas bag collection methods. *Br. J. Anaesth.* **2015**, *114*, 261–268.
24. Schoffelen, P.F.M.; Plasqui, G. Classical experiments in whole-body metabolism:

open-circuit respirometry—diluted flow chamber, hood, or facemask systems. *Eur. J. Appl. Physiol.* **2017**.
25. Tissot, S.; Delafosse, B.; Bertrand, O.; Bouffard, Y.; Viale, J.P.; Annat, G. Clinical validation of the Deltatrac monitoring system in mechanically ventilated patients. *Intensive Care Med.* **1995**, *21*, 149–153.
26. Donahoo, W.T.; Levine, J.A.; Melanson, E.L. Variability in energy expenditure and its components. *Curr. Opin. Clin. Nutr. Metab. Care* **2004**, *7*, 599–605.
27. Galgani, J.E.; Castro-Sepulveda, M.A. Influence of a Gas Exchange Correction Procedure on Resting Metabolic Rate and Respiratory Quotient in Humans. *Obesity* **2017**, *25*, 1941–1947.
28. Sanchez-Delgado, G.; Alcantara, J.; Ortiz-Alvarez, L.; Xu, H.; Martinez-Tellez, B.; Labayen, I.; Ruiz, J. Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. *Clin. Nutr.* **2018**, *37*, 1618–1624.
29. Chen, K.Y.; Smith, S.; Ravussin, E.; Krakoff, J.; Plasqui, G.; Tanaka, S.; Murgatroyd, P.; Brychta, R.; Bock, C.; Carnero, E.; et al. Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0): A Guide to Conducting and Reporting Human Whole-Room Calorimeter Studies. *Obesity* **2020**, *28*, 1613–1625.
30. McAnena, O.J.; Harvey, L.P.; Katzeff, H.L.; Daly, J.M. Indirect calorimetry: comparison of hood and mask systems for measuring resting energy expenditure in healthy volunteers. *JPEN. J. Parenter. Enteral Nutr.* **1986**, *10*, 555–557.
31. Leff, M.L.; Hill, J.O.; Yates, A.A.; Cotsonis, G.A.; Heymsfield, S.B. Resting metabolic rate: Measurement reliability. *J. Parenter. Enter. Nutr.* **1987**, *11*, 354–359.
32. Darren M. Roffey; Nuala M. Byrne; and Andrew P. Hills Day-to-Day Variance in Measurement of Resting Metabolic Rate Using Ventilated-Hood and Mouthpiece & Nose-Clip Indirect Calorimetry Systems. *J. Parenter. Enter. Nutr.* **2006**, *30*, 426–432.
33. Armour Forse, R. Comparison of Gas Exchange Measurements With a Mouthpiece, Face Mask, and Ventilated Canopy. *J. Parenter. Enter. Nutr.* **1993**, *17*, 388–391.
34. Cooper, B.G.; McLean, J.A.; Taylor, R. An evaluation of the Deltatrac indirect calorimeter by gravimetric injection and alcohol burning. *Clin. Phys. Physiol. Meas.* **1991**, *12*, 333–341.
35. Miodownik, S.; Melendez, J.; Carlon, V.A.; Burda, B.; Melendez, J.; Arslan, V.; Quantitative, B.B. Quantitative methanol-burning lung model for validating gas-exchange measurements over wide ranges of FIO2. *J. Appl. Physiol.* **1998**, *84*, 2177–2182.
36. Horner, N.K.; Lampe, J.W.; Patterson, R.E.; Neuhouser, M.L.; Beresford, S.A.; Prentice, R.L. Indirect Calorimetry Protocol Development for Measuring Resting Metabolic Rate as a Component of Total Energy Expenditure in Free-Living Postmenopausal Women. *J. Nutr.* **2001**, *131*, 2215–2218.
37. Da Rocha, E.E.M.; Alves, V.G.F.; Da Fonseca, R.B. V Indirect calorimetry: Methodology, instruments and clinical application. *Curr. Opin. Clin. Nutr. Metab. Care* **2006**, *9*, 247–256.
38. Simonson, D.C.; DeFronzo, R.A. Indirect calorimetry: methodological and interpretative problems. *Am. J. Physiol. Metab.* **1990**, *258*, E399–E412.
39. McClave, S.A.; Spain, D.A.; Skolnick, J.L.; Lowen, C.C.; Kleber, M.J.; Wickerham, P.S.; Vogt, J.R.; Looney, S.W. Achievement of steady state optimizes results when performing indirect calorimetry. *J. Parenter. Enter. Nutr.* **2003**, *27*, 16–20.
40. Ferrannini, E. The theoretical bases of indirect calorimetry: A review. *Metabolism* **1988**, *37*, 287–301.
41. Irving, C.J.; Eggett, D.L.; Fullmer, S. Comparing Steady State to Time Interval and Non-Steady State Measurements of Resting Metabolic Rate. *Nutr. Clin. Pract.* **2017**, *32*, 77–83.
42. Borges, J.H.; Guerra-Júnior, G.; Gonçalves, E.M. Methods for data analysis of resting energy expenditure measured using indirect calorimetry. *Nutrition* **2019**, *59*, 44–49.
43. Johnstone, A.M.; Murison, S.D.; Duncan, J.S.; Rance, K.A.; Speakman, J.R. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am. J. Clin. Nutr.* **2005**, *82*, 941–948.
44. Melanson, K.J.; Saltzman, E.; Russell, R.; Roberts, S.B. Postabsorptive and Postprandial Energy Expenditure and Substrate Oxidation Do Not Change during the Menstrual Cycle in Young Women. *J. Nutr.* **1996**, *126*, 2531–2538.
45. Ferraro, R.; Lillioja, S.; Fontvieille, A.M.; Rising, R.; Bogardus, C.; Ravussin, E. Lower sedentary metabolic rate in women compared with men. *J. Clin. Invest.* **1992**, *90*, 780–784.

CONCLUSIONS

GENERAL CONCLUSIONS

The results of the present Doctoral Thesis show differences in the performance of several metabolic carts for assessing RMR and RER in healthy humans. The Omnical metabolic cart seems to be the most valid system among the six metabolic carts studied for assessing both, RMR and RER. On the other hand, the long TI methods provided more reproducible RMR and RER results using the Q-NRG and the Omnical metabolic carts. The influence of the methods on the RMR and RER biological reproducibility seems to depend on the metabolic cart, and thus, the method for gas exchange data selection yielding the best results should be determined for each metabolic cart. Finally, the post-calorimetric correction procedure does not improve neither the RMR nor the RER day-to-day biological reproducibility.

SPECIFIC CONCLUSIONS

Section 1: Metabolic carts for assessing resting metabolic rate

- The alcohol burning and gas infusions tests show that the Omnical metabolic cart provides more accurate and precise estimations of RMR and RER. The Q-NRG might be also consider a valid option for assessing the RMR.

- The measurement error determined by the gas infusions and burning tests are comparable. Consequently, both methods can be comparably used to validate the metabolic cart systems.

- Four out of the six metabolic carts (i.e. the Q-NRG, the Vyntus CPX, the Omnical and the Ultima CardiO2) analyzed provide similar day-to-day RMR and RER biological reproducibility in young healthy adults, although yielded non-comparable RMR and RER estimations. Conversely, the CCM and the MGU provide lower day-to-day RMR and RER biological reproducibility in young healthy adults.

- The post-calorimetric correction procedure does not improve the day-to-day RMR and RER biological reproducibility neither improve the comparability among the four metabolic carts analyzed. Moreover, it worsened the association of RMR with its classical predictors in the Q-NRG, the Omnical and the Ultima CardiO2 metabolic carts

- The individual's characteristics likely play a more significant role on the assessed RMR biological variability than the metabolic cart used.

Section 2: Methods for gas exchange data selection in resting metabolic rate assessments

- The methods for gas exchange data selection influenced the RMR and RER estimates when using the CCM, the RMR estimates when using the MGU and the Vyntus, and the RER when using the Q-NRG metabolic cart respectively. Conversely, methods did not influence the RMR and RER estimates for the Omnical and the Ultima metabolic carts.

- The long TI methods for gas exchange data selection (i.e. the 6-25 min and 6-30 min) show the best day-to-day RMR and RER biological reproducibility results. Nevertheless, whenever possible, RMR and RER day-to-day biological reproducibility should be tested for each method and metabolic cart.

ANNEXES

PAPERS DERIVED FROM THE THESIS

- **Alcantara JMA**, Sanchez-Delgado G, Martinez-Tellez B, Merchan-Ramirez E, Labayen I, Ruiz JR. Congruent validity and inter-day reliability of two breath by breath metabolic carts to measure resting metabolic rate in young adults. ***Nutr Metab Cardiovasc Dis***. 2018 Sep;28(9):929-936. PMID: 29739678.

- **Alcantara JMA**, Sanchez-Delgado G, Amaro-Gahete FJ, Galgani JE, Ruiz JR. Impact of the Method Used to Select Gas Exchange Data for Estimating the Resting Metabolic Rate, as Supplied by Breath-by-Breath Metabolic Carts. ***Nutrients***. 2020 Feb 14;12(2):487. PMID: 32075052.

- **Alcantara JMA** et al. Validity of four commercially available metabolic carts for assessing resting metabolic rate and respiratory exchange ratio in healthy humans. *In preparation.*

- **Alcantara JMA** et al. Influence of the method used to select the indirect calorimetry gas exchange data on the day-to-day biological reproducibility. *In preparation.*

SHORT CURRICULUM VITAE

Personally

Juan Manuel Alcántara Alcántara
Born 12th June 1991, La Carlota, Córdoba, Spain
alcantarajma@ugr.es

Academic social networking site profiles:

1. **Web of Science Researcher ID:** AAY-3879-2020
2. **ORCID:** 0000-0002-8842-374X
3. **Publons:**

   Juan MA Alcantara, profile link:
   https://publons.com/researcher/3882875/juan-ma-alcantara/

4. **Research Gate:**

   Juan Manuel Alcántara, profile link:
   https://www.researchgate.net/profile/Juan_Alcantara4

5. **Google Scholar:**

   Juan Manuel Alcántara Alcántara, profile link:
   https://scholar.google.com/citations?user=nyGdZhsCBooC&hl=es

Education

2016-2020	PhD Student in Biomedicine, University of Granada, Spain.
2015	Master's Degree in Researching in Physical Activity and Sports, University of Granada, Spain.
2014	Bachelor's Degree in Physical Activity and Sport Sciences, University of Universidad Católica de Valencia San Vicente Mártir, Spain.
2012	Bachelor's Degree in Teaching (Physical Education), University of Cordoba, Spain.

International Internships

2019	University of Pontificia Universidad Católica de Chile, Santiago de Chile, Chile. Department of Nutrition, Diabetes and Metabolism, Faculty of Medicine. Prof. José E. Galgani's lab. *3-month stay as PhD Student.*
2018	University of Leeds, United Kingdom. Human Appetite Research Unit, School of Phycology, Faculty of Medicine and Health. Prof. John Blundell's lab. *3-month stay as PhD Student.*

Previous Positions

2016-2020	Predoctoral FPU Research Fellow. Department of Physical Education and Sports, School of Sport Sciences, University of Granada, Granada, Spain.
2016-2016	Part time 5-month research contract linked to the SPORTEUS project. Fundación General UGR-Empresa. University of Granada, Granada, Spain.

Participation in Research Projects

2020	Inter-day reliability of the oral glucose tolerance test using indirect calorimetry. *Researcher*.
2020	Effect of the Addition of Medium Chain Triglycerides to a Mixed Macro Nutritional Meal on the Oxidation of Postprandial Macronutrients. *Researcher*.
2018-2020	The SmartMove project: Exercise in the prevention and treatment of obesity and insulin resistance: Smart analysis-smart interventions. Funded by Spanish Ministry of Economy and competitiveness: 100000€. *Researcher.*
2014-2017	ACTIBATE: Activating Brown Adipose Tissue through Exercise. Effects of an exercise intervention on activity and quantity of Brown adipose tissue: A Randomized Controlled Trial. Funded by the Spanish Ministry of Economy and competitiveness among others: ≈600000€. *Researcher.*
2015-2016	SPORTEUS: Effects of the intake of a protein enriched smoothie on recovery of muscular function and muscle damage after an acute bout of high intensity exercise. Funded by Lactalis-Puleva S.L.: 190000€. *Researcher.*

Publications

1. Plaza-Florido A, **Alcantara JMA**, Amaro-Gahete FJ, Sacha J, Ortega FB. Cardiovascular risk factors and heart rate variability: impact of the level of the threshold-based artefact correction used to process the heart rate variability signal. ***J. Med. Syst.*** 2020, *in press*.
    - [**IF** JCR 2019:**3.06**. Rank 23/102. **Q1**. HEALTH CARE SCIENCES & SERVICES]
2. Sanchez-Delgado G, **Alcantara JMA**, Acosta FM, Martinez-Tellez B, Amaro-Gahete FJ, Merchan-Ramirez E, Löf M, Labayen I, Ravussin E, Ruiz JR. Energy Expenditure and Macronutrient Oxidation in Response to an Individualized Nonshivering Cooling Protocol. ***Obesity (Silver Spring)***. 2020 Sep 27. Epub ahead of print. PMID: 32985119.
    - [**IF** JCR 2019:**3.74**. Rank 28/89. **Q2**. NUTRITION & DIETETICS].
3. Plaza-Florido A*, **Alcantara JMA***, Migueles JH, Amaro-Gahete FJ, Acosta FM, Mora-Gonzalez J, Sacha J, Ortega FB. Inter- and intra-researcher reproducibility of heart rate variability parameters in three human cohorts. ***Sci Rep***. 2020 Jul 9;10(1):11399. PMID: 32647148.
    - * Equally contributed: Abel Plaza-Florido and **Juan M. A. Alcantara**.
    - [**IF** JCR 2019:**3.99**. Rank 17/71. **Q1**. MULTIDISCIPLINARY SCIENCES].
4. **Alcantara JMA**, Sanchez-Delgado G, Amaro-Gahete FJ, Galgani JE, Ruiz JR. Impact of the Method Used to Select Gas Exchange Data for Estimating the Resting Metabolic Rate, as Supplied by Breath-by-Breath Metabolic Carts. ***Nutrients***. 2020 Feb 14;12(2):487. PMID: 32075052.
    - [**IF** JCR 2019:**4.55**. Rank 17/89. **Q1**. NUTRITION & DIETETICS].
5. **Alcantara JMA**, Plaza-Florido A, Amaro-Gahete FJ, Acosta FM, Migueles JH, Molina-Garcia P, Sacha J, Sanchez-Delgado G, Martinez-Tellez B. Impact of Using Different Levels of Threshold-Based Artefact Correction on the Quantification of Heart Rate Variability in Three Independent Human Cohorts. ***J Clin Med***. 2020 Jan 23;9(2):325. PMID: 31979367.
    - [**IF** JCR 2019:**3.30**. Rank 36/165. **Q1**. MEDICINE, GENERAL & INTERNAL].
6. Amaro-Gahete FJ, Sanchez-Delgado G, **Alcantara JMA**, Martinez-Tellez B, Acosta FM, Merchan-Ramirez E, Löf M, Labayen I, Ruiz JR. Energy expenditure differences across lying, sitting, and standing positions in young healthy adults. ***PLoS One***. 2019 Jun 12;14(6):e0217029.
    - [**IF** JCR 2019:**2.74**. Rank 27/71. **Q2**. MULTIDISCIPLINARY SCIENCES].
7. Martinez-Tellez B, Perez-Bey A, Sanchez-Delgado G, Acosta FM, Corral-Perez J, Amaro-Gahete FJ, **Alcantara JMA**, Castro-Piñero J, Jimenez-Pavon D, Llamas-Elvira JM, Ruiz JR. Concurrent validity of supraclavicular skin temperature measured with iButtons and infrared thermography as a surrogate marker of brown adipose tissue. ***J Therm Biol***. 2019 May;82:186-196. PMID: 31128647.
    - [**IF** JCR 2019:**2.36**. Rank 38/93. **Q2**. BIOLOGY].
8. **Alcantara JMA**, Sanchez-Delgado G, Martinez-Tellez B, Labayen I, Ruiz JR. Impact of cow's milk intake on exercise performance and recovery of muscle function: a systematic review. ***J Int Soc Sports Nutr***. 2019 May 6;16(1):22. PMID: 31060583.
    - [**IF** JCR 2019:**5.09**. Rank 6/85. **Q1**. SPORT SCIENCES].
9. Amaro-Gahete FJ, Sanchez-Delgado G, **Alcantara JMA**, Martinez-Tellez B, Acosta

FM, Helge JW, Ruiz JR. Impact of data analysis methods for maximal fat oxidation estimation during exercise in sedentary adults. ***Eur J Sport Sci***. 2019 Oct;19(9):1230-1239. Epub 2019 Mar 28. PMID: 30922184.
- [**IF** JCR 2019:**2.78**. Rank 24/85. **Q2**. SPORT SCIENCES].

10. Amaro-Gahete FJ, Sanchez-Delgado G, **Alcantara JMA**, Martinez-Tellez B, Muñoz-Hernandez V, Merchan-Ramirez E, Löf M, Labayen I, Ruiz JR. Congruent Validity of Resting Energy Expenditure Predictive Equations in Young Adults. ***Nutrients***. 2019 Jan 22;11(2):223. PMID: 30678176.
    - [**IF** JCR 2019:**4.55**. Rank 17/89. **Q1**. NUTRITION & DIETETICS].
11. Sanchez-Delgado G, Martinez-Tellez B, Garcia-Rivero Y, Acosta FM, **Alcantara JMA**, Amaro-Gahete FJ, Llamas-Elvira JM, Gracia-Marco L, Ruiz JR. Association between brown adipose tissue and bone mineral density in humans. ***Int J Obes (Lond)***. 2019 Aug;43(8):1516-1525. Epub 2018 Dec 5. PMID: 30518823.
    - [**IF** JCR 2018:**4.51**. Rank 29/145. **Q1**. ENDOCRINOLOGY & METABOLISM].
12. Sanchez-Delgado G, Martinez-Tellez B, Garcia-Rivero Y, **Alcantara JMA**, Acosta FM, Amaro-Gahete FJ, Llamas-Elvira JM, Ruiz JR. Brown Adipose Tissue and Skeletal Muscle ^{18}F-FDG Activity After a Personalized Cold Exposure Is Not Associated With Cold-Induced Thermogenesis and Nutrient Oxidation Rates in Young Healthy Adults. ***Front Physiol***. 2018 Nov 16;9:1577. PMID: 30505277.
    - [**IF** JCR 2018:**3.20**. Rank 25/81. **Q2**. PHYSIOLOGY].
13. Martinez-Tellez B, Sanchez-Delgado G, **Alcantara JMA**, Acosta FM, Amaro-Gahete FJ, Osuna-Prieto FJ, Perez-Bey A, Jimenez-Pavon D, Llamas-Elvira JM, Gil A, Aguilera CM, Rensen PCN, Ruiz JR. Evidence of high ^{18} F-fluorodeoxyglucose uptake in the subcutaneous adipose tissue of the dorsocervical area in young adults. ***Exp Physiol***. 2019 Feb;104(2):168-173. Epub 2018 Dec 18. PMID: 30468689.
    - [**IF** JCR 2018:**2.62**. Rank 33/81. **Q2**. PHYSIOLOGY].
14. Sanchez-Delgado G, **Alcantara JMA**, Acosta FM, Martinez-Tellez B, Amaro-Gahete FJ, Ortiz-Alvarez L, Löf M, Labayen I, Ruiz JR. Estimation of non-shivering thermogenesis and cold-induced nutrient oxidation rates: Impact of method for data selection and analysis. ***Clin Nutr***. 2019 Oct;38(5):2168-2174. Epub 2018 Sep 18. PMID: 30297258.
    - [**IF** JCR 2018:**6.40**. Rank 6/87. **Q1**. NUTRITION & DIETETICS].
15. 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. Association of Objectively Measured Physical Activity With Brown Adipose Tissue Volume and Activity in Young Adults. ***J Clin Endocrinol Metab***. 2019 Feb 1;104(2):223-233. PMID: 30137350.
    - [**IF** JCR 2019:**5.39**. Rank 21/143. **Q1**. ENDOCRINOLOGY & METABOLISM].
16. Acosta FM, Berchem J, Martinez-Tellez B, Sanchez-Delgado G, **Alcantara JMA**, Ortiz-Alvarez L, Hamaoka T, Ruiz JR. Near-Infrared Spatially Resolved Spectroscopy as an Indirect Technique to Assess Brown Adipose Tissue in Young Women. ***Mol Imaging Biol***. 2019 Apr;21(2):328-338. PMID: 29956118.
    - [**IF** JCR 2019:**2.93**. Rank 43/134. **Q2**. RADIOLOGY, NUCLEAR MEDICINE & MEDICAL IMAGING].
17. **Alcantara JMA**, Sanchez-Delgado G, Martinez-Tellez B, Merchan-Ramirez E, Labayen I, Ruiz JR. Congruent validity and inter-day reliability of two breath by breath metabolic carts to measure resting metabolic rate in young adults. ***Nutr Metab Cardiovasc Dis***. 2018 Sep;28(9):929-936. Epub 2018 Mar 30. PMID: 29739678.
    - [**IF** JCR 2018:**3.34**. Rank 30/89. **Q2**. NUTRITION & DIETETICS].
18. Acosta FM, Martinez-Tellez B, Sanchez-Delgado G, **Alcantara JMA**, Acosta-Manzano P, Morales-Artacho AJ, Ruiz JR. Physiological responses to acute cold exposure in young lean men. ***PLoS One***. 2018 May 7;13(5):e0196543. PMID: 30001395.
    - [**IF** JCR 2018:**2.78**. Rank 24/69. **Q2**. MULTIDISCIPLINARY SCIENCES].
19. Martinez-Tellez B, Sanchez-Delgado G, Garcia-Rivero Y, **Alcantara JMA**, Martinez-Avila WD, Muñoz-Hernandez MV, Olza J, Boon MR, Rensen PCN, Llamas-Elvira JM, Ruiz JR. A New Personalized Cooling Protocol to Activate Brown Adipose Tissue in Young Adults. ***Front Physiol***. 2017 Nov 2;8:863. PMID: 29163207.
    - [**IF** JCR 2017:**3.39**. Rank 20/83. **Q1**. PHYSIOLOGY].
20. Martinez-Tellez B, Sanchez-Delgado G, Acosta FM, **Alcantara JMA**, Boon MR, Rensen PCN, Ruiz JR. Differences between the most used equations in BAT-human studies to estimate parameters of skin temperature in young lean men. ***Sci Rep***. 2017 Sep 5;7(1):10530. PMID: 28874709.

- [**IF** JCR 2017:**4.12**. Rank 12/64. **Q1**. MULTIDISCIPLINARY SCIENCES].

21. Sanchez-Delgado G, **Alcantara JMA**, Ortiz-Alvarez L, Xu H, Martinez-Tellez B, Labayen I, Ruiz JR. Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. ***Clin Nutr***. 2018 Oct;37(5):1618-1624. Epub 2017 Aug 5. PMID: 28826698.
    - [**IF** JCR 2017:**5.50**. Rank 8/83. **Q1**. NUTRITION & DIETETICS].
22. Sanchez-Delgado G, Martinez-Tellez B, Olza J, Aguilera CM, Labayen I, Ortega FB, Chillon P, Fernandez-Reguera C, **Alcantara JMA**, Martinez-Avila WD, Muñoz-Hernandez V, Acosta FM, Prados-Ruiz J, Amaro-Gahete FJ, Hidalgo-Garcia L, Rodriguez L, Ruiz YA, Ramirez-Navarro A, Muros-de Fuentes MA, García-Rivero Y, Sanchez-Sanchez R, de Dios Beas Jimenez J, de Teresa C, Navarrete S, Lozano R, Brea-Gomez E, Rubio-Lopez J, Ruiz MR, Cano-Nieto A, Llamas-Elvira JM, Jimenez Rios JA, Gil A, Ruiz JR. Activating brown adipose tissue through exercise (ACTIBATE) in young adults: Rationale, design and methodology. ***Contemp Clin Trials***. 2015 Nov;45(Pt B):416-425. Epub 2015 Nov 3. PMID: 26546068.
    - [**IF** JCR 2015:**2.05**. Rank 75/124. **Q3**. MEDICINE, RESEARCH & EXPERIMENTAL].

Invited Lectures and Conferences

2019	Lecture in a seminar (5 hours): "*Jornadas de Actualización en Evaluación Cardiometabólica*", University of Valparaíso, Chile.
2018-2019	Lecture in Master Degree (4 hours): Máster Propio en Alimentación, Ejercicio y Deporte para la Salud, University of Granada, Spain. Title of lecture: "*Calorimetría: pruebas de gasto metabólico"*.
2017-2018	Lecture in Master Degree (4 hours): Máster Propio en Alimentación, Ejercicio y Deporte para la Salud, University of Granada, Spain. Title of lecture: "*Calorimetría indirecta y pruebas de esfuerzo máximo y submáximo*".

Teaching experience

2020-present	Physical Activity and Health. Degree in Physical Activity and Sport Sciences (6 ECTS Credits) University of Granada, Spain.
2019-2020	Physical Activity and Health. Degree in Physical Activity and Sport Sciences (6 ECTS Credits) University of Granada, Spain.
2018-2019	Design and organization of sport events and activities. Degree in Physical Activity and Sport Sciences (4 ECTS Credits) University of Granada, Spain.
2017-2019	Bases of sports III: Football, Judo and Gymnastics. Degree in Physical Activity and Sport Sciences (6 ECTS Credits) University of Granada, Spain.

Accepted congress communications as first author

2018	Title: "Efecto del consumo de leche y productos lácteos sobre el rendimiento físico y la recuperación de la función muscular: una revisión sistemática". *VI Simposio EXERNET. Investigación en Ejercicio, Salud y Bienestar: "Exercise is Medicine"*. Pamplona, Comunidad Foral de Navarra, Spain. Authors: **Juan M.A. Alcantara**; Guillermo Sánchez-Delgado; Borja Martinez-Tellez; Idoia Labayen; Jonatan R Ruiz. *Poster*.
2018	Title: "Diferencias en gasto energético en reposo: efecto de la posición al realizar la medición". *III Jornadas/I Congreso Nacional de Investigadores en Formación (JIFFI)*. Granada, Andalucía, Spain. Unversity of Granada. Authors: **Juan M.A. Alcantara**; Francisco J. Amaro-Gahete; Guillermo Sánchez-Delgado; Borja Martinez-Tellez; Francisco Acosta-Manzano; Jonatan R Ruiz. *Poster*.
2017	Title: "Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis". *4th Recent Advances & Controversies in Measurement of Energy Metabolism (RACMEM)*. Friburg, Switzerland.

Unviesrity of Fribourg.
Authors: **Juan M.A. Alcantara**; Guillermo Sanchez-Delgado; Yolanda García-Rivero; Wendy D. Martinez-Avila; Victoria Muñoz-Hernandez; Lourdes Ortiz-Alvarez; Huiwen Xu; Borja Martinez-Tellez; Idoia Labayen; Jonatan R. Ruiz.
Poster.

2017 Title: "Evaluación del gasto metabólico basal en adultos jóvenes: Impacto de alcanzar el estado estable sobre la fiabilidad de la medida". *II Jornadas de Investigadores en Formación (JIFFI)*. Granada, Andalucía, Spain. University of Granada.
Authors: **Juan M.A. Alcantara**; Guillermo Sanchez-Delgado; Francisco J Amaro-Gahete; Huiwen Xu; Lourdes Ortiz-Alvarez; Jonatan R Ruiz.
Poster.

2016 Title: Modelo de ejercicio excéntrico para provocar daño muscular: estudio de validez y Fiabilidad. *Simposio EXERNET. Investigación en Ejercicio, Salud y Bienestar: "Exercise is Medicine"*. Cádiz, Andalucía, Spain.
Authors: **Juan M.A. Alcantara**; Martinez-Tellez, B; Sanchez-Delgado, G; Ruiz, JR.
Poster.

2016 Title: "Fiabilidad de dos analizadores de gases en la medición del Gasto Metabólico Basal: resultados preliminares del estudio piloto ACTIBATE".
I Jornadas de Investigadores en Formación (JIFFI). Granada, Andalucía, Spain. University of Granada.
Authors: **Juan M.A. Alcantara**; Guillermo Sánchez Delgado; Borja Manuel Martínez Téllez; Guillermo Sánchez Delgado.
Oral Presentation.

2015 Title: "Validación y fiabilidad de un ejercicio en pendiente negativa para provocar daño muscular". *VII Seminario Sociedad Andaluza de Medicina del Deporte (SAMEDE): "Entrenamiento y rendimiento deportivo: actualización en métodos y medios para el entrenamiento y valoración del rendimiento deportivo"*. Jaén, Andalucía, Spain. Sociedad Andaluza de Medicina del Deporte (SAMEDE).
Authors: **Juan M.A. Alcantara**; Borja Manuel Martínez Téllez; Guillermo Sánchez Delgado; Jonatan Ruiz Ruiz.
Oral Presentation.

Other merits

2019 Internship grant: "Estancia breve en el extranjero financiada por el programa estatal de promoción del talento y su empleabilidad subprograma estatal de movilidad: estancias breves y traslados temporales FPU". From the "Ministerio de Ciencia, Innovación y Universidades". 4260€. University of Pontificia Universidad Católica de Chile, Santiago de Chile, Chile. Department of Nutrition, Diabetes and Metabolism, Faculty of Medicine. Prof. José E. Galgani's lab.

2018 Moderator in the VI Simposio EXERNET. Investigación en Ejercicio, Salud y Bienestar "Exercise is Medicine". Pamplona, Spain.

2018 Member of the Scientific Committee of the International Symposium: Role of brown adipose tissue in human health. Granada, Spain.

2018 Internship grant: "Plan propio de movilidad internacional de estudiantes de programas de doctorado de la Universidad de Granada". From the University of Granada. 2075€. University of Leeds, United Kingdom. Human Appetite Research Unit, School of Phycology, Faculty of Medicine and Health. Prof. John Blundell's lab.

2015 – Co-author of 24 congress communications, including national and international conferences (without taking into account the aforementioned communications).

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS
AGRADECIMIENTOS

La verdad es que no sé muy bien cómo afrontar esta sección... tengo que reconocer que he hecho una breve revisión sistemática de la literatura utilizando diferentes tesis doctorales de compañer@s. Aunque hay tanta heterogeneidad a la hora de abordar esta sección que en lugar de poder llegar a una conclusión más o menos clara de cómo hacerlo, esta revisión me ha hecho "*petar cual palomita*"... Así que, como dijo Jack el destripador, "*vayamos por partes*".

Como soy una persona poco propensa a abrirse y mostrar sus sentimientos (menos aún por escrito), y también dicho sea de paso soy algo desastre, empiezo mis agradecimientos pidiendo disculpas. En primer lugar quiero pedir perdón si alguna vez no he demostrado (ya sea verbalmente, con mensajes, cartas, gestos, emojis, GIFs...) todo lo que aquí dejo por escrito, pero bueno, siempre puedo escudarme en que "*las palabras se las lleva el viento*" y esto se quedará aquí escrito "*pa toa la vida*". Muchísimas personas han formado parte (en mayor o menor medida) en todo el proceso de mi formación personal, académica y de la tesis doctoral en sí, por lo que también aprovecho para pedir disculpas si con la emoción del momento me he dejado a alguien atrás (ya decía antes que soy algo desastre para estas cosas)... Por lo tanto, toda aquella persona que me quiera y que se preocupa por mí sabe que el sentimiento es recíproco y, que por tanto, aunque no vea su nombre aquí escrito porque "*se me haya ido la pinza*" que sepa que le doy las gracias de corazón (y que le debo una cerveza por daños y prejuicios).

La verdad, pensando y preparando esta sección me he dado cuenta de muchas cosas que había pasado por alto en algún momento, y es que tengo que reconocer que soy todo un privilegiado... ya no sólo porque he disfrutado de unas condiciones muy propensas para realizar mi doctorado, también por la calidad humana que había a mi alrededor, como dice mi sobri, *"¡buaaauuh! es increíble"*. De hecho, he tenido la suerte de tener financiación para permitirme una dedicación completa para realizar mi doctorado (hecho - lamentablemente - harto difícil en España), la suerte de tener unos directores fantásticos y unos (compañeros)amigos del trabajo impresionantes. Por si fuese poco, todo esto se ha visto complementado por unos amigos y una familia espectaculares que me han apoyado incondicionalmente en todo momento. Gracias a tod@s.

GRACIAS a mis directores.

Sin vuestra ayuda tanto en lo académico como en lo personal nada de esto habría sido posible. Si como directores sois unos *"bicharracos"* como personas ya ni os cuento... La verdad es que además de unos buenos directores habéis sido unos maravillosos amigos.

Jonatan Ruiz Ruiz, a día de hoy es imposible olvidar aquellas maravillosas palabras cuando nos enteramos de que iba a ser FPU y que podría hacer mi doctorado contigo... la verdad es que aún resuenan en mi cabeza y me emocionan... *"Cabr*n, ¿el teléfono para qué lo tienes?"*. Jonatan, gracias por haberte volcado y haber luchado lo imposible para que pudiese quedarme en el grupo y hacer la tesis bajo tu dirección, gracias por todo lo que has hecho (y sigues haciendo por mí). Me reafirmo cuando hago el símil y te digo que te considero como mi *"padre académico"* ya que me estás orientando (y dejando que me de mis golpes, como hace un buen padre) para que aprenda cómo es esto de la vida académica. Gracias por tu guía, consejos y palabras de ánimo cuando lo necesitaba, también por *"haberme apretado las clavijas"* cuando ha hecho falta. De corazón, gracias por todo el tiempo que me has dedicado, por toda la paciencia que has tenido conmigo, por la confianza que depositaste en mi desde el momento en el que *"el tío del piercing"* llamó a la puerta de tu despacho diciendo no sé qué de un TFM y no sé qué de una tesis... espero haber estado a la altura de tus expectativas. Por mi parte ha sido todo un orgullo y siempre podré decir que hice la tesis con el Dr. Jonatan Ruiz. También me gustaría darte las gracias por ser más que un director y un mentor, te agradezco que hayas sido un amigo.

Guillermo Sánchez Delgado, gracias por las charlas filosóficas, las discusiones sobre ética personal y profesional, gracias por tu apoyo incondicional y por la oportunidad que me has brindado al aceptar formar parte de todo este proceso. Gracias también por todas las discusiones sobre temas

diversos, por esos cafés *"de la vida"* que al final del día son los que *"dan vida"* y gracias también por todos esos consejos. Siempre he sentido que había una cierta conexión entre los dos, que teníamos formas similares de pensar y de afrontar la vida (aprendida a *"base de tortas*"). Creo que por eso he recurrido a ti en muchas ocasiones en las que necesitaba apoyo o una palmadita en la espalda, por eso te doy las gracias por no haberme dejado nunca que arrojase la toalla (mentiría si no reconociese que he estado a punto en varias ocasiones). Guille, eres un ejemplo de talento, de aptitud y de actitud (aunque no de puntualidad, para que veas que estoy siendo sincero 100% y no haciendo la pelota). Eres una persona optimista por naturaleza, lo cual se agradece y se contagia. Gracias por permitirme la posibilidad de trabajar contigo como compañeros de laboratorio, como director-doctorando y ojalá podamos seguir haciéndolo durante muchos años más.

GRACIAS a mi "mentor del lado oscuro".

Borja M. Martínez Téllez, ojalá algún día llegue a tener la pasión y las *"pilotes"* que tienes socio (y también tu tolerancia al alcohol). Eres la definición viviente de superación, perseverancia y resiliencia. Eres el ejemplo de que trabajando duro y luchando a muerte, al final, la vida no tiene más remedio que darte lo que mereces, y tú te mereces muchísimo. Nuestra amistad, aunque ha tenido sus momentos, se mantiene y espero que así sea por siempre. Sé que en ocasiones no he estado a la altura de lo que necesitabas o esperabas de mí (me he dado cuenta a posteriori) porque la verdad, yo soy *"de llevarlo por dentro"* y de *"rumiación lenta"* (no es una buena combinación para según qué cosas), pero eso no quita que te quiera mucho *"bro"*. Gracias por haberme apoyado cuando toqué fondo y haber estado ahí siempre que me hacía falta. Recuerdo (aún estábamos sobrios) en esas cruces cuando me preguntaste si era consciente del cambio que había sufrido mi vida y lo que me quedaba por descubrir… Gracias también por los miles de millones de neuronas que me has matado a base de alcohol, chupitos de Jägermeister y por dejarme dormido de pie *"sujetando"* farolas. Ojalá podamos seguir descubriendo juntos qué nos depararán las siguientes etapas (obviamente, con sus celebraciones pertinentes).

GRACIAS amig@s del equipo ACTIBATE.

¡Gracias a tod@s! Esta tesis no habría sido posible sin el esfuerzo, dedicación, estrés, caída de pelo, canas, arrugas de expresión, llantos, gritos, euforia, fiestas y borracheras… en definitiva, no habría sido posible sin el equipazo humano que tenemos. **Victoria** (la **hermana**) y **Weeeeendy** (leer con entonación mexicana), llegamos prácticamente a la vez al iMUDS (a día de hoy somos oficialmente *los perros viejos del iMDUS*) y no sabíamos lo importante que sería esta experiencia para nosotros… Tampoco sabía que iba a conocer a dos personas tan especiales. Gracias por haberme cuidado desde el principio y por ser tan geniales. **Fran Amaro**, *Mindy* muchas gracias por tus consejos y tu ayuda siempre que la he necesitado, gracias también por aguantarme en el cubículo de al lado (sé que en ocasiones era duro porque me encanta hacerte rabiar…) y por regañarme cuando me despistaba; **Eli**, otra de *"las viejas"* del iMUDS. Eres una gran compañera y aún mejor persona. Gracias por ser como eres, no cambies nunca; **Fran Acosta**, eres un ejemplo de buen investigador y de buena persona (a pesar de que vienes de Motril…). Gracias por tantas charlas filosóficas y por no traer más veces a tu amigo a comer carne a la piedra; **Lucas J.**, recuerdo cuando llegaste por primera vez a iMUDS a hacer tus prácticas… con lo tranquilito que parecías, no veas socio… tienes más peligro que una caja de bombas… Te agradezco todo tu apoyo siempre que lo he necesitado, y sabes que aquí estoy para lo que te haga falta. **Pepa**, gracias por mostrarnos el apasionante mundo del cuello, por transmitirnos tu locura en la pista de baile y por todos esos buenos momentos; **Manu Dote**, el *guaperas* del iMUDS. Rebosas *"style"* y flequillo, así como capacidad de trabajo. Sigue así y llegarás muy lejos (y muy delgado) gracias al TRE. Por cierto, ponte chándal para las prácticas en INEF ¡*po favó!*; **Javier**, *"er niño de LuSena Sity"*. Eres una persona muy peculiar, inquieta y curiosa, y muy capaz, lo cual te hace muy especial. Me gustaría poder seguir compartiendo experiencias (tanto en estado de embriaguez como de sobriedad) contigo. De hecho, tenemos varias charlas pendientes; **Lucas G., Carmen Quinn, Miguel A., Juanlo, Laura, Joern, Nico, José C., Sonsoles, Rober, Esther, Pepe, Andrea, Pascual, Anayara, Mau, Unai, Rachel**… parte de esta tesis también es vuestra. Muchas gracias por todo. Mi consejo personal con los nuevos fichajes, aprovechad el tiempo en el grupo porque no es fácil encontrar algo así. Para el final me dejo a **mis ñiñas**, también

conocidas como **Lourdes y Hui, Hui y Lourdes, la china y la amiga**... etc. etc. Os doy las gracias de corazón por todo vuestro cariño y apoyo durante este largo proceso. Gracias por esos momentos de coche *"camino a"* o *"camino del"* iMUDS. Gracias por regalarme CDs de música cuando llegaba la etapa de Carnavales porque estabais hasta la *"pepitilla"*, gracias por las comidas, meriendas *"de gorduras"* y las cenas, gracias por los regalitos de cumple, de Reyes, y por estar siempre pendientes de mí (aunque yo sea terrible para eso). Sois unas personas maravillosas y estoy súper feliz porque me llevo a unas grandísimas amigas *"pa toa la life"*. Os quiero un montón.

Por último, agradecer a los grandísimos compañer@s y amig@s de Medicina Nuclear del Hospital Virgen de las Nieves (pieza clave para el estudio ACTIBATE - sin ellos nada de esto habría sido posible). Muchísimas gracias **Dr. Llamas** y **Yolanda**, así como al resto de miembros del servicio.

GRACIAS a los PROFITHeroles y demás compañer@s investigadores.

Gracias compañeros. Gracias por formar parte de esta tan maravillosa (y dura) etapa pre-doctoral.

Gracias a los ActiveBrainers, porque aunque el ACTIBATE molase más, sois muy grandes y especiales. Gracias a **Irene E.** por ser capaz de *"infartarte"* el corazón (anda que no nos has quitado años de vida con los gritos...) y demostrar tanta pasión y esfuerzo por hacer las cosas bien, cosa que se transmite; gracias también a **Pablo Molina**. Has sido un apoyo en muchas ocasiones tanto dentro como fuera del terreno de juego de la investigación, me quedo con tus frases célebres tales como: *"hay que darlo todo [...] aunque sea en el barro"* o *"la Vogue nunca defrauda, siempre hay que darle una oportunidad"*. Gracias por tantas risas y *"pabladas"* (no se me olvidará la *"cucharadita de café"*); gracias también a **Pepe Mora, Pato, Jairo, Cristina C., J.J., Lucía, Miguelón, Luis Gracia, Mireia de Castellón, Fer, María, Pontus, Manu H**... sois muy grandes. Gracias a **Juan Pablo** por su maravilloso recibimiento en su casa cuando estuve en Chile y por enseñarme tantísimas cosas junto a nuestro magnífico guía **Iñaki**. Aquí tengo que hacer un alto en el camino para agradecer a mi compañero de batallas, un apasionado investigador, Abelino (el*"hache-ere-uve"*). **Abel**, también es conocido por ser el *"terror de las ladies"*. Aunque también hay que reconocer que es la única persona que he conocido con una vocación tan grande por lo que hace que tiene la ocurrencia de, estando más morado que un puñetazo, ponerse a preguntar a todas y cada de una de las chicas con las que se cruzaba en la Vogue (un sitio que me ha contado Javi que está cerca de la Biblioteca de Derecho) si quería que le midiese la HRV. Si eso no es pasión por la investigación que baje Dios y lo vea. Ahora enserio, gracias Abel por esos cafés, esas charlas, esa amistad y apoyo y por todo lo que me has enseñado sobre HRV porque no tenía ni idea (ahora sigo sin tener mucha, pero bueno, algo se me va quedando poco a poco). Gracias también a **Fran Ortega**, **Palma** y **Virgi** por vuestras palabras y consejos que en más de una ocasión han llegado en el momento justo y han *"venido de perlas"*. Gracias también al resto de compañeros **Alberto S., Blanca, Elia, Milkana, Pedro A., Artacho, Javi F., Cou, Javi L.** Gracias también a **Alex de la O**. Hemos compartido parte de esta última etapa juntos *"mesa con mesa"* y también lo hemos sufrido juntos. Espero poder leer pronto el resultado de tu grandísimo trabajo y esfuerzo.

I am also very grateful to those who helped and taught me and for their valuable patient during my internship in Leeds (UK). Dr. **John E Blundell**, thank you for offering me a different point of view and perspective of science and researching. I would also thank Dr. **Graham Finlayson** for his support and valuable help during my whole period there. Thank you for all what I have learned about the interrelation between biological processes and behavior. I am also grateful to Dr. **Catherine Gibbons**, to Dr. **Kristine Beaulieu**, and to **Pauline Oustric** and **Nuno Casanova**.

Muchas gracias también a todos aquellos que me habéis cuidado tantísimo durante mi estancia en Chile. Para el Dr. **José E Galgani** no tengo más que palabras de agradecimiento. Muchas gracias por darme la oportunidad de visitaros y compartir vuestro centro. Gracias por todo lo que he aprendido estando allí con vosotros y por todo el tiempo que me has dedicado para discutir, organizar ideas, y un larguísimo etc. Sinceramente, creo que ha sido una estancia perfecta en un momento perfecto que ha cambiado mi forma de entender la ciencia. Gracias también al Dr. **Rodrigo Fernández** por las charlas científicas, por todo lo que me ha enseñado durante este periodo de tiempo y por recibirme en su casa como a uno más desde el primer día. Si vuelves por Granada te llevaré a comer bocadillos de tortilla de patatas que sé que son tus preferidos. Estoy deseando de seguir trabajando y colaborando con vosotros. Gracias también al resto del equipo **Juan, Lore**... por lo bien que me habéis tratado durante esos 3 meses.

GRACIAS a los Torrenteros.

La verdad es que esta etapa en Valencia ha influido muchísimo en que hoy pueda estar donde estoy. Aunque nos conocimos en Córdoba estudiando Magisterio, fue en Torrent donde nos conocimos mejor y compartimos dos años geniales. Gracias a **Edu**, a los "Pedros": **Pedro C.** y **Pedro S.**, y a **Claudio**. Gracias por ser unos freaks y tener el piso más empollón de Valencia entera (por esto conseguí mi beca para hacer el doctorado). Gracias también por los buenos ratos, las risas, las bromas, y todos esos momentos que se quedarán ahí para siempre.

En Valencia también conocí a dos niñas maravillosas. Una de ellas es la mallorquina más andaluza del mundo mundial, **Ainhoa** (mi morena) y la otra es la, como a mí me gusta llamarla, Albóndiga (también conocida como **Alba**). Aunque me llamases *"zanahorio"* yo sé que siempre estaré *"en to lo arto"* del top de andaluces. Muchas gracias a tod@s vosotr@s por tan maravillosa experiencia que me cambió la vida. Ainhoa gracias por todos los momentos, risas, cariño, apoyo y amor que me has dado durante el tiempo que vivimos juntos en Granada, eres una persona increíble, no cambies nunca. También te doy las gracias por presentarme a **Manuel Padilla** (Manel para los amigos). Manel, aunque al principio no me querías contigo porque pensabas que era un fiestero y que estaba loco perdido (y lo peor es que tenías razón), me diste la oportunidad de formar parte de *"vuestro piso"*, convivir y forjar una bella amistad que espero que perdure. También te doy gracias por tu paciencia en mis días locos y por tirar la basura (o ponerme la bolsa delante de la puerta para que no se me olvidase) además de por ayudarme a hacer rabiar a Ainhoa (*"¿Dónde está la silla?"* – al final la encontró). Gracias también por todo el esfuerzo que has depositado en tratar de que esta tesis se *"quedase bonita"* y por el pedazo de portada que te has currado.

GRACIAS a mis amig@s de La Carlota City y todas aquellas personas que han estado ahí de una u otra forma.

Gracias, gracias y gracias. Son muchas las personas que en uno u otro sentido me han marcado y que por tanto han hecho que la persona que hoy está aquí sea como es.

Si hay una persona a la que tengo que nombrar en primer lugar porque supuso un cambio en mi mentalidad, en mi forma de ser, de ver y de afrontar la vida, así como una persona que dejó un vacío que nunca se llenará por completo… esa persona eres tú. Y es que **Cristóbal** (**Gaty** para tus amigos) eras la mejor persona que alguna vez me he podido echar a la cara, no me cansé de decírtelo en su momento y, es que no podías ser más bueno… Recuerdo como si fuese ayer aquel día en el que te cargué y te llevé en mi hombro, recuerdo cuando te dijimos adiós (desde entonces no me gusta usar adiós y prefiero el hasta luego) y con 20 y pocos años fui consciente de lo que estaba pasando y entendí de una forma brutal que la vida no siempre es justa y que a las buenas personas también les pasan cosas malas. Ojalá pudiese ver hoy tu sonrisa (porque socio… como contagiabas felicidad) y tu orgullo cuando me vieses defender mi tesis. Ojalá tantas otras cosas que hablábamos aquellos últimos días (sin ser conscientes de que los eran). Te doy las gracias por haberme enseñado tantísimo en aquellas interminables charlas (pipas en mano)… *"Juanma tenemos que aprovechar el momento…"*, *"Juanma no nos podemos olvidar de vivir porque en algún momento puede pasar algo malo, si no mírame a mí…"*, *"Juanma, que luego nos arrepentimos cuando ya es tarde…"*; qué razón tenías. Quiero que sepas que estés donde estés te llevo siempre conmigo, que siempre estás y estarás muy presente y que mis logros siempre serán tuyos pues en mi hay una parte de ti. Te quiero muchísimo amigo, te echo mucho de menos… que persona tan maravillosa se ha perdido el mundo; si todos fuésemos más como tú eras… todo sería mucho más fácil.

Cuando pienso en Gaty, me viene a la mente el *"team"* **Antonio** (**Percha**) y **Maxi**. **Percha**, gracias por ser un amigo de los de verdad y de los que quedan muy pocos, de los de estar ahí siempre que hace falta, de escuchar y de guardar confidencias. Porque aunque los dos no seamos de estar hablando 24/7 tenemos la certeza de que, si el otro nos necesita, no hay excusas, distancia, ni nada, *allá que va el tío*. Recuerdo ese viaje que nos pegamos por la costa andaluza y me encantaría repetir experiencia (queda pendiente el tour por el norte). Gracias por todo, de verdad. **Maxi**, eres muy grande. Y que sepas que no lo digo porque midas cerca de 1.85 y peses (tirando a lo muy bajo) 100 kg…, lo digo porque eres una bellísima persona y un buen amigo de tus amigos. Espero

que esos ajos y esas cebollas que me ibas a dar hace 4 o 5 años estén llegando ya a Granada, si no tendremos que llamar a los mensajeros a ver si se han perdido (guiño, guiño). Además, quién nos iba a decir que mientras estoy escribiendo estas líneas tú estás cuidando a *"tus mujercitas"*, Ángela cuida tu también del bulto (*bultaco* más bien) con ojos que tienes por marido. **José**, conocido como el **Diablo Grande**, gracias por sacar un hueco para mí cada vez que piso el pueblo, gracias por nuestras charlas y confidencias, gracias por ser como eres. **Gloria**, gracias por contagiarme tu locura cada vez que nos vemos, me ha hecho súper feliz que hayamos retomado de nuevo el contacto en esta última etapa. Por último, quiero hacer una mención especial a mi carlo-belga favorito, **Alfonso** (**Orejitas**). Te doy las gracias por todo lo que has hecho por mí siempre, por quererme y apreciarme tanto, por vernos en España, en Bélgica y donde haga falta siempre con cerveza en mano. Te pido perdón por no haber podido acompañarte cuando me has necesitado. Espero que podamos vernos y abrazarnos (te lo debo) de nuevo muy pronto.

En Santiago de Chile me encontré a una cordobesa de las de *"los pies a la cabeSa"*. Y es que la Fuensanta (la **Fuen**) no puede parar quieta... Para colmo, como Santiago es una ciudad pequeña (entiéndase la ironía), resulta que vivíamos los unos de los otros separados por un par de bloques... Si es que, el mundo es un pañuelo... **Fuen, Sergi** (el Master chef) y **Zero** (el inagotable), gracias por habernos cuidado tantísimo a Ana y a mí, por habernos reído y compartido tantos momentos. De verdad, ha sido un placer compartir parte de nuestra experiencia con vosotros... tenemos pendiente vernos ahora que estáis de vuelta.

No me olvido de mi gran amigo *"del poblao"* **Enrique Montenegro**. Parece que era ayer cuando salía corriendo para cerrar la puerta de mi casa a grito de *"¡Mamá, que viene Enrique!"* porque venías corriendo a quitarme algún juguete (tengo que reconocer que siempre te llevabas los rotos)... También me acuerdo de cuando te quedabas en mi casa porque echabas de menos tu antigua casa y estabas en *"fase de adaptación"*... algo ha llovido ya... Y ahora mira, cada vez que hablamos y me cuentas tus logros, tus objetivos e intenciones futuras me siento orgulloso de ver cómo vas avanzando. Ya te he dicho que tienes mucho potencial, sigue formándote, aprendiendo y currando como lo estás haciendo y ya verás que todo tendrá su recompensa. Sabes que siempre que me necesites estaré ahí porque me siento en deuda contigo, has sido una parte muy importante de mi proceso de crecimiento personal y eso nunca se olvida. Muchas gracias amigo. También aprovecho para agradecer a **Mari Paqui** y a **Enrique** sénior por todo lo que han hecho por mí, por mi hermana y por mi familia. No tengo palabras de agradecimiento para vosotros porque sabéis, igual que yo, que tenéis muchísima *"culpa"* de que hoy esté aquí defendiendo mi tesis. Aunque *"sólo"* fuésemos vecinos siempre nos habéis cuidado y considerado como de la familia. De corazón, os estaré eternamente agradecido. Gracias también por tener unos hijos tan maravillosos.

Por último, quiero dar las gracias a mis *"nuevos"* **amigos durqueñ@s**. Perdonad que no os nombre a todos porque *"kopón"*... es que sois un millón y la lista sería más larga *"kel kali"* (esto es para que veáis que me estoy aplicando con el durqueño profundo - cuando termine la tesis me presento al B1). Fuera bromas, gracias por haberme recibido con los brazos abiertos desde el primer día, por aceptarme en todos vuestros planes (aunque no he podido estar en todos los que me habría gustado) y, en definitiva, por ser tan geniales. Tenéis un grupo de amigos de los que ya no quedan (o quedan muy pocos). Seguid cuidándolo porque *"quién tiene un amigo tiene un tesoro"*, y la verdad es que vosotros no sabéis el tesoro que tenéis entre manos (no busquéis el doble sentido, marran@s).

GRACIAS a mi familia política.

Porque como el buen turrón de chocolate (chocolate, cosa rica y que nunca falta en vuestra casa – punto a favor para los suegris e indirecta) llegué allí por Navidad, que si uno lo piensa ahora en frío... ¿en qué momento se nos ocurrió?

Pero tengo que decir que me tratasteis como a uno más de la familia desde el primer momento en el que crucé el umbral de vuestra puerta. Me acuerdo de aquella charla pre-cena, sentados en el patio, al solecito, bebiendo cerveza y comiendo jamón. **Antonio y Merki**, muchísimas gracias por todo y por hacerme sentir como en casa. Tampoco puede faltar aquí el resto de la *family*. **Sonia, Pirri y Emma**; **Marta, David y Víctor**. No tengo más que palabras de agradecimiento para todos vosotros. Gracias por

tratarme como a uno más de la familia desde el primer día y gracias por dejarme jugar con los sobris. Me siento muy feliz de la nueva familia que he encontrado y que tengo en Dúrcal (y alrededores).

GRACIAS a mi cari.

Lo pienso y me da la risa, nos teníamos *face-to-face* durante tantísimo tiempo y resulta que todo surge a distancia… si es que… *"donde no hay mata, no hay papa*"…

La verdad es que aquel curso de doctorado de Macros en Excel (en el que, por cierto, estaba cazando moscas) me permitió que nos viésemos después de habernos perdido la pista durante cerca de un año. Tengo que reconocer que la formación te cambia la vida, así que consejo, nunca dejéis de formaros y aprender… Ya en serio, **Ana**, gracias por todo lo que has hecho por mí. Sin tu cariño y sin tu apoyo seguramente hoy no estaría donde estoy. Has sabido manejar muy bien mis idas y venidas, mis subidas y bajadas, mis altos y mis bajos. Gracias por aguantar tanto *no, no, no* y gracias por no tenerme en cuenta tantos planes en los que te he dejado sola. Gracias por todo *love*, haces que lo cotidiano se vuelva especial y que lo especial sea aún más maravilloso de lo que ya es. Te quiero siempre conmigo para seguir viviendo nuevas aventuras y etapas juntos.

GRACIAS a toda mi familia.

De corazón os doy las gracias a todos/as y cada uno/a de vosotros/as.

Muchas gracias a todos mis **primos y primas**, a mis **titos y titas**. Gracias por haber contribuido en mayor o en menor medida en el proceso. En especial me gustaría hacer mención a mi **Tío Miguel**. Gracias por todas las charlas filosóficas, cerveza en mano (cosa fundamental para ser un buen Alcántara de la "*estirpe Monte Alteña*"), las no filosóficas de mareados esas que no sirven para nada, las risas a carcajada limpia con ese dolor que da en los mofletes y en la barriga cuando no puedes parar, por los chistes malos y los chistes malísimos… y por supuesto, gracias por el "*¿nos vamos a ir sin convidarnos*? – *niño, llena aquí*". Deseando de que podamos juntarnos otra vez.

Gracias a mis **abus**, **Manuel, Juan** e **Isabel**, los que seguro me ven henchidos de orgullo desde allá donde estén. Todo esto también os pertenece. **Abu Manuel**, me da mucha pena no haberte conocido, pero según las historias que me cuentan eras una persona maravillosa y estoy seguro de que hoy estarás orgulloso de tu nieto porque todo esto también es un logro de tu hijo. **Abus Juan e Isabel**, gracias por tanto cariño, tanto amor, tantas risas, tanto chocolate a escondidas, tantos eurillos y centimillos dados como el que está traficando con droga… Gracias por vuestros consejos que en ocasiones me faltan ahora que soy más viejo, la verdad es que ahora lo pienso y desearía haber pasado más tiempo con vosotros. Os quiero mucho. Por último, infinitas gracias a mi **abuela Rosario** (conocida comúnmente como la "**agüela**" o la "**aweeeeeeeeela**") que me estará viendo a través de una pantalla (siempre y cuando no esté la copla o el Juan y medio – esperemos no coincidir en horario). **Agüela**, espero que sigan siendo muchos últimos años los que estás con nosotros (llevas diciendo que "*este año es el último*" desde que yo tenía 10 años y espero escucharlo muchos, muchísimos más…), porque siendo honestos, ¿qué haría yo sin los "*jayuyos*" (el pan élfico como yo le llamo)? O sin los churros caseros en las vacaciones. Además, todavía tienes que contarnos muchos *"chascarrillos"* y regañarme muchas veces cuando me paso con el anís en Navidad… Te quiero abu.

A mi segunda madre, **Mi Madrina**, **La Madrina**. La vida nos privó de tu amor y de tu sonrisa mucho antes de lo que debería, pero no hay un instante en el que no te llevemos con nosotros… Daría todo lo que tengo y más porque pudieses estar aquí con todos nosotros, viendo todo lo que estamos consiguiendo… Me encantaría que pudieses ver a tu *Chiquitín*, el que jugaba en la tierra de tu naranjo, convertirse en doctor y que me dijeses "*chiquitín, chiquitín… enhorabuena*". Aunque hayan pasado algunos años aún recuerdo perfectamente el sonido de ese "*chiquitín*" en mi cabeza y también recuerdo todo lo que me hacía sentir. Quiero que sepas que aunque pasan los años siempre estás, estarás y formarás parte de todos mis/nuestros logros porque gracias a ti soy/somos como soy/somos. Gracias por todo Madrina, te quiero y te echo mucho de menos. Gracias también por las maravillosas hijas que tienes, y es que mis (primas)**hermanas Rosario** e **Isa** son increíbles, "*sus quiero mucho idiotas"*.

Tata Ro, Tata Isa, ¿qué os digo yo que no sepáis? Aunque no hayamos nacido de la misma madre sois, y seréis siempre, mis hermanas… nuestras madres tenían un vínculo especial que han transmitido a sus hijos. Gracias por haber apoyado a vuestro *Chiquitín* en todo momento, haber confiado en él y haberle demostrado vuestro amor incondicional. Sé que soy una persona despegada por naturaleza así que agradezco aún más si cabe todo lo atentas que estáis y lo mucho que me queréis… aunque en la mayoría de ocasiones no lo demuestre físicamente (si Isa, es una indirecta a tus quejas de que no te doy abrazos), prometo que trataré de hacerlo más a menudo. Recién incorporadas a la familia y, como aquel que dice "*en oferta*" (pack 2x1) no puedo dejarme atrás a las **mellis**. **Carla y Silvia**, aunque no he tenido la oportunidad de pasar mucho tiempo con vosotras porque la distancia, el confinamiento, el COVID, los cierres perimetrales y su PM en almíbar (no os asustéis con PM me refiero a otra cosa, nunca pondría put* madre en una tesis doctoral) no me lo han permitido, tomaré todo esto como un aprendizaje. Así que intentaré pasar todo el tiempo posible con todos mis seres queridos cuando tenga de nuevo la oportunidad. No veo el momento de poder pasar más tiempo con vosotras, revolcarme por el suelo, jugar, haceros reír y rabiar (sobre todo rabiar). **Mellis**, también os doy las gracias por haber calmado el *síndrome del nido vacío* (guiño, guiño, mamá, guiño, guiño, ¿indirecta captada?) que había por aquellos lares con tantísimas dosis de felicidad… Gracias también al (*"primo"*) **Sánchez** por todas tus muestras de afecto y por los abrazos cada vez que nos vemos, somos muy afortunados de tenerte con nosotros, os deseo lo mejor en vuestra próxima etapa (guiño, guiño). No penséis que me iba a olvidar de "*la otra perra"* de la familia. **Nala**, gracias por morderme los pies cuando voy de visita, porque todos sabemos que así demuestras tu amor. Espero que celebremos muchos más cumpleaños perrunos todos juntos. Amo a mi familia y amo que busquemos cualquier excusa para juntarnos (digo excusa para disimular, porque la gente que no nos conozca puede pensar que estamos locos… pero nosotros sabemos que no, que somos *"tan nodmadeds"*).

A mi hermana, **Lau**, gracias por todo tu apoyo sin ti no sería hoy la persona que soy. Como casi toda relación de hermanos en sus orígenes hemos tenido nuestra etapa de *amor máximo de hermanos*, y después nuestra etapa de *"querernos como perros y gatos"* (pero de los de campo, no de esos que se crían juntos en casas y se llevan genial). Quiero que sepas que estoy orgulloso de tus logros y que me siento afortunado de que seas mi hermana y formes parte de mi vida. Lucha por tus metas y por tus objetivos y no dejes que nadie, nadie, te ponga techo. Tú tienes el techo que quieras tener. Te lo he dicho muchas veces, eres de las personas más inteligentes que he conocido nunca. Es verdad que eres floja (como tu hermano, no lo vamos a negar) pero tienes una capacidad brutal para hacer todo lo que te pretendas. Dale caña a tu nueva etapa, disfrútala y a por todo lo que venga. Estaré siempre a tu lado para ayudarte en todo lo que necesites. Te quiero *sister*.

A mis padres, **Manuel** y **Josefa**, por muchas veces que os de las gracias nunca llegarán a ser suficientes. Gracias por el enorme esfuerzo que habéis tenido que hacer para que hoy pueda estar escribiendo estas líneas y defendiendo esta tesis doctoral (entre otras tantísimas cosas que he podido hacer gracias a vosotros). Recuerdo aquel día en el que salí de casa con un sentimiento agridulce, cargando maletas y bultos hasta en las pestañas, rumbo a Valencia, a una nueva aventura en la que ninguno de nosotros teníamos mucha idea de cuánto duraría. Al final, tuvimos suerte, y con algo de sacrificio, un poco de sacrificio y mucho sacrificio (porque somos familia de clase obrera) logramos tirar para adelante con los dientes apretados, y, mirad ahora donde estamos… Gracias a los dos por vuestra infinita generosidad, por haberos sacrificado y dejado de hacer y de vivir tantísimas cosas como habéis hecho para poder darnos a la hermana y a mí tanto como nos habéis dado. Gracias por brindarnos de una educación tanto personal como académica inmejorables. Gracias por nunca haberme quitado las ganas de estudiar, es más, gracias por haberme animado siempre a ello. Gracias por no haberme dejado tirar la toalla en el instituto y haberme hecho que *"apretase los nacasones"* para salir adelante a base de coraje y cabezonería. Gracias por haberme apoyado en los buenos y sobre todo en los malos (y en los muy malos) momentos. Gracias por ser mi apoyo incondicional y nunca haberme fallado. Gracias por vuestras palabras de ánimo, de consuelo, de apoyo, de cariño, de regañina, y de "*Juan Manuel Alcántara (y a veces también el segundo Alcántara – denotando y detonando el enfado descomunal y mastodóntico que la situación requería) me-cago-en-argshasdrujnvk*" con los dientes apretados porque el niño era… pues era como era. De verdad, gracias, gracias y millones de veces gracias. Me siento muy afortunado de tener los padres que tengo. Y la respuesta siempre será si, **os elegiría una y mil veces como padres**, os quiero muchísimo y estoy súper feliz y orgulloso de tener los padres que tengo. Sólo espero estar siempre a la altura de lo que merecéis.

Además, me voy a permitir el lujo de citar las letras de un gran comparsista carnavalero que falleció el año pasado, D. Juan C. Aragón (1967-2019), ya que creo que estas líneas reflejan fielmente una gran parte de lo que siento por mis padres (podéis buscar los vídeos en Youtube, guiño, guiño):

[...]
Por más sed que calme el agua del rio
Más calor que de la llama del fuego
Por más hondo que un dolor haya sido
Y mayor el desconsuelo
Por más que se pueda perder
Y más que se pueda ganar
Donde se ponga una madre
Que quiten el bien
Y que quiten el mal
[...]
No hay alegría más bella
Ni primavera más grande
Que la que te da una madre
Al sentírtela a tu vera
El que dice sin pensar
[...]
Que nadie es imprescindible
O nunca tuvo mama
[...]
O es que no sabe lo que dice
Por más que la vida
Y los años pasen
La mujer se va haciendo un gigante
Desde que el vientre se le abre
Mira si será grande
Y divino su amor
Que para hacerse hombre hasta el mismo Dios
Necesitó del amor de una **madre**.

"Por anchos que sean los mares", Comparsa Los Mafiosos, Semifinal COAC 2018

Un amigo es un amigo me dijo un amigo mío
Y era tan amigo mío y tanta amistad la nuestra
Que no supe que pensar, pero le dije mu *"dolío"*
Un amigo de verdad no lo dice y lo demuestra
Un amigo-amigo no te dice *"Un amigo está pa algo"*
Un amigo-amigo está contigo en los momentos más amargos
Un amigo-amigo de verdad no dice *"Quiero ser tu amigo"*
Pero si es tu amigo de verdad tu muerte la muere contigo
La amistad es regalar el corazón de un caballero
A un caballero
[...]
Por eso los corazones
De los amigos cañones
Son corazones de oro
Oro por el que te digo
Que los mejores amigos
Son los mayores tesoros
Y esos tesoros no tienen
Reputaciones ni bienes
Ni huecos en los altares
Que los altares se adoran
A la semana una hora
Y otra hora en los bares
Por eso sé lo que digo
"Na" más que tengo un amigo
Y es mi **padre**

"Un amigo es un amigo",
Comparsa Los Condenaos, Semifinal COAC 2001

GRACIAS A TODOS Y A TODAS POR VUESTRA AYUDA, AMOR Y PACIENCIA CONMIGO. SIN CADA UNO DE VOSOTROS Y VOSOTRAS NADA DE ESTO HABRÍA SIDO POSIBLE. POR FAVOR, TOMAOS UNA/S CERVEZA/S A MI SALUD YA QUE LA SITUACIÓN ESTA NO NOS LO PERMITE...

... Y llegados a este punto aquí se cierra una etapa que como ya he dicho ha sido muy bonita y muy dura a la vez... parecía que no lo conseguiría (he dudado muchas veces de ello) pero...

"Imposible nada es.
Difícil, muchas cosas son."

Maestro Yoda

Obesity is one of the most important risk factors for developing cardiovascular disease, type 2 diabetes and some types of cancer. Consequently, numerous research groups around the world are studying human energy balance regulation. To this end, the accurate and precise assessment of energy expenditure is mandatory to understand energy homeostasis and determining energy intake requirements. In this regard, indirect calorimetry is nowadays considered the reference method to assess energy expenditure and it also allows to quantify different energy substrates utilization.

Metabolic carts are the most extended indirect calorimetry systems. Unfortunately, most of the commercially available metabolic carts provide unacceptable accuracy, precision or biological reproducibility of human measurements results. Although one metabolic cart was for long time considered the gold standard, that system is no longer manufactured, and therefore, nowadays no metabolic cart is recognized as the referent. Consequently, determining the accuracy, precision and reproducibility of diverse commercially available metabolic carts is of vital importance. Aiming to improve metabolic carts results, a novel technique called 'post-calorimetric correction' has been proposed, although research is needed to understand the extent to which it can improve the indirect calorimetry assessments. Another debated issue in indirect calorimetry methodology is the adequacy of different methods for the selection of gas exchange data. In fact, the method for gas exchange data selection is a key factor that may influence the results obtained by indirect calorimetry and may also impact the biological reproducibility of human measurements results.

The present Doctoral Thesis aimed to determine the accuracy, precision, biological reproducibility and comparability of six different commercially available metabolic carts to assess resting metabolic rate and respiratory exchange ratio in healthy adults, and to examine the influence of different methods for gas exchange data selection on the resting metabolic rate and respiratory exchange ratio estimates, as supplied by different metabolic carts. Lastly, the effect of a post-calorimetric correction procedure was also studied in four of the metabolic carts.