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**ΣΧΟΛΗ ΤΕΧΝΟΛΟΓΙΑΣ ΓΕΩΠΟΝΙΑΣ ΚΑΙ ΤΕΧΝΟΛΟΓΙΑΣ
ΤΡΟΦΙΜΩΝ**

ΤΜΗΜΑ ΔΙΑΤΡΟΦΗΣ ΚΑΙ ΔΙΑΙΤΟΛΟΓΙΑΣ

Η αξιοπιστία του VYNTUS CPX

The reliability of the VYNTUS CPX

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ABSTRACT

Objective: The purpose of this study was to assess for the first time the reliability of a new indirect calorimeter, the Vyntus CPX in measuring O₂ consumption and CO₂ production to estimate resting metabolic rate (RMR) and to assess the effect of not calibrating on its reliability.

Methods: Twenty four healthy adults (12 males and 12 females) were measured four times with the indirect calorimeter Vyntus CPX in canopy mode in the morning for two consecutive days (2 measurements each day). Each measurement lasted 20 minutes in which the first 5 minutes were discarded. A standardized protocol was used for all the individuals. Each day before the first measurement the device was warmed up for at least 15 minutes, gas and volume calibrated according to the manufacturer's instructions. The second measurement was randomly carried out after gas and volume calibration on one day and without gas and volume calibration on the other day.

Statistical analysis included non - parametric tests, intraclass correlation coefficients, Bland-Altman plots and calculation of the standard error of the measurement (SEM) for the assessment of reliability.

Results: Subjects ranged in age from 19 to 57 years (28.5 ± 11.3 years) and in body mass index from 18.4 to 36.5 (23.8 ± 4.1 kg/m²). Means of RMR were not significantly different in between days ($p = 0.91$) and within the same day ($p = 0.61$). In between days, the intraclass correlation coefficient of RMR was questionable ($r = 0.78$), but a good intraclass correlation coefficient of RMR was found ($r = 0.83$) within the same day. However, high limits of agreement and high SEM were found for RMR in between days (± 476 kcal·day⁻¹, SEM = 183 kcal·day⁻¹) and within the same day (± 435 kcal·day⁻¹, SEM = 156 kcal·day⁻¹). Similar results were found for VO₂ and VCO₂ in between days and within the same day respectively. At last, means of RMR were not significantly different comparing the calibrated and not calibrated measurements within the same day ($p = 1.00$). The intraclass correlation coefficient of RMR showed good correlation ($r = 0.82$). In addition, good correlation was found for VO₂, but questionable for VCO₂. However, high limits of agreement and high SEM were found for RMR (± 417 kcal·day⁻¹, SEM = 146 kcal·day⁻¹ respectively). High limits of agreement and high SEM were indicated for the variables VO₂ and VCO₂ as well.

Conclusion: The variables VO_2 , VCO_2 and RMR were not significantly different in between days and within the same day. However, the high limits of agreement and the SEM of VO_2 , VCO_2 and RMR on the three reliability tests indicate low reliability of the Vyntus CPX in measuring O_2 consumption, CO_2 production and estimating RMR in healthy individuals. Furthermore, our results do not show an effect of calibration before each measurement indicating that calibration before each measurement is not beneficial in providing more reliable VO_2 , VCO_2 and RMR results, although it is still preferable to calibrate between each measurement.

Keywords: energy expenditure, resting metabolic rate, indirect calorimetry, Vyntus CPX, reliability, oxygen consumption, carbon dioxide production

ΠΕΡΙΛΗΨΗ

Σκοπός: Η παρούσα μελέτη είχε ως στόχο να εκτιμηθεί για πρώτη φορά η αξιοπιστία ενός νέου έμμεσου θερμιδομετρητή, του Vynthus CPX σε ότι αφορά τη μέτρηση κατανάλωσης O_2 και παραγωγής CO_2 και υπολογισμού του βασικού μεταβολικού ρυθμού ηρεμίας (RMR), καθώς και στην εκτίμηση της επίδρασης της μη βαθμονόμησης του οργάνου στην αξιοπιστία του.

Μεθοδολογία: Είκοσι τέσσερις υγιείς ενήλικες (12 άντρες και 12 γυναίκες) μετρήθηκαν τέσσερις φορές με τον έμμεσο θερμιδομετρητή Vynthus CPX με τη χρήση ειδικού θόλου (canopy) το πρωί για δύο συνεχόμενες ημέρες (2 μετρήσεις κάθε ημέρα). Κάθε μέτρηση διήρκησε 20 λεπτά εκ των οποίων τα πρώτα 5 λεπτά απορρίπτονταν. Για όλα τα άτομα χρησιμοποιήθηκε ένα κοινό πρωτόκολλο. Κάθε μέρα πριν από την πρώτη μέτρηση γινόταν ζέσταμα (warm-up) του μηχανήματος για τουλάχιστον 15 λεπτά και βαθμονόμηση του σύμφωνα με τις οδηγίες του κατασκευαστή. Η δεύτερη μέτρηση πραγματοποιούταν με τυχαία επιλογή μετά από βαθμονόμηση την μία ημέρα και χωρίς βαθμονόμηση την άλλη ημέρα.

Η στατιστική ανάλυση συμπεριλάμβανε μη παραμετρικά τεστ, συντελεστές συσχέτισης intraclass, διαγράμματα Bland-Altman και τον υπολογισμό του τυπικού σφάλματος της μέτρησης (SEM) για την εκτίμηση της αξιοπιστίας.

Αποτελέσματα: Τα άτομα ήταν μεταξύ ηλικίας 19 και 57 χρονών (28.5 ± 11.3 χρονών) με δείκτη μάζας σώματος από 18.4 μέχρι 36.5 (23.8 ± 4.1 kg/m^2). Οι μέσοι όροι των RMR δεν ήταν σημαντικά διαφορετικοί σε διάστημα μίας ημέρας ($p = 0.91$) και εντός της ίδιας ημέρας ($p = 0.61$). Σε διάστημα μίας ημέρας, ο συντελεστής συσχέτισης intraclass των RMR ήταν αμφισβητήσιμος ($r = 0.78$), ενώ εντός της ίδιας ημέρας ο συντελεστής συσχέτισης intraclass των RMR ήταν καλός ($r = 0.83$). Ωστόσο, βρέθηκαν υψηλά όρια εμπιστοσύνης και υψηλό SEM των RMR σε διάστημα μίας ημέρας (± 476 $kcal \cdot day^{-1}$, SEM = 183 $kcal \cdot day^{-1}$) και εντός της ίδιας ημέρας (± 435 $kcal \cdot day^{-1}$, SEM = 156 $kcal \cdot day^{-1}$). Παρόμοια αποτελέσματα βρέθηκαν για τις μεταβλητές VO_2 και VCO_2 σε διάστημα μίας ημέρας και εντός της ίδιας ημέρας αντίστοιχα. Τέλος, οι μέσοι όροι των RMR δεν ήταν σημαντικά διαφορετικοί συγκρίνοντας τις μετρήσεις μετά από βαθμονόμηση του οργάνου με εκείνες χωρίς βαθμονόμηση εντός της ίδιας ημέρας ($p = 1.00$). Ο συντελεστής συσχέτισης intraclass των RMR έδειξε καλή συσχέτιση ($r = 0.82$). Επιπλέον, καλή συσχέτιση βρέθηκε για τη VO_2 , αλλά αμφισβητήσιμη για τη VCO_2 . Ωστόσο, βρέθηκαν

υψηλά όρια εμπιστοσύνης και υψηλό SEM των RMR ($\pm 417 \text{ kcal}\cdot\text{day}^{-1}$, SEM = 146 $\text{kcal}\cdot\text{day}^{-1}$ respectively). Επίσης, δείχτηκαν υψηλά όρια εμπιστοσύνης και υψηλό SEM για τις μεταβλητές VO_2 και VCO_2 .

Συμπεράσματα: Οι μεταβλητές VO_2 , VCO_2 και RMR δεν ήταν σημαντικά διαφορετικές σε διάστημα μίας ημέρας και εντός της ίδιας ημέρας. Ωστόσο, τα υψηλά όρια εμπιστοσύνης και το υψηλό SEM των VO_2 , VCO_2 , RMR στα τρία τεστ αξιοπιστίας υποδεικνύουν χαμηλή αξιοπιστία του Vynthus CPX να μετράει την κατανάλωση O_2 και την παραγωγή CO_2 και να υπολογίζει το RMR σε υγιή άτομα. Επιπλέον, τα αποτελέσματα μας δεν δείχνουν κάποια επίδραση της βαθμονόμησης του οργάνου πριν από κάθε μέτρηση υποδεικνύοντας ότι η βαθμονόμηση του οργάνου πριν από κάθε μέτρηση δεν δίνει πιο αξιόπιστα αποτελέσματα των VO_2 , VCO_2 και RMR, αν και είναι προτιμότερο να γίνεται βαθμονόμηση μεταξύ των μετρήσεων.

Λέξεις Κλειδιά: ενεργειακή δαπάνη, μεταβολικός ρυθμός ηρεμίας, έμμεση θερμιδομετρία, Vynthus CPX, αξιοπιστία, κατανάλωση οξυγόνου, παραγωγή διοξειδίου του άνθρακα.

INTRODUCTION

Total energy expenditure (TEE) is the energy required by the organism daily and is divided into three components: basal metabolic rate (BMR), activity energy expenditure (AEE) and diet-induced thermogenesis (DIT) with BMR being the largest component of TEE for most people (Volp A.C.P., et al., 2011, IOM 2002). In practice, resting metabolic rate (RMR) is usually measured instead of BMR, because RMR measurement is conducted under less strict conditions (Volp A.C.P., et al., 2011, Koletzko B., et al., 2005). However, the procedures for measuring RMR are very similar to those for BMR (Volp A.C.P., et al., 2011). RMR is measured at rest in a thermos-neutral environment, but after at least 5 hours fast and not immediately after awakening (Compher C., et al., 2006, Koletzko B., et al., 2005). Therefore, usually, RMR is up to 10% higher than BMR, because of thermogenesis and more recent activity and represents 70 to 80% of the calories used by the body (TEE) (Sion-Sarid R., et al., 2013, Porter C., Cohen N.H., 1996). Measurement of RMR constitutes an essential step in determining optimal nutrition and can be measured by five different methods: a) direct calorimetry b) indirect calorimetry c) predictive equations d) thermodilution (Fick method) e) $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ doubly labeled water (Oshima T., et al., 2016, Graf S., 2013). Direct calorimetry, thermodilution and doubly labeled water method are invasive, cumbersome and costly (Oshima T., et al., 2016). Therefore, in clinical practice, RMR is determined either by using predictive equations or by actual measurement using indirect calorimetry (Sion-Sarid R., et al., 2013). Many predictive equations have been suggested e.g. Harris-Benedict, Mifflin-St. Jeor, Cunningham, and Owen (Cunningham J.J., 1991, Mifflin M.D., 1990, Owen O.E., 1986, Harris A.J., Benedict F.G., 1919). These equations are based on individual's anthropometric measures including height, weight, gender, and age or body composition measurements in order to measure RMR (Sion-Sarid R., et al., 2013, Cunningham J.J., 1991). However, many reports have shown that predictive equations are not accurate enough, especially in pathological states, since values can be either under or overestimated in a wide range of actual RMR (Blond E., et al., 2010, Alves V.G., et al., 2009).

RMR is often calculated using indirect calorimetry, an efficient tool for measuring RMR with accuracy and precision under standardized conditions (Haugen H.A., et al., 2007, Compher C., et al., 2006). The principle of indirect calorimetry is derived from the fact that the human body burns available sources of fuel using O_2 while producing CO_2 . In this model, all the O_2 consumed is completely used and the CO_2 that is expired is derived from

complete oxidation of fuels (Sion-Sarid R., et al., 2013). It is known that 1 liter of O₂ consumed generates 3.9 kcal and 1 liter of CO₂ produced generates 1.1 kcal. The Weir equation then is used to calculate RMR (Weir J.B., 1949). Indirect calorimetry is applied both at outpatient population for weight management and at inpatient population to optimize caloric provision, especially in complicated patients or patients with high risks for under or overfeeding to achieve faster recovery and decrease the risk of developing clinical complications (Haugen H.A., et al., 2007). However, all measurements of instruments might be subjected to some degree of measurement error. Typically, the amount of measurement error is expressed in terms of a coefficient of reliability. Reliability means the extent to which repeated measurements, taken under the same conditions, are similar to one another (Fitzmaurice G., 2002). There are many technical factors which could influence indirect calorimetry's accuracy, precision and reproducibility such as the O₂, CO₂ analyzers and the flowmeter, but also factors related to the methodology of indirect calorimetry measurements (Oshima T., et al., 2016, Compher C., et al., 2006). DeltatracII is a well investigated indirect calorimeter which is considered as the reference tool validated for indirect calorimetry measurements, but is no longer produced (Cooper J.A., et al., 2009, Alam D.S., et al., 2005). Other well studied indirect calorimeters such as the Quark RMR (Cosmed, Italy) and the CCMexpress (MedGraphic, USA) have not corresponded so far whether they are accurate in measuring the RMR, even in healthy individuals. A study on 24 healthy individuals compared VO₂, VCO₂ and RMR obtained by the Quark RMR performing the canopy dilution technique and the CCMexpress using three different methods of gas collection including canopy, face tent and facemask, to the DeltatracII performing the canopy dilution technique. This study highlighted the need for refining the accuracy both Quark RMR and CCMexpress. However, the measurements were performed for 10 minutes and the subjects were not in fasting state and did not eat the same breakfast before the measurements (Graf S., et al., 2013). Another study of 30 healthy subjects comparing VO₂, CO₂ and RMR obtained by the Quark RMR and the DeltatracII, showed that the Quark RMR using the canopy dilution technique is a valid system to measure RMR in resting and post-prandial conditions in healthy subjects. In this study the subjects had an overnight fast after standardized evening meals and had to lay at complete physical rest, alone, and undisturbed in a quiet room for 30 minutes before the measurements. Each individual was measured for three periods of 45 minutes and could read or watch movies during the measurement (Blond E., et al., 2010). Additionally, Cooper et al. compared the reliability of 3 indirect calorimeters, that measure O₂

consumption and CO₂ production to estimate RMR, including the MedGraphics CPX Ultima, Vmax Encore 29 System and the TrueOne 2400 to the reliability of the DeltatraccII in estimating RMR. Reliability assessment for RMR revealed that none of the devices can be considered adequately reliable for use in a research setting. However, this study was collaboration between three institutions and there were some site-to-site variations in study protocol and subject characteristics (Cooper J.A., et al., 2009).

Considering these studies, there is strong evidence that the already known and investigated indirect calorimeters are not accurate in measuring O₂ consumption, CO₂ production to estimate RMR in healthy individuals. The development of a new calorimeter is needed to provide practical solutions and make available a calorimeter corresponding to the requirements by clinicians for in and outpatients, featuring accuracy, ease of use and affordable cost (Oshima T., et al., 2016). The Vyntus CPX is a new indirect calorimeter that measures ventilation, VO₂, VCO₂ to determine functional capacity (Vyntus CPX Brochure). The Vyntus CPX has an easy calibration and there is no need for a manual 3 Liter syringe, as the Quark RMR, because it is equipped with a unique, fully automatic volume calibration unit (Blond E., et al., 2010, Vyntus CPX Brochure). One click in the sentry suite software and the volume sensor calibration will be automatically performed using the integrated blower (Vyntus CPX Brochure). With the twin tube sample line and fresh air flush system, moving the sample line to a calibration port is not necessary. The “click and play” fully automatic 2-point gas calibration of the O₂/CO₂ analyzers determines the delay and response times in the same procedure for exact synchronization with the volume signal (Vyntus CPX Brochure). The Vyntus CPX provides the “auto flow control” setting, while the blower flow of the Quark RMR is continuously monitored with a turbine flowmeter, and makes the flow automatically controlled until a Pred-FeCO₂ concentration of 0.9 (+/- 0.3%) has been reached (Blond E., et al., 2010, Vyntus CPX Brochure). Usually, this takes about 2-3 minutes (Vyntus CPX Brochure). However, it is unknown whether the Vyntus CPX is a reliable and valid device in measuring O₂ consumption and CO₂ production in estimating RMR. This study aims to assess for the first time the reliability of the Vyntus CPX using the canopy dilution technique in measuring O₂ consumption and CO₂ production to estimate RMR in healthy individuals.

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1. HUMAN ENERGY REQUIREMENTS

1.1 Definition of Energy Requirements

Energy requirement is the amount of food energy needed to balance total energy expenditure (TEE) in order to maintain body size, body composition and a level of necessary and desirable physical activity consistent with long-term good health. This includes the energy needed for the optimal growth and development of children, for the deposition of tissues during pregnancy, and for the secretion of milk during lactation consistent with the good health of mother and child. Growth requires energy for synthesis of tissues. In the first three months of life, growth uses about 35% of total energy needs. This falls to 5% at the 12 months, less than 2% in the second year of life, 1-2% until mid-adolescence and zero by 20 years of age (FAO, WHO, UNU, 2001). Additional energy is also needed in pregnancy and lactation to cover the needs of the growing fetus, the placenta and expanding maternal tissues and additional maternal effort at rest and in physical activity, as well as the production of breast milk (NHMRC, 2006). Energy requirements and recommended levels of intake are often referred to as daily requirements or recommended daily intakes. The recommended level of dietary energy intake for a population group is the mean energy requirement of the healthy, well-nourished individuals who constitute that group (FAO, WHO, UNU, 2001).

1.2 Components of Total Energy Expenditure

Human energy requirements are estimated from measures of TEE. Recommendations for dietary energy intake from food must satisfy these requirements for the attainment and maintenance of optimal health, physiological function and well-being. Energy balance is achieved when input (i.e. dietary energy intake) is equal to output (i.e. TEE). In practice TEE depends and can be divided into three main components: basal metabolic rate (BMR), activity energy expenditure (AEE) and diet induced thermogenesis (DIT) (FAO, WHO, UNU, 2001). In particular, TEE depends on: the requirement for maintenance of normal body structure, function and metabolic integrity – the basal metabolic rate (BMR), the energy required for work and physical activity - activity energy expenditure (AEE) and the energy cost of synthesizing reserves of fat and glycogen and the increase in protein synthesis in the fed state - dietary-induced thermogenesis (DIT), which includes energy expended in the digestion, absorption and transport of nutrients (Weekes E.C., 2007, Bender D.A., 2004).

Table 1.1: Components of total energy expenditure in healthy subjects and diseased individuals (Oshima T., et al., 2016, Haugen H.A., et al., 2007)

Total energy expenditure (TEE)	RMR + AEE
Basal metabolic rate (BMR)	Energy expended in fasting state, resting in lying position at neutral ambient temperature, free of physical and psychological stress. It is the energy required to maintain the body's basic cellular metabolic activity and organ functions
Diet – induced thermogenesis (DIT)	Oxidation of energy substrates during oral, enteral or intravenous energy intake
Resting metabolic rate (RMR)	BMR + DIT
Activity energy expenditure (AEE)	Energy expenditure to support physical activity

1.2.1 Basal Metabolic Rate

BMR is the energy requirement for the maintenance of metabolic integrity and activity, including respiration, heartbeat, maintenance of body temperature nerve, muscle tone and circulation (Weekes C.E., 2007, Bender D.A., 2004). This component of TEE must be measured under standardized ambient conditions such as controlled temperature and humidity. Subject must be at complete rest after at least 8 hours of sleep and after 12-18 hours overnight fast (Volp A.C.P., et al., 2011, Koletzko B., et al., 2005). In other words, BMR is the energy expenditure by the body at rest, but not asleep, under controlled conditions of thermal neutrality and about at least 12 after the last meal (Bender D.A., 2004). Therefore, BMR is generally measured immediately after the patient awakens in the morning (Porter C., Cohen N.H., 1996). During the measurement, subject must be kept fully awake, lied down quietly, completely relaxed and breathing normally (Volp A.C.P., et al., 2011). It is essential at the estimation of the BMR that the subject is awake, as some people show an increased metabolic rate, while others have a reduced metabolic rate and a slight fall in body temperature when they fall asleep (Bender D.A., 2004). Normally, BMR ranges from about 0.8 to 1.2 kcal/ minute in healthy women and men and is most closely associated with lean body mass (FFM). Approximately 2/3 of the BMR represents energy needed for maintenance of cell membrane pumps and for protein synthesis in the liver, brain, heart and kidney (Porter C., Cohen N.H., 1996). The BMR contributes for 60 to 70% of daily energy requirement for most sedentary individuals and nearly 50% for those physically active (Volp A.C.P., et al., 2011). There are three basic components of BMR: body weight, age and gender. Body weight affects BMR because there is a greater amount

of metabolically active tissue in a larger body. Age affects BMR because increasing age is due to changes in body composition. With increasing age, even when body weight remains constant, there is a loss of muscle tissue and replacement by adipose tissue, which is metabolically very much less active, as 80% of the weight of adipose tissue consists of reserves of triacylglycerol. Similarly, the gender difference, as women have a significantly lower BMR than do men of the same body weight, is accounted for by the differences in body composition. The proportion of body weight that is adipose tissue reserves in lean women is considerably higher than in men (Bender D.A., 2004). Other individual factors that may affect BMR include smoking habits, diet, physical activity, menstrual period and fasting. Room's conditions (temperature, noise and time of resting) and technical factors related to the equipment used may also affect the measurement of BMR. Additionally, factors which may affect BMR at different levels are thyroid and sexual hormones, growth, fever, sleep, metabolic stress and diseases (Volp A.C.P., et al., 2011).

1.2.2 Activity Energy Expenditure

The most useful way of expressing the AEE is as multiple of BMR (Bender D.A., 2004). AEE is the most variable determinant of energy needs and is the second largest user of energy after BMR (NHMRC, 2006). The physical activity ratio (PAR) is the ratio between energy expenditure corresponding to a sedentary or a physical activity (kcal/min) and BMR (kcal/min) (Department of Health, 1991). In other words, it is the energy cost of an activity per unit of time (usually a minute or an hour expressed as a multiple of BMR (Bender D.A., 2004). The sum of energy cost of all the activities by the subject during the 24 hours constitute the TEE (Lazzer S., et al., 2009). The ratio between TEE and BMR is the physical activity level (PAL) (Department of Health, 1991). For adults, a PAL above 1.75 is considered to be compatible with a healthy lifestyle. This value of 1.75 may also be relevant for adolescence but it is not certain whether it applies to childhood, particularly early childhood (NHMRC, 2006). The energy cost of physical activity is obviously affected by body weight, because more energy is required to move a heavier body (Bender D.A., 2004).

Table 1.2: Physical activity ratio (PAR) in different kind of activities (Bender D.A., 2004)

PAR	Activities
1.0 – 1.4	Lying, standing or sitting at rest, e.g. watching television, reading, writing, eating, playing card and board games
1.5 – 1.8	Sitting: sewing, knitting, playing piano, driving Standing: preparing vegetables, washing dishes, ironing, general office and laboratory work
1.9 – 2.4	Standing: mixed household chores, cooking, playing snooker or bowls
2.5 – 3.3	Standing: dressing undressing, showering, making beds, vacuum cleaning Walking: 3-4 km/h, playing cricket Occupational: tailoring, shoemaking, electrical and machine tool industry, painting and decorating
3.4 – 4.4	Standing: mopping floors, gardening, cleaning windows, table tennis, sailing Walking: 4-6 km/h, playing golf Occupational: motor vehicle repairs, carpentry and joinery, chemical industry, bricklaying
4.5 – 5.9	Standing: polishing furniture, chopping wood, heavy gardening, volley ball Walking: 6-7 km/h Exercise: dancing, moderate, hoeing, road construction, digging and shoveling, felling trees
6.0 – 7.9	Walking: uphill with load or cross-country, climbing stairs Exercise: jogging, cycling, energetic swimming, skiing, tennis, football

1.2.3 Diet-Induced Thermogenesis

There is a considerable increase in metabolic rate in response to a meal (Bender D.A., 2004). Diet-induced thermogenesis (DIT) or thermic effect of food (TEF) is the component of TEE related to the energy required for the digestion, absorption, usage and storage of nutrients after food intake (Volp A.C.P., et al., 2011). A small part of this is the energy cost of secreting digestive enzymes and the energy cost of active transport of the products of digestion. The major part is the energy cost of synthesizing body reserves of glycogen and

triacylglycerol, as well as the increased protein synthesis that occurs in the fed state. The cost of synthesizing glycogen from glucose is about 5% of the ingested energy, whereas the cost of synthesizing triacylglycerol from glucose is about 20% of the ingested energy (Bender D.A., 2004). The DIT represents 5 to 15% of the TEE, and has an essential role in the regulation of energy balance and body weight. The thermic effect of food on TEE varies according to the type of macronutrient intake: 0-3% for lipids, 5-10% for carbohydrates and 20-30% for proteins. DIT is higher for proteins because their synthesis requires at least for high-energy phosphate bonds (ATP) per amino acid incorporated into a protein molecule, with the dispense of 0.75 kcal/g of synthesized protein, and the high metabolic cost of ureagenesis and gluconeogenesis (Volp A.C.P., et al., 2011). Depending on the relative amounts of fat and carbohydrate is in the diet, and the amounts of triacylglycerol and glycogen being synthesized, this DIT may account for 10% or more of the total energy yield of a meal (Bender D.A., 2004). DIT can be divided into two distinct phases: the cephalic and the gastrointestinal. The first one is related to sympathetic nervous system action which is activates by food sensory properties, while the second is characterized by ATP consumption during the absorption and utilization of nutrients. There are some factors that may influence and modulate DIT, such as stimulus to the autonomic nervous system, hormones, diet palatability, physical activity (PA), body composition, adiposity, and the most important, diet composition (Volp A.C.P., et al., 2011).

1.2.4 Growth, Pregnancy and Lactation

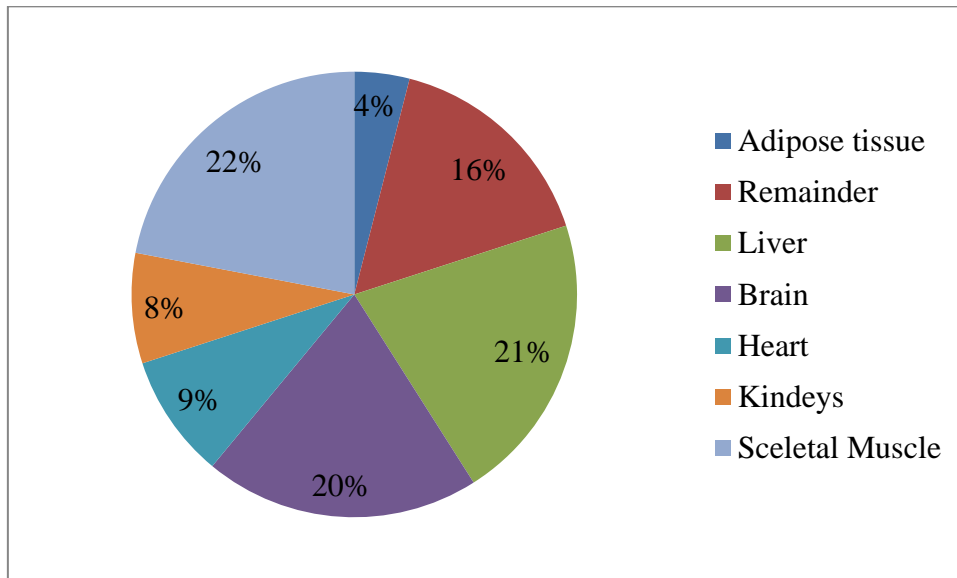
Growth: The energy cost of growth has two components: the energy needed to synthesize growing tissues and the energy deposited in those tissues. The energy cost of growth is about 35% of TEE during the first three months of age, falls rapidly to about 5% at 12 months and about 3% in the second year, remains at 1-2% until mid-adolescence, and is negligible in the late teens.

Pregnancy: During pregnancy, extra energy is needed for the growth of the foetus, placenta and various maternal tissues, such as in the uterus, breasts and fat stores, as well as for changes in maternal metabolism and the increase in maternal effort at rest and during physical activity.

Lactation: The energy cost lactation has two components: the energy content of the milk secreted and the energy required producing that milk. Well-nourished lactating women can

derive part of this additional requirement from body fat stores accumulated during pregnancy (Bender D.A., 2004).

Figure 1.1: Percentage of Total Energy Expenditure by Different Organs of the Body (Bender D.A., 2004)



1.3 Estimation of Resting Metabolic Rate

RMR can be measured by five different methods: a) direct calorimetry b) indirect calorimetry c) predictive equations d) thermodilution (Fick method) e) $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ doubly labeled water (Oshima T., et al., 2016, Blond E., et al., 2010). However, in clinical practice, RMR is determined either by using predictive equations or by actual measurement using indirect calorimetry (Sarid R.S., et al., 2013)

1.3.1 Direct Calorimetry

RMR can be determined directly, by measuring the heat production from the body. This principle is based on the phenomenon that all energy substrates, upon oxidation, produce heat. The subject needs to be confined in an insulated chamber to measure the heat production (Oshima T., et al., 2016). This is a thermally insulated chamber, in which the temperature can be controlled so as to maintain the subject's comfort and in which it is possible to measure the amount of heat produced – for example by the increase in temperature of water used to cool the chamber (Haugen H.A., et al., 2007). The subjects also have to be able to maintain a complete resting rate during the measurement in order to avoid extra heat production by physical activity. Therefore, the conditions are unrealistic

for clinical use, and the availability is limited only for research use (Oshima T., et al., 2016).

1.3.2 Indirect Calorimetry

Most estimates of RMR are based on indirect measurements – either measurement of O₂ consumption and CO₂ production or indirect assessment of CO₂ production by use of dual isotopically labelled water which will be described further below. From the results of a number of studies in which EE in different activities has been measured, it is possible to calculate total energy expenditure (TEE) from the time spent in each type of activity (Bender D.A., 2004).

1.3.3 Doubly Labeled Water

The doubly water method is regarded as the gold standard for measuring the RMR (Westerterp K.R., Plasqui G., 2004). Water containing non-radioactive isotope labeled hydrogen and oxygen atoms (²H/¹H and ¹⁸O/¹⁶O) is given orally, after a baseline evaluation of the body liquids: urine, saliva and blood. The evaluation of the body liquids is repeated after 7-12 days to calculate the variations of concentrations of the isotopes over time. CO₂ production can be calculated by observing the elimination rates of the isotopes from the body liquids. RMR can be calculated by assuming a given RQ. The calculations are based on several assumptions such as steady-state CO₂ and H₂O turnover, and constant body water pool size during the measurement period. These assumptions may not be applicable for critically ill patients, as fluid volume shifts together with large changes in CO₂ production are frequently observed (Fraipont V., Preiser J.C., 2013). The costs of the doubly labeled water and of mass spectrometry measurements are very high. Therefore, even this method allows accurate calculating of RMR, this method is only applicable in research (Oshima T., et al., 2016).

1.3.4 Predictive Equations

Predictive equations are easily accessible without the need to use specialized equipment and appliances (Blond E., et al., 2010). Depending on the patient population and clinician preferences, these equations include Harris-Benedict, Mifflin-St., Jeor and Owen (Mifflin M.D., 1990, Owen O.E., 1986, Harris A.J., Benedict F.G., 1919). These equations are based on individual's anthropometric measures including height, weight, gender, and age in order to measure RMR (Sion-Sarid R., et al., 2013). Cunningham, based on previous studies on RMR equations and on data relating RMR to free fat mass (FFM), suggested a

new RMR equation based on individual's FFM (Cunningham J.J., 1991). However, many reports have shown that predictive equations are not accurate enough, especially in pathological states, since values can be either under or overestimated in a range from 70 to 140% of actual RMR (Blond E., et al., 2010, Alves V.G., et al., 2009). Understanding how these equations are derived and their limitations provide the insight clinicians need to determine when estimation of RMR with population-based predictive equations is adequate and when an individualized measurement is warranted (Haugen H.A., et al., 2007).

1.3.5 Thermodilution – Fick Method

The Fick method required a pulmonary artery catheter to measure the cardiac output, using the thermodilution method. Arterial and mixed venous O₂ contents must also be measured. After measuring the O₂ content in arterial and mixed venous blood from the pulmonary artery, VO₂ can be calculated using the Fick equation. The RMR is calculated by assuming a fixed RQ. Several problems limit its use in clinical practice. First, only few patients have pulmonary artery catheters and the insertion of the catheter only for RMR measurements would be invasive. Second, the VO₂ calculated by this method is only a snapshot of the moment of the measurement, while the error of the thermodilution method is about 15% due to cardiac output variation over the respiratory cycle. Furthermore, mixed venous O₂ concentration may be overestimated because of the shunting of arterial blood from bronchial vessels, thus leading to underestimation of VO₂ and subsequently the RMR (Oshima T., et al., 2016).

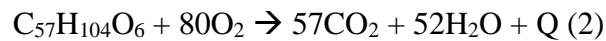
1.4 Metabolic Fuels

Energy for the metabolic and physiological functions of humans is derived from the chemical energy found in food (FAO, WHO, UNU, 2001). The nutrients in food that provide this chemical energy are the macronutrients: carbohydrates, fats, proteins and alcohol (ethanol), which act as substrates or fuels (Raman A., Schoeller D.A., 2005, FAO, WHO, UNU, 2001). After food is ingested, its chemical energy is released and converted into thermic, mechanical and other forms of energy (FAO, WHO, UNU, 2001). The chemical process that releases the chemical energy is respiration, in which each of these macronutrients is combined with O₂ to produce CO₂ and H₂O (Raman A., Schoeller D.A., 2005). These metabolic fuels can be converted to the same end-products chemically, by burning in air. Although the process of metabolism in the body is more complex and includes an enzymatic process, it is a fundamental law of chemistry that, if the starting material and end-products are the same, the energy yield is the same, regardless of the

route taken. Therefore, the energy yield of foodstuffs can be determined by measuring the heat produced when they are burnt in air, making allowance for the extent to which they are digested and absorbed from foods (Raman A., Schoeller D.A., 2005, Bender D.A., 2004). For example, one molecule of sugar (glycose) breaks down as follows:



During this reaction, six molecules of CO₂ are produced and six molecules of O₂ are consumed. Therefore, the ratio of CO₂ to O₂ has a value of 1.0. This ratio is commonly called the respiratory quotient (RQ) or respiratory exchange ratio (RER). However, sometimes the term RER is used when it is applied to a whole body measurement. Similarly, when one molecule of fat is broken down completely, the chemical reaction is:



During this reaction, 57 molecules of CO₂ are produced while 80 molecules of O₂ are consumed. This yields an RER of 0.71. When only carbohydrate and fat are oxidized to support energy expenditure, this difference in RER makes it possible to calculate what percentage of RMR is being supported by each of the two energy substrates. However, there is a third macronutrient that is oxidized to produce energy. That macronutrient is protein which is more difficult to describe on a chemical basis, because a protein is made from a mixture of amino acids, and for each dietary protein the number and composition of amino acids differ. The breakdown of the average dietary protein, however, can be described by the chemical reaction:



During this reaction, 87 molecules of CO₂ are produced while 105.3 molecules of O₂ are consumed. This yields an RER of 0.83. Although this RER value is intermediate between carbohydrate and fat, protein is unique among the three energy substrates because it is the only one to contain nitrogen. As such, urinary nitrogen can be assayed to obtain an estimate of protein oxidized by an individual (Raman A., Schoeller D.A., 2005).

Table 1.3: Oxygen consumption and carbon dioxide production in oxidation of metabolic fuels (Bender D.A., 2004)

	Kcal/ g	kJ/ g	Oxygen consumed (L/g)	Carbon dioxide produced (L/g)	Respiratory quotient (CO₂/O₂)
Carbohydrate	4	16	0.829	0.829	1.0
Protein	4	17	0.966	0.782	0.809
Fat	9	37	2.016	1.427	0.707

1 kcal = 4.186 kJ

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2. INDIRECT CALORIMETRY

2.1. The Basics of Calorimetry

All living organisms require a source of energy for survival. This energy is provided in the form of chemical energy in the nutrients they consume, which are converted to other forms of energy through respiration. This conversion is subject to the same laws of thermodynamics that govern all energy systems. The first law of thermodynamics states that energy can neither be created nor destroyed and it can only be exchanged from one system to another. Hence, the chemical energy consumed in the form of food is converted into mechanical energy for work performed by the body, thermal energy for maintenance of body temperature, or stored as chemical energy in tissues as fat, protein, or a small fraction as carbohydrates. This conservation of energy can be stated mathematically as:

$$\text{Energy}_{\text{in}} = \text{Energy}_{\text{work}} + \text{Energy}_{\text{heat}} + \text{Energy}_{\text{stored}}$$

The sum of energy converted to work and heat is defined as metabolism. Although metabolism constitutes thousands of chemical reactions occurring at the same time throughout the body that cannot be individually measured, their sum can be measured as either the sum of work and heat energy or, in the absence of any measurable work, the rate of heat production by the body. This is based on the assumption that all the cellular events ultimately result in heat (Raman A., Schoeller D.A., 2005).

2.1.1 Definition of Calorimetry

Calorimetry is the process of measuring heat produced by the body during combustion of substances or nutrients in animals or humans. There are two methods of calorimetry, the direct and the indirect calorimetry. The term “direct calorimetry” is used when the rate of heat production is directly measured by placing a person in a thermally isolated chamber. The term ‘indirect calorimetry’ is used when heat production is not measured directly, but it is calculated from the measurement of the rates of O₂ consumption (VO₂) and CO₂ production (VCO₂). In both measurements, the rate of metabolism is commonly referred to as the rate of EE, which in the absence of work output is the rate at which chemical energy in food is converted to heat (Raman A., Schoeller D.A., 2005).

2.2 Definition of Indirect Calorimetry

Indirect calorimetry measures the O₂ consumption and the CO₂ production, which correspond to the cellular respiration and allows the calculation of RMR. This is possible because heat production is tightly correlated with O₂ consumption and CO₂ production according to the type of energy substrate is oxidized (Weir J.B.V., 1949). This gas exchange reflects the nutrient metabolism in the body (Blond E., et al., 2010). Measurement of O₂ consumption is quite simple using a spirometer. Such instruments are portable, so people can carry on more or less normal activities for several hours at a time, while their EE is being estimated. Measurement of O₂ consumption and CO₂ production at the same time is a simple process using a spirometer and provides information on the mixture of metabolic fuels being metabolized. (Bender D.A., 2004).

2.2.1. Measurement of Resting Metabolic Rate by Indirect Calorimetry

RMR and relative proportions of energy derived from oxidation of carbohydrate, protein, and fat can be measured by indirect calorimetry. When carbohydrate, protein and fat are oxidized, there is a known arithmetic relationship between moles of reactant consumed (substrate and O₂) and moles of products produced (H₂O, CO₂, urea, adenosine triphosphate (Porter C., Cohen N.H., 1996). The principle of indirect calorimetry is derived from the fact that the human body burns available sources of fuel using O₂, while producing CO₂. In this model, all the O₂ that is consumed is completely used and the CO₂ that is expired is derived from complete oxidation of fuels (Sarid R.S., et al., 2013). Indirect calorimetry requires the measurement of inspired and expired O₂ and expired CO₂ concentrations, as well as the volume of expired gas per minute to calculate the VO₂ (L/min) and VCO₂ (L/min). Then VO₂ and VCO₂ are used to calculate the RMR (kcal/day) using the Weir's equation: $RMR = [(VO_2 \cdot 3,941) + (VCO_2 \cdot 1.11) + (uN_2 \cdot 2.17)] \cdot 1.44$, where VO₂ is the O₂ consumption (L/min), VCO₂ is the CO₂ production (L/min) and uN₂ is the urinary nitrogen (gr/day) (Oshima T., et al., 2016).

2.3. Technology of Indirect Calorimeters

Calorimeters are designed to measure spontaneously breathing patients or mechanically ventilated patients. Devices designed for spontaneous breathing use various collection methods such as a ventilated canopy, masks, and mouthpieces/nose clips (Haugen H.A., et al., 2007). However, the different techniques predetermine the limitations of their performances (Oshima T., et al., 2016). There are handheld devices that are capable of measuring VO₂ to determine RMR. These devices are only appropriate for spontaneously

breathing patients. Additionally, they assume a constant RQ (e.g., 0.8) for all patients and measure only VO_2 (VCO_2 is estimated [$\text{VCO}_2 = \text{RQ} / \text{VO}_2$]) to determine EE (Haugen H.A., et al., 2007). This type of assumption is acceptable and used in healthy subjects on balanced nutrition. However, it is not recommended for patients, because their substrate oxidation may change according to the type of disease and nutrition (Oshima T., et al., 2016). Indirect calorimeters are divided in two different circuit systems, the closed- and the opened-circuit systems. In the open-circuit systems, the patient breathes room air or air supplied from a mechanical ventilator and expires into a gas sampling system, which eventually vents the expired air back into the room. The difference between inspired and expired gas concentrations and minute ventilations is measured to estimate VO_2 . Both open- and closed-circuit systems require devices to measure concentration of O_2 and CO_2 , gas volume or flow, temperature and time. Some systems also measure barometric pressure, while other systems require to be entered manually (Matarese L.E., 1997).

2.3.1 Closed-Circuit Systems

Closed-circuit indirect calorimetry involves the recirculation of the same air through a sealed reservoir. These circuit systems are designed so that the patient breathes from a reservoir of 100% O_2 . The decrease in the volume of O_2 over time then is used to calculate VO_2 . The recirculated air is kept breathable by removing the CO_2 produced by the subject and replacing the O_2 consumed by subject. The replacement of O_2 is controlled by continuously monitoring the change in the volume of the gas in the closed breathing circuit. As the subject consumes O_2 , a sensor detects the decrease in volume and a signal is sent to an external source to release constant calibrated pulses of O_2 back into the system to restore the original values. The rate of O_2 consumption is measured by recording the amount of O_2 that is added to the air during recirculation. The CO_2 produced by the subject is removed from the recirculated air by an absorber attached to the system and the CO_2 production is measured from the increased weight of the absorber (Raman A., Schoeller D.A., 2005). The decrease in the volume of the O_2 over time is used to calculate VO_2 . The major advantage of the closed system is that inspired minute ventilation can be measured rather than calculated using the Haldane equation: $\text{VO}_2 = [(1 - \text{FeO}_2 - \text{FeCO}_2) \cdot (\text{FiO}_2 - \text{FeO}_2) \cdot \text{Ve}] / (1 - \text{FiO}_2)$, where VO_2 (L/min) is the O_2 consumption, FeO_2 and FeCO_2 are the fraction of expired O_2 and fraction of expired CO_2 respectively, FiO_2 is the fraction of inspired O_2 and Ve is the expired volume (L). This method is used to calculate inspired gas volume by the ratio of the inspired and expired nitrogen concentrations, to calculate the

inspired gas volume thus simplifying the flow or volume measurements (Oshima T., et al., 2016, Matarese L.E., 1997). In ventilated patients with high forced inspiratory oxygen (FiO_2), the Haldane equation becomes less accurate as the denominator ($1-\text{FiO}_2$) approaches zero. Therefore, there is FiO_2 upper limit with a closed system. Fluctuation in FiO_2 is not a great concern, because O_2 analyzers are not required. However, breathing resistance is increased, inspiratory time is prolonged, and the work of breathing may be increased as much as 10% in closed systems. A respiratory therapist must be present during the test to make adjustments to the ventilator to overcome the resistance and make certain that the patient receives the appropriate therapy. The system is less versatile, because canopy measurements cannot be performed. Closed-circuit systems are affected by changes in lung volume or leaks in the system. Any changes in lung volume are displayed into the spirometer. Since O_2 consumption is determined by the volume loss per unit time, any leak will be interpreted as a change in VO_2 . Leaks can be so significant that they result in obvious erroneous data (Matarese L.E., 1997).

2.3.2 Opened-Circuit Systems

Most systems use open-circuit technology to measure RMR as part of a nutrition assessment program. Open-circuit systems are versatile and can be used in a variety of clinical conditions. Thus, RMR of both ventilated and spontaneously breathing can be measured (Matarese L.E., 1997). Open-circuit indirect calorimetry involves a system in which both ends of the breathing system are open to the atmosphere. The inspired and expired airs are kept separate by means of a three-way respiratory valve or non-breathing mask. The expired gases are collected into an air-tight bag or are frequently sampled or continuously analyzed for O_2 and CO_2 content (Raman A., Schoeller D.A., 2005). There are many collection systems that can be used e.g. canopy, mask or mouthpiece and nose clip. The open-circuit systems do not increase the work of breathing. However, they should not be used for patients receiving FiO_2 greater than 60%, because the Haldane transformation is used in the calculations. Since O_2 analyzers are employed, open-circuit systems are susceptible to fluctuations in FiO_2 . The FiO_2 that is selected and delivered through the ventilator blender is not consistent either within or between breaths. Many of the open circuit-systems provide a display of O_2 , CO_2 , and flow to permit direct monitoring of leak detection, FiO_2 stability and system functioning. Breath by breath systems that measure FiO_2 in each breath minimize changes caused by fluctuating FiO_2 , because of the frequent sampling. However, there are some technical considerations. It is essential in an

opened-circuit measurement to prevent leaks in the system. Patients with incompetent endotracheal tube cuffs, leaking chest tubes, or bronchopleural fistulas should not be measured because complete gas collection is probably not possible. Water vapors must be eliminated from the gas, before it reaches the analyzers, which may be accomplished by heating or cooling the sample or using desiccants, water traps, or special tubing. The gas collection systems for open circuit calorimeters include breath-by-breath, mixing chamber, or dilution systems (Matarese L.E., 1997).

2.3.2.1 Breath by Breath System

The breath-by-breath method samples VO_2 and VCO_2 at each breath and then averages the data over time (Matarese L.E., 1997). In spontaneously breathing subjects a ventilated canopy hood, a fitted face mask or a mouthpiece is used to collect the inspired and expired gas (Oshima T., et al., 2016, Raman A., Schoeller D.A., 2005). Conducting a measurement with a mask makes often difficult to obtain an airtight seal without excessive pressure at the site of contact with the mask and face (Raman A., Schoeller D.A., 2005). The measurements using a mouthpiece are similar to ventilated canopy systems in principle, but instead of placing a hood over the subject's head, the subject wears a mouthpiece connected to the analyzer and nose clips to prevent breathing through the nose. Also, breathing through the mouthpiece often causes untrained subjects to involuntarily hyperventilate leading to inappropriate O_2 and CO_2 rate. However, the mouthpiece systems are generally used for studies of gas exchange and energy metabolism during exercise and provide a shorter measurement response time than the ventilated hood systems (Raman A., Schoeller D.A., 2005). In breath by breath system the respiratory gas composition and flow are measured continuously by connecting the gas analyzers to the ventilator circuit. The signals received by the gas analyzers and flow meters are synchronized to calculate the O_2 consumption (VO_2 L/min) and CO_2 production (VCO_2 L/min) as the difference between the volumes of inhaled and exhaled O_2 and CO_2 per breath by integral calculations. The Haldane transformation is used to calculate the inhaled gas volume from exhaled gas volume measurement. The Weir's equation is used to calculate EE (kcal/d) per breath, and averaged for the duration of the measurement (Oshima T., et al., 2016)

2.3.2.2 Mixing Chamber System

With the mixing chamber system, inspired gas is directed into the mixing chamber, and analyzers sample the gas collection at factory-selected intervals. This system works well for steady-state metabolic testing. A mixing chamber system measures the inhaled and

exhaled gasses separately, to detect the global change in the inhaled and exhaled gas. The expired volume is usually measured by a separate flow meter, or by a dilution technique using a constant flow chamber to calculate the volume. The O_2 concentration of F_iO_2 is first measured. Exhaled gas is collected into the mixing chamber, where it is physically averaged and analyzed for O_2 (F_eO_2) and CO_2 (F_eCO_2) concentrations. The collected gas is eliminated through an independent chamber where the gas flow (Q) is kept constant at 40-45 L/min, to dilute the exhaled gas from the mixing chamber with the ambient air. CO_2 in the diluted gas (F_dCO_2) is measured to calculate the CO_2 production (V_{CO_2} , L/min) by multiplying the concentration by the flow ($V_{CO_2} = F_dCO_2 \cdot Q$). An equation using Haldane transformation, allows the calculation of the respiratory quotient (RQ) from the measured O_2 and CO_2 values ($RQ = (1 - F_iO_2) / [(F_iO_2 - F_eO_2) / (F_eCO_2 - F_iO_2)]$), and thus enables the calculation of the O_2 consumption (V_{O_2} , L/min) $V_{O_2} = V_{CO_2} / RQ$. Devices with a mixing chamber generate more stable measurements, because the gases are physically “averaged” before being analyzed, allowing the gas analyzers to generate very accurate analysis. The mixing chamber typically occupies 3-5 L of space, precluding the making of a small device. The capacity to make reliable measurements in a short duration (e.g. 3-5 minutes) is also limited as it takes just as much time for the gas concentration in the mixing chamber to stabilize (Oshima T., et al., 2016).

2.3.2.3 Dilution System

Dilution systems take the expired air, dilute it with room air, and shunt the gases into a mixing chamber for analysis. This system works best for canopy measurements in spontaneously breathing patients (Matarese L.E., 1997). Open-circuit indirect calorimeter systems, using the so called “canopy dilution technique”, are the most common devices for measuring RMR (Blond E., et al., 2010). The canopy is used to measure RMR in spontaneously breathing subjects (Oshima T., et al., 2016). The subject is placed under a clear canopy with a plastic drape to avoid air leakage. Calorimeters feature constant flow generator to create an outward flow through the canopy. F_iO_2 enters from the surrounding environment (room air) and the exhaled O_2 and CO_2 content is measured for calculation of O_2 consumption and CO_2 production (Blond E., et al., 2010). The breath by the subject is diluted by the constant flow Q (L/min), and collected by the calorimeter for gas analysis (F_dO_2 , F_dCO_2). This process enables calculations of V_{O_2} and V_{CO_2} . F_iO_2 and F_iCO_2 are either assumed as ambient air values or measured, depending on the calorimeter. These values are used to calculate RMR using the Weir’s equation (Oshima T., et al., 2016).

2.4 Protocol of Indirect Calorimetry Measurements

There are several factors that may alter apparent RMR during measurement with indirect calorimetry. These factors are: the thermic effect of food, alcohol, nicotine, physical activity, the resting period before a measurement, the body position during the measurement, the environmental conditions, and the type of gas collection (Compher C., et al., 2006).

2.4.1 Thermic effect of food

Metabolic rate is associated and increased in conditions such as digestion, absorption and metabolism of dietary nutrients. This increase is referred as TEF. The typical pattern is that of a rapid rise to peak levels followed by a gradual return to baseline RMR. Several studies showed that the peak in TEF occurs between 60-180 minutes in most individuals and that people with obesity and older people tend to peak later than no obese and younger people. A minimum fast of 5 hours after meals or snacks and 4 hours after small meals is recommended if longer fast is clinically inappropriate (Compher C., et al., 2006). Additionally, one study tested the hypothesis that a mixed meal rich in fat can lead to energy saving compared with a meal rich in carbohydrates with the same amount of proteins and calories. Therefore, they measured the TEF after a low fat (20% fat, 68% carbohydrates and 12% protein) and the TEF after a high fat (48% fat, 40% carbohydrates and 12% protein) and they found out that TEF, expressed as a percentage of energy intake was significantly higher after the low fat meal than after the high fat meal. These results highlight that a high fat and low carbohydrate diet is able to induce lower thermogenesis than a low fat meal with the same amount of proteins and calories (Maffeis C., et al., 2001)

2.4.2 Alcohol

As with food, alcohol can also increase metabolic rate. Based on limited available studies, individual RMR increases of 1.1% to 13.6% have been reported over 95 minutes after ingestion of alcohol in healthy men and mean RMR increases of 9% have been recorded 90 to 100 minutes post ingestion in women. One study examined the duration of TEF after different amounts of alcohol consumption and they found a small raise in RMR even after 90 minutes (Weststrate J., et al., 1990). Hence, a minimum abstention from alcohol for 2 hours is recommended (Compher C., et al., 2006).

2.4.3 Nicotine

Several studies have tested the effect of nicotine on RMR (Compher C., et al., 2006). A study showed that smoking is associated with an increased RMR per kg fat free mass and an increased RMR adjusted for fat free mass, compared with never smoking. However, this study indicated no difference in RMR per kg fat free mass or RMR adjusted for fat free mass between never and former smokers or between never and occasional smokers, which implies that smoking has primarily acute effects on RMR (Blauw L.L., et al., 2015). The initial thermic effect of nicotine peaks at 10 to 60 minutes after exposure and subsequent exposure can lead to additional peaks. Although RMR elevations occur within 10 minutes with the first exposure, they are of short duration and RMR returns to baseline 2 hours later (Compher C., et al., 2006).

2.4.4 Caffeine

There is strong evidence that caffeine can lead to increases in RMR. One meta-analysis claimed a significant effect on RMR after the consumption of mixtures with caffeine and catechins, but also after only caffeine consumption (Hursel R., et al., 2010). In general, a thermic response to caffeine can be measured between 30 and 150 minutes after ingestion. There is no direct evidence to determine when metabolic rate returns to true resting levels following caffeine consumption. The increase in metabolic rate was sustained at 3 hours, but one study reported that after overnight abstinence from caffeine, RMR had returned to baseline levels. This suggests that a maximum of 12 hours of abstinence will eliminate the thermic effect of caffeine, but 3 hours of abstinence come close to baseline RMR (Compher C., et al., 2006).

2.4.5 Physical activity

RMR increases with physical activity in proportion to the amount of work performed. Following activity, RMR returns toward baseline resting levels, but the recovery time varies as a function of the type, intensity, and duration of the activity, and the physical fitness level of the individual. Thus, it is important to allow ample recovery time following physical activity to obtain an accurate measure of RMR. After walking or jogging on a treadmill at low to moderate intensity for 20 to 30 minutes, metabolic rate returns to baseline RMR in 30 to 90 minutes. In a different study, the recovery time after 15 minutes of high intensity exercise was 14 ± 6.5 minutes for high intensity exercise and 5.7 ± 4.9 minutes for low intensity exercise. Performing resistance exercise also elevates metabolic rate following cessation of exercise. In a study they found out increased metabolic rate

even 14 hours and 30 minutes after a workout with resistance exercises. Hence, a minimum abstention from moderate aerobic or anaerobic exercise for 2 hours and abstention of at least 14 hours for vigorous resistance exercise is needed before an indirect calorimetry measurement (Compher C., et al., 2006).

2.4.6 Resting Period before the RMR measurement

In healthy adults, a minimum rest of 10 to 20 minutes of resting period before initiating an RMR measurement is stated as an adequate testing condition (Compher C., et al., 2006).

2.4.7 Body position during the RMR measurement

Body position may influence the metabolic rate during an indirect calorimetry measurement. Certain postures require increased muscle tone and may influence the measurement of RMR. Therefore, each individual should be physically comfortable with the measurement position during the test and repeated measures should be in the same position (Compher C., et al., 2006). One study indicated a higher RMR when subjects were sitting upright motionless than when the subjects were at supine position (Levine J.A., Schleusner S.J. and Jensen M.D., 2000).

2.4.8 Environmental Conditions

Environment conditions include humidity, noise and ambient temperature. No primary research studies addressed the effects of ambient noise and lighting on RMR in healthy adults. Two narrative reviews suggest that the room should be quiet and lighting mild when measuring RMR for patients in critical care settings (McClave S.A., Snider H.L., 1992, Feurer I.D., Mullen J.L., 1986). These conditions logically extend to other settings. According to ambient temperature, RMR is affected to variable degrees by moderate cold exposure or ambient room temperatures outside a comfortable zone (20 to 25 degrees) (Compher C., et al., 2006).

2.4.9 Type of Gas Collection

Forse R.A. investigated the effect of different gas collection systems on O₂ consumption, CO₂ production, RMR and RQ. In this study mean RMR was 7% higher for facemask and 9% higher for mouthpiece than the canopy measurements (Forse R.A., 1993). However, further studies comparing modern gas collection devices are needed in healthy and clinical populations (Compher C., et al., 2006).

2.4.10 Variation in Oxygen Consumption Carbon Dioxide Production

To obtain accurate RMR measurement, attention must be given to ensure steady –state conditions, defined by the degree of variation in VO_2 and VCO_2 over a set time period (Compher C., et al., 2006). If steady state is achieved, one measure is adequate and if not, two to three nonconsecutive measurements improve accuracy. In healthy individuals, reliable RMR measurements can be obtained with the use of a 10 minute protocol in which the first 5 minutes of data are discarded and the remaining 5 minutes of data have a CV of no more than 10% for VO_2 and VCO_2 (Horner N.K., et al., 2001).

2.4.11 Resting Metabolic Rate Measurements at Various Times of the Day and on Different Days

It has been observed from several studies that repeated measurements vary 3% to 5% over 24 hours and up to 10% over weeks to months (Compher C., et al., 2006). In addition, some studies have reported within subject that day to day CVs range from 2% to 10% of RMR (Ventham J.C and Reilly J.J., 1999).

2.4.12 Respiratory Quotient

Respiratory quotient (RQ) measurements < 0.70 or > 1 suggest protocol violations or inaccurate gas measurement. Under typical metabolic conditions with stable respiratory function, the range of RQ in human metabolism is approximately 0.7 to 1. Under atypical metabolic and respiratory conditions, RQ can be <0.70 or > 1 and so RQ might aid in the assessment of the validity of some indirect calorimetry measurements of RMR. Prolonged fasting, recent or excessive food consumption, and ethanol consumption before RMR measurement may affect RQ. Food consumption increases RQ depending on the amount and composition of the meal (Compher C., et al., 2006). One study claimed that individual RQ values ranged from 0.72 to 0.80 after 16 hours of fasting but sometimes it drops below 0.70 in fasts lasting 22 hours (Romijn J.A., et al., 1990).

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3. RELIABILITY

3.1 Definition of Reliability

All the measurement instruments are fallible and subject to at least some degree of measurement error (Fitzmaurice G., 2002). Measurement error is the difference between the true value and the observed value estimated by an instrument (Bruton A., Conway J.H., Holgate S.T., 2002). The main components of measurement error are systematic bias (e.g. general learning or fatigue effects on the test) and random error due to biological or mechanical variation (Atkinson G., Nevill A.M., 1998). Reliability means the extent to which repeated measurements, taken under the same conditions are similar to one another (Fitzmaurice G., 2002). Another common topic is the assessment of the validity of a particular measurement tool. Validity is the ability of a measurement tool to reflect what it is designed to measure. However, reliability should be tested for first in a new measurement tool, since it will never be valid if it not adequately consistent in whatever value it indicates from repeated measurements. Terms that have been used interchangeably with “reliability”, in the literature are “repeatability”, “reproducibility”, “consistency”, “agreement”, “concordance” and “stability” (Atkinson G., Nevill A.M., 1998). Baumgarter identified in 1989 two types of reliability: relative reliability and absolute reliability.

3.1.1 Relative Reliability

Relative reliability is the degree to which individuals maintain their position in a sample over repeated measurements. This type of reliability is usually assessed with some type of correlation coefficient (Atkinson G., Nevill A.M., 1998).

3.1.2 Absolute Reliability

Absolute reliability is the degree to which repeated measurements vary for individuals i.e. the less they vary, the higher the reliability (Bruton A., Conway J.H., Holgate S.T., 2002). This type of reliability is expressed either in the actual units of measurement or as proportion of the measured values (dimensionless ratio). Methods used to describe ‘absolute reliability’ include the standard error of measurements (SEM), coefficient of variation (CV) and limits of agreement (LOA) (Atkinson G., Nevill A.M., 1998).

3.1.2.1 Standard Error of Measurement

The standard deviation of measurement errors is a reflection of the reliability of the test response, and it is known as the standard error of measurement (SEM) (Bruton A., Conway

J.H., and Holgate S.T., 2002). One aspect of the SEM is that it is unaffected by the range of measurements. The value for the SEM will vary from subject to subject, but there are equations for calculating a group estimate, e.g. $SEM = SD \cdot \sqrt{1 - ICC}$ where SD is the sampled standard deviation and ICC is the calculated intraclass correlation coefficient (Atkinson G., Nevill A.M., 1998). The SEM is a measure of absolute reliability and is expressed in the actual units of measurement, making it easy to interpret, i.e. the smaller the SEM, the greater the reliability (Bruton A., Conway J.H., and Holgate S.T., 2002). SEM is only appropriate for use with interval data since with ratio data the amount of random error may increase as the measured values increase (Bruton A., Conway J.H., and Holgate S.T., 2002, Atkinson G., Nevill A.M., 1998). Additionally, the use of the SEM requires a normally distributed population, no carry-over effect between the repeated tests and that heteroscedasticity is not present in the data. However, there is lack of clarity over an acceptable SEM (Atkinson G., Nevill A.M., 1998).

3.1.2.2 Coefficient of Variation

The coefficient of variation (CV) is an often quoted estimate of measurement error. Similarly with the SEM, CV is not affected by the range of measurements. There are various methods of calculating CV. The simplest form of the CV is calculated as the standard deviation (SD) of the data, divided by the mean and multiplied by 100 to give a percentage score. This expresses the SD as a proportion of the mean, making it unit independent. Moreover, CV methods should be used only if the variability depends on the magnitude of the mean values (heteroscedasticity). However, as Bland pointed out in 1987, the problem with expressing the error as a percentage is that the percentage of the smallest observation will differ markedly from percentage of the largest observation (Atkinson G., Nevill A.M., 1998). Chinn suggested in 1991 that is preferable to use the intraclass correlation (ICC), which will be described below, rather than CV, as the first relates the size of the error variation to the size of the variation of interest (Atkinson and Neville, 1998, Chinn S., 1991).

3.1.2.3 Limits of Agreement

The method “limits of agreement” (LOA) is an indicator of absolute reliability such as SEM and CV. The main difference between these statistics seems to be that the LOA assume a population of individual test-retest differences, while SEM and CV involve an assumed population of repeated measurements around a ‘true’ value for each individual (Atkinson and Neville, 1998). LOA represent the test-retest differences for 95% of a

population. The first step in the LOA analysis is to present and explore the test-retest individual subject differences between the tests plotted against the respective individual means. This is possible by using the Bland Altman plot (Bland J.M., Altman D.G., 1995). In addition, it is important to observe whether there is any heteroscedasticity in the data (whether the differences depend on the magnitude of the mean). In case of heteroscedasticity with the correlation being close to zero and the differences being normally distributed, one may proceed to calculate the limits of agreement (LOA). The process of calculating the LOA is as it follows. Firstly, the SD of the differences between test 1 and test 2 is calculated. If there is no significant systematic bias (identified by a paired t-test) then there is rationale for expressing the LOA as \pm this value. However, one of the drawbacks of the t-test that will be discussed subsequently is the fact that significant bias cannot be detected if they are accompanied by large random errors. One could quote the random error with the bias to form the LOA, even if it is not statistically significant. Expressed this way, the LOA are actually a measure of ‘total error’ which is the sum of bias and random error (Atkinson and Neville, 1998).

3.1.3 Reliability Defined in Terms of the Source of Measurement Error

Apart of the relative and absolute types of reliability, reliability is also defined in terms of the source of measurement error:

Internal consistency reliability is the variability between repeated trials within a day.

Stability reliability is defined as the day to day variability in measurements and it is the most common type of reliability analysis.

Rater reliability or objectivity is the degree to which different observers agree on the measurements. This type of reliability assessment is relevant to measurements that might be administered by different clinicians over time (Atkinson G., Nevill A.M., 1998).

3.2 Systematic Bias and Random Error

Irrespective of the type of reliability that is assessed (internal consistency, stability, objectivity) there are 2 components of variability associated with each assessment of measurement error. These are systematic bias and random error. The sum total of these components of variation is known as total error (Atkinson G., Nevill A.M., 1998).

3.2.1 Systematic Bias

Systematic bias refers to a general trend for measurements to be different in a particular direction (either positive or negative) between repeated tests (Atkinson G., Nevill A.M., 1998). These bias are predictable errors, occurring in one direction only, constant and biased (Bruton A., Conway J.H., Holgate S.T., 2002). There might be a trend for a retest to be higher than a prior test due to a learning effect being present. Bias may also be due to there being insufficient recovery between tests. In this case, a retest would show a “worse” score than a prior test. It may be that, after a large number of repeated tests, systematics bias due to training effects (if the test is physically challenging) or transient increases in motivation become apparent (Atkinson G., Nevill A.M., 1998).

3.2.2 Random Error

Random error is the second component of variability between repeated tests (Atkinson G., Nevill A.M., 1998). Random errors are due to chance and unpredictable. Hence, they are the basic concern of reliability (Bruton A., Conway J.H., Holgate S.T., 2002). Large amounts of random differences could arise due to biological or mechanical variation, or inconsistencies in the measurement protocol. Whilst such obvious sources of error as protocol variation can be controlled, the random error component is still usually larger than that due to bias. Unfortunately, researchers can do relatively little to reduce random error once the measurement tool has been purchased, especially if it is due wholly to inherent mechanical (instrument) variation (Atkinson G., Nevill A.M., 1998).

3.3 Estimation of Reliability

Although, the calculation of true reliability is not possible, reliability can be estimated, based on the statistical concept of variance, i.e. a measure of the variability of differences among scores within a sample (Bruton A., Conway J.H., and Holgate S.T., 2002). It is recommended that sports clinicians and researchers to cite and interpret a number of statistical methods for assessing reliability. Many statistical tests have been proposed in the sport science literature for the appraisal of measurement issues. The most common methods involve the use of hypothesis tests (paired t-tests, ANOVA) and/or correlation coefficients (Pearson's, intraclass correlation). Other methods cited in the literature involve regression analysis, coefficient of variation (CV) or various methods that calculate “percentage” variation (Atkinson G., Nevill A.M., 1998).

3.3.1 Bland and Altman plot

An issue in sport and exercise reliability studies is how the measurement error relates to the magnitude of the measured variable. When the amount of random error increases as the measured values increase, the data are said to be heteroscedastic (Atkinson G., Nevill A.M., 1998). Heteroscedasticity means that the individuals who score the highest values on a particular test also show the greatest amount of measurement error (in the units of measurement). It is also likely that these high-scoring individuals show the smallest changes (in the units of measurement) in response to a certain experimental intervention (Schultz R.W., 1989). When there is no relation between the error and the size of the measured value, the data is described as homoscedastic. Such characteristics of the data influence how the described error is eventually expressed and analyzed. The Bland-Altman plot has been suggested, because it shows the measurement error schematically and assists to identify the presence of heteroscedasticity. Heteroscedasticity can be examined formally by plotting the absolute differences against the individual means and calculating the correlation coefficient. In case of heteroscedasticity with the correlation being close to zero and the differences being normally distributed, one may proceed to calculate the limits of agreement (LOA) by conducting the process described before (Atkinson G., Nevill A.M., 1998).

3.3.2 Paired t-Test

The paired t-test, and analysis of variance techniques are statistical methods for detecting systematic bias between groups of data. However, they give information only about systematic bias between the means of two sets of data, not about individual differences and random variation between tests (Bruton A., Conway J.H., and Holgate S.T., 2002, Atkinson G., Nevill A.M., 1998). Therefore, such tests should not be used in isolation, but be complemented by other methods, e.g. Bland and Altman agreement tests (Bland J.M., Altman D.G., 1986). Specifically, because of the nature of the formula employed to calculate the t-value, significant systematic bias will be less likely to be detected if it is accompanied by large amounts of random error between tests. It should be noted that the correlation between test and retest may not, in all data sets, be a good indicator of the amount of absolute random error present, which is the basis of the denominator in the paired t-test equation. The use of a t-test may still be recommended in a measurement study that investigates a simple test and retest, since it will detect large systematic bias (relative to the random error), and the terms in the formula for the t-value can be used in

the calculation of measures of random error (e.g. limits of agreement) (Atkinson G., Nevill A.M., 1998).

3.3.3 Correlation Coefficients

Correlation coefficients (r) give information about the degree of association between two sets of data, or the consistency of position within the two distributions (Bruton A., Conway J.H., and Holgate S.T., 2002). However, the use of the correlation coefficient cannot on its own assess systematic bias and it depends greatly on the range of values in the sample (Atkinson G., Nevill A.M., 1998). Hence, it is possible to have two sets of scores that are highly correlated, but not highly repeatable. Correlation only tells how two sets of scores vary together and not the extent of agreement between them (Bruton A., Conway J.H., and Holgate S.T., 2002). Additionally a high correlation coefficient reflects adequate relative reliability for use of the measurement tool in particular population that has been investigated. This seems sensible, since the more homogeneous a population is, the less the measurement error would need to be in order to detect differences between individuals within that population. The Pearson's correlation coefficient has been the most common technique for assessing reliability. The idea is that if a high (>0.8) and statistically significant correlation coefficient is obtained, the equipment is deemed to be sufficiently reliable (Atkinson G., Nevill A.M., 1998).

3.3.4 ANOVA

ANOVA with repeated measures has been used for comparing more than one retest with a test. With appropriate a priori or post hoc multiple comparisons (e.g. Tukey tests), it can be used to assess systematic bias between tests. However, the sole use of ANOVA is associated with exactly the same drawback as the paired t-test in that the detection of systematic bias is affected by large random (residual) variation. It should be noted once more that a correlation coefficient (intraclass in the case of ANOVA) may not be as sensitive an indicator of this random error as an examination of the residual mean squared error itself in the ANOVA results table. As with the t-test, ANOVA is useful for detecting large systematic errors and the mean squared error term from ANOVA can be used in the calculation of indicators of absolute reliability (Atkinson G., Nevill A.M., 1998).

3.3.5 Intraclass Correlation

Intraclass correlation (ICC) methods have been a quite good choice of statistics in reliability studies and they are an attempt to overcome some of the limitations of the

classic correlation coefficients (Bruton A., Conway J.H., and Holgate S.T., 2002, Atkinson G., Nevill A.M., 1998). ICC is a single index calculated using variance estimates obtained through the partitioning of total variance into between and within subject variance (known as analysis of variance or ANOVA). Therefore, it reflects both degree of consistency and agreement among ratings (Bruton A., Conway J.H., and Holgate S.T., 2002). The most common methods of ICC are based on the terms used in the calculation of the F-value from repeated measures ANOVA. The main advantages of this statistic over Pearson's correlation are maintained to be that the ICC is univariate rather than bivariate and it can be used when more than one retest is being compared with a test. The ICC can also be calculated in such a way that it is sensitive to the presence of systematic bias in the data. However, there are at least 6 ways of calculating an ICC, all giving different results. Researchers must detail exactly how this choice is made and how an ICC has been calculated in reliability study (Atkinson G., Nevill A.M., 1998). Intraclass correlation coefficient ranges from 0 to 1, with values closer to one representing the higher reliability (Bruton A., Conway J.H., and Holgate S.T., 2002). Whatever the type of ICC that is calculated, it is suggested like Pearson's r , that ICC close to 1 indicates "excellent reliability" (Atkinson G., Nevill A.M., 1998). Although, there are various categories of agreement based on the ICC, ranging from "questionable" (0.7 to 0.8) to high (>0.9), as with other reliability coefficients, there is no standard acceptable level of reliability using the ICC (Bruton A., Conway J.H., and Holgate S.T., 2002, Atkinson G., Nevill A.M., 1998). However, Chinn recommended that any measure should have an intraclass correlation coefficient of at least 0.6 to be useful (Chinn S., 1991). Any reliability coefficient is determined as the ratio of variance between subjects to the sum of error variance and subject variance. If the variance between subjects is sufficiently high i.e. the data come from a heterogeneous sample, then reliability will inevitably appear to be high. Thus, if the ICC is applied to data from group of individuals demonstrating a wide range of the measured characteristic, reliability will appear to be higher than when applied to a group demonstrating a narrow range of the same characteristic (Bruton A., Conway J.H., and Holgate S.T., 2002).

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4. RESEARCH – THE RELIABILITY OF THE VYNTUS CPX

4.1 Objective

The objective of this study is to assess in between days reliability, within one day reliability and the effect of not calibrating in between 2 measurements on reliability of the Vyntus CPX, a new indirect calorimeter, in measuring O₂ consumption and CO₂ production to estimate RMR.

4.2 Methods

All procedures were carried out in the research center of Nutrition and Dietetics department in The Hague University of Applied Sciences (THUAS).

4.2.1 Subjects

In this study 24 healthy individuals participated with a sex ratio of one half, a wide range of BMI classes (normal weight ($18.5 < \text{BMI} \leq 25 \text{ kg/m}^2$) overweight ($25 < \text{BMI} \leq 30 \text{ kg/m}^2$) and obese ($30 < \text{BMI} \leq 35 \text{ kg/m}^2$) and a wide range of age.

Excluding criteria were: smoking, claustrophobic subjects, pregnant or breast feeding women, subjects with acute infections, chronic inflammatory disease or taking medication which could interfere with metabolic rate.

4.2.2 Protocol

Indirect calorimetry measurements were performed with an open-circuit system, the Vyntus CPX in canopy mode for 20 minutes each and included the measurement of VO₂, VCO₂ and RMR. Two different Vyntus CPX devices were studied. Sixteen individuals were measured with a Vyntus CPX device and eight individuals were measured with an other Vyntus CPX. In total 4 measurements were conducted for each individual. All the measurements took place in the morning (08:00-12:00 am) and there was a divergence of maximum 2 hours between the time of the measurements for each individual in between days to facilitate individuals' food abstention and reduce the effect of diurnal variation. Additionally, the measurements of each individual were conducted in 2 consecutive days to avoid body composition changes in the subjects that could influence the results of the research. Each measurement lasted 20 minutes in which the first 5 minutes were discarded. Each individual had a minimum rest of 10 minutes before initiating a measurement and was comfortable with the measurement position during the test. The individuals had the same body position (supine position) in all the measurements. Each day before the first

measurement the device was warmed up for at least 15 minutes, gas and volume calibrated according to the manufacturer's instructions. With regard to the second measurement on each day, 12 individuals were measured after gas and volume calibration and the other 12 individuals were measured without carrying out gas and volume calibration. This selection was performed randomized by drawing a sealed envelope containing the day in which the second measurement would be without carrying out gas and volume calibration. Aim of this process was to identify whether calibration is necessary before each measurement to improve the reliability of the Vyntus CPX in measuring O₂ consumption, CO₂ production and estimating RMR.

The participants were in fasting state, including abstention from alcohol, caffeine, nicotine, drugs and medicines for 10 hours before the measurements. Furthermore, they had a minimum abstention from moderate aerobic or anaerobic exercise for 2 hours and at least 14 hours abstention from vigorous resistance exercise prior to the measurements.

4.2.3 Anthropometric evaluation

Prior to the first RMR measurement, participants underwent measurements of body weight (± 100 gr) in light indoor clothes, without shoes and of height (± 0.5 cm) without shoes as well. Then Body Mass Index (BMI) was calculated (± 0.1 kg/m²).

4.2.4 Environmental Characteristics

The measurements took place in a quiet room with mild lighting and a temperature of 17-20 Celsius degrees.

4.2.5 Statistical Analysis

Statistical Package for the Social Sciences (SPSS for Windows, release 17) was used for the statistical analysis to assess in between days reliability, within one day reliability and the effect of not calibrating on reliability of Vyntus CPX in measuring O₂ consumption, CO₂ production and RMR. The assumptions for reliable analysis underlying the test for normally distributed data and homoscedasticity. Normality was examined using the Skewness and kurtosis z-values, Shapiro-Wilk normality test, histograms, normal Q-Q plots and Box plots for the variables VO₂, VCO₂, RMR. The non-parametric Wilcoxon test was conducted for analysing differences between measurements of VO₂, VCO₂ and RMR. Test-retest reliability was calculated using Intraclass correlation coefficients (r) for VO₂, VCO₂ and RMR. The differences and the means were calculated for each of the three variables and the Bland and Altman plot was used to show the bias and the limits of

agreement schematically. Homoscedasticity was examined by plotting the absolute differences against the individual means and calculating the correlation coefficient. Standard error of measurement (SEM) was calculated with this equation: $SEM = SD \cdot \sqrt{1 - r}$, where SD is the standard deviation of $\dot{V}O_2$, $\dot{V}CO_2$ and RMR and r is the calculated intraclass correlation coefficient respectively (Atkinson G., Nevill A.M., 1998).

5. RESULTS

Twenty four individuals (12 males and 12 females) completed the measurements. Subject characteristics are reported in Table 5.1, with data summarized for age, height, weight and body mass index (BMI). Age ranged from 19 to 57 years and BMI ranged from 18.4 kg/m² to 36.5 kg/m² with 17 subjects having a BMI < 25 kg/m², 4 subjects a BMI of 25 to 29.9 kg/m² and 3 subjects a BMI ≥ 30kg/m².

Table 5.1: Subject Characteristics for Males (n=12) and Females (n=12) Subjects (mean ± SD)

	Unit	Total Group	Males	Females
Age	Year	28.5 ± 11.3	22.9 ± 3.1	34.2 ± 13.7
Weight	Kg	71.1 ± 12.6	76.0 ± 10.7	66.1 ± 12.7
Height	Cm	171.8 ± 10.1	178.9 ± 7.2	164.7 ± 7.1
BMI	Kg/m ²	23.8 ± 4.1	23.4 ± 2.4	24.2 ± 5.4

O₂ consumption, CO₂ production and RMR data of the 2 tests are presented in Table 5.2. Test 1 of both days was used for the assessment of between days reliability.

Table 5.2: Comparison of oxygen consumption (VO₂), carbon dioxide production (VCO₂) and resting metabolic rate (RMR) values between 2 tests in 2 consecutive days (n=24 subjects)

	Day 1	Day 2	p-value	r	Limits of Agreement	SEM
	Test 1	Test 1				
	Mean ± SD	Mean ± SD				
VO ₂ (mL·min ⁻¹)	207 ± 55	213 ± 47	0.88	0.78	67	26
VCO ₂ (mL·min ⁻¹)	178 ± 53	183 ± 41	0.77	0.75	66	27
RMR (kcal·day ⁻¹)	1428 ± 390	1466 ± 330	0.91	0.78	476	183

Mean differences for VO₂, VCO₂ and RMR were 6 mL·m⁻¹, 5 mL·m⁻¹ and 38 kcal·day⁻¹ respectively. The variables were not normally distributed and t-tests could not be conducted. Thus, the non – parametric Wilcoxon test was conducted for analysing differences between measurements of VO₂, VCO₂ and RMR. Wilcoxon test indicated not statistically significant mean differences in between days for VO₂ (p-value = 0.88), VCO₂ (p-value = 0.77) and RMR (p-value = 0.91). The intraclass correlation coefficient of VO₂ was r = 0.78, for VCO₂ was r = 0.75 and for RMR r = 0.78. The correlation between VO₂,

VCO₂ and RMR of the first tests on day 1 and day 2 is showed in graphs (Figure 5.1, 5.2 and 5.3). Bland and Altman plot showed high limits of agreement for VO₂ and VCO₂ ($\pm 67\text{mL}\cdot\text{m}^{-1}$, $\pm 66\text{mL}\cdot\text{m}^{-1}$ respectively) (Figure 5.4 and 5.5). Similarly, Bland and Altman plot indicated high limits of agreement for RMR ($\pm 476\text{kcal}/\text{day}$) (Figure 5.6). In addition, Bland and Altman plots showed that there is no presence of heteroscedasticity. The standard error of measurement between days for each variable VO₂, VCO₂ and RMR was SEM = $26\text{ mL}\cdot\text{min}^{-1}$, $27\text{ mL}\cdot\text{min}^{-1}$ and $183\text{ kcal}\cdot\text{day}^{-1}$ respectively.

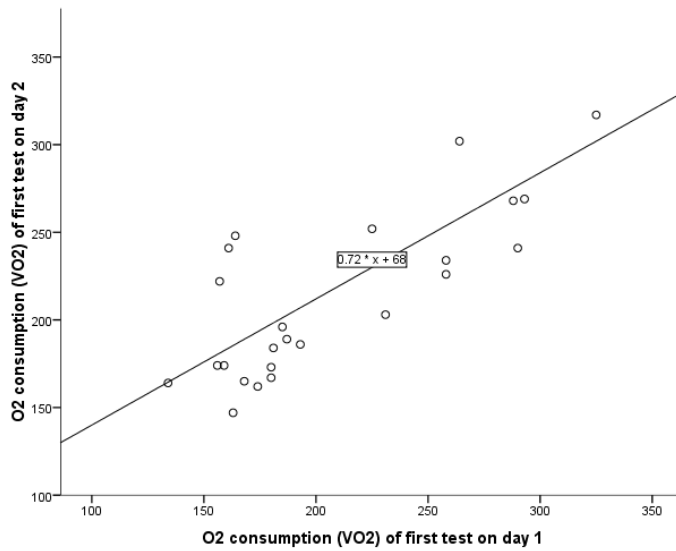


Figure 5.1: Graph showing the correlation between oxygen consumption (VO₂) values of first tests on day 1 and day 2

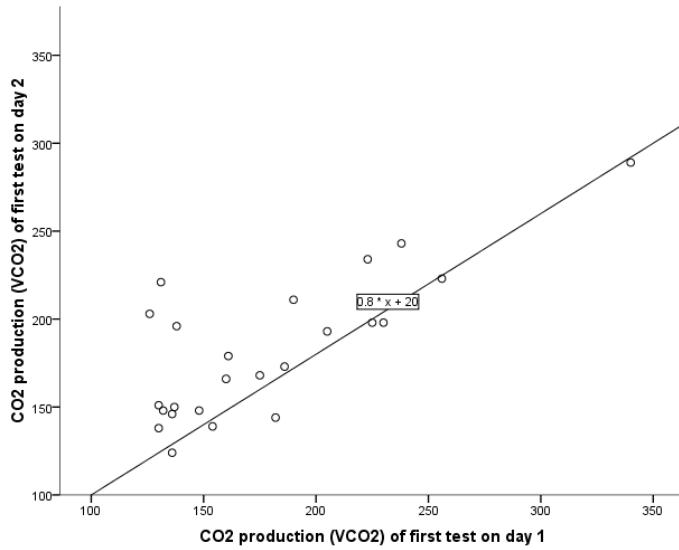


Figure 5.2: Graph showing the correlation between carbon dioxide production (VCO₂) values of first tests on day 1 and day 2

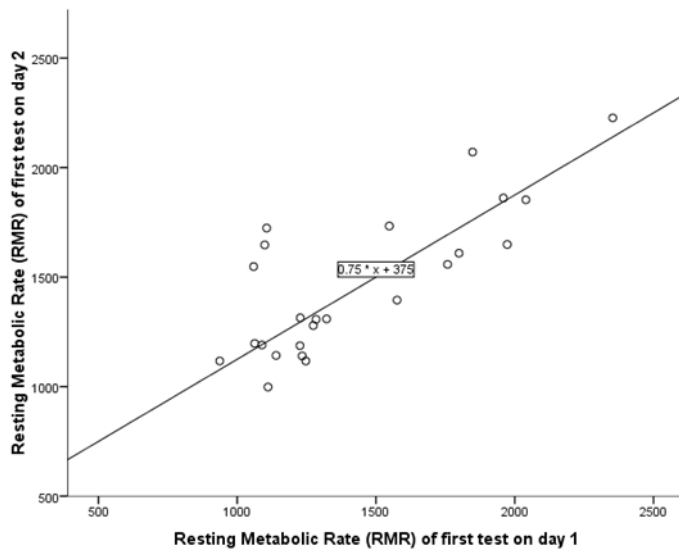


Figure 5.3: Graph showing the correlation between resting metabolic rate (RMR) values of first tests on day 1 and day 2

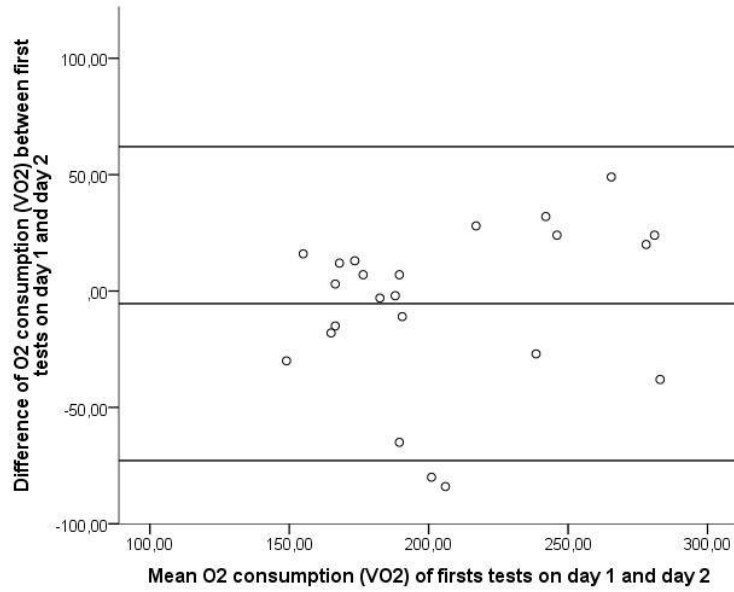


Figure 5.4: Bland-Altman plot depicting differences of oxygen consumption (VO₂) values of first tests on day 1 and day 2 versus mean VO₂ values (n=24) and limits of agreement for VO₂

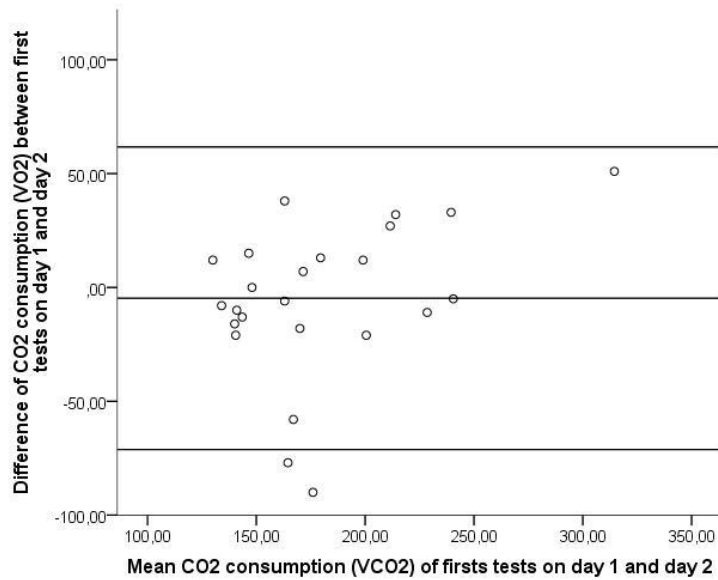


Figure 5.5: Bland-Altman plot depicting differences of carbon dioxide production (VCO₂) values of first tests on day 1 and day 2 versus mean VCO₂ values (n=24) and limits of agreement for VCO₂

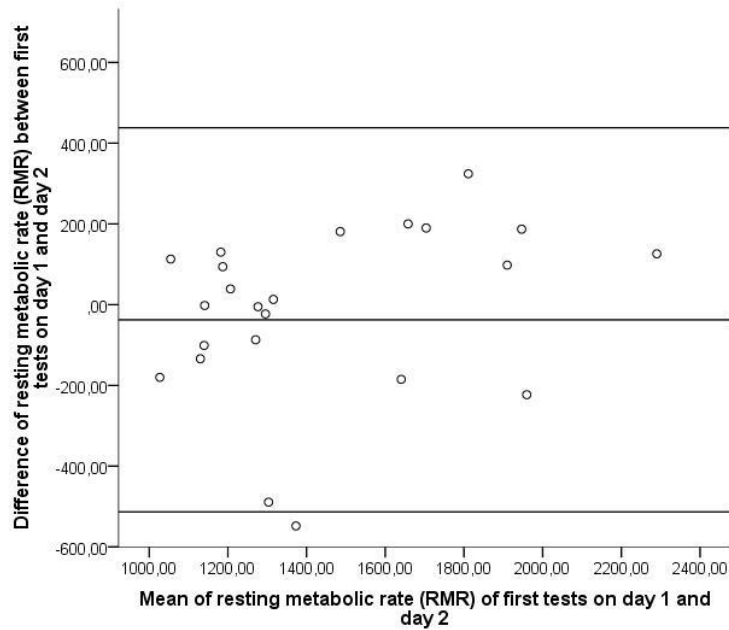


Figure 5.6: Bland-Altman plot depicting differences of resting metabolic rate (RMR) values of first tests on day 1 and day 2 versus mean RMR values (n=24) and limits of agreement for RMR

Table 5.3 summarizes O_2 consumption, CO_2 production and RMR values for the assessment in within day reliability. Day A was used to assess within one day reliability.

Table 5.3: Comparison of oxygen consumption (VO_2), carbon dioxide production (VCO_2) and resting metabolic rate (RMR) values between 2 tests within the same day (n=24 subjects)

	Day A		p-value	r	Limits of Agreement	SEM
	Test 1	Test 2				
	Mean \pm SD					
VO_2 ($mL \cdot min^{-1}$)	212 \pm 54	208 \pm 53	0.69	0.82	64	23
VCO_2 ($mL \cdot min^{-1}$)	182 \pm 46	178 \pm 43	0.42	0.83	51	19
RMR ($kcal \cdot day^{-1}$)	1459 \pm 379	1432 \pm 366	0.61	0.83	435	156

Day A = the day which both tests were calibrated

Mean differences for VO_2 , VCO_2 and RMR were $4 mL \cdot min^{-1}$, $4 mL \cdot min^{-1}$ and $27 kcal \cdot day^{-1}$ respectively. No statistical differences were indicated for VO_2 (p-value = 0.69), VCO_2 production (p-value = 0.42) and RMR (p-value = 0.61). The intraclass correlation coefficient of VO_2 was $r = 0.82$, of VCO_2 was $r = 0.83$ and for RMR was $r = 0.83$. The

correlation between VO_2 , VCO_2 and RMR of test 1 and test 2 on day A is showed in graphs, respectively (Figure 5.7, 5.8 and 5.9). Bland and Altman plot was conducted by the difference and the mean of VO_2 and VCO_2 within the same day and showed high limits of agreement ($\pm 64\text{mL}\cdot\text{m}^{-1}$ and $\pm 51\text{mL}\cdot\text{m}^{-1}$ respectively) (Figure 5.10 and 5.11). Similarly, Bland and Altman plot was conducted by the difference and the mean of RMR indicating high limits of agreement ($\pm 435 \text{ kcal/day}$) (Figure 5.12). Additionally, Bland and Altman plots showed there is no presence of heteroscedasticity. The standard error of measurement for each parameter VO_2 , VCO_2 and RMR was $\text{SEM} = 23 \text{ mL}\cdot\text{min}^{-1}$, $19 \text{ mL}\cdot\text{min}^{-1}$ and $156 \text{ kcal}\cdot\text{day}^{-1}$ respectively.

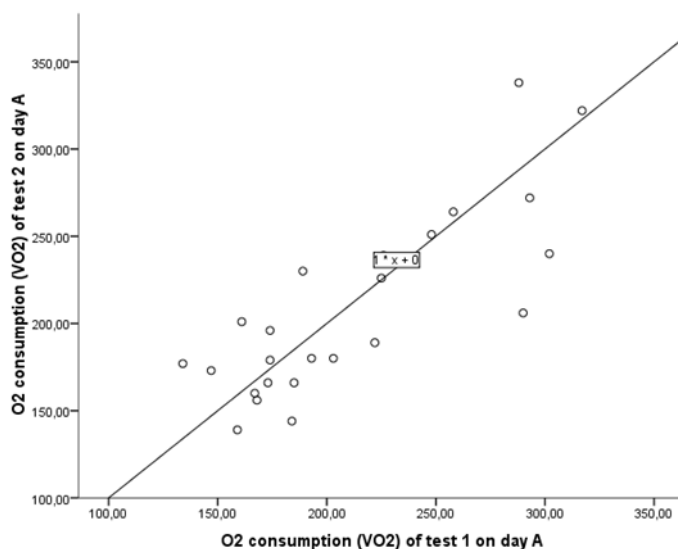


Figure 5.7: Graph showing the correlation between oxygen consumption (VO_2) values of test 1 and test 2 on day A

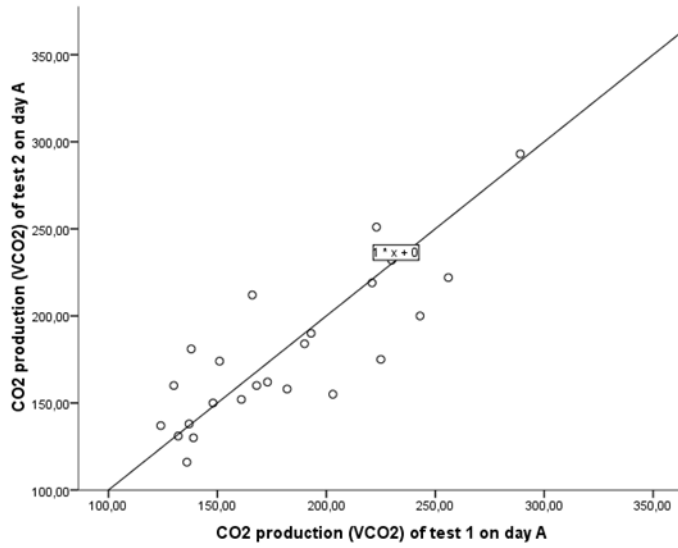


Figure 5.8: Graph showing the correlation between carbon dioxide production (VCO₂) values of test 1 and test 2 on day A

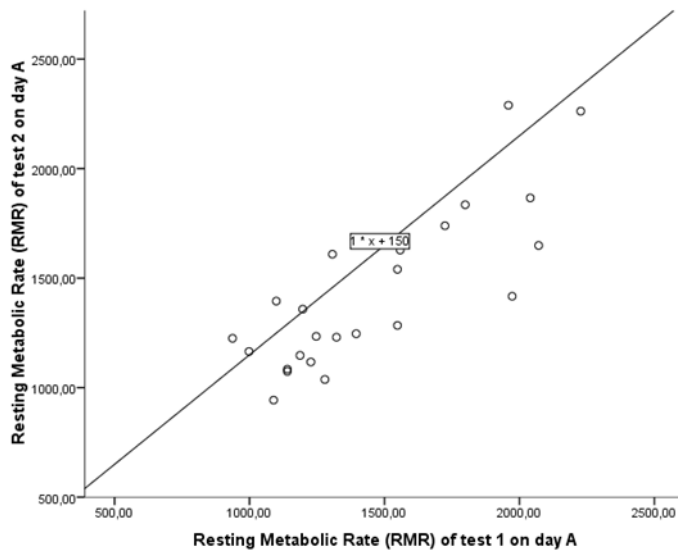


Figure 5.9: Graph showing the correlation between resting metabolic rate (RMR) values of test 1 and test 2 on day A

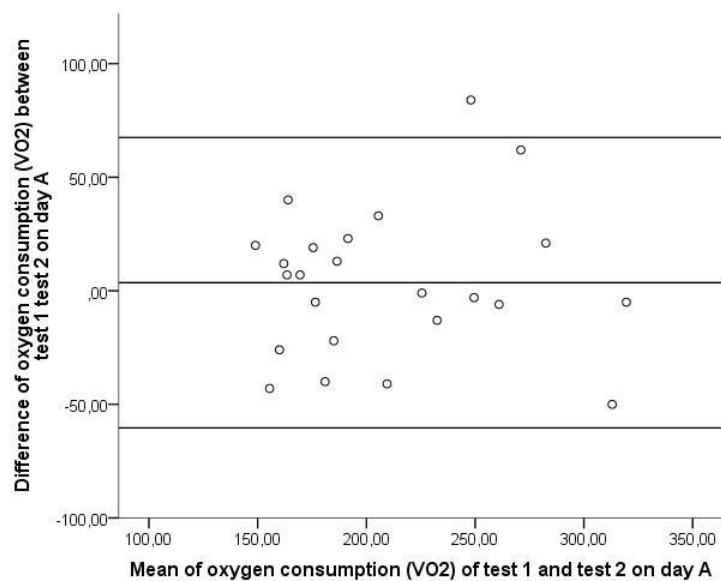


Figure 5.10: Bland-Altman plot depicting differences of oxygen consumption (VO₂) values of test 1 and test 2 on day A versus mean VO₂ values (n=24) and limits of agreement for VO₂

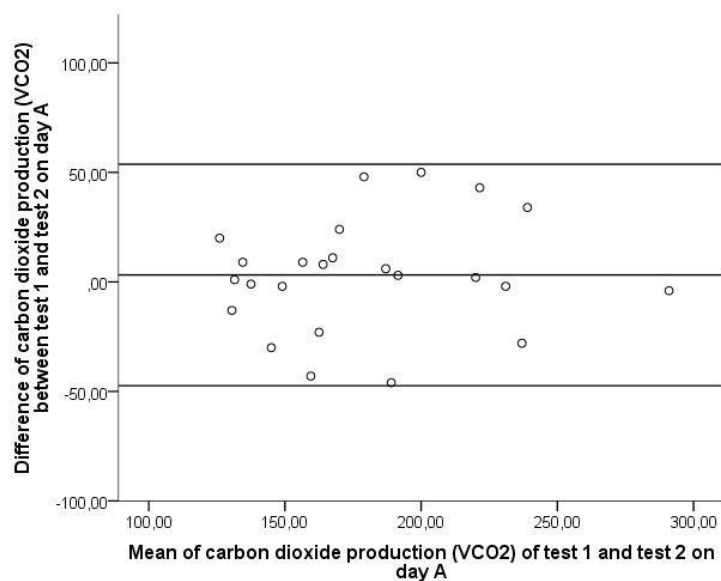


Figure 5.11: Bland-Altman plot depicting differences of carbon dioxide production (VCO₂) values of test 1 and test 2 on day A versus mean VCO₂ values (n=24) and limits of agreement for VCO₂

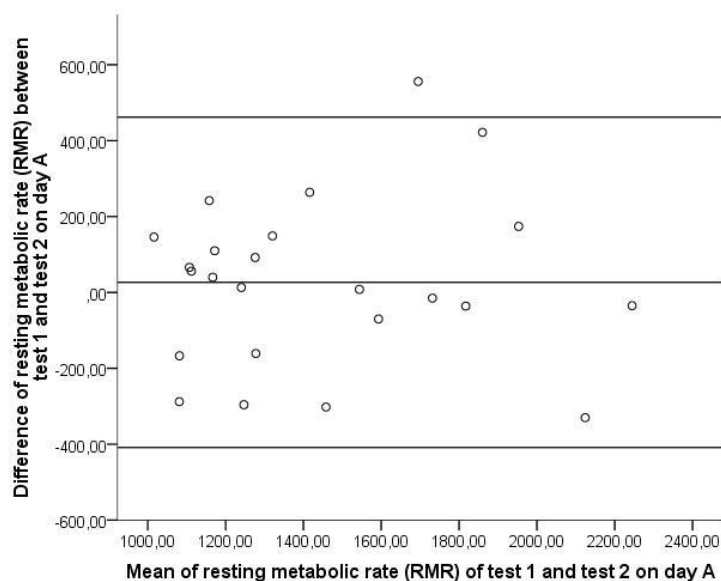


Figure 5.12: Bland-Altman plot depicting differences of resting metabolic rate (RMR) values of test 1 and test 2 on day A versus mean RMR values (n=24) and limits of agreement for RMR

Table 5.4 summarizes O_2 consumption, CO_2 production and RMR values for the assessment of the effect of not calibrating on reliability. Day B was used for the assessment of the effect of not calibrating on reliability.

Table 5.4: Comparison of oxygen consumption (VO_2), carbon dioxide production (VCO_2) and resting metabolic rate (RMR) values between the 2 with and without calibration tests within the same day (n=24 subjects)

	Day B		p-value	r	Limits of Agreement	SEM
	Test 1	Test 2				
	Mean \pm SD					
VO_2 ($mL \cdot min^{-1}$)	208 \pm 47	209 \pm 52	0.76	0.81	61	23
VCO_2 ($mL \cdot min^{-1}$)	179 \pm 49	186 \pm 43	0.52	0.76	63	24
RMR ($kcal \cdot day^{-1}$)	1435 \pm 343	1448 \pm 353	1.00	0.82	417	146

Day B = the day which the test 1 was calibrated and the test 2 was not calibrated

Mean differences for VO_2 , VCO_2 and RMR were $1 mL \cdot min^{-1}$, $7 mL \cdot min^{-1}$ and $13 kcal \cdot day^{-1}$ respectively. There were not statistically significant mean differences for VO_2 (p-value = 0.76), VCO_2 (p-value = 0.52) and RMR (p-value = 1.00). Intraclass correlation coefficient of VO_2 was $r = 0.81$ and of VCO_2 $r = 0.76$. The correlation between VO_2 , VCO_2 and RMR

of test 1 and test 2 on day B is showed in graphs, respectively (Figure 5.13, 5.14 and 5.15). Similarly correlation coefficient of RMR was calculated $r = 0.82$. Bland and Altman plot was conducted by the difference and the mean of VO_2 and VCO_2 and showed high limits of agreement ($\pm 61\text{mL}\cdot\text{m}^{-1}$ and $\pm 63\text{mL}\cdot\text{m}^{-1}$ respectively) (Figure 5.16 and 5.17). Similarly, Bland and Altman plot was conducted by the difference and the mean of RMR and indicated high limits of agreement ($\pm 417\text{kcal}/\text{day}$) (Figure 5.18). Bland and Altman plots also showed there is no presence of heteroscedasticity. The standard error of measurement for each variable VO_2 , VCO_2 and RMR was $\text{SEM} = 23 \text{ mL}\cdot\text{min}^{-1}$, $24 \text{ mL}\cdot\text{min}^{-1}$ and $146 \text{ kcal}\cdot\text{day}^{-1}$ respectively.

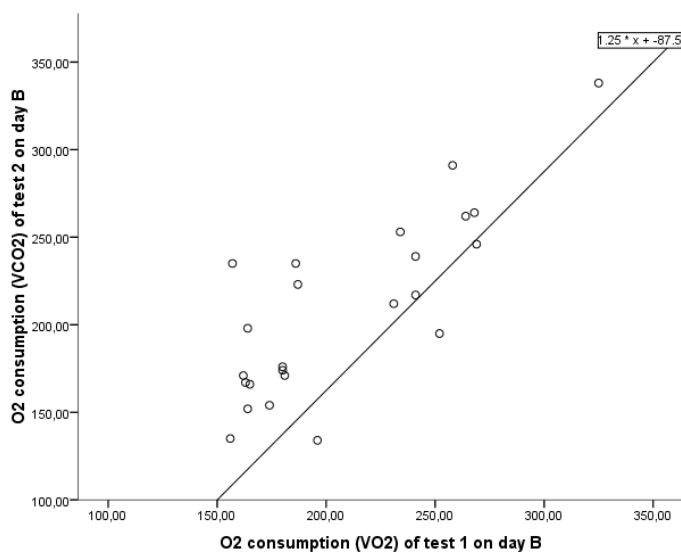


Figure 4.13: Graph showing the correlation between oxygen consumption (VO_2) values of test 1 and test 2 on day B

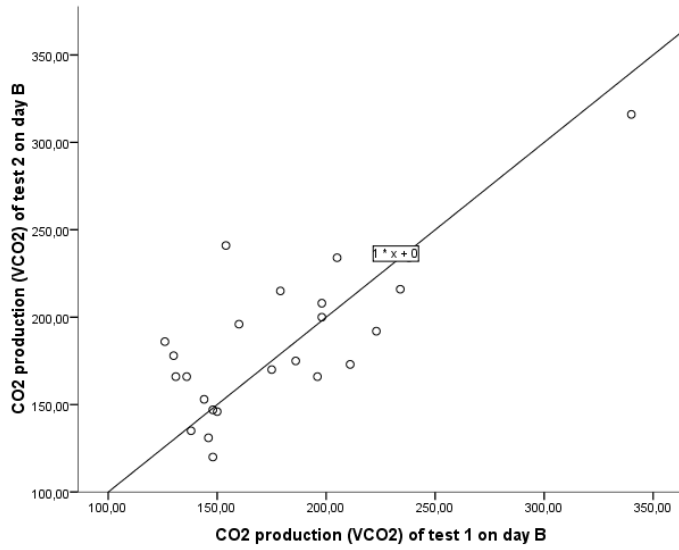


Figure 5.14: Graph showing the correlation between carbon dioxide production (VCO₂) values of test 1 and test 2 on day B

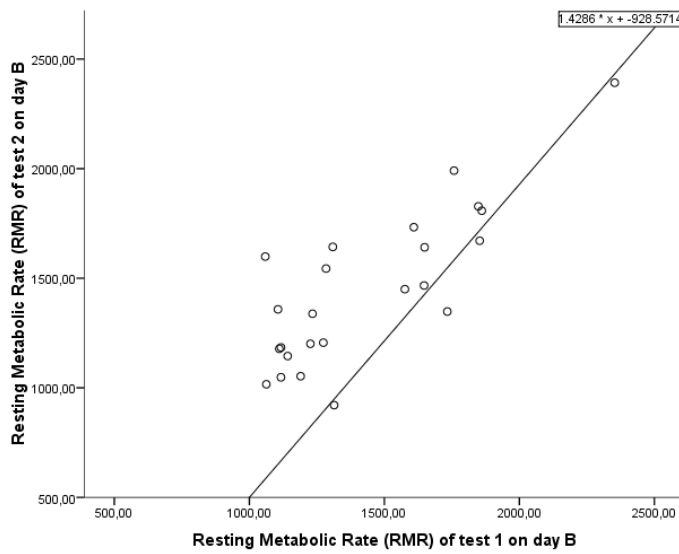


Figure 5.15: Graph showing the correlation between resting metabolic rate (RMR) values of test 1 and test 2 on day B

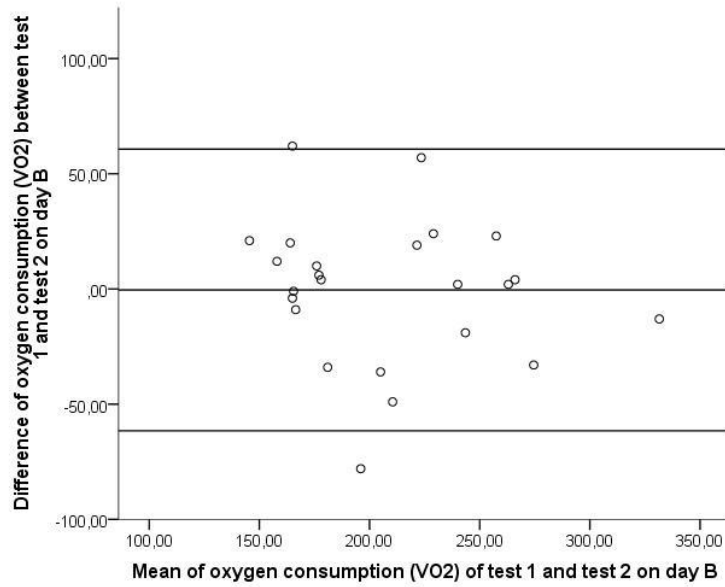


Figure 5.16: Bland-Altman plot depicting differences of oxygen consumption (VO_2) values of test 1 and test 2 on day B versus mean VO_2 values (n=24) and limits of agreement for VO_2

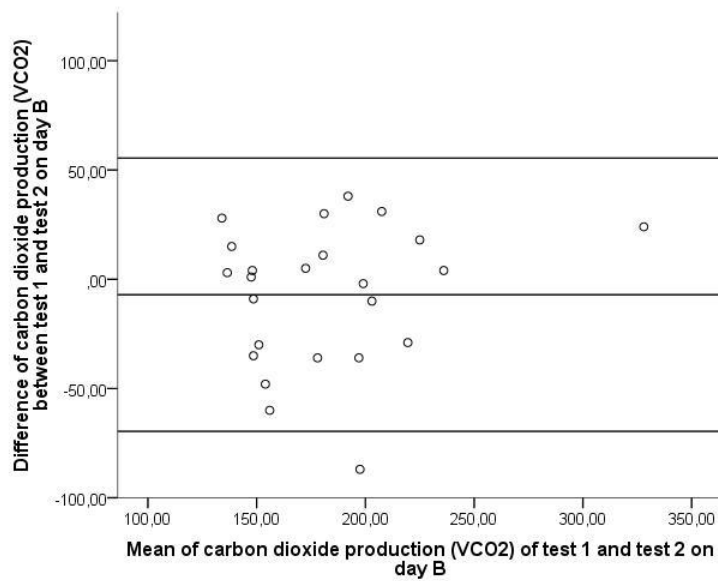


Figure 5.17: Figure 4: Bland-Altman plot depicting differences of carbon dioxide production (VCO_2) values of test 1 and test 2 on day B versus mean VCO_2 values (n=24) and limits of agreement for VCO_2

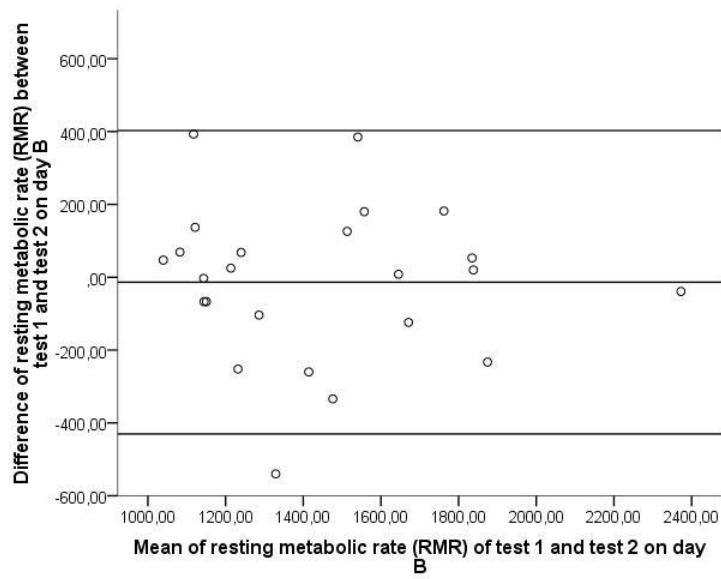


Figure 5.18: Bland-Altman plot depicting differences of resting metabolic rate (RMR) values of test 1 and test 2 on day B versus mean RMR values (n=24) and limits of agreement for RMR

Considering possible differences between the two devices we also assessed the reliability of each device individually. P-values for differences, intraclass correlation coefficients, limits of agreement and SEM for both devices. The results are summarized in the Table 5.5, 5.6 and 5.7.

Table 5.5: Comparison of oxygen consumption (VO₂), dioxide production (VCO₂) and resting metabolic rate (RMR) between the 2 Vyntus CPX devices (n=24) for the assesment of between days reliability

	Test 1 on day 1	Test 1 on day 2	p-value	r	Limits of agreement	SEM
VO₂	Mean ± SD					
Vyntus CPX 1	192 ± 44	190 ± 31	0.45	0.69	± 60	24
Vyntus CPX 2	238 ± 64	258 ± 41	0.21	0.72	± 76	34
VCO₂						
Vyntus CPX 1	162 ± 34	164 ± 27	0.84	0.55	± 58	23
Vyntus CPX 2	209 ± 71	221 ± 37	0.33	0.73	± 83	37
RMR						
Vyntus CPX 1	1318 ± 298	1308 ± 219	0.41	0.67	± 424	171
Vyntus CPX 2	1649 ± 475	1781 ± 291	0.26	0.73	± 547	247

Table 5.6: Comparison of oxygen consumption (VO₂), dioxide production (VCO₂) and resting metabolic rate (RMR) between the 2 Vyntus CPX devices (n=24) for the assesment of within one day reliability

	Test 1 on day A	Test 2 on day A	p-value	r	Limits of agreement	SEM
VO₂	Mean ± SD					
Vyntus CPX 1	193 ± 43	186 ± 37	0.42	0.73	± 57	22
Vyntus CPX 2	250 ± 58	252 ± 54	0.67	0.77	± 78	28
VCO₂						
Vyntus CPX 1	166 ± 35	162 ± 31	0.31	0.85	± 36	14
Vyntus CPX 2	214 ± 49	212 ± 44	0.83	0.70	± 74	27
RMR						
Vyntus CPX 1	1326 ± 294	1280 ± 256	0.41	0.76	± 376	144
Vyntus CPX 2	1725 ± 406	1737 ± 375	0.78	0.76	± 557	199

Table 5.7: Comparison of oxygen consumption (VO₂), dioxide production (VCO₂) and resting metabolic rate (RMR) between the 2 Vyntus CPX devices (n=24) for the assesment of not calibrating on reliability

	Test 1 on day B	Test 2 on day B	p-value	r	Limits of agreement	SEM
VO₂	Mean ± SD					
Vyntus CPX 1	190 ± 33	189 ± 45	0.76	0.79	± 52	15
Vyntus CPX 2	245 ± 52	248 ± 43	0.89	0.66	± 81	30
VCO₂						
Vyntus CPX 1	160 ± 26	174 ± 37	0.15	0.54	± 57	18
Vyntus CPX 2	216 ± 63	210 ± 48	0.58	0.82	± 70	27
RMR						
Vyntus CPX 1	1300 ± 223	1319 ± 299	0.80	0.79	± 343	102
Vyntus CPX 2	1705 ± 394	1707 ± 321	0.78	0.71	± 563	212

VO₂, VCO₂ and RMR measured with the Vyntus CPX 1 were lower than measured with the Vyntus CPX 2, due to difference in gender (71% female for Vyntus CPX 1 and 75% male for Vyntus CPX 2). Regarding the reliability, devices were comparable.

6. DISCUSSION

This is the first study assessing the reliability of the Vyntus CPX in measuring O₂ consumption and CO₂ production to estimate RMR in healthy individuals. Three different reliability tests were conducted, one for the assessment of reliability in between days, one for the assessment of reliability within one day and one to assess the effect of not calibrating on reliability. Mean differences of VO₂, VCO₂ and RMR were small and there was no statistically significant difference in between days and within the same day. The intraclass correlation coefficients indicated questionable correlation coefficients (0.7 to 0.8) of VO₂, VCO₂ and RMR in between days, but good correlation coefficients for the three variables within the same day (Bruton A., Conway J.H., and Holgate S.T., 2002, Atkinson G., Nevill A.M., 1998). However, the limits of agreement showed wide variation for the three variables between the measurements in between days and within the same day. Furthermore, high standards errors of measurement were indicated for the three variables in between days and within the same day. Similar results were found for VO₂, VCO₂ and RMR between the calibrated tests within the same day and between the calibrated tests and the not calibrated tests within the same day. However, the use of the SEM requires a normally distributed population and the SEM of this study has to be interpreted with caution (Atkinson G., Nevill A.M., 1998).

Similar results were reported in studies assessing the reliability of different indirect calorimeters in measuring O₂ consumption and CO₂ production to estimate RMR in healthy individuals and mechanically ventilated patients. Cooper et al. calculated the coefficient of variation (CV) for repeated measurements in three indirect calorimeters including MedGraphics CPX Ultima, Vmax Encore 29 System and the TrueOne 2400. In this study they showed high CV of RMR for the three devices. This study indicated that none of these devices can be considered adequately reliable for use in a research setting. However, in this study there were some site-to-site variations in study protocol and subject characteristics (Cooper J.A., et al., 2009). Allingstrup et al. compared the CCM Express and the Quark RMR to a gold standard in mechanically ventilated patients. They also calculated the CV for repeated measurements in the CCM Express and the Quark RMR and they indicated high CV for O₂. The results of this study were obtained by hemodynamic, respiratory and metabolically stable, mechanically ventilated patients and indicated low reliability of the CCM Express and the Quark in measuring O₂ and estimating RMR in mechanically ventilated patients (Allingstrup M.J., et al., 2016).

There are several strengths in this study. Twenty four individuals with a sex ratio of one half, a wide range of age and BMI were measured. The measurements were conducted in the morning under the same standardized protocol for all the individuals including fasting state, abstention from alcohol, caffeine, nicotine, drugs, medicines, exercise for a couple of hours before the measurements and a resting period before initiating an RMR measurement. Furthermore, the individuals had the same body position (supine position) in all the measurements. All the measurements were conducted in a quiet room, including small constant range of temperature and mild lighting. Each individual was measured twice in 2 consecutive days to avoid body composition changes in the subjects that could affect the results of this study with a divergence of maximum 2 hours between the time of the measurements for each individual in between days to reduce the effect of diurnal variation. Additionally, the first 5 minutes of each measurement were discarded each time, because some individuals may do not feel comfortable and relaxed enough at the beginning of the measurement. However, with any study, certain limitations exist. Although we gave clear instructions about the protocol the individuals should follow before the measurements, the observance of the protocol could not be assured. This could affect the reliability in between days. Additionally, the measurements were carried out in a room with mechanical ventilation, while humidity was not measured in this study. These factors could possibly affect our results.

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7. CONCLUSION

There is a high evidence that available and studied indirect calorimeters are not reliable and valid in measuring O_2 consumption and CO_2 production to estimate resting metabolic rate (RMR) in healthy individuals. Thus, a new indirect calorimeter is needed in order to provide reliable and valid RMR measurements. We assessed for the first time the reliability of the new indirect calorimeter, Vyntus CPX in measuring O_2 consumption and CO_2 production to estimate RMR in healthy individuals. In fact, we assessed in between days reliability, within one day reliability and the effect of not calibrating on reliability of the Vyntus CPX in measuring O_2 consumption and CO_2 production to estimate RMR. Similar means for VO_2 , VCO_2 and RMR, but high limits of agreement and high standard error of the measurement were showed for the three variables in the three reliability tests. This indicates limited repeat in measuring O_2 consumption, CO_2 production and estimating RMR in between days and within the same day in healthy individuals. However, as expected, the higher reliability appears on measurements conducted within the same day. At last, our results do not show an effect of calibration before each measurement indicating that calibration before each measurement is not beneficial in providing more reliable VO_2 , VCO_2 and RMR results, although it is still preferable to calibrate between each measurement.

8. APPENDIX

8.1 Construction of a Bland and Altman plot in SPSS

Bland and Altman plot is a graphical method used to compare 2 measures. It plots the differences between the 2 measurers against the averages between the 2 measures. It is a method to quantify agreement between two quantitative measurements by constructing limits of agreement. These statistical limits are calculated by using the mean and the standard deviation of the differences between two measurements (Giavarina D., 2015). The steps to construct a Bland and Altman plot in SPSS are the following:

1. Compute two new variables. The first one represents the difference between the 2 measures (A-B) and the second one represents the average between the 2 measures $((A+B)/2)$.
2. Calculate the mean and the standard deviation of the differences.
3. The limits of agreement (LoA) can be calculated:
4. Upper LoA = Std. deviation \cdot 1.96 + Mean of the differences
5. Lower LoA = Std. deviation \cdot (-1.96) + Mean of the differences
6. The resulting graph is a scatter plot XY, in which the Y axis shows the difference between the two paired measurements (A-B) and the X axis represents the average of these measures $((A+B)/2)$. In other words, the difference of the two paired measurements is plotted against the mean of the measurements (Giavarina D., 2015). Finally, the limits of agreement can be added at Y axis.

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