

Silva, A. M. O., Santos, D. A., Matias, C. N., Judice, P. B., Magalhães, J. P. P., Ekelund, U., Sardinha, L. B. (2015). Accuracy of a combined heart rate and motion sensor for assessing energy expenditure in free-living adults during a double-blind crossover caffeine trial using doubly labeled water as the reference method. *European Journal of Clinical Nutrition*, 69, 20-27.

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**Accuracy of a Combined Heart Rate and Motion Sensor for Assessing Energy
Expenditure in Free-Living Adults during a Double-blind Crossover Caffeine Trial
using Doubly Labeled Water as the Reference Method**

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Running Title: **Motion sensor validity under a caffeine trial**

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Abstract

Background/Objectives: A combined heart rate (HR) and motion sensor (Actiheart®) has been proposed as an accurate method for assessing total (TEE) and physical activity energy expenditure (PAEE). However the extent to which factors such as caffeine may affect the accuracy by which the estimated HR-related PAEE contribution will affect TEE and PAEE estimates is unknown. Therefore, we examined the validity of Actiheart® in estimating TEE and PAEE in free-living adults under a caffeine trial compared with doubly labeled water (DLW) as criterion.

Subjects/Methods: Using a double-blind crossover trial (ClinicalTrials.govID: #NCT01477294) with two conditions (4-day each with a 3-day washout period) randomly ordered as caffeine (5mg/kg/day) and placebo (malt-dextrine) intake, TEE was measured by DLW in 17 physically active males (20-38 years), non-caffeine users. In each condition, resting energy expenditure (REE) was assessed by indirect calorimetry and PAEE calculated as $[TEE - (REE + 0.1TEE)]$. Simultaneously, PAEE and TEE were estimated by Actiheart® using an individual calibration ($ACC + HR_{step}$).

Results: Under caffeine, $ACC + HR_{step}$ explained 76% and 64% of TEE and PAEE from DLW, respectively; corresponding results for the placebo condition were 82% and 66%. No mean bias was found between $ACC + HR_{step}$ and DLW for TEE (caffeine:468 kJ/day; placebo:407 kJ/day) though PAEE was slightly underestimated (caffeine:-856 kJ/day; placebo:-1150 kJ/day). Similar limits of agreement were observed in both conditions ranging from -2066 to 3002 and from -3488 to 1776 kJ/day for TEE and PAEE.

Conclusions: Regardless of caffeine intake, the combined HR and motion sensor is valid for estimating free-living energy expenditure in a group of healthy males but is less accurate for an individual assessment.

Key Words: Physical Activity; Indirect calorimetry; Heart rate monitoring, Accelerometry; Methods

Introduction

Adequately measuring physical activity (PA) is important for determining trends in PA levels over time, for evaluating the effect of PA interventions and for determining PA health benefits. Poor measurement of PA may hinder detection of important associations or effects. In fact energy expenditure resulting from PA (PAEE) may differ substantially and an accurate assessment is determinant to established dose-response relationship with health outcomes. Indeed, higher levels of PAEE have been reported to decrease the risk of all-cause mortality in elderly people¹, blood pressure in adults², abdominal obesity³, and risk of weight gain^{4,5}. Nevertheless, the amount of PAEE required for disease prevention and health promotion remains unclear given the intrinsic difficulties in assessing the intensity, duration, and frequency of all types of activities during free-living condition in large population studies⁶.

The doubly labeled water (DLW) method is the most accurate method for measuring total energy expenditure (TEE) in free-living conditions. Unfortunately, this method is expensive and requires a specialized laboratory for sample analysis. Indeed the DLW technique is a stable isotope technique used for determining human energy metabolism of healthy and clinical populations through biological markers⁷. Combined with the measurement of resting energy expenditure (REE) this technique is considered the criterion measure of PAEE in free-living individuals. Due to the confinement and intrusiveness of direct calorimetry and the cost of DLW, objective methods such as heart rate (HR) monitoring and accelerometers, have been developed to estimate TEE and PAEE. While the latter has been shown to considerably underestimate PA given the accelerometers limitations in capturing several activities⁸ the evaluation of PA based on HR relies on a linear HR and physical activity intensity (PAI) relationship. This relationship is known to vary according to age, sex, and fitness level and an individual calibration is required⁹. A new generation of PA motion sensors that combines an

accelerometer with a HR monitor has been suggested by many to offer greater measurement validity of PA than either method used in isolation⁹⁻¹⁴. So far only two studies assessed the validity of a commercially available device (Actiheart, CamNtech Limited, UK). Using participants of rural and urban areas Assah et al¹⁴ found no significant mean bias between PAEE estimated from the combined monitor or measured by DLW in free-living conditions. Recently, Villars et al¹⁰ observed a good level of agreement between the Actiheart® estimates and DLW-measured PAEE in lean and overweight men with varying fitness levels, including the changes in free-living PAEE in response to an exercise intervention.

While combining HR monitoring to movement registration improves the validity of PAEE estimates, difficulties in accurately evaluate HR may also compromise PAEE assessment. For instances, the previous mentioned linear HR-PAI relationship may also be dependent on the use of ergogenic substances, such as caffeine, that is proposed to act by increasing sympathomimetic activity (and thus heart rate)¹⁵. As a result, the advantage of obtaining PAEE by adding HR measures to a motion sensor may be also dependent on the accuracy by which the estimated HR-related PAEE contribution is independent of expected increases in sympathomimetic activity. Hence, the validity of this combined monitor in accurately assessing TEE and PAEE under the effect of a moderate dose of caffeine intake in non-regular users is still unknown.

The aim of this study was to analyze the validity of the combined monitor in estimating TEE and PAEE in free-living male adults during a double-blind crossover caffeine trial compared with doubly labeled water (DLW) as the reference method.

Methods

Participants

A total of 30 healthy non-smoking males, recruited through advertisements around the institution, volunteered to participate in this study, as described in the Consort flow diagram¹⁶ (**Figure 1**).

Participants were non-athletes and were engaged in daily general fitness activities up to 2 h. Inclusion criteria were: age 20-39 years; body mass index (BMI) 18.5-29.9 kg/m²; not taking any medication or dietary supplement; physically active, defined as ≥ 30 minutes/day of moderate to vigorous PA, according to the recommendations of the World Health Organization and corresponding policy actions for the promotion of PA¹⁷; and low-caffeine users (<100 mg/day)¹⁸. The daily consumption of caffeine was estimated based on a 7-day self-report of the daily intakes of coffee, tea, caffeinated sodas, chocolate, and other dietary sources, according to a list provided by two sources^{19, 20}. All participants were informed about the possible risks of the investigation before giving their written informed consent to participate. All procedures were approved by the ethics Committee of the Faculty of Human Kinetics, Technical University of Lisbon, and were conducted in accordance with the declaration of Helsinki for human studies²¹.

Figure-1

Experimental Design

Participants were enrolled in a double-blind crossover experiment with two conditions administered in random order: caffeine (5 mg/kg/day) and malt-dextrin as placebo, both through capsules.

Caffeine/placebo conditions were randomized by an automated computer-generated randomization scheme and assigned to specific study days. Staff involved on data collection and capsules deliver as well as participants and were blinded to the condition allocation. A laboratorial technician, responsible

for preparation of the doses, was the only person aware of the randomization code during the trial. Each condition lasted for 4 days and participants were instructed to keep the same eating patterns and level of PA. There was a washout period of 3 days between each condition²². Moreover, to reduce the variability of individual PA patterns during the week, both conditions were performed on the same weekdays while the washout period always included the weekend days. Evaluations occurred from January to March 2011 and were performed at 3 time points: 1) first visit: baseline data collection; 2) second visit: 4 days after baseline, for collecting the final measurements of the first randomly assigned condition (placebo or caffeine); and 3) third visit: 7 days after the end of the second condition, including the 3-day washout period, for collecting the final measurements of the second randomly assigned condition (placebo or caffeine).

Participants were required to fast for at least 12 h prior to each visit, refrain from vigorous exercise for at least 15 h, refrain from caffeine and alcohol consumption for 24 h, and consume a normal evening meal the night before the visit. All measurements were carried out in the morning of the same week day. In brief, the procedures are described as follows:

Caffeine and placebo intake

After weighing the participants, the dose was individually prepared to assure that a 5 mg of caffeine/kg of body mass/day was administered. The dose of caffeine was divided into two equal parts (2.5 mg/kg, specifically divided in labeled containers) to be orally consumed through capsules in the morning and after lunch. An equivalent dose (5 mg/kg/day) and number of placebo capsules, of the same color as the caffeine capsules, containing malt-dextrin were provided for the placebo condition. Daily phone calls to participants ensured compliance with treatment conditions.

Anthropometry

Subjects wearing a bathing suit and without shoes were weighed to the nearest 0.01 kg on an electronic scale connected to a plethysmograph computer (BOD POD[®], COSMED, Rome, Italy). Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany) according to the standardized procedures described elsewhere²³.

Fat mass (FM) and fat free mass (FFM)

A dual energy X-ray absorptiometry whole-body scan was used to estimate FM and FFM (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, Waltham, USA). Based on test-retest using ten subjects, the coefficient of variation (CV) for the FM and FFM are 1.7% and 0.8%, respectively²⁴.

Resting Energy Expenditure (REE) assessed by indirect calorimetry

REE was assessed in the morning in the same room at an environmental temperature and humidity of $\pm 22^{\circ}\text{C}$ and 40-50% respectively. The MedGraphics CPX Ultima (Medical Graphics Corp, St Paul, MN, with Breeze suite software) indirect calorimeter was used to measure breath by breath oxygen consumption ($\dot{V}\text{O}_2$) and carbon dioxide production ($\dot{V}\text{CO}_2$) using a facial mask. Participants lay down supine for 30 minutes and the calorimeter device was then attached to the mask for collecting breath by breath $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ during an additional 30-min. The first and the last 5-min of data collection were discarded and the mean of a 5-min steady state interval between the 5 and the 25 minute with RQ between 0.7 and 1.0 was used to calculate REE. Steady state was defined as a 5-min period with $\leq 10\%$ CV for $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ ²⁵. Mean $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ of 5-min steady states were used in Weir's equation²⁶

with the lowest REE period considered. The CV for REE, based on test-retest using 7 young active adults, was 4.0 %²⁷.

Total energy expenditure (TEE) from doubly labeled water

TEE was measured by an established procedure using deuterium oxide and 18-Oxygen²⁸. Doubly labeled water (DLW) was administered in the morning of the first visit (baseline). Briefly, subjects were weighed in the morning and baseline urine was collected. An oral dose of 2.7 g/kg of TBW of a 10 atom% (AP) solution of H₂¹⁸O (Taiyo Nippon Sanso Corporation, Tokyo, Japan), assuming that TBW is 0.61xbody mass, and 0.24 g/kg of TBW of a 99.9 AP solution of ²H₂O (Sigma-Aldrich, Co, St Louis, Mo, USA), diluted in 50 ml of water and administered to the subjects at 7.00 a.m.. Post-dose urine samples of the first visit day were taken and stored from voids at 4 and 5 hours. Morning urine samples and 1 hour after were collected on day 4 (end of first condition), after the washout period (day 7), and at the end of the second condition (last day). Urine samples were prepared and filled with the equilibration gas. The equilibration period lasted for 3 days and 8h, respectively for ²H and ¹⁸O. Samples were analyzed in duplicates and calibrated against standard mean ocean water (SMOW), using Hydra isotope ratio mass spectrometer (PDZ, Europa Scientific, UK). A two-point sample method was used to evaluate the elimination constants (kd and ko, respectively for deuterium and 18-oxygen) over the first and the second 4-day periods (condition 1 and 2, respectively). For analyzing condition 2, urine samples collected after the washout period (day 7) and on the last day of the trial (day 11) were considered to evaluate the elimination constants. A similar procedure was used elsewhere²⁹. TEE by the DLW method was calculated from a modified Weir's equation, including the food quotient obtained by dietary intake records²⁶. The CV for TEE, based on test-retest using 10 elite athletes, was 4.3 %²⁷.

PAEE was calculated as the difference between TEE and the sum of REE with $0.1 \times \text{TEE}$ (assuming the thermic effect of food is ~10% of TEE) while physical activity level (PAL) was determined as the ratio between TEE to REE.

Dietary-record analysis

Food intake was assessed throughout the trial using 24-h diet records. Participants were instructed regarding portion sizes, food preparation methods, and others aspects pertaining to an accurate recording of their food intake. Accuracy of the food intake recordings was ascertained by the study nutritionist at the second study visit (4 days after baseline). At the last visit, records were turned in and reviewed for macronutrient composition and energy intake. Diet records were analysed using a software package (Food Processor SQL, ESHA Research Salem, OR, USA).

Energy expenditure (EE) from combined HR and motion sensor

EE were evaluated using a combined HR and motion sensor monitor (Actiheart, CamNtech Limited, UK). The monitor was worn on a polar band placed on the chest. Initially, participants performed an 8-min step test at a step height of 215 mm, the stepping speed ramps linearly increased from 15 to 33 step cycles/min. Step test provided individual calibration of HR-PAI. Subsequently, the device was started at the long term mode to record HR and acceleration with 60-sec epochs. The participants were asked to wear the monitor at all times throughout the trial, along with the DLW assessment.

Data from the monitors were downloaded into to the commercial software (version 4.0.99). The camNtech software algorithm allowed data cleaning, recovering, and interpolation of missing and noisy HR.

PAEE was estimated, separately for the caffeine and placebo condition, using different energy models, available in the commercial software (Actiheart software version 4.0.99, CamNtech Limited, UK):

ACC+HR_{step}: using the individual HR calibration model (Group Cal JAP2007/StepHR³⁰), with HR and accelerometry data;

ACC+HR_{group}: using the group HR calibration model (Group Cal JAP2007³⁰) with HR and accelerometry data;

HR_{flex}: using the individual HR calibration model (Group Cal JAP2007/StepHR³⁰), with HR data;

ACC: using accelerometry data.

Overall, Actiheart counts and HR data are initially used to estimate separate acceleration and HR_{flex} models. The counts are converted to ACC-PAEE estimates using group-calibrated accelerometry equations derived from walking and running accelerations in studies conducted with the Actiheart³⁰. The HR_{flex} was estimated with a sleeping HR-based regression equation¹³ while the related PAEE assessment was calculated using the individual calibrated HR-PAEE relationships above the HR_{flex} (established at 0 for minutes below that point). Then, minute-by-minute HR- and ACC-PAEE estimates were combined in a branched equations model to calculate daily PAEE as described elsewhere³⁰. In the branched equations model, the relative contribution of accelerometry and HR for PAEE estimation is weighted epoch by epoch according to different counts and HR thresholds. Briefly, when both ACC and HR values are low, the ACC-PAEE estimates have more weight, while when ACC and HR values are high, the HR-PAEE estimates are the predominant contributor to the minute-by-minute PAEE estimates.

The TEE was estimated by adding to the PAEE the thermic effect of food (~10% of TEE) and the REE, estimated using the Schofield equation³¹, as suggested by the commercial software.

Statistical Analysis

Descriptive statistics including means and standard deviations (SD) were calculated for all outcome measurements. Normality was tested using Shapiro–Wilk test. Comparisons between methods were analysed using paired sample T-test or the non-parametric Wilcoxon test.

Simple linear regressions were performed to calculate the relationship between TEE and PAEE estimated by the reference DLW method and the combined monitor models.

The concordance coefficient correlation (CCC) was analysed to evaluate the degree to which pairs of observations fall on the 45° line through the origin³². The CCC (ρ_c) contains a measurement of precision ρ and accuracy ($\rho_c = \rho C_b$) where ρ is the Pearson correlation coefficient, which measures how far each observation deviates from the best-fit line, and is a measure of precision, and C_b is a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin, and is a measure of accuracy.

Agreement between methods was assessed³³, including the 95% limits of agreement, including the trend between the mean and the difference of both methods.

Data analysis was performed using IBM SPSS Statistics version 19.0, 2010 (SPSS Inc., an IBM Company, Chicago, Illinois, U.S.A.) and the MedCalc Statistical Software version 11.1.1.0 (MedCalc Software, Mariakerke, Belgium). For all tests, statistical significance was set at $p < 0.05$.

The sample size estimated for the primary outcome of this clinical trial (effects of caffeine on EE) was calculated based on prior normally distributed data on EE mean differences of 322 kJ/day with a standard deviation of 502 kJ/day³⁴. To reject the null hypothesis that this difference is zero with a power of 80% and a type I error probability of 0.05, 21 pairs of participants were necessary. To assure that drop-outs or equipment/technique failure would not compromise our results we enrolled 30 participants.

Results

The participant's characteristics are shown in **table 1**.

Table-1

The mean and standard deviations for HR, REE, TEE, and PAEE from the reference and alternative methods are summarized in **table 2**.

Table-2

Under both conditions, TEE values from HR_{flex} significantly overestimated the DLW method, while an underestimation was observed for the ACC model. The PAEE was underestimated using the individual calibration (ACC+HR_{step}) under placebo and the ACC whereas using the HR_{flex} model the reference method was overestimated.

We further tested if the order of treatment both as a main effect and its interaction with each EE models explained the variability of the reference TEE and PAEE values, under placebo and caffeine treatment conditions. Neither the order of treatment nor the interaction terms were significant predictors of the reference PAEE and TEE, for both conditions.

Accuracy and agreement of the combined monitor in estimating TEE and PAEE from the reference method under both conditions are presented in **tables 3 and 4**.

Tables-3 and4

Under placebo treatment, the combined monitor models explained between 43% (ACC) to 82% (ACC+HR_{step}) of TEE values obtained from DLW whereas for PAEE the monitor explained between 17% (ACC) and 66% (ACC+HR_{step}) of the variability in the PAEE values obtained from the reference method. During caffeine treatment, the combined monitor models explained between 50% (ACC) to 76% (ACC+HR_{step}) of the TEE values obtained from DLW. For PAEE the monitor explained between 39% (ACC) to 53% (ACC+HR_{step} and HR_{flex}) of the variance in the reference PAEE values.

The higher CCC values were obtained for the ACC+HR_{step} model, ranging from 0.67 (PAEE) to 0.89 (TEE) under placebo and from 0.71 (PAEE) to 0.84 (TEE) during the caffeine treatment. Lower CCC values were observed for the ACC model, ranging from 0.10 (PAEE) to 0.33 (TEE) under placebo and from 0.13 (PAEE) to 0.32 (TEE) during caffeine intake.

For both conditions, lower limits of agreement were found for TEE and PAEE values obtained by the ACC+HR_{step} model with no trend between the mean and the difference of the methods. **Figure 2** displays the regression analysis and Bland-Altman plots for PAEE values assessed by the ACC+HR_{step} model and the criterion method, under both conditions.

Figure-2

We further tested the sensitivity of the ACC+HR_{step} model in detecting changes occurred in PAEE from placebo to caffeine condition using DLW as the reference. The changes in PAEE estimated by the monitor explained 45% of the variability of the PAEE changes from DLW with a standard error of estimation of 1520 kJ/day. The slope (0.848) and intercept (181) did not differ from 1 and 0, respectively. The CCC value was 0,64 (precision of 0.67 and accuracy of 0.96) and the limits of agreement ranged

from -3212 to 2631 kJ/day with no trend ($r=-0.308$, $p=0.229$) between the mean and the difference of the methods.

Discussion

This is the first study to address the validity of a combined HR and motion sensor monitor in assessing EE during a double-blind crossover caffeine trial using DLW as the reference criteria. Given the limitations of relying on either HR or accelerometer only to estimate TEE and PAEE, this device is a new generation of PA monitors that combines both measurements. Therefore this study tested the extent to which factors such as a moderate dose of caffeine in non-regular users would affect the accuracy by which the estimated HR-related PAEE contribution would affect TEE and PAEE estimates.

Regardless of caffeine or placebo intake and order of treatment, TEE values from the ACC+HR_{step} did not differ from DLW whereas a slight underestimation was found for PAEE under the placebo condition. It should be noted that this result may be due to a previously-found underestimation (~800kJ/day) of REE using the MedGraphics CPX Ultima in relation to Deltatrac Metabolic Monitor (VIASYS Healthcare Inc, SensorMedics, Yorba Linda, CA), one of the most popular indirect calorimetry systems for measuring resting metabolic rate in human subjects³⁵. If in fact REE is being underestimated, a resulted overestimation of PAEE is expected when using the combined DLW and indirect calorimetry techniques. Therefore, if higher REE and lower PAEE would have been observed from the criterion method, the true PAEE calculated from DLW would be lower and closer to the Actiheart predicted PAEE values, instead of showing a considerable underestimation. The HRflex and the ACC model over and underestimated both TEE and PAEE values in both conditions. The models were significantly related with the reference DLW values in estimating TEE and PAEE, with the exception of the ACC model. We further observed that both for TEE and PAEE, the individual calibration combined model was overall

more accurate and precise whereas the accelerometry-based model was the least accurate and precise in estimating TEE and PAEE from DLW.

So far, only two studies^{10, 14} tested the validity of this particular combined monitor (Actiheart) in free-living conditions used DLW as the reference method. In fact, our findings seem to extend those observed by Villars et al¹⁰ that tested the validity of the combined ACC and HR in estimating PAEE in 11 lean active, 12 lean sedentary, and 12 overweight sedentary participants aged 18-55 years.

According to this study PAEE estimates based on both recordings combined in a weighed branched model correlate better with DLW reference measures in free-living conditions than estimates from HR or ACC alone. Using the group calibrated equations the agreement in estimating PAEE from the reference method is modest but when the authors used the individual calibration of the HR, an improved PAEE prediction of the combined model was observed, accounting for 71% of the total variance in the reference method, with no significant bias and a reduction by one-third of the standard error of estimation. On the other hand, Assah et al¹⁴ analysed the accuracy of the *Camtech* combined monitor in rural and urban participants during free-living conditions using DLW as the reference criterion. The authors observed a significantly higher PAEE in rural compared to urban participants, reporting higher associations between methods for urban participants, ranging from 0.40 (for HR_{flex}) to 0.70 (for ACC) in urban and from 0.25 (for HR_{flex}) to 0.45 in rural (for ACC). Our results suggested stronger associations between the combined monitor with DLW ranging from 0.04 (for ACC) to 0.90 (for ACC+HR_{step}) under placebo and from 0.58 (for ACC) to 0.86 (for ACC+HR_{step}) under caffeine. Agreement analysis indicated a non-significant trend between the mean and the difference of the methods in estimating TEE and PAEE for the ACC+HR_{step}. However for the remaining models, specifically HR_{flex} and ACC, a significant association between the difference and the mean of the methods was observed meaning that the magnitude of the combined monitor error is dependent on the individual TEE and

PAEE, when using these EE models. These findings are in accordance with those reported by Assah et al¹⁴ that observed less accurate results in the rural sub-sample with higher TEE and PAEE. Using group calibration these authors¹⁴ reported a non-significant mean bias between PAEE between the combined monitor and the reference method with limits of agreement similar to ours. However PAEE from the combined sensor only accounted for 16% of the variance in the combined DLW and indirect calorimetry for PAEE assessment. In fact our findings are close to those reported by Villars et al¹⁰ that observed a better accuracy and agreement between the individual calibration model and the reference method. It is possible that the higher correlations observed in our study and the data reported by Villars et al¹⁰ could be related to the similar characteristics of the sample living in an European urban environment, favouring more acceleration-dependent and less weight bearing activities than rural participants. In fact Assah et al¹⁴ did report a better accuracy for the participants living in the urban sub-Saharan area.

Regardless of caffeine consumption, tighter limits of agreement for TEE and PAEE were observed using ACC+HR_{step} whereas the ACC model displayed wider limits of agreement. The significant underestimation of both TEE and PAEE and the poor individual accuracy observed for the ACC model is in accordance with the findings reported by Brage et al³⁰ suggesting that PA intensity is underestimated, mainly due to the variability of the sources of movement and the assumptions about the efficiency of the work performed. It is expected that accelerometers underestimate EE as the linearity between counts and aerobic intensity cannot always be assumed at moderate to high velocities³⁶. Previous studies observed that the accelerometer component of the combined monitor presents a poor performance compared to other hip worn equipment, particularly at higher intensities during level walking and level jogging³⁷. Therefore, the accuracy of accelerometer models from combined sensing may be limited due to the position of the monitor on the sternum³⁸. Also the use of HR for EE prediction is not error free as its relation with PA intensity may be affected by several factors like age, sex, training state, mental stress,

ambient temperature, hydration, muscle mass involved in the activity, among others^{39, 40}. In the current trial we were expecting that caffeine would increase HR and bias the results for PAEE and TEE. Though slightly higher PAEE and TEE values were observed under caffeine intake, our results suggested that there was no difference in either measured or estimated EE during the two different conditions. The combined monitor includes a built-in step-test protocol that is used for deriving the individual HR-PAI relationship in the field and EE is then calculated using $ACC+HR_{step}$. When the step test is not performed it is possible to select a model that uses the group calibration, which is an approximation for a range of individual fitness levels³⁰.

From the initial 30 participants, we lost data from 13 participants since ~11 complete days were required to validate this equipment under both conditions and we only included those with complete and valid days. Our power calculation suggested we would need at least 21 individuals with complete data at both time points to detect an effect of caffeine intake on energy expenditure. Thus, the lower sample size with complete data (N=17) may affect the possibility to detect absolute differences in TEE and PAEE between conditions. However, when comparing estimated TEE and PAEE from combined sensing with that measured by DLW at both conditions the mean differences were fairly small and approximately 8% for TEE and 11% for PAEE when using group calibration. While a larger sample size would allow detection of statistically significant differences between methods given the absolute mean differences are unchanged we consider the fairly small absolute and relative differences between methods as clinically acceptable. Further, the magnitude of differences between methods were unaffected by the caffeine treatment. Finally, even though we used a polar band placed on the chest (recently commercialized by *CamNtech*) instead of the standard ECG electrodes used in the aforementioned studies^{10, 14} these missing data may question the usefulness of this equipment for measuring PAEE during free-living for period longer than a week.

Conclusions

Overall, the ingestion of a moderate dose of caffeine in low-users did not affect the validity of the combined ACC with HR, specifically the individual calibration in assessing TEE and PAEE in physically active males. Nevertheless, given the relatively larger limits of agreement, some caution should be used to interpret individual energy expenditure requirements.

Acknowledgements

We would like to express our gratitude to the participants for their time and effort. This work was supported by the Portuguese Institute of Hydration and Health and the Portuguese Foundation for Science and Technology.

The authors do not have any conflict of interest.

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Figures

Figure 1 - Flow diagram of the progress through the phases of the cross-over randomized trial.

Figure 2 - Upper panels: Regression between methods in assessing physical activity energy expenditure (PAEE) from the reference method ($PAEE_{ref}$) and PAEE using individual HR calibration from the combined motion sensor with heart rate monitoring ($PAEE_{ACC+HRstep}$). Lower panels: Bland-Altman analysis of the agreement between methods in assessing PAEE, including the trend (r , coefficient of correlation) between the mean and the difference of both methods.

Table 1 – Baseline participant’s characteristics

N=17	Mean ± SD	Range
Age (years)	24.9 ± 4.8	20.0 - 38.0
Height (cm)	176.0 ± 7.1	164.0 - 186.9
Body weight (kg)	72.0 ± 8.5	62.0 - 90.2
BMI (kg/m²)	23.2 ± 2.4	20.2 - 26.8
FM (Kg)	11.9 ± 4.0	6.6 - 20.6
FM (%)	16.5 ± 4.1	10.3 - 23.5
FFM (kg)	59.3 ± 5.9	51.9 - 70.1

Abbreviations: N, number of participants; SD, standard deviation; BMI, body mass index; FM, fat mass; FFM, fat-free mass.

Table 2. Energy expenditure under caffeine or placebo treatments using the reference and the alternative methods

N=17	Placebo	Caffeine
Heart Rate from indirect calorimetry (beats/min)	52 ± 6	54 ± 7
REE indirect calorimetry (kJ/day)	5876 ± 956	6112 ± 1125
REE Schofield (kJ/day)		7389 ± 519
PAL from DLW	2.22 ± 0.45	2.28 ± 0.48
TEE from reference † (kJ/day)	12875 ± 2373	13616 ± 2198
TEE from Actiheart* (kJ/day)		
ACC+HR _{step}	13282 ± 2178 (p=0.606)	14084 ± 2642 (p=0.578)
ACC+HR _{group}	13921 ± 2923 (p=0.261)	14701 ± 3180 (p=0.256)
HR _{flex}	16806 ± 3578 (p<0.001)	17895 ± 4443 (p=0.001)
ACC	10774 ± 1261 (p<0.001)	11082 ± 1396 (p<0.001)
PAEE from reference ‡ (kJ/day)	5712 ± 1747	6142 ± 1835
PAEE from Actiheart* (kJ/day)		
ACC+HR _{step}	4565 ± 1516 (p=0.049)	5286 ± 2198 (p=0.227)
ACC+HR _{group}	5140 ± 2442 (p=0.438)	5842 ± 2709 (p=0.708)
HR _{flex}	7736 ± 2994 (p=0.022)	8717 ± 3807 (p=0.017)
ACC	2397 ± 1042 (p<0.001)	2585 ± 1023 (p<0.001)

Abbreviations: N, number of participants; PAL, physical activity level; REE, resting energy expenditure; TEE, total energy expenditure; PAEE, physical activity energy expenditure

† TEE assessed by doubly labeled water

‡ PAEE assessed by doubly labeled water and indirect calorimetry, assuming that the thermogenic effect of food is 10% of TEE (PAEE = TEE – (REE+0.1TEE))

*Models of energy expenditure prediction from Actiheart: ACC+HR_{step} - individual HR calibration model (Group Cal JAP2007/Step HR.) with HR and accelerometry data; ACC+HR_{group} - group HR calibration model (Group Cal JAP2007) with HR and accelerometry data; HR_{flex} – using the individual HR calibration model (Group Cal JAP2007/Step HR) with HR data; ACC - ACC: using accelerometry data.

1 **Table 3.** Validity of the energy expenditure models from the combined HR and motion sensor monitor, under placebo treatment

	<i>Regression analysis</i>		<i>CCC analysis</i>			<i>Agreement analysis</i>				
	<i>r</i> ²	<i>see</i>	<i>CCC</i>	<i>ρ</i>	<i>C_b</i>	<i>Bias</i>	<i>Limits</i>	<i>Trend</i>		
<i>Total Energy Expenditure (kJ/day)*</i>										
ACC+HR _{step}	0.82 ^{a)}	1028	0.89	0.9077	0.9797	406.6	-1545.7	2359.0	-0.20	0.439
ACC+HR _{group}	0.73 ^{a)}	1268	0.77	0.8558	0.9046	1045.1	-1929.5	4019.8	0.38	0.137
HR _{flex}	0.80 ^{a)}	1098	0.44	0.8941	0.4874	3930.1	397.2	7462.9	0.69	0.002
ACC	0.43 ^{a)}	1847	0.33	0.6576	0.5022	-2101.8	-5655.0	1451.5	-0.67	0.003
<i>Physical Activity Energy Expenditure(kJ/day)*</i>										
ACC+HR _{step}	0.66 ^{a)}	1214	0.67	0.8106	0.8229	-1146.9	-3457.3	1163.5	-0.26	0.314
ACC+HR _{group}	0.50 ^{a)}	1465	0.67	0.7073	0.9481	-572.3	-4000.3	2855.7	0.27	0.297
HR _{flex}	0.63 ^{a)}	1265	0.55	0.7922	0.6929	2024.1	-1267.2	5675.5	0.56	0.020
ACC	0.17	1886	0.10	0.4152	0.2492	-3315.2	-6917.6	287.2	-0.61	0.009

2 *Models of energy expenditure prediction from Actiheart: ACC+HR_{step} - individual HR calibration model (Group Cal JAP2007/Step HR, with HR and accelerometry data; ACC+HR_{group} - group HR
3 calibration model (Group Cal JAP2007) with HR and accelerometry data; HR_{flex} – using the individual HR calibration model (Group Cal JAP2007/Step HR) with HR data; ACC - ACC: using
4 accelerometry data.

5 ^{a)} Significant associations (p<0.05)

6 Abbreviations: r², coefficient of determination; see, standard error of estimation; CCC, concordance correlation coefficient; ρ, precision; C_b, accuracy)

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Table 4. Validity of the energy expenditure models from the combined HR and motion sensor monitor, under caffeine treatment

	<i>Regression analysis</i>		<i>CCC analysis</i>			<i>Agreement analysis</i>				
	<i>r²</i>	<i>see</i>	<i>CCC</i>	<i>P</i>	<i>C_b</i>	<i>Bias</i>	<i>Limits</i>	<i>Trend</i>		
<i>Total Energy Expenditure (kJ/day)*</i>										
ACC+HR _{step}	0.76^{a)}	1107	0.84	0.8730	0.9644	467.9	-2066.4	3002.2	0.35	0.163
ACC+HR _{group}	0.64^{a)}	1368	0.69	0.7980	0.8632	1085.6	-2729.9	4901.0	0.53	0.028
HR _{flex}	0.74^{a)}	1147	0.38	0.8629	0.4436	4279.4	-1166.0	9724.8	0.83	<0.001
ACC	0.50^{a)}	1603	0.32	0.7081	0.4513	-2533.9	-5592.3	524.4	0.55	0.021
<i>Physical Activity Energy Expenditure(kJ/day)*</i>										
ACC+HR _{step}	0.64^{a)}	1155	0.71	0.7928	0.8986	-856.0	-3487.5	1775.5	0.26	-0.261
ACC+HR _{group}	0.51^{a)}	1333	0.65	0.7110	0.9204	-300.1	-4038.5	3438.3	0.49	-0.044
HR _{flex}	0.64^{a)}	1133	0.45	0.8014	0.5611	2574.4	-2486.4	7635.1	0.80	<0.001
ACC	0.39^{a)}	1484	0.13	0.6220	0.2102	-3557.7	-6383.5	-731.8	-0.62	0.008

*Models of energy expenditure prediction from Actiheart: ACC+HR_{step} - individual HR calibration model (Group Cal JAP2007/Step HR, with HR and accelerometry data; ACC+HR_{group} - group HR calibration model (Group Cal JAP2007) with HR and accelerometry data; HR_{flex} - using the individual HR calibration model (Group Cal JAP2007/Step HR) with HR data; ACC - ACC: using accelerometry data.

^{a)} Significant associations (p<0.05)

Abbreviations: r², coefficient of determination; see, standard error of estimation; CCC, concordance correlation coefficient; p, precision; C_b, accuracy)