Methodological considerations for meal-induced thermogenesis

Measurement duration and reproducibility

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Abbreviations: MIT: Meal Induced Thermogenesis, RMR: Resting Metabolic Rate, DXA: dual-energy X-ray absorptiometry, VAS: visual analogue scales, %FM: percent fat mass, RER: Respiratory Exchange Ratio

ABSTRACT

Meal-Induced Thermogenesis (MIT) research findings are highly inconsistent, in part, due to the variety of durations and protocols used to measure MIT. We aimed to determine: 1) the proportion of a 6 h MIT response completed at 3, 4 and 5 h; 2) the associations between the shorter durations and the 6 h measure; 3) whether shorter durations improved the reproducibility of the measurement. MIT was measured in response to a 2410 KJ mixed composition meal in ten individuals (5 male, 5 female) on two occasions. Energy expenditure was measured continuously for 6 h post-meal using indirect calorimetry and MIT was calculated as the increase in energy expenditure above the pre-meal RMR. On average, 76%, 89%, and 96% of the 6 h MIT response was completed within 3, 4 and 5 h respectively, and the MIT at each of these time points was strongly correlated to the 6 h MIT (range for correlations, $r = 0.990$ to 0.998; $p < 0.01$). The between-day CV for the 6 h measurement was 33%, but was significantly lower after 3 h of measurement (CV = 26%, p = 0.02). Despite variability in the total MIT between days, the proportion of the MIT that was complete at 3, 4 and 5 h was reproducible (mean CV: 5%). While 6 h is typically required to measure the complete MIT response, 3 h measures provide sufficient information about the magnitude of the MIT response and may be applicable for measuring individuals on repeated occasions.

Introduction

Meal-Induced Thermogenesis (MIT) is the energy expended consequent to the consumption of a meal, and reflects the energy required for the processing and digestion of the food and/or drink consumed. The contribution of MIT to total daily energy expenditure is commonly stated to be approximately 10% [1, 2]. This value has been noted to vary considerably between individuals, or within individuals with changes in energy balance. However, the extent of this variation is not consistent among studies [3-10]. Differences in protocols, for example meal size and the duration of the post-meal measurement period, as well as the methods used to calculate MIT, may contribute to the discrepancies between studies.

The total MIT response may take as long as 8-10 h following the ingestion of larger meals (> 4184 KJ) [11, 12]; however, in the majority of studies using meals of between 1674 and 4184 KJ, MIT has been measured for between 3 and 6 h, and is often incomplete at the end of the measurement period [5, 9, 10, 13-16]. Individual differences in the rate of gastric emptying, and nutrient digestion and storage may affect the duration of the MIT response [17], and larger meal size, a greater ratio of fat to protein, and protein to carbohydrate, as well as greater adiposity, may extend the MIT response and also delay the peak in energy expenditure [11-13, 18, 19]. However, long measurement durations place considerable burden on the participant, so it is important to determine whether shorter measurement durations can accurately reflect the total MIT response, and capture a similar proportion of the total response in the same individuals on repeated tests days.

In addition to the challenge of interpreting findings from studies using very different measurement protocols, the reported day-to-day variability in the measurement is high, with CVs typically in the range of 15 to 29% [20-24], but as high as 42% [21]. This high variability may be associated with the method used to calculate MIT. In the majority of studies, MIT is calculated as the difference between total post-meal energy expenditure and the resting metabolic rate (RMR) measured in a fasted state immediately prior to meal ingestion [4, 5, 9, 10, 13, 14, 25]. As such, even if the meal-induced component of the measurement was identical between days, differences in the pre-meal RMR can affect the calculated value for MIT. In an attempt to minimise the effects of any between-day variability in RMR, several studies have used a single value for RMR (“fixed” RMR) to calculate each subsequent MIT [20, 23, 26]. While this approach has tended to reduce day-to-day variability, a substantial degree of between-day variability in MIT remains unexplained.

Therefore the aims of this study were: 1) to determine whether shorter measures of MIT will correlate with the total MIT response; 2) to determine the reproducibility of the total MIT response and the proportions of the response complete at 3, 4 and 5 h; and 3) to determine whether shorter measurement durations and the use of a fixed RMR result in lower day-to-day variability.

Methods

Participants

Ten participants (5 male, 5 female) were recruited for the study. All participants were weight stable for the last 6 months (within 2 kg), did not smoke, were euthyroid, and free from food allergies. Participants were also excluded if currently pregnant, lactating, post or currently menopausal or on medications that may affect metabolic rate or heart rate. Participants self-reported their average weekly physical activity along a scale, with participants ranging from the lowest ranking of less than 1 h/wk to the highest ranking of greater than 6 h/wk. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Human Research Ethics Committee at the Queensland University of Technology, Brisbane. Written informed consent was obtained from all participants prior to commencement.

Experimental design

All participants completed three Test Days over a maximum 4 wk period: Test Day 1 was used to collect anthropometric and body composition data and served as a familiarisation session. On Test Days 2 and 3 participants undertook repeat 6 h measurements of MIT. All testing was conducted in the morning following an overnight fast ($\geq$ 8 h). Participants were instructed to consume the same meal on the evening before Test Days 2 and 3, to abstain from exercise for 48 h prior to all test days, and to minimise activity on the mornings of each test day. Participants were asked to arrive to the laboratory by car to minimise activity prior to the measurement.

Anthropometry and body composition

Height was measured to the nearest 0.1 cm on Test Day 1. Body weight was measured to the nearest 0.05 kg at the start of each Test Day to ensure that participants were weight stable for the duration of the study. Body composition was measured using dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, Lunar Corp, Madison, WI USA). The scans were analysed using the DPX-L adult software (Encore adult software, version 9, Lunar prodigy, Madison, WI). Quality assurance was assessed regularly by analysing a phantom spine and calibrations were undertaken before each scan using a calibration block provided with the equipment.

Measurement of MIT

Familiarisation session (Test Day 1). Height, weight, and body composition were measured, followed by a 30 min measurement of RMR to familiarise participants with the ventilated hood and measurement procedure. To ensure participants were relaxed, they were permitted to listen to music during their RMR measurement.

MIT measurements (Test Days 2 and 3). An overview of the protocol for Test Days 2 and 3 is shown in Fig. 1. Upon arrival at the laboratory, body weight was measured and then RMR was measured in a fasted state for 30 min. This RMR measurement served as the "baseline" energy expenditure for the calculation of MIT. Immediately after this baseline RMR, participants consumed a fixed test meal within 15 min. The metabolic measurement was then resumed and postprandial energy expenditure was measured for a total of 6 h, with two, 10 min "comfort breaks" at 3 and 4.5 h. These breaks were mandatory and all participants were required to walk along the corridor to the restrooms on both Test Days 2 and 3. For all metabolic measurements, participants were in a semi-reclined position in a lounge chair and the position of the participant was consistent between test days. Prior to commencing the pre-meal RMR, before and after breakfast, and then at 45 min intervals during the 6 h postprandial measurement, participants completed subjective alertness and comfort sensations using visual analogue scales (VAS). These were conducted to evaluate levels of restlessness and alertness both throughout the test and between days to consider their contribution to MIT variability. Participants were able to watch movies for the duration of the metabolic measurements.

Test meal composition

The standard fixed breakfast meal on Test Days 2 and 3 represented a typical breakfast and consisted of muesli with milk, toast with margarine and jam, and a glass of orange juice. The meal provided 2410 KJ and comprised of 20.1 g of fat, 78.2 g of carbohydrate, and 20.6 g of protein, giving relative energy contributions of 32% fat, 54% carbohydrate, and 14% protein.

Measures of resting and postprandial energy expenditure

Resting and postprandial energy expenditure was measured using indirect calorimetry with a ventilated hood and canopy system (Parvo Medics, TrueOne 2400, Sandy, Utah, USA). The rate of airflow being pumped through the hood was manually adjusted to maintain a constant carbon dioxide level in the hood between 1.00 and 1.20%. Oxygen concentration in the hood was measured by a paramagnetic oxygen analyser and carbon dioxide concentration was

measured with an infrared single beam single wavelength carbon dioxide analyser. Oxygen consumption and carbon dioxide production were calculated as the difference between the expired air in the hood and room air. Energy expenditure was calculated automatically by the software (Parvo Medics, TrueOne 2400 Metabolic Measurement System OUSW 4.3) from VO_2 and VCO_2 measured continuously throughout the testing using the Weir formula which calculates the non-protein caloric equivalent for oxygen: Energy expenditure (KJ) = [(1.106 x RER) + 3.941] x VO_2 x 4.184 [27]. The metabolic cart was calibrated before each measure for flow, using a standard 3 L calibration syringe, and gas concentration using a two-point calibration procedure using room air and a standardised calibration gas (16.0% O_2, 1.0% CO_2). To control for any drift within the gas analysers, automated 30 s calibrations were performed at 5 min intervals throughout the measurements.

Data handling and calculations

Pre-meal RMR. To ensure that pre-meal RMR was measured during a stable period, the initial 10 min of the 30 min RMR measurement was discarded and RMR was calculated as the lowest 10 min average during the final 20 min of the 30 min measurement period. The CV for VO2 over the final 20 min of the tests averaged 5 ($_{SD}$ 2) %. Estimated RMRs were calculated for each participant using the Schofield equations based on height and body weight [28] to determine whether measured RMRs were consistent with predicted values of RMR.

Postprandial energy expenditure. Data from the 6 h postprandial measurement period were averaged over 15 min intervals and plotted against time. To minimise the effect of movement on the measurement of energy expenditure, the first 5 min of the postprandial measurement (i.e. immediately following breakfast), and 5 min periods following each of the prescribed breaks at 3 and 4.5 h were excluded from the calculations. The peak postprandial energy expenditure was defined as the 15 min interval with the highest rate of energy expenditure, and the time of peak was defined as the time that corresponded with this 15 min maximum. MIT on each Test Day was calculated, as the energy expenditure above RMR. This was calculated by averaging all postprandial 15 min data points where the rate of energy expenditure was greater than that during the baseline RMR, subtracting RMR, and then multiplying the 'net' energy expenditure by the time taken for energy expenditure to return to the baseline RMR for a minimum of 30 min. The MIT was also expressed as a percentage of the total energy of the test meal. To minimise the effect of day-to-day variability in RMR on the calculation of MIT, MIT was also calculated for both Test Days using the lower of the two pre-meal RMRs measured on Test Days 2 and 3 (a "fixed" baseline). The lowest RMR

was chosen as it was considered to represent participants in their most rested state and more likely to reflect participants' RMR after prolonged resting during their MIT measurement. RMR SD was calculated over the 10 min period used for each participant's RMR. Participants' MIT responses were considered to have returned to baseline if their energy expenditure returned to within 1 SD of their RMR for a minimum of 30 min.

Subjective ratings of alertness and comfort

Questions were administered on a laptop to measure subjective ratings of alertness and comfort using VAS. Participants were asked to rate their intensity of alertness and comfort by moving a cursor along a 100 mm continuous line on a laptop with the two extremes of each question at either end. The questions asked included, 'How content are you right now?,' 'How alert are you right now?,' 'How comfortable are you right now?,' and 'How great is your desire to move right now?'. The minimum and maximum VAS scores were 0 mm and 100 mm respectively.

Calculation of VAS ratings

The subjective alertness and comfort sensations were expressed in millimetres and calculated by averaging the VAS scores from each 45 min period after the fixed meal.

Statistical Analysis

SPSS version 18 for Windows (SPSS Inc. Chicago, USA), was used for all statistical analysis. A value of $p < 0.05$ was considered statistically significant. All values are reported as mean ($_{SD}$). Data was verified as normally distributed using Shapiro-Wilk tests of normality prior to analysis. A RM-ANOVA was used to test for changes in body weight between days and a one way ANVOA was used to compare the differences in characteristics and RMR between males and females. To determine the extent to which measured RMRs varied from Schofield predictions, measured RMRs were subtracted from predicted RMRs and expressed as a percentage difference from the predicted RMRs. Paired t-tests were used to determine significant differences between predicted and measured RMRs. To assess for the reproducibility of the response curve, the cumulative MIT at 3, 4 and 5 h were calculated and expressed as a percentage of the MIT measured at 6 h. Paired t-tests were used to assess for any significant changes in the peak postprandial energy expenditure (KJ) and the time of this peak. Paired t-tests and Coefficient of Variation (CV) were used for determining differences between days and the variability of the pre-meal RMRs, MIT in both absolute terms (total KJ

over the time period) and relative terms (the proportion of the total response complete in the time period), and MIT (calculated with the "fixed" baseline method). A Bland Altman plot was used to depict the mean difference and 95% limits of agreement in the MIT response between days. A paired t-test was used to compare the CV of the standard MIT calculation method and the "fixed" baseline calculation method, and a RM ANOVA was used to compare the CV between the MIT calculated after 3, 4, 5 and 6 h. Minimal detectable change (MDC), which is defined as the minimal amount of change that is not due to variation in measurement noise [29] were calculated for MIT. Scores at or above the MDC level can be attributed to the intervention rather than measurement error. Measurement error includes expected or typical variability in participant physiology over repeated tests where tests are undertaken under the same conditions [29]. MDC scores were calculated for MIT using the following formula MDC_{90} = SEM x 1.65 x √2, where SEM is calculated using the equation: SEM = sd x √(1 – r) [29, 30]. In these equations SD is the standard deviation of the measure, r is the intraclass correlation coefficient (ICC) for the subject group, 1.65 represents the z-score at the 90% confidence interval and 1.65 is multiplied by the square root of 2 to account for errors associated with repeated measures. Pearson's correlations were used to assess the relationship between the MIT (KJ) calculated at 3, 4 and 5 h and the response measured at 6 h. A 2 x 2 RM ANOVA with the test day and each 45 min VAS rating as the repeated measures were used to determine differences in the VAS variables between days and changes in the VAS variables across the MIT test duration and CV was used to determine VAS between-day variability. The CV was calculated to determine the variability of the fasting, average postprandial, and peak postprandial Respiratory Exchange Ratio (RER). Pearson's correlations were used to determine any relationship between MIT and RER, and MIT and RMR.

Results

Table 1 provides the participant characteristics. Compared with females, males were heavier and taller, but these differences did not reach statistical significance. Further, males and females did not differ significantly in terms of age, BMI or %FM. Participants maintained a stable weight across the study period (Day 1: 65.2 ($_{SD}$ 11.3) kg, Day 2: 65.1 ($_{SD}$ 11.2) kg, Day 3: 65.2 ($_{SD}$ 10.7) kg; p = 0.87). There were no differences between the RMR determined as the lowest 10 min average and the predicted RMRs using the Schofield equations on either test day one (Measured RMRs 1.1 ($_{SD}$ 8.9) % below predicted; p = 0.72) or day 2 (Measured RMRs 0.9 ($_{SD}$ 11.3) % above predicted; p = 0.77).

Fig 2 illustrates the average MIT response for MIT1 and MIT2. Participants' peak rate of energy expenditure above RMR was similar between tests (MIT1: 1.09KJ/min ($_{SD}$ 0.28) KJ/min *vs.* MIT2: 1.15 ($_{SD}$ 0.47) KJ/min; p = 0.64). Although the peak tended to occur earlier for participants during MIT1 than MIT2, this difference was not significant (MIT1: 68 ($_{SD}$ 38) min *vs.* MIT2: 95 ($_{SD}$ 55) min; p = 0.31). At the end of the 6 h measurement, energy expenditure returned to baseline (i.e. within 1_{SD} of pre-meal RMR) for 6 participants during MIT1 and 6 participants during MIT2. Five of these were the same participants. For the remaining participants, the average energy expenditure over the final 30 min of the measurement period averaged 0.46 ($_{SD}$ 0.25) KJ/min above baseline.

The cumulative MIT completed within 3, 4, and 5 h was calculated for MIT1 and MIT2 and expressed as a percentage of the MIT response measured at 6 h for each test (Table 2). On average, the proportion of the response completed at 3, 4, and 5 h was 76, 89, and 96%. The between-day variability of the percent of the 6 h response complete within 3, 4 and 5 h is provided in Table 2.

With respect to the reproducibility of the MIT response, the pre-meal RMR was not significantly different between days (MIT1 RMR: 4.52 ($_{SD}$ 0.84) KJ/min; MIT2 RMR: 4.60 ($_{SD}$ 0.88) KJ/min: p = 0.66). The mean between-day CV was 9% ($_{SD}$ 6%). As outlined in Table 2, there was no significant difference in the total MIT response between days (p = 0.83) and the CV was significantly lower at 3 h and 4 h compared to at 6 h (3 vs. 6 h CV: p = 0.02, 4 vs. 6 h CV: p = 0.03).

To minimise the potential effect of between-day variability of RMR on variability in the MIT, both MIT1 and MIT2 were also calculated using a "fixed" RMR value (the lower of the two pre-meal RMRs) for each participant. Using this approach, the between-day CV for the 6 h measurement was 33% ($_{SD}$ 12%) which was not significantly different from the standard approach ($p = 0.98$).

The individual between-day differences are illustrated with a Bland Altman plot (Fig. 3). While the mean difference between days was -9 KJ (-0.4%) ($p = 0.83$), the 95% limits of agreement were wide, ranging from 239 KJ to -257 KJ (9.9 to -10.7% of energy intake). However, as shown in Fig. 3, this large between-day difference was heavily influenced by two individuals who experienced marked differences (+236 KJ (9.8%) and -246 KJ (-10.2%) between MIT1 and MIT2. The remaining eight participants had between-day differences in MIT within ±87 KJ (3.2% of energy intake). While there was no clear reason for excluding data from these two participants from the analysis, calculations performed without these two responses resulted in a similar mean between-day difference of -10 KJ (-0.4%) but reduced the 95% limits considerably to between 115 KJ and -135 KJ (4.8% to -5.6%). Day-to-day variability determined using the ICC was 0.382. The MDC_{90} was calculated as 122.1 KJ.

The relationship between the MIT measured for 6 h, and the cumulative MIT values measured after 3, 4, and 5 h are illustrated in Fig. 4. The MIT measured after 3, 4, and 5 h was strongly correlated with MIT measured over 6 h during both MIT1 and MIT2 (Correlations reported in Fig. 4).

Despite large variation in the MIT response between days, the between-day CV for the VAS variables was relatively low. There were no significant differences in contentment ($p = 0.68$), level of comfort ($p = 0.14$), or desire to move ($p = 0.60$) between days, with between-day CVs of 11%, 10%, and 11% respectively. Although level of alertness was significantly higher in MIT2 compared to MIT1 ($p = 0.04$), the between-day CV was only 7%. Ratings of alertness did not change across the duration of the test ($p = 0.93$). However, there was a significant decrease in contentment and comfort, and a significant increase in the desire to move over the 6 h postprandial measurement period ($p < 0.001$ for all variables). This was the result of significant changes over the first 3 h (all variables $p < 0.001$), with no significant changes between 3 and 6 h (contentment: $p = 0.159$; comfort: $p = 0.59$; desire to move: $p = 0.80$).

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291 There was no between-day differences in fasting RER (MIT1: 0.79 ($_{SD}$ 0.05); MIT2: 0.79 ($_{SD}$
292 0.05); $p = 0.66$), average 6 h postprandial RER (MIT1: 0.80 ($_{SD}$ 0.02); MIT2: 0.80 ($_{SD}$ 0.04);
293 $p = 0.98$) and peak postprandial RER (MIT1: 0.86 ($_{SD}$ 0.03); MIT2: 0.86 ($_{SD}$ 0.04); $p = 0.99$).
294 The between-day variability for these RER variables was low with CVs of 5%, 4% and 3%
295 respectively. There was no relationship between MIT and average 6 h postprandial RER
296 (MIT 1: $p = 0.576$; MIT 2: $p = 0.334$) or MIT and peak postprandial RER (MIT 1: $p = 0.699$;
297 MIT 2: $p = 0.622$). Further analysis revealed no relationship between the size of participants'
298 MIT response and their RMR (MIT 1: $p = 0.319$; MIT 2: $p = 0.405$).

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DISCUSSION

The primary findings from the present study were that the magnitude of MIT measured for 3, 4, or 5 h strongly correlated with the magnitude of the 6 h MIT, and the proportion of the 6 h MIT response complete at 3, 4, or 5 hours was reproducible between days. In addition, while measurements made over shorter durations were more reproducible, using a "fixed" RMR did not reduce the day-to-day variability in the measurement.

MIT is a small but important component of total daily energy expenditure. MIT is typically measured for up to 6 h [4-7], which places a large burden on the participant, and thus it is important to consider whether shorter measurement durations may be used. The initial few hours of the MIT measurement contain a considerable amount of information about the total MIT response. In the present study, peak postprandial energy expenditure occurred at 67 and 94 min in MIT1 and MIT2 respectively, which was within the range of 30-120 min previously reported in studies using similar sized meals (2510 to 4054 KJ) [9, 15, 31]. Furthermore, 76%, 89%, and 96% of the 6 h response was complete at 3, 4 and 5 h respectively, which is similar to, albeit slightly higher than, the 60%, 78% and 91% reported by Reed and Hill at the same time points [18]. The inclusion of larger meals in the Reed and Hill study (2711 to 5807 KJ) compared to the 2410 KJ test meal in the present study, may have contributed to this difference because larger meals may delay the peak response and lengthen the MIT total duration, and therefore result in a greater proportion of the MIT occurring later in the measurement [11, 18, 19].

The use of either a relative (to RMR) or a standard meal size remains equivocal. While several studies have used meals relative to body weight or RMR [5, 7, 8, 24], a large number provide standard meals sizes for all participants [2, 6, 9, 13, 15, 17, 20, 22, 26, 31]. D'Alessio et al. (1988) compared four energetic loads in lean and obese individuals and found that MIT remained proportional to energy intake for each individual regardless of meal size [12], indicating that meal size is inconsequential when measuring the entire MIT response. However, as larger meals have been shown to prolong the MIT response [11, 18, 19], providing relative meal sizes may result in longer and more delayed responses in individuals with a greater body mass. This raises the concern of measuring different proportions of the total MIT response over a fixed measurement period, either between individuals or pre and post weight loss, unless the entire MIT response is measured. A standard meal size was

chosen for this study to minimise variation in the timing of the response in order to provide tighter estimates of the proportion of the chosen meal complete within 3, 4 and 5 h.

In the present study, the magnitude of MIT measured for 3, 4, and 5 h was strongly correlated with the 6 h measurement, which is also in line with previous findings [18]. Furthermore, the proportion of the response completed at 3, 4, and 5 h was consistent between days (Table 2), indicating the temporal profile of the response is reproducible. As such, for an individual whose 6 h MIT response was smaller on one test day, their response was proportionally smaller at 3, 4 and 5 h. Hence, shorter measurement durations reflected the magnitude of each individual's total MIT and the proportion of their total response captured at these time points was comparable between test days. This suggests that while measurement durations of ~6 h may be required to quantify the entire MIT response to a meal, shorter measures may provide sufficient information to perform between-group and within-subjects comparisons across time. For the former, this assumes that the timing of the response is the same between the groups and for the latter, that the test meal (i.e. energy and composition) is held constant.

In the present study, MIT reproducibility was measured using two approaches. The CV was used to determine the variability relative to the size of the response to allow a comparison to previous studies, and a Bland Altman plot with 95% limits of agreement was used to illustrate the extent of individual between-day variation. Previously, studies measuring MIT with a ventilated hood and using the standard pre-meal RMR to calculate the response, have reported average CVs for MIT between 15 and 29% [20-23]; however CVs as high as 42% have been reported within some groups, even under highly controlled conditions [21]. While there was no significant difference in the group MIT between days (Fig.2), there was considerable within-individual variability with an average between-day CV of 33% for the 6 h measurement, which is at the upper end of the previously reported range. Although the variability was significantly decreased when MIT was measured over 3 and 4 h, it was still 26-29%. The reasons for the high CV in the present study are not clear, especially since consistent and standardised approaches were undertaken to minimise variability. Despite the between-day variability of MIT, the variability of the fasting RER, 6 h postprandial average RER and peak postprandial RER values was low. The low RER varibaility despite a much greater MIT variability is similar to previous findings [21, 23], and indiciates that while the MIT may be greater on any particular test day, the substrates oxidized increase proportionally.

To minimise the effect of day-to-day differences in RMR and glycogen stores, both of which may affect the magnitude of the MIT response [32], pre-test conditions were controlled, with participants asked to avoid exercise outside of their daily work requirements for a minimum of 48 h prior to the test days and to replicate their evening meal preceding both test days. Greater control of diet in the days preceding the measurement may have improved reproducibility. However, it seems unlikely that pre-test diet can explain the variability in MIT given that Weststrate [21] found no improvement in the reproducibility of MIT even when controlling antecedent diet for four days prior to MIT measurements. Where facilities allow, accommodating participants at the testing centre on the night preceding their RMR and MIT tests may offer an additional means of minimising variability through more stringent control over activity and meal choices in the evening prior, and morning of, the test days.

It is also critical to control conditions during the test. Relative to total energy expenditure, MIT is small, and is therefore easily obscured by any noise in the measure. Boredom and a lack of entertainment can increase restlessness and fidgeting [33], and this may “artificially” increase RMR [34], and contribute to variability during the measurement [33]. In the present study, participants were able to watch movies of their choice for the duration of the measurement, and were provided with two opportunities for brief breaks in the measurement. Despite this, participants became more restless over the 6 h measurement period, with significantly lower ratings of contentment and comfort, and significantly higher ratings of desire to move, at 3 and 6 h compared to the start of the measurement. It is worth considering how this may affect the measured response within a test and the day-to-day variability. While energy expenditure had returned to baseline in six of the ten participants by 6 h, it remained slightly elevated in the remaining four. While this may represent a true biological response in these individuals, it is also possible that increased restlessness in the latter parts of the test contributed to the sustained elevation in metabolic rate. On the other hand, given that the between-day CV for all these variables was between 7 and 11% and, with the exception of alertness, there were no significant differences between tests days, differences in comfort or level of arousal are unlikely to explain the high day-to-day variability in MIT.

While eight of the ten participants had differences in MIT values within 115 KJ and -135 KJ between the two tests, the remaining two participants had very large between-day differences (Fig. 3). Both participants were female and because some females were tested in different

phases of the menstrual cycle, this may have contributed to the large differences. However, Melanson et al [35] compared women in the luteal and follicular phases of their menstrual cycle and reported no differences in the MIT between the two phases. Furthermore, while one of the participants with high between-day differences was in different phases of the menstrual cycle for the two MIT tests, the other was in the luteal phase on both test days suggesting that the large day-to-day variability was not due to the menstrual cycle alone. In addition, both participants complied with the pre-test meal and exercise requirements and there were no further obvious reasons for their variability.

The between-day CV for RMR was 9% in the present study, and while this may have contributed to the between-day variability of MIT, the use of a "fixed" RMR did not result in a reduction in variability. This is in contrast with previous research in which the use of "fixed" RMRs has resulted in a significant reduction in day-to-day variability [20, 23]. A possible explanation for this discrepancy is the method used to calculate CV. In studies reporting that the use of a fixed RMR improved reproducibility, the fixed RMR has tended to produce substantially higher MIT values. Because the same absolute between-day difference in MIT (e.g. 60 KJ) will result in a smaller CV with large MIT values (e.g. Test 1: 170 and Test 2: 230 KJ; CV: 21%) compared to a small MIT values (e.g. Test 1: 70 and Test 2: 130 KJ; CV: 42%), the apparent improvement in MIT with a "fixed" RMR may be more a mathematical function rather than a reflection of a true reduction in biological variability between days.

It is important to note that the findings from this study apply to this 'typical' mixed breakfast meal (2410 KJ: 20.1 g fat, 78.2 g carbohydrate, and 20.6 g protein) with a relative energy contribution of 32% fat, 54% carbohydrate, and 14% protein. In situations with markedly different meal composition, there is the possibility of an altered temporal function of gastric transit, and therefore MIT [13, 17, 19]. A limitation of the study was the small sample size with 10 participants underpowered to be able to determine significant differences. However, the data from this study allow determination of variability and measures of MDC to inform future studies. Based on the findings from the MDC_{90}, we may suggest that a difference of $\geq$122 KJ between test days would be required to be confident the difference was from the intervention rather than measurement variability. Retrospective power calculations indicated that to detect a difference of 9 KJ, as was the average difference between days in this study, a sample of 1567 participants would have been required. While the small sample size in the

current study may not provide a population wide example of ICC and variability for calculating MDC_{90}, this study does suggest that the differences we noted between days would not have been found to be statistically significant in most experimental or clinical studies. Should the study of N=1567 be undertaken, based on the results from this study the Cohen's *d* calculation of effect size 0.08, indicates the difference is trivial, even if it would reach statistical significance. Additionally while the homogeneity of the study population limits the findings to those with similar characteristics, the advantage was the ability to provide a standardized test meal to all participants and therefore provide tighter guidelines on the time requirements for MIT measurement for an average sized meal. However, some research has indicated a delayed MIT response in obese individuals [12, 18, 26]. Therefore, further research is necessary to determine the proportion of MIT captured within shorter measurement durations in a wider range of individuals including obese individuals and in a larger variety of meal compositions.

In conclusion, the proportion of the total MIT measured over 3, 4 or 5 h is reproducible between days, and the magnitude of the response measured over shorter durations is strongly related to the magnitude of an individual’s total response. Furthermore, important elements of the response, for example the peak in energy expenditure, occur early in the measurement period, and thus measurements as short as 3 h provide valuable information about an individual’s response to meal ingestion. Therefore, given the substantial participant burden, and potential for the confounding effects of restlessness associated with long measurements, shorter measurement durations may provide a practical option for repeated measurements of MIT. Given that factors such as body weight, meal size and meal composition may alter the timing of the MIT response curve [11-13, 18, 19], further investigation is recommended to determine the applicability of shorter measurement durations in a wider population and for different meals.

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TABLES

TABLE 1

Descriptive characteristics of participants

	Males (n = 5)		Females (n = 5)		All (n = 10)		*P*
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	32.0	10.5	28.4	3.4	30.2	7.6	0.49
Height (cm)	176.2	10.1	164.5	6.9	170.4	10.2	0.07
Weight (kg)	71.8	10.7	58.7	8.1	65.2	11.3	0.06
BMI (kg/m²)	23.0	2.0	21.6	1.7	22.3	1.9	0.27
% FM (DXA)	25.8	5.9	29.4	5.8	27.6	5.9	0.36
RMR (KJ/day)	7395.1	459.8	5699.9	568.2	6547.5	514.0	0.10

P values for comparisons between male and female participants

RMR is calculated as the average pre-meal RMR of both test days

TABLE 2

MIT calculated after 3, 4, 5 and 6 h reported in absolute terms (KJ above RMR) and as a percent of the test meal energy. The cumulative MIT completed at 3, 4 and 5 h is expressed as a percentage of the 6 h measurement. The CV of the cumulative MIT and the CV of the percentage of the 6 h MIT that was complete within 3, 4 and 5 h are also provided.

	3 h		4 h		5 h		6 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MIT Day 1 (KJ)	142	45	172	66	191	83	203	94
MIT day 2 (KJ)	149	66	180	87	198	108	212	128
Mean MIT (KJ)	146	46	176	63	194	78	207	92
Mean MIT (% of meal)	6	2	7	3	8	3	9	4
CV (%)	26	18	29	20	32	19	33	20
MIT1 % Complete	75	14	88	9	95	5		
MIT2 % Complete	78	16	90	11	96	6		
Mean % complete	76	14	89	9	96	5		
CV of percent complete	9	7	5	5	1	2		

FIGURES

FIGURE 1

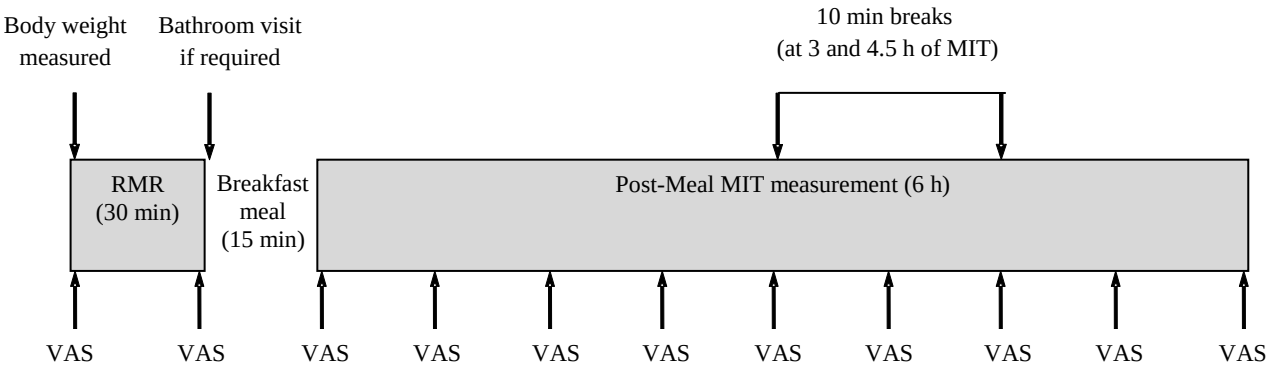


FIGURE 2

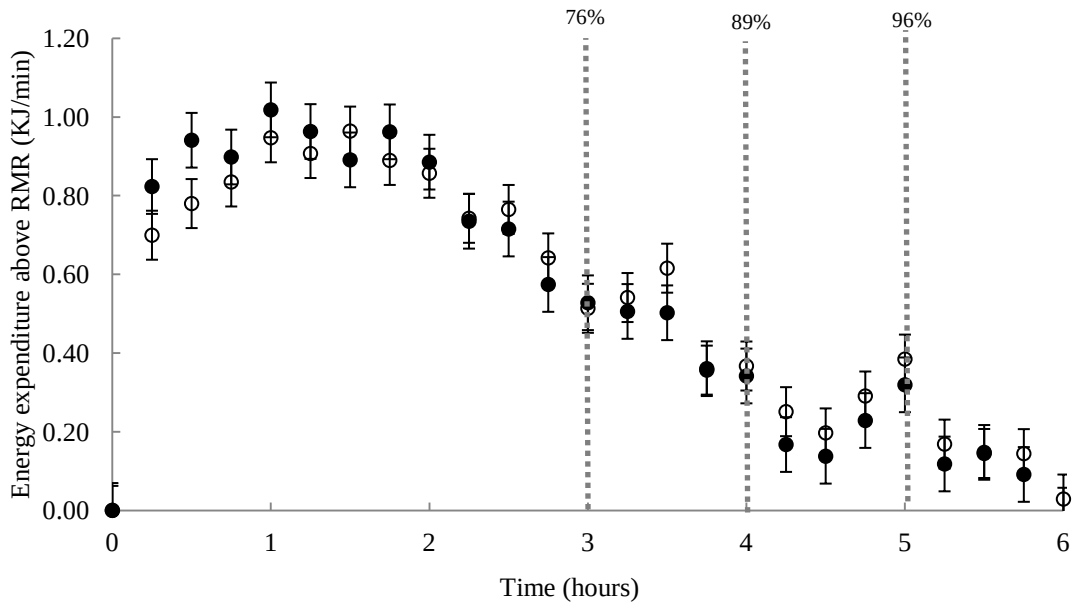


FIGURE 3

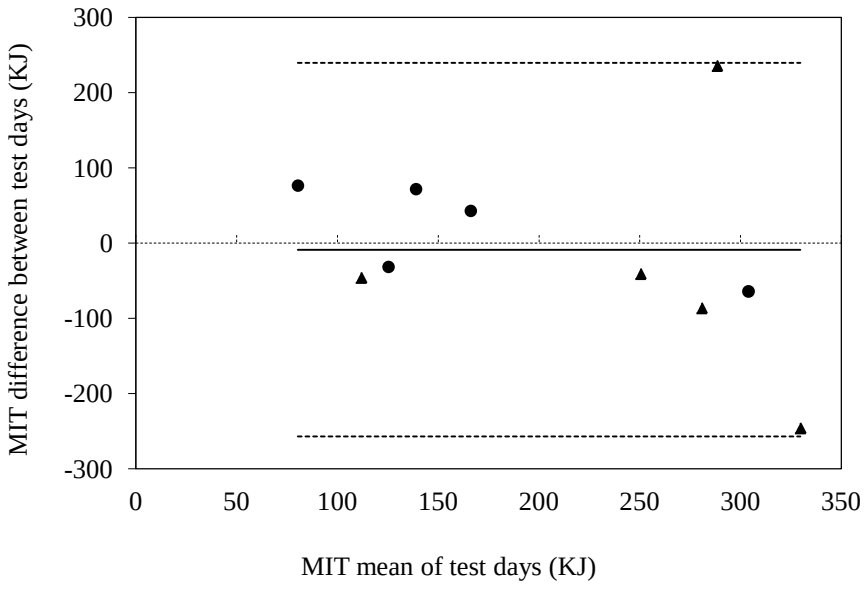
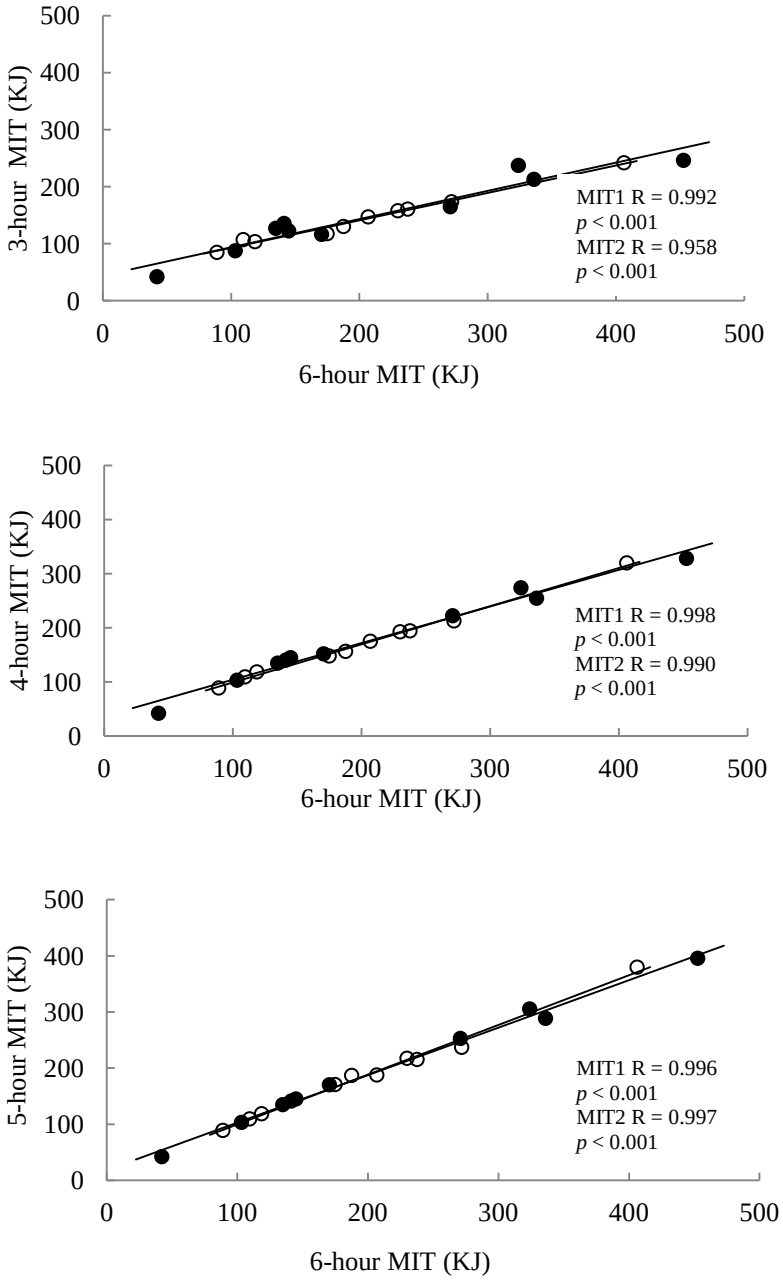


FIGURE 4


3-hour MIT (KJ)
6-hour MIT (KJ)
MIT1 R = 0.992
p < 0.001
MIT2 R = 0.958
p < 0.001
4-hour MIT (KJ)
6-hour MIT (KJ)
MIT1 R = 0.998
p < 0.001
MIT2 R = 0.990
p < 0.001
5-hour MIT (KJ)
6-hour MIT (KJ)
MIT1 R = 0.996
p < 0.001
MIT2 R = 0.997
p < 0.001

Legends for figures

Figure 1: Procedure for MIT test days. The VAS ratings were administered at 45 min intervals throughout the MIT test.

Figure 2: Average MIT response. MIT1 is shown as open circles (○) and MIT2 is shown as filled circles (●). Data points are representative of 15 min averages of energy expenditure post-meal consumption above baseline-RMR (KJ/min). The first data point for each series represents the pre-meal RMR. MIT is calculated as the total energy expenditure above RMR until energy expenditure returns to the RMR value. Error bars represent the SEM. The percent of the 6 h response complete after 3, 4 and 5 h was 76 %, 89% and 96% respectively, as illustrated on the graph.

Figure 3: Bland Altman plot of individual differences in MIT (KJ) between MIT1 and MIT2. (———) indicates mean bias and (- - - - -) represents the upper and lower 95% limits of agreement. The upper 95% limits of agreement was 239 KJ and the lower 95% limits of agreement was -257KJ. The mean difference was -9 KJ. Difference calculated as MIT1 minus MIT2. Males are shown as circles (●) and females are shown as filled triangles (▲)

Figure 4: Regression line of the KJ measured over 3, 4 and 5 h compared to the KJ measures over 6 h. MIT1 is shown as open circles (○) and MIT2 is shown as filled circles (●). The correlation coefficients are included in the bottom right corner of each figure.