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Randomized Control Trials

Effects of protein quantity and type on diet induced thermogenesis in overweight adults: A randomized controlled trial

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SUMMARY

Background & aims: Protein content of a meal is hypothesized to drive DIT dose-dependently. However, no single meal study exists comparing two different doses of protein on DIT. In addition, the source of protein, particularly whey protein, was shown to have a higher DIT than casein and soy in the acute setting, however the mechanism behind this difference is not yet clear. The aim of the present work is therefore to evaluate the efficacy of two different doses and types of protein (whey protein and casein) on DIT in overweight adults.

Methods: Randomized, double blind crossover including seventeen overweight men and women assigned to four isocaloric study treatments where protein and carbohydrate were exchanged: control, 30 g of whey protein microgels (WPM30), 50 g WPM (WPM50) or 50 g micellar casein (MC50). Energy expenditure was measured by indirect calorimetry. Blood, breath and urine samples were collected in order to measure substrate oxidation, amino acid profile, glucose and insulin, protein turnover and other metabolic parameters.

Results: DIT was 6.7 ± 3.7%, 13.0 ± 5.0%, 18.0 ± 5.0% and 16.0 ± 5.0% for control, WPM30, WPM50 and MC50, respectively. There was a significant difference between WPM50 and WPM30 ($p < 0.005$) and a trend was observed between WPM50 and MC50 ($p = 0.06$). WPM50 resulted in the highest total, essential, and branched-chain plasma amino acid concentrations when compared with the other study treatments ($p < 0.005$) and a higher insulin concentration than MC50 ($p < 0.005$). Protein oxidation was higher for WPM50 than MC50. Protein turnover was significantly correlated with DIT through total leucine oxidation ($r = 0.52$, $p = 0.005$).

Conclusions: Our findings show that DIT does increase at a dose beyond 30 g of WPM and that the type of dairy protein may have an effect on DIT with WPM tending towards a higher DIT than casein. Although further research is required to understand the mechanism behind the effect of different protein sources on thermogenesis, we suggest that amongst the components of protein turnover, protein oxidation may be an important driver of thermogenesis at doses higher than 30 g. These results have concrete implications when choosing a dose of protein to optimize its thermogenic effect.

Clinical trial registry number: NCT02303080 www.clinicaltrials.gov.

Abbreviations: BCAA, Branched chain amino acids; CHOox, Carbohydrate oxidation; DIT, Diet induced thermogenesis; EAA, Essential amino acids; Fatox, Fat oxidation; HOMA, Homeostatic model assessment; Leuox, Leucine oxidation; MC, Micellar casein; MPS, Muscle protein synthesis; NOLD, Non-oxidative leucine disposal; Post Leuc Bal, Post-prandial leucine balance; Ra, Rate of appearance; Rd, Rate of disappearance; REE, Resting energy expenditure; TAA, Total amino acids; WPM, Whey protein microgels.

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1. Introduction

High protein diets have been repeatedly linked to better weight loss outcomes when compared with other lower protein diets [1], through its effect on both components of energy balance: energy expenditure [2] and food intake [3]. The impact of different protein contents in the diet on energy expenditure and diet induced thermogenesis (DIT) has been studied before and shown to be significant. In fact Soenen et al. reported a clear advantage of a diet containing 1.2 g/kg over one containing 0.8 g/kg on resting energy expenditure and lean body mass [4]. However, in terms of a single meal effect of protein on DIT, the data are scarce. In 1986, Belko et al., compared three meals with different protein contents (15%, 30% and 45% energy) and found that energy expenditure reached a plateau beyond the dose a 30% protein [5]. On the other hand, a meta-analysis by Ravn et al. pooled the results from 5 single meal studies each testing one dose of protein ranging from 11 to 30% of energy, and reported a strong positive association between protein energy intake and DIT [6]. The effect of high protein meals on DIT has been hypothesized to be mediated by the increase of protein turnover (synthesis and degradation) and ureagenesis, energy costly processes of which whole body protein synthesis (WBPS) is a large contributor (about 250–350 kcal/day of heat for a WPBS of 250–350 g/d) [7,8]. Muscle protein synthesis (MPS) has been suggested in some reports to reach a plateau at higher intakes of protein neighboring 20–30 g in healthy adults [9,10]. Therefore, although there seems to be a strong association between DIT and the protein content of a meal, it is not known whether the DIT response will reach a plateau at doses higher than 30 g. Protein source may also have an effect on energy balance. Casein and whey protein have typically been referred to as “slow protein” and “fast protein” respectively, with reference to the rate at which amino acids appear in the blood after their ingestion, the implications of which on protein synthesis, were hypothesized to explain a rise in DIT [11–14]. Although data in the literature on body weight, food intake and body composition are conflicting, clinical trials have reported a greater loss of fat mass and gains in muscle mass after a weight loss diet supplemented with whey protein versus casein when coupled with exercise [15,16]. Soy, casein and whey protein were compared, showing a distinctive effect on food intake (reduced with casein), diet induced thermogenesis (increased with soy) and respiratory quotient (decreased with whey) in a more recent study over 7 days [17]. Acheson et al. [18], tested 3 protein sources in an acute trial on 23 healthy volunteers. Results from the study suggested that whey protein has a metabolic advantage over casein and soy protein, the advantage being a higher diet induced thermogenesis for whey (14%) than casein (12%) and soy (12%) relative to control.

The protein content and source in a meal are thus two factors to take into consideration to optimize the DIT response to a meal. Therefore, the primary objective of the present study was to evaluate the effect of 2 doses of whey protein microgels (WPM; 30 and 50 g), a micellar form of whey protein [19], and a high dose (50 g) of micellar casein on DIT in overweight adults. As a secondary objective, we compared the thermogenic effect of the highest dose of WPM to the same dose of micellar casein (MC) in order to explore the potential mechanisms behind the effect of protein source on DIT. Accordingly, the secondary outcomes included plasma kinetics of amino acids, insulin, glucose, as well as whole body protein turnover using stable isotopes in a subset of the study sample.

2. Methods

2.1. Subjects

Seventeen subjects 20–45 years old were recruited through advertisement in the area of Lausanne, Switzerland. Inclusion criteria were a BMI ranging between 25 and 32 kg/m^2, and a percent fat in or above the overweight range [20]. Subjects had to be otherwise healthy based on the results of a medical visit and blood biochemistry conducted by the clinical unit medical staff. Main exclusion criteria were: history of disease affecting nutrient metabolism such as diabetes, cardiovascular disease, liver disease, malabsorption disorders, significant weight loss (more than 5%) in the last 3 months, food allergies especially cow milk protein, special diets such as vegetarian or high protein (set at >1.6 g/kg), pregnancy, smoking and high physical activity level defined as more than 2 h of moderate to intense physical activity per week. Protein intake was assessed using a food frequency questionnaire validated for protein intake in a Swiss population [21]. Interested subjects were first screened by questionnaire, then invited to return for a screening visit where a blood test determined their final eligibility. After signing an informed consent form, eligible subjects were invited back for their first baseline visit.

2.2. Study design

The clinical trial was a randomized double blind, placebo-controlled, single center, crossover design with four treatment groups. The randomization schedule was generated by the study statistician using William's design with 4 products. The library *Crossdes* in the statistical software R version 2.8.1 was used to generate the experimental design. The schedule was then communicated in sealed envelopes to the metabolic unit staff who enrolled subjects and assigned them to their respective coded treatments. The trial was carried out at the Metabolic Unit of the Nestlé Research Center in Lausanne, Switzerland. Ethical approval was obtained from the ethical board of the Canton Vaud, “Commission Cantonale d’Ethique de Recherche sur l’Etre Humain” (Ref: 269/10). The trial was registered in clintrial.gov under the identifier NCT02303080.

2.2.1. Study protocol

The study design was a crossover including 4 treatment phases separated by a 30-day washout, in order for female volunteers to be tested during the follicular phase of their menstrual cycle. Each treatment phase was comprised of 2 days: a preparation day (Day 1), and a test day (Day 2). On day 1, the subject was invited to the metabolic unit for anthropometric measurements (weight, DEXA) and to fill in questionnaires. During the day the subject consumed 3 standard meals (breakfast, lunch and dinner) and spent the night at the unit to ensure compliance with the standard diet and minimal physical activity. The standard meals were designed according to a standard 2000 kcal and 3000 kcal menu for women and men respectively with a macronutrient breakdown of 55% carbohydrate, 30% fat and 15% protein. On day 2, the subject was woken up at 6:30 am, after a 12 h overnight fast, and prepared for testing. Preparation included weighing, fasting urine collection as well as the insertion of 2 catheters in the subject's forearm and hand for isotope administration and blood collection respectively. The detailed protocol for Day 2 is illustrated in Fig. 1. After collecting total fasting urine (urine collected in the morning and overnight if any) the subject was comfortably placed in a recumbent position, in a small indirect calorimetric chamber (600L) in which respiratory exchange measurements were made. The total duration of the measurement protocol was 420 min (7:00 h) during which the subject rested quietly while watching television or reading. At 90 min, either the control or one of the three protein drinks was given to the subjects who were instructed to consume it within 5 min. At time point 180 min (T180), the subject exited the chamber for a second urine collection and upon re-entry into the chamber, respiratory exchange measurements were continued for a further 210 min. At the end of the 420 min test the subject exited the chamber and a third urine collection was made.

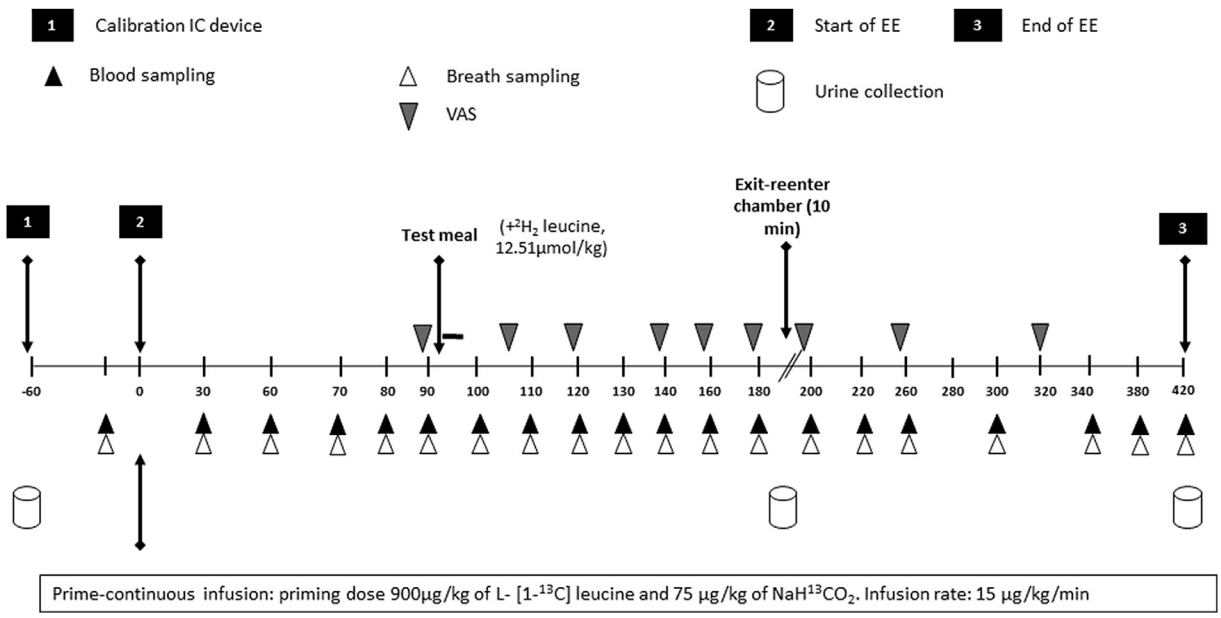

Fig. 1. Testing protocol (Day 2). EE: energy expenditure; IC: indirect calorimetry; VAS: visual analog scales. Time points between 60 and 90 min used to calculate baseline EE. Time points 60, 80 and 90 min (prior to meal ingestion but after VAS) were used to calculate baseline for other parameters (e.g. glucose, insulin, amino acids). Time points between 90 and 420 min were used to calculate post-prandial EE and other parameters.

Water was allowed ad libitum throughout the test. Resting energy expenditure (REE) was obtained from 0 to 90 min and diet induced thermogenesis was measured from 90 to 420 min.

Blood sampling was performed by inserting a catheter in a retrograde fashion into a dorsal vein of the hand which was placed in an infrared-heated glove (Powerlet, USA) to arterialize the venous blood. Multiple blood and breath samples were collected at baseline before meal ingestion at T60, T70, T80 and T90. Upon meal ingestion samples were taken every 10 min until T140 and thereafter every 20 min until the end of the test. A subgroup of subjects (n = 7, 5 males and 2 females) was investigated for whole body protein metabolism, whereby tracers kinetics were measured through the administration of a primed continuous intravenous infusion of [1−^{13}C]-Leucine (Euriso-top, Saint-Aubin Cedex, France) along with a prime of NaH$^{13}CO_3$ (Euriso-top, Saint-Aubin Cedex, France), through a catheter inserted in the other hand. The primer bolus consisted of 900 µg/kg of [1−^{13}C]-Leucine and 75 µg/kg of NaH$^{13}CO_3$, followed by a continuous infusion rate of 15 µg/kg/min of [1−^{13}C]-Leucine. In addition, the test meals were enriched in [$^2H_3$]-Leucine (Euriso-top, Saint-Aubin Cedex, France) for leucine oxidation measurements.

Subjects were asked to rate their hunger and satiety using visual analog scales (VAS). This was done using a Pocket PC (Dell Technologies, Texas) placed in the calorimeter during testing. Hunger/satiety ratings were recorded 5 min before, and 20, 30, 50, 70, 90 and 110, 170 and 230 min after test meal consumption and were scheduled not to coincide with blood sampling times.

2.3. Dietary intervention

The investigational products were produced by the Nestlé Research Center Pilot Plant, and delivered to the investigational site after Ethical Committee's approval.

The test meals included a control containing 50 g of maltodextrin and 3 protein beverages containing: 30 g of WPM (WPM30), 50 g of WPM (WPM50) and 50 g of casein (MC50). The whey protein was incorporated into a shelf-stable drink, in the form of whey protein microgels (WPM). The use of WPM allows the incorporation of large quantities of whey protein in liquid matrices without affecting stability, taste or texture of the product. The macronutrient composition of the test meals is detailed in Table 1a and the amino acids composition is given in Table 1b. Microbiological tests were conducted on a regular basis to insure the safety of study beverages. The sensory profile of the product was developed and monitored using an internal panel at the Nestlé Research Center Experimental Kitchen. Both subjects and investigators were blinded to the test meals, which had similar volumes (400 mL), similar sensory properties, packaged in the same way, and coded.

2.4. Biological measurements

2.4.1. Energy expenditure, Diet induced thermogenesis (DIT) and substrate oxidation

Energy expenditure and substrate utilization were measured in a ventilated-hood, open-circuit, indirect calorimeter (MAX II metabolic system, AEI Technologies, Inc, Naperville, USA). After a stabilization phase of 20–30 min, oxygen consumption (L/min) and carbon dioxide (L/min) production were continuously measured using a Max-II analyzer system (AEI Technologies, Pittsburgh, PA, USA) calibrated before each session. The airflow going through the system was setup at around 30 L/min and

Table 1a
Energy and macronutrient composition of study drinks.

	Control (maltodextrin)	WPM30	WPM50	MC50
Mass (g)	368.9	370.4	375.9	374.9
Energy (kcal)	269.7	284.2	291.7	293.1
Protein (g)	0.0	30.0	50.0	50.0
Protein (% energy)	0.0	42.0	69.0	68.0
Fat (g)	4.4	4.8	4.5	4.9
Fat (% energy)	15.0	15.0	14.0	15.0
Carbohydrates (g)	57.5	30.2	12.8	12.3
Carbohydrates (% energy)	85.0	43.0	18.0	17.0
Fibre (g)	<0.5	<0.5	<0.5	<0.5

Table 1b
Amino acids composition of the investigational products.

Amino acid	Unit	control	WPM30	WPM50	CM50
L-cystine	g/100 g	<0.010	0.242	0.375	0.063
L-Methionine	g/100 g	<0.010	0.193	0.314	0.337
L-aspartic acid	g/100 g	<0.006	0.955	1.566	0.847
L-Threonine	g/100 g	<0.006	0.440	0.721	0.525
L-Serine	g/100 g	<0.006	0.390	0.637	0.698
L-Glutamic acid	g/100 g	<0.006	1.470	2.424	2.674
L-Proline	g/100 g	<0.003	0.469	0.757	1.229
Glycine	g/100 g	<0.006	0.156	0.260	0.214
L-Alanine	g/100 g	<0.006	0.418	0.688	0.358
L-Valine	g/100 g	<0.006	0.474	0.777	0.738
L-Isoleucine	g/100 g	<0.006	0.464	0.763	0.601
L-Leucine	g/100 g	<0.006	1.059	1.742	1.134
L-Tyrosine	g/100 g	<0.006	0.312	0.541	0.641
L-Phenylalanine	g/100 g	<0.006	0.327	0.531	0.595
L-Lysine	g/100 g	<0.006	0.868	1.432	0.985
L-Histidine	g/100 g	<0.006	0.173	0.287	0.330
L-Arginine	g/100 g	<0.006	0.243	0.404	0.435
L-Tryptophane	g/100 g	<0.006	1.19	1.87	1.90

measured using a Pneumotachometer (Hans Rudolph, Shawnee, KS, USA). The concentration of oxygen and carbon dioxide in inhaled and exhaled air were determined respectively by paramagnetic and infra-red analyzers. The Standard Temperature and PressureDry (STPD) correction was applied. Before each test session, these two analyzers were calibrated using pure nitrogen and a mix of 21% O_2 and 5% CO_2 into nitrogen (Classe Saphir ±1–2% relative, Carbagas, Lausanne, Switzerland). Urine collection was used to measure urinary nitrogen content and calculate substrate oxidation.

Energy expenditure was taken as the area under the curve of EE values calculated by the software (Max-II software 3.1.05) and expressed in kcal/min. Diet induced thermogenesis (DIT), corresponding to the extra energy expenditure following beverage ingestion was calculated as the ratio of the area under the EE curve from 90 min to 420 min adjusted for baseline, over the caloric content of the test meal.

$$DIT = \frac{\text{AUC EE}(90-420) - \text{AUC EEbase}}{\text{calorie content of meal}} * 100$$

The nitrogen content of the collected urine was determined using the Kjeldahl method (Agrobio, Rennes, France). Energy expenditure (kcal/min), total protein oxidation, net carbohydrate and fat utilizations (mg/min) were calculated from the respiratory exchange data and urinary nitrogen excretion, using standard equations [22,23] detailed below:

$$\text{Protein}_{Ox}\,(\text{mg/min}) = \text{Urinary Nitrogen excretion}\,(\text{mg/min}) \times 6.25 \quad (1)$$

Where Protein_{Ox} is the amount of protein oxidized per minute and 6.25 is the conversion factor from nitrogen to protein.

$$PVO_2 = \text{Protein}_{Ox}\,(\text{mg/min}) \times 1.01031\,(\text{ml/mg}) \quad (2)$$

Where PVO_2 is the volume of O_2 consumed for protein oxidation and 1.01031 is the volume of O_2 consumed to oxidize 1 g of protein

$$PVCO_2 = \text{Protein}_{Ox}\,(\text{mg/min}) \times 0.84361\,(\text{ml/mg}) \quad (3)$$

Where $PVCO_2$ is the volume of CO_2 produced for protein oxidation and 0.84361 is the volume of CO_2 produced through oxidation of 1 g of protein

$$\left.\begin{array}{l} NPVO_2 = \text{Total } VO_2 - PVO_2 \\ NPVCO_2 = \text{Total } VCO_2 - PVCO_2 \end{array}\right\} \; NPRQ = \frac{NPVCO_2}{NPVO_2} \quad (4)$$

Where $NPVO_2$ is the volume of O_2 consumed for non-protein oxidation and $NPVCO_2$ is the volume of CO_2 produced for non-protein oxidation.

NPRQ is the respiratory quotient relative to carbohydrate and fat oxidation

$$NPRQcho = \frac{NPRQ - 0.707}{0.293} \quad (5)$$

$$NPRQfat = \frac{NPRQ - 1.000}{0.293} \quad (6)$$

Where NPRQcho is the proportion of the RQ coming from carbohydrates, NPRQfat is the proportion of the RQ coming from fat, 0.707 is the NPRQ for 100% fat and 1.000 is the NPRQ for 100% CHO

$$CHO_{ox}\,(\text{mg}) = (NPVO_2 \times RQ_{CHO}) + 1000/828.21 \quad (7)$$

$$Fat_{ox}\,(\text{mg}) = (NPVO_2 \times RQ_{fat}) + 1000/2014.94 \quad (8)$$

Where CHO_{ox} is the amount of carbohydrate oxidized and Fat_{ox} is the amount of fat oxidized. 828.21 is the volume of O_2 consumed by 1000 mg of glucose. 2014.94 is the volume of O_2 consumed by 1000 mg of fat.

2.4.2. Whole body protein turnover

Plasma [^{13}C] and [$^2H_3$]-Leucine (Leu) and ketoiscocaproic acid (KIC) enrichments and concentrations were measured by gas chromatography-mass spectrometry (GC–MS), (Agilent Technologies, Santa Clara, USA). Breath CO_2 isotopic enrichment was determined by gas chromatography-isotope ratio mass spectrometry (GC-IRMS), (Thermo, Bremen, Germany). For breath isotopic analysis, the data were expressed as $\delta^{13}C$ vs. Vienna Pee Dee Belemnite (VPDB), in ‰ and further transformed into atom percent excess (APE). In order to measure the isotopic enrichments of Leu and KIC, plasma samples were prepared with two different protocols. Plasma Leu isotopic enrichments were measured using N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide as derivative reagent [6]. The ions used to measure Leu isotopic enrichments were *m/z* 200, *m/z* 201 and *m/z* 202 for [$^2H_3$]-Leu and *m/z* 302, *m/z* 303 for [1–^{13}C]-Leu. Plasma KIC isotopic enrichments were determined using O-phenylenediamine and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide derivative reagents [12,14]. The ions used to measure the isotopic enrichments were *m/z* 259, *m/z* 260, *m/z* 261 and *m/z* 262 for [^{13}C]–KIC and [$^2H_3$]–KIC, respectively. Further corrections were applied for ^{13}C and $^2H_3$ enrichments for Leu and KIC according to Biolo et al. [24].

Leucine (Leu) fluxes were calculated from the time evolution data of plasma Leu and KIC enrichments, concentration of plasma Leu, and breath CO_2 isotopic enrichment using non-steady state equations according to the dual tracer approach described by Boirie et al. [12].

2.4.3. Amino acid profile

Total, branched-chain and essential amino acids were analyzed by gas chromatography (GC) (Agilent 6980 GC, Hewlett Packard) connected to a quadrupole mass spectrometer (MS) (from Micro mass, Waters, UK) using 20 μL of plasma heparin sample. For the quantitation, plasma samples were spiked with a mixture of stable isotope-labelled amino acids solution (U–^{13}C, U–^{15}N cell free amino acid mix produced by algae from Euriso-top, Saint-Aubin Cedex, France). The

MS was operated in single ion monitoring (SIM) mode. For each amino acid and for its labeled counterpart (used as internal standard), specific ions were monitored to perform the quantitation with results expressed in nmol/mL based on linear regression analysis.

2.4.4. Glycemic response

Glucose was measured in the plasma (250 μL heparin plasma) using an autoanalyser (Dimension X-PAND, Siemens). Briefly, the method measures the absorbance due to the formation of NADH, produced during glucose oxidation. Insulin was measured in plasma (50 μL EDTA plasma) by a highly specific and sensitive two-site enzyme-linked immunosorbent assay (ELISA Insulin, IBL, Germany).

2.4.5. Other metabolic parameters

Total bile acids were measured in plasma using an enzymatic kit (Trinity Biotech, USA) and analyzed using Cobas C111 (Roche). Free fatty acid concentrations were measured in plasma (250 μL, heparin plasma) using a calorimetric enzymatic method adapted for the autoanalyser (Dimension X-PAND, Siemens). Beta-hydroxybutyrate was measured in plasma (EDTA plasma) via a standardized kit (Autokit 3HB, Wako Chem, Germany) and run through a Cobas C111.

2.4.6. Subjective ratings of hunger and satiety

The satiety questions were based on Hill & Blundell's [25] motivational rating questionnaire using the Dietary Ratings Tracker (DRT) software. This software allows the collection of numerical data using the visual analogue scale (VAS). To record an answer, DRT computes the distance between the left of the scale and the point where the subject draws a mark along the line with a stylus. The distance is recorded in millimeters and transformed to a ratio of 100 mm. This method was validated at Nestlé Research Center and showed that the answers using DRT are comparable in accuracy and reproducibility to those obtained with traditional paper scales. The questions asked to the subjects were:

- How strong is your desire to eat? ('very weak – very strong')
- How hungry do you feel? ('not hungry at all – as hungry as I ever felt')
- How full do you feel? ('not full at all – very full')
- How thirsty do you feel? ('not at all thirsty – very thirsty')
- How much do you think you could eat? ('nothing at all – a large amount')
- How much did you like this beverage? (not at all – very much')

After consolidation of the results, and the overall satiety score, the Composite Satiety Score (CSS) was calculated by introducing the results of the individual questions into the following equation:

$$CSS = \frac{\text{Fullness} + (100 - \text{Desire to eat}) + (100 - \text{Hunger}) + (100 - \text{PFC})}{4} \quad (9)$$

2.5. Statistical analyses

Sample size calculation was based on the estimated difference in effect between the 2 doses of WPM, from the study of Acheson et al., 2011 [18], and a power of 80%. The effect size in DIT between WPM30 and WPM50 was estimated to be 2.8% and the within standard deviation to 2. One-sided testing procedure was used with a significance level of 0.025. To allow two other comparisons at least as large as 2.8% (WPM30 vs Control and WPM50 vs Control), Bonferroni correction was applied resulting in a level $\alpha = 0.025/3$. The required sample size was thus calculated to be 15 subjects. In order to accommodate a possible high drop-out rate in a 4 period cross-over study, 20 subjects were planned to be enrolled and randomized.

All primary and secondary endpoints were analyzed using a linear mixed model adjusting for baseline with the product and the visit as fixed effects. Subject-specific random effect was added to account for the correlated observations coming from a crossover design and all efforts via suitable transformations were made to achieve normality of the response variables. Non-parametric methods suited for the analysis of crossover studies described in Putt et al. [26] were also used.

Multiple comparisons procedures were conducted and each parameter analyzed, single-step method was used.

To compute the AUCs, individual curves were first smoothed using local quadratic regression. The curve characteristics (AUC, C_{max} and t_{max}) were retrieved from this smoothed function. Cmax was defined as the highest peak in the curve and Tmax as the time to peak. The trapezoidal rule was used on the smoothed data to compute the AUCs. The adjustment for baseline was performed by subtracting the baseline AUC (AUC EEbase) from the total AUC considered. The baseline EE was calculated as a weighted mean of the data collected 30 min before ingestion with more weight applied to the data just before the ingestion. The primary endpoint, DIT was calculated as explained above in the Energy Expenditure section of the methods. Carbohydrate and fat oxidation were treated in a similar manner, comparing AUC adjusted to baseline. Protein oxidation was compared at the 2 main post-ingestion urine collection timepoints described earlier, and data was adjusted to fasting urine nitrogen.

Secondary endpoints including amino-acids (branched-chain, essential, leucine and total), glucose and insulin, free fatty acids, bile acids, hydroxybutyrate as well as CSS were measured using post-ingestion adjusted AUC with the baseline as the mean of measurements before ingestion at 60, 80 and 90 min.

The association between DIT and whole body protein turnover was explored by plotting protein synthesis, leucine oxidation, and postprandial leucine balance against DIT. The correlation analysis was performed by Spearman rank correlation. Adjusted areas under the curve were used for this analysis. Statistical significance was set at $p = 0.05$. For trend, a level of 0.1 was considered.

Results are expressed as mean ± SEM unless stated otherwise. Statistical analyses were performed by R 3.0.1 or higher.

3. Results

3.1. Baseline characteristics

Overall, 17 overweight subjects were enrolled in the study, 65% male and 35% women. The subjects' mean age was 32.7 ± 7.5 years, BMI was 27.8 ± 1.5 kg/m^2 and body fat was 32.4 ± 6.3%. Insulin resistance was assessed at baseline via HOMA-IR, the average value was 3.7 ± 1.0.

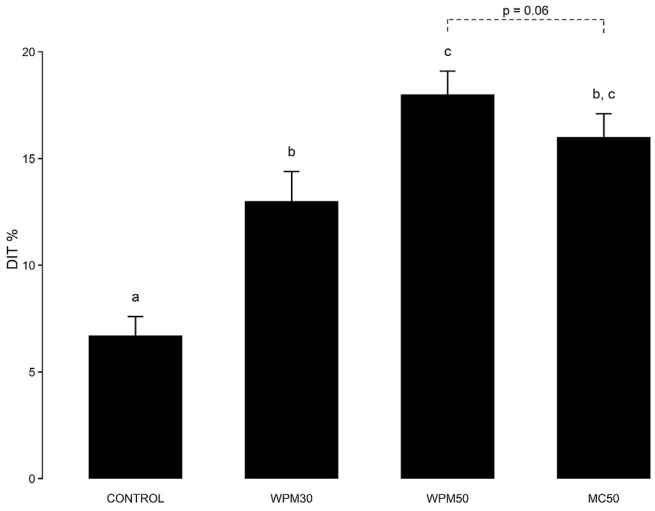

Fig. 2. Baseline adjusted diet-induced thermogenesis (DIT). Data reported as means ± SEM. A linear mixed model adjusting for baseline was used. Treatment groups with different superscript letters are significantly different, $p < 0.05$. Trend observed between WPM50 and MC50 ($p = 0.06$). No significant difference observed between MC50 and WPM30. WPM50: 50 g whey protein microgels, WPM30:30 g whey protein microgels, MC50: 50 g micellar casein.

3.2. Energy expenditure (EE) and diet induced thermogenesis (DIT)

Diet-induced thermogenesis, primary outcome of the trial, was significantly higher ($p < 0.0001$) in the WPM30 (13.0 ± 5.0%), WPM50 (18.0 ± 5.0%) and MC50 (16.0 ± 5.0%) than control (6.7 ± 3.7%) (Fig. 2). The relative difference was calculated to compare protein groups. As such, DIT for WPM50 was significantly higher ($p = 0.006$) than WPM30 by 34%. The difference between WPM50 and MC50 did not reach significance, however, there was a trend ($p = 0.062$) towards a higher DIT for WPM50 versus MC50 by 16%.

Baseline-adjusted energy expenditure AUC values are presented in Table 2. Briefly, there was a significant difference between the protein groups and control ($p < 0.0001$) as well as between WPM30 and WPM50 ($p < 0.05$).

Energy expenditure rose after the intake of the 4 test meals, with a significantly lower peak ($p < 0.005$) for control when compared with the 3 protein test meals. When adjusting to baseline REE, peak EE ($cmax_{adj}$) for WPM30 (0.21 ± 0.07 kcal/min) was lower and significantly different ($p = 0.044$) from WPM50 (0.25 ± 0.08 kcal/min), and WPM50 was significantly higher ($p = 0.011$) than MC50 (0.20 ± 0.06 kcal/min). Energy expenditure after MC50 intake maintained a plateau until the end of the trial whereas EE tended towards baseline in the other groups. In terms of the time to peak (Tmax), WPM50 and MC50 were both delayed when compared with control (51.8 ± 15.7 min and 39.6 ± 15.7 min respectively), however no difference was observed between the two protein groups.

3.3. Substrate oxidation

Substrate oxidation adjusted values are presented in Table 2. The AUC for CHO_{ox} was significantly lower ($p < 0.0001$) than control in the three protein groups. CHO_{ox} was 183% lower for WPM50 than WPM30 ($p < 0.05$) however the significance was lost when using non-parametric tests. No significant difference was observed between WPM50 and MC50. The baseline-adjusted AUC for Fat_{ox} was

Table 2
Energy expenditure (EE) substrate oxidation and plasma metabolic parameters.

	Control (n = 17)	WPM30 (n = 17)	WPM50 (n = 17)	MC50 (n = 17)
EE (AUC_{adj}; kcal)	18.1 ± 2.4[a]	38.2 ± 3.9[b]	52.6 ± 3.2[c]	45.3 ± 3.2[b#c*]
EE $Cmax_{adj}$ (kcal/min)	0.12 ± 0.05[a]	0.21 ± 0.07 [b]	0.25 ± 0.08[c]	0.20 ± 0.06[b]
EE Tmax (min)	172.8 ± 58.3[a]	188.1 ± 17.6[ac]	224.9 ± 30.2[b]	211.0 ± 71.2[bc]
CHOox (AUC_{adj}; mg)	11,144.3 ± 1284.9[a]	2359.2 ± 974.4[b]	−1964.4 ± 1004.3[b£c]	−4172.2 ± 1149.3[c]
CHO $Cmax_{adj}$ (mg/min)	77.3 ± 23.1[a]	42.0 ± 17.6[b]	15.9 ± 19.2[c]	5.0 ± 19.0[c]
CHO Tmax (min)	205.9 ± 23. 4[a]	202.9 ± 30.0[a]	192.0 ± 22.2[a]	188.1 ± 41.4[a]
Fatox (AUC_{adj}; mg)	−3588.1 ± 675.7[a]	1804.4 ± 785.5[b]	4115.3 ± 769.3[b£]	4463.3 ± 739.1[c]
Fatox $Cmax_{adj}$ (mg/min)	12.2 ± 14.[a]	19.9 ± 11.9[ab]	25.1 ± 11.2[b]	24.3 ± 10.3[ab]
Fatox Tmax (min)	172.8 ± 58.3[a]	265.8 ± 127.3[a]	281.6 ± 92.9[a]	249.7 ± 112.2[a]
Protox post-ingestion 1 (mg)	56.41 ± 3.6[a]	56.0 ± 5.6[a]	57.2 ± 5.4[a]	53.6 ± 3.2[a]
Protox post-ingestion 2 (mg)	44.70 ± 3.3[a]	60.76 ± 6.8[b]	69.8 ± 4.5[b&]	58.5 ± 5.9[b]
Plasma glucose (AUC_{adj}; mmo/l × min)	190.0 ± 47.8[a]	−4.9 ± 28.5[b]	−49.6 ± 21.0[b]	−41.6 ± 20.5[b]
Glucose $Cmax_{adj}$ (mmol/l)	3.2 ± 1.5[a]	0.72 ± 0.76[b]	0.06 ± 0.33[b£]	0.17 ± 0.48[b]
Glucose Tmax (min)	170.0 ± 65.6[a]	181.6 ± 83.6[ab]	226.0 ± 83.7[b]	186.2 ± 76.3[ab]
Plasma insulin (AUC_{adj}; µIU/ml × min)	8666.0 ± 959.2[a]	7384.5 ± 715.3[a]	5731.6 ± 608.5[a£b]	3588.1 ± 389.3[c]
Insulin $Cmax_{adj}$ (µIU/ml)	86.0 ± 28.0[a]	85.5 ± 25.3[a]	54.7 ± 15.5[b]	34.5 ± 24.4[c]
Insulin Tmax (min)	152.8 ± 8.2[a]	148.6 ± 6.3[a]	146.0 ± 5.6[a§*]	192.3 ± 82.0[a&#]
Plasma amino acids				
Total (AUC_{adj}; µmol/l × min)	47,692.5 ± 5708.8[a]	361,765.2 ± 11,334.4[b]	569,413.3 ± 11,664.8[c]	470,772.4 ± 1,9408.5[b#c*]
$Cmax_{adj}$ (µmol/l)	−71.3 ± 122.1[a]	1487.2 ± 245.1[b]	2285.0 ± 357.3[c]	1540.8 ± 393.6[b]
Tmax (min)	199.5 ± 154.3[a]	183.2 ± 9.2[a]	198.9 ± 18.9[a]	178.7 ± 18.1[a]
Essential (AUC_{adj};µmol/l × min)	1691.2 ± 2489.3[a]	212,271.8 ± 6030.2[b]	371,819.0 ± 8979.6[c]	254,330.0 ± 1,1060.9[b#]
$Cmax_{adj}$ (µmol/l)	−68.4 ± 70.0[a]	994.8 ± 153.2[b]	1609.6 ± 240.5[c]	892.5 ± 214.7[b#]
Tmax (min)	296.9 ± 159.3[a]	185.62 ± 10.0[a]	208.7 ± 20.9[a]	195.5 ± 61.0[a]
Branched-chain (AUC_{adj};µmol/l × min)	−4383.5 ± 1377.7[a]	115,242.2 ± 3585.0[b]	219,844.77 ± 6116.5[c]	145,163.24 ± 7653.7[b#]
$Cmax_{adj}$ (µmol/l)	−25.0 ± 37.7[a]	552.9 ± 90.8[b]	951.9 ± 144.4[c]	509.6 ± 126.0[b]
Tmax (min)	278.2 ± 163.5[a]	186.3 ± 10.5[a]	217.5 ± 21.6[a]	198.7 ± 61.7[a]
Leucine (AUC_{adj};µmol/l × min)	941.6 ± 504.6[a]	53,893.3 ± 1618.9[b]	99,081.5 ± 2615.7[c]	54,696.7 ± 2474.5[b]
$Cmax_{adj}$ (µmol/l)	2.7 ± 12.0[a]	264.4 ± 42.2[b]	444.3 ± 67.7[c]	203.0 ± 43.2[b#]
Tmax (min)	376.1 ± 109.4[a]	187.0 ± 10.5[b]	217.6 ± 21.0[b]	193.6 ± 63.1[b]

All data is presented as AUC_{adj} and $Cmax_{adj}$ adjusted to baseline, except for protein oxidation. All data presented as mean ± SEM. Values with different superscript letters are significantly different.
*Trend between MC50 and WPM50, £Trend between WPM30 and WPM50, #Trend between MC50 and WPM30, § Trend between WPM50 and control, & Trend between MC50 and control.
AUC: area under the curve, Protox post-ingestion 1 and post-ingestion 2: protein oxidation as measured by indirect calorimetry, urine collection at time points 180 min and 420 min respectively.

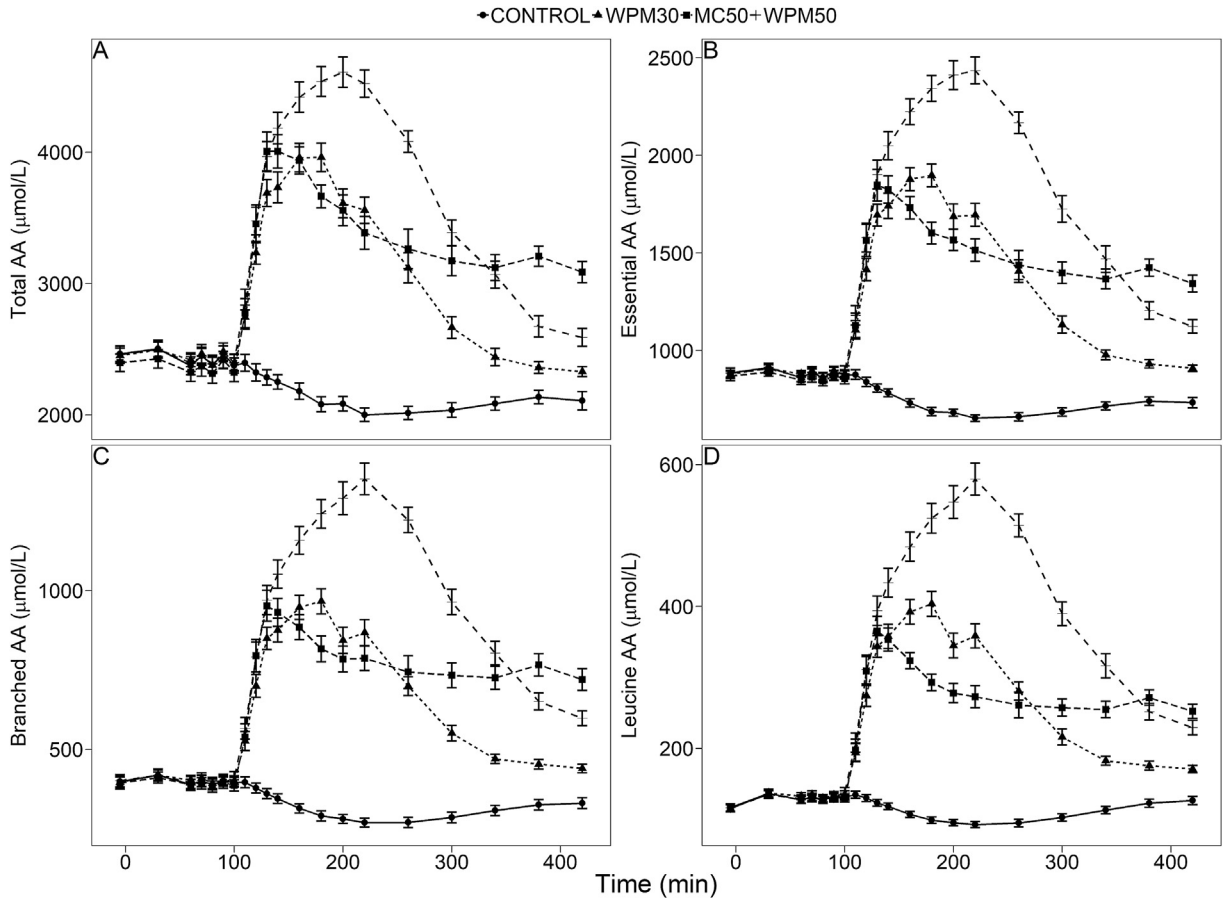

Fig. 3. Plasma amino acid (AA) concentrations as a function of time. Plasma total AA (**A**), essential AA (**B**), branched chain AA (**C**) and leucine (**D**) after WPM30, MC50, WPM50, and control meal (time of meal ingestion: 90 min) measured by GC–MS. Data presented as means ± SEM. A linear mixed model adjusting for baseline was used. $Cmax_{adj}$ for plasma aa (A, B, C, D) for WPM50 significantly higher than other groups. $Cmax_{adj}$ significantly higher for WPM30 than MC50, $p < 0.05$. WPM50: 50 g whey protein microgels, WPM30:30 g whey protein microgels, MC50: 50 g micellar casein.

significantly higher than control by 150%, 215% and 224% for WPM30, WPM50 and MC50 respectively. Fat_{ox} was significantly higher ($p < 0.05$) by 128% for WPM50 relative to WPM30, however the significance was lost with the non-parametric test. MC50 and WPM50 groups were not significantly different. Endpoint protein oxidation (baseline-adjusted) was higher ($p < 0.05$) after the intake of the three protein test meals compared to control. The difference with control versus WPM50 was higher than that with WPM30 by 44%, however, the difference between WPM50 and WPM30 was not significant ($p = 0.09$). There was a slight trend observed towards a higher protein oxidation in the case of WPM50 versus MC50 by approximately 56% ($p = 0.06$).

3.4. Plasma amino acids

Total amino acid (TAA) concentrations increased significantly over baseline for WPM30, WPM50 and MC50 meals and when compared with the control (Fig. 3). The baseline-adjusted AUC was highest ($p < 0.0001$) for WPM50 amongst the protein groups, higher by 92% and 28% than WPM30 and MC50 respectively. The same pattern was observed for branched chain amino acids (BCAA), essential amino acids (EAA) and leucine whereby WPM50 resulted in the highest plasma concentration when compared with other protein groups. In terms of BCAA, plasma concentrations (AUC_{adj}) were higher ($p < 0.0001$ in linear model and $p < 0.05$ in non-parametric test) for WPM50 than WPM30 and MC50 by 112% and 60% respectively. As for leucine AUC, WPM50 was higher ($p < 0.0001$) than WPM30 and MC50 by 52% and 47% respectively.

3.5. Glucose and insulin response

The baseline-adjusted AUC of plasma insulin for WPM30, WPM50 and MC50 were 22.8 ± 8.5, 17.6 ± 7.5 and 11.2 ± 5.1 μIU/ml respectively. WPM50 thus incurred a 57% higher ($p < 0.01$) insulin concentration than MC50, and 22% lower ($p < 0.04$) than WPM30. Postprandial glycemic response was lower for the protein test meals than control however there were no significant differences in AUC_{adj} for glucose between the protein test meals. Glucose and insulin concentrations as a function of time are represented in Fig. 4.

3.6. Whole body protein turnover

At baseline before test meal ingestion total rate of Leu appearance (total Leu Ra) and total rate of Leu disappearance (total Leu Rd) were not significantly different amongst the 4 test meal conditions. Total Leu Ra values (mean ± SEM, $n = 7$) were calculated at 1.22 ± 0.08, 1.40 ± 0.10, 1.43 ± 0.09 and 1.32 ± 0.12 μmol/kg/min for the control, WPM30, WPM50 and MC50 meals, respectively. During the first 90 min, the isotopic steady state was reached (e.g. slope of ^{13}C-KIC was not significantly from zero, $P = 0.53$, data not reported) allowing to study postprandial Leu kinetics using a second tracer (e.g. [$^{2}H_3$]-Leu) that was incorporated into the different test meals. Total Leu Ra and Rd measured in this study before meal ingestion are in

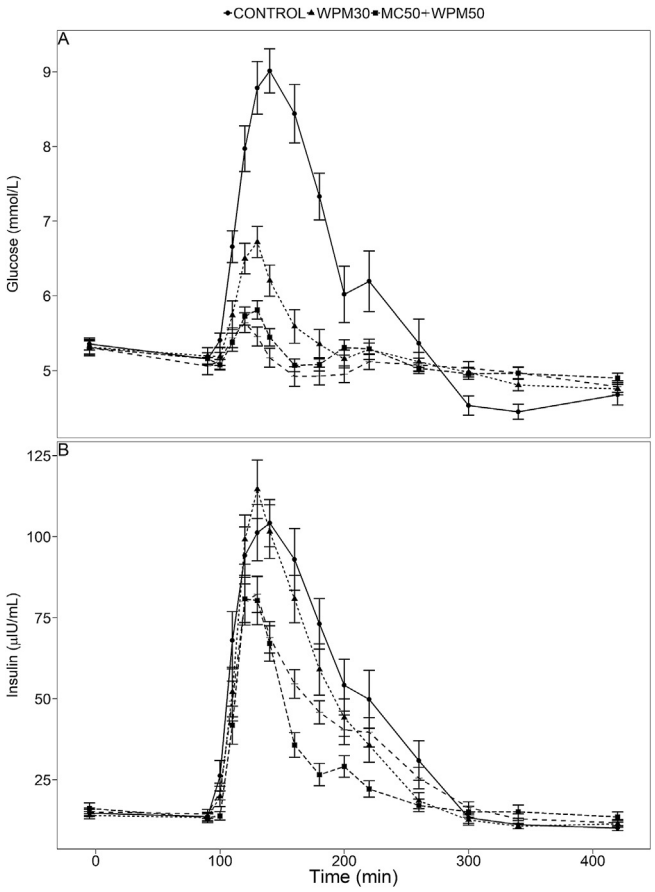

Fig. 4. Plasma glucose (A) and Insulin (B) from baseline to 420 min. Data presented as Mean ± SEM. Time of meal ingestion: 90 min. A linear mixed model adjusting for baseline was used. (**A**) Blood glucose: WPM30, WPM50 and MC50 different from Control; $Cmax_{adj}$ WPM30 significantly different from WPM50; (**B**) Insulin: $Cmax_{adj}$ significantly different amongst WPM30, WPM50 and MC50, $p < 0.05$. WPM50: 50 g whey protein microgels, WPM30:30 g whey protein microgels, MC50: 50 g micellar casein.

agreement with the published data of Boiry et al. in healthy subject (i.e. total Leu Ra about 1.49 ± 0.13 µmol/kg/min at baseline) [12].

After each protein meal ingestion a significant ($p < 0.05$) and rapid increase of total Leu Ra and total Leu Rd was observed compared to the control, with WPM50 resulting in the highest rates ($p < 0.05$) when compared with WPM30 and MC50. Only a trend was observed suggesting a higher rate for WPM30 relative to MC50. The corresponding calculated AUCs for non-oxidative leucine disposal (NOLD), total leucine oxidation (Leu Ox) and postprandial leucine balance (Post Leu Bal) are shown in Fig. 5. The AUCs of Total Leu Ox (mean ± SE, expressed in µmol/kg) were 209.1 ± 15.6, 334.9 ± 39 and 170.3 ± 5.4 for WPM30, WPM50 and MC50, respectively. Significant differences were observed between WPM30 and MC50 ($P < 0.05$), between WPM50 and MC50 ($p < 0.05$) and between WPM50 and WPM30 ($p < 0.05$) (Fig. 5).

For NOLD representing the total protein synthesis, no significant differences were observed between WPM30, WPM50 and MC50 meals. The AUCs of NOLD (mean ± SE, expressed in µmol/kg) were 174.9 ± 36.1, 204.3 ± 61.7 and 164.0 ± 23.56 for WPM30, WPM50 and MC50, respectively.

Total postprandial Leu balance calculated over a period of 420 min was modulated by the type of meal ingested (Fig. 5). Postprandial Leu balance calculated as AUC were 171.5 ± 6.3, 294.1 ± 19.9 and 240.9 ± 18.8 and µmol/kg for WPM30, WPM50 and MC50, respectively. Significant differences were observed between WPM30 and MC50 ($p < 0.05$) and between WPM50 and WPM30 ($p < 0.05$), whereas no significant change was found between WPM50 and MC50 ($p > 0.05$).

Based on the subset analysis of whole body protein turnover measured in 7 subjects, the correlation between protein turnover and DIT was explored and the strongest correlation with DIT was found to be for total leucine oxidation ($r = 0.52$, $p = 0.0053$) and post-prandial leucine balance ($r = 0.59$ $p = 0.0011$). Protein synthesis was also significantly correlated with DIT however the correlation was weaker ($r = 0.39$, $p = 0.043$).

3.7. Other metabolic markers

There were no significant differences amongst protein meals in terms of plasma free fatty acids, hydroxybutyrate or bile acids.

3.8. Subjective ratings of hunger and satiety

Visual analog scales results showed no significant difference in terms of composite satiety score (CSS) and other components of the satiety/hunger subjective ratings.

4. Discussion

Our results show an increase in diet induced thermogenesis of 11% from baseline, for the highest dose of WPM (50 g) relative to control. This is in line with the increase in energy expenditure resulting from protein ingestion seen in previous studies [5,18] since protein-rich meals have a higher DIT than carbohydrate rich meals. A mixed diet is known to have a DIT between 5 and 15% of total daily energy intake and is expected to be higher with a greater protein ratio [27]. Even though we cannot accurately compare different studies, the energy expenditure following WPM50 intake in the present study was higher than responses previously recorded [5,18]. This is likely to be attributed to the higher protein/energy ratio of 0.7 of the meal in the current trial. Studies showed that protein content is strongly associated with DIT, as reported in a meta-analysis by Ravn et al. [6]. The present results agree with these findings whereby we demonstrate that 50 g WPM incurred a 34% relative difference in DIT when compared with 30 g WPM. This has definite implications when choosing a dose of whey protein to optimize its thermogenic effect. The concept of “slow and fast” protein stipulates that the higher rate of protein digestion and absorption drives protein synthesis and retention [11,12,14,28], an energy–costly process which in turn results in higher DIT [29]. Therefore, even though there was reason to believe that DIT might reach a plateau at doses of 20–30 g as was observed for muscle protein synthesis outcomes [9,10], we show here that the administration of 50 g protein still leads to an incremental effect when compared with the 30 g dose. In the light of these findings, we can suggest that protein synthesis is not the only determinant of DIT.

The relationship between DIT and other parameters such as amino acid kinetics, whole body protein turnover and insulin response have been reported in the previous studies mentioned above [11,12,14,28,29]. In addition, in the present study, these parameters were explored as part of the second objective to further understand the drivers of DIT, by comparing WPM and casein (50 g) known to have different effects on protein synthesis and turnover [11,12]. The main findings from this comparison are a trend towards a higher DIT ($p = 0.06$ for difference in AUC and cmax significantly different, $p < 0.05$), a significantly higher postprandial amino-acidemia, protein turnover (namely leucine oxidation) and insulin response for WPM50.

The effect of protein type on DIT, in the literature, is inconclusive. In a review of the evidence on metabolic effects of dairy protein by

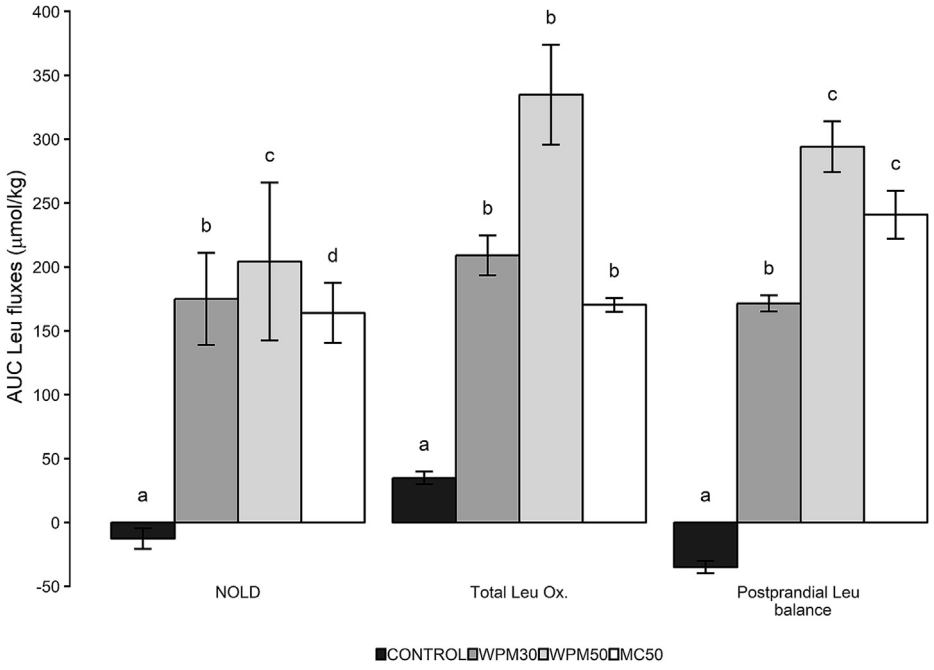

Fig. 5. Effect of WPM and casein meal ingestion on whole body protein turnover and postprandial Leucine balance. Data presented as means ± SEM, n = 7, NOLD (protein synthesis), Total Leu oxidation and postprandial Leu balance expressed as AUC after meal intake. Values with different superscript letters are significantly different. A non-parametric Rank-based test was used. $p < 0.05$. As compared to control group, NOLD, Total Leu Ox, postprandial Leu balance values were significantly different after mixed meals containing protein. AUC: area under the curve, NOLD: non-oxidative leucine disposal, Leu Ox: leucine oxidation, WPM50: 50 g whey protein microgels, WPM30:30 g whey protein microgels, MC50: 50 g micellar casein.

Bendtsen et al. [30], the authors state that there is no conclusive evidence for the superiority of one protein source over another in terms of DIT. In addition, to date, only four studies have examined the direct comparison between whey and casein, the conclusions of which are not in agreement. Acheson et al. [18] reported a difference in DIT of 2% between whey and casein, in an acute trial on 23 lean young men. Bendtsen et al. [31] and Lorenzen et al. [32] administered whey versus casein to overweight and obese individuals showing no significant difference in DIT between protein groups, in the acute and 24 h setting respectively. Finally a 7 day study by Alfenas et al. [17] where casein, whey and soy protein given to normal weight subjects did not incur significantly different DIT post intervention. The dissimilarities in protein doses, duration of trial and study populations amongst the 4 studies, render the findings difficult to compare. The design of the present study is closest to that of Acheson et al., 2011 [18], however the 2.6% difference in DIT between WPM and casein did not reach significance as we only observed a trend ($p = 0.06$). This could be attributed to various factors such as the weight status of subjects and the micellar structure of whey administered in our study. However we believe that the most relevant factor is an insufficient sample size. In fact, our sample size ($n = 17$) was not calculated to see a difference between WPM and casein but rather between 2 doses of WPM (primary outcome). The former may have required a larger number of subjects. Indeed, our retrospective sample size calculation shows that 39 subjects would have been required to observe a significant difference in DIT of 2.6% between WPM50 and MC50. The weight status of subjects is unlikely to have been the major contributor to the lack of significance as obese and lean subjects were previously shown to have a similar DIT response to different treatments [33]. As for the whey micellar structure, the large difference in amino acid profile between WPM and MC would not support a blunted difference in DIT between these two groups. Therefore we believe that a greater number of participants would have possibly allowed us to see a significant difference between WPM50 and MC50.

Given the hypothesis that thermogenesis is stimulated by amino acidemia, the energy expenditure response to the intake of protein is expected to be consistent with the relevant amino acid profile in the blood. Our results clearly show that for the duration of the test, WPM50 incurred the highest response (measured as AUC) in terms of BCAA and Leu. This matches the trend in DIT observed in favor of WPM50. However our results cannot predict whether the difference between WPM50 and MC50 would be maintained beyond the duration of the test as amino acid and energy expenditure levels did not go back to baseline 5.5 h post-meal. Although it is a limitation of the study not to be able to measure EE for a longer duration, we could not impose a longer testing period to the subjects in our study conditions. Nevertheless, and following the threshold hypothesis, it is more likely that the surge in aminoacidemia would drive thermogenesis rather than low persistent levels of amino acids such as the case for casein.

At the protein turnover level, our subset analysis showed that the higher levels of total, essential, branched chain and leucine did not translate into a higher protein synthesis for WPM50 when compared with MC50. In fact, even WPM30 and WPM50 were not different despite the wide gap in their respective amino acid profiles. On the other hand, protein oxidation was higher as a result of WPM50 in comparison with MC50, as well as with WPM30. This is demonstrated both by the significant difference in leucine oxidation and the trend seen in endpoint protein oxidation measured using indirect calorimetry. In addition, given the stronger correlation of DIT with leucine oxidation, we can hypothesize that at high doses of protein (30 g and beyond), the contribution of protein oxidation to DIT may be greater than that of protein synthesis. The latter potentially reaches a plateau above a certain concentration of plasma amino acids, as was already demonstrated for muscle protein synthesis [9,10]. Beyond this point, the increase in thermogenesis would be driven by protein oxidation.

Another protein turnover component to consider is postprandial leucine balance, also positively correlated with DIT in our

study. Post-prandial leucine balance represents the net anabolic effect of protein intake and was previously shown to be higher as a result of casein ingestion versus whey when adjusting for leucine content [14]. However when given as isonitrogeneous doses, the results were reversed with a larger treatment difference seen in the elderly versus the young population. Our results agree with Dangin et al. [12] whereby MC50 had a higher leucine balance than WPM30 (similar leucine content) whereas the difference between MC50 and WPM50 did not reach significance, but was in favor of WPM50.

Finally, whey protein was shown to be more insulinogenic than casein in the literature [18,34,35]. Post-prandial insulin response is believed to contribute to diet induced thermogenesis, as it shifts substrate oxidation from fat to carbohydrates resulting in a slightly higher thermogenesis [27]. The stimulating effect of insulin may have also contributed to the trend in DIT observed between WPM50 and MC50. However in our study we did not observe a difference in carbohydrate and fat oxidation between WPM50 and MC50. Since a shift from fat to carbohydrate oxidation is the main mechanism mediating the effect of insulin on thermogenesis, we cannot conclude on the contribution of post-prandial insulin to the difference in DIT in our study.

The present study was conducted as a proof of concept in the acute setting. Results thus cannot predict whether supplementation of WPM over the long term would lead to a sustained increase in energy expenditure or DIT. On the other hand these findings give an indication of the percent increase in energy expenditure that would be induced by a change in the whole diet through protein quantity or quality. As such, a difference in DIT which seems clinically irrelevant in the acute setting could contribute to a significant calorie deficit on the long term.

5. Conclusion

This study is the first to evaluate the effect of WPM on thermogenesis in overweight adults. Our results show an incremental thermogenic effect beyond 30 g of WPM, as well as a trend towards a thermogenic advantage for WPM over casein. We suggest that the quantity and type-driven difference in DIT may be mediated by a difference in protein oxidation rather than protein synthesis beyond the 30 g dose. In order to better understand the link between whole body protein turnover, thermogenesis, protein amount and source, a dose response study based on amino acid profile should be conducted with the aim of measuring protein turnover and its impact on thermogenesis.

Conflict of interest

The authors declare that they have no competing interests. All authors are employees of Nestec at the Nestlé Research Center, Lausanne, Switzerland.

Authors' contribution

Amira Kassis and Jean-Philippe Godin designed the research and wrote the paper.

Sophie E. Moille conducted the clinical trial, analyzed the data and contributed to the writing of the paper.

Corinne Nielsen-Moennoz, Sylvianne Oguey-Araymon, Fabienne Praplan, Karine Groulx, Frederik Delodder conducted the clinical trial.

Maurice Beaumont supervised clinical trial implementation.

Irina Monnard, Anne-France Kapp, Corinne Ammon-Zufferey, Nathalie Frei, Laurence Guignard analyzed biological samples.

Julien Sauser conducted statistical analyses.

Katherine Mace was involved in the design and supervision of the research.

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