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Impact of Phenylketonuria Type Meal on Appetite, Thermic Effect of Feeding and Postprandial Fat Oxidation

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Abbreviations: PKU, phenylketonuria; EE, energy expenditure; EI, energy intake; TEF, thermic effect of feeding; RMR, resting metabolic rate; RER, respiratory exchange ratio; GLP-1, glucagon like peptide-1; PYY, peptide YY.

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Study registration: This trial was registered at ClinicalTrials.gov as NCT02440932.

SUMMARY

Background: Dietary management of phenylketonuria (PKU) requires the replacement of natural protein-containing foods with special low protein foods. The effect of a PKU type diet on factors contributing to energy balance requires investigation.

Objective: To investigate the impact of a PKU type meal on appetite ratings, gut appetite hormones, thermic effect of feeding (TEF) and fat oxidation.

Methods: Twenty-three healthy adults (mean ± SD age: 24.3 ± 5.1 years; BMI: 22.4 ± 2.5 kg/m^2) participated in a randomized, crossover design study. Each participant conducted two (PKU and Control) experimental trials which involved consumption of a PKU type meal and protein substitute drink or an isocaloric and weight matched ordinary meal and protein-enriched milk. Appetite, metabolic rate, fat oxidation measurements and blood collections were conducted for the duration of 300 minutes. On the completion of the measurements *ad libitum* buffet dinner was served.

Results: Responses of appetite ratings, plasma concentrations of GLP-1 and PYY (P>0.05, trial effect, two-way ANOVA) and energy intake during *ad libitum* buffet dinner (P>0.05, paired t-test) were not significantly different between the two trials. The TEF (PKU, 10.2 ±1.5%; Control, 13.2 ± 1.0%) and the total amount of fat oxidized (PKU, 18.90 ± 1.10 g; Control, 22.10 ± 1.10 g) were significantly (P<0.05, paired t-tests) lower in the PKU than in the Control trial. The differences in TEF and fat oxidation were significant (P<0.05, paired t-tests) for the post-meal period.

Conclusions: Consumption of a meal composed of special low protein foods has no detrimental impact on appetite and appetite hormones but produces a lower TEF and postprandial fat oxidation than an ordinary meal. These metabolic alterations may contribute to the increased prevalence of obesity reported in patients with PKU on contemporary dietary management.

Clinical trial registration: The trial has been registered in ClinicalTrials as NCT02440932.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder of phenylalanine (PHE) metabolism resulting from the dysfunction of the enzyme phenylalanine hydroxylase and leading to accumulation of PHE to neurotoxic levels [1]. PHE levels are controlled by a PKU diet [2, 3] which does not permit intake of natural protein foods rich in PHE, such as meat, cheese, poultry, eggs and milk, and restricts plant or vegetable proteins such as potatoes and cereals to small amounts depending on disease severity and patient's compliance to the dietary treatment [4]. Thus, the diet of patients with PKU comprises predominantly of foods low in natural protein and prescribed special low protein foods, which are high in carbohydrate (CHO) and fat [5], and PHE-free protein substitutes [6-8].

Despite the very restrictive nature of the PKU diet, prevalence of overweight and obesity in patients with PKU is similar to general population with more female PKU patients developing obesity than males [9-11]. From an energy balance point of view, increased body fatness in patients with PKU should mainly relate either to enhanced energy intake (EI) and/or reduced energy expenditure (EE). With respect to EI, it has been reported that PKU special low protein foods, such as bread, pasta, flour and breakfast cereals, provide more energy than their protein-containing equivalent amount of food [8, 12]. However, the aetiology of obesity in individuals with PKU may be also associated with effects of the PKU dietary regimen on a wider range of factors contributing to energy balance regulation.

Previous research in healthy people suggested that, in comparison to meals providing the recommended protein amounts, high protein meals enhance satiety and postprandial responses of anorexigenic hormones such as PYY, GLP-1 and CCK [13-15]. It is also known that protein has the highest and most prolonged thermic effect of all macronutrients, followed respectively by carbohydrate and fat [16, 17]. In addition, enhanced protein content of the meal was reported to upregulate fat oxidation [15, 17, 18]. Thus, meals based on PKU special low protein foods

can be expected to have a detrimental impact on appetite and satiety, leading to an enhanced EI, and simultaneously attenuate TEF and fat oxidation, factors known to contribute independently to increased obesity risk [19].

This is a mechanistic study aimed at investigation of the impact of meal based on PKU special low protein foods on subjective appetite scores, gut appetite hormones, thermic effect of feeding and postprandial fat oxidation in healthy individuals.

2. Materials and methods

Participants

Participants of this study were essentially healthy women and men with body mass index (BMI) < 27 kg/m^2, recruited by advertisements and word of mouth. Study participants were required to be non-smokers, to have stable body weight for one month prior to study enrolment, and were not taking any medication, nutritional supplement or being on a special diet. Female participants were not pregnant and had no disturbances in menstrual cycle. Exclusion criteria included chronic illness, eating disorders and history of gastrointestinal operations which could interfere with the results of the study. Written informed consent were obtained prior commencing the study. The Ethics Committee of the College of Medical, Veterinary and Life Sciences, Glasgow University approved the study.

Study design

The study applied a randomized, crossover design with two sequenced experimental trials, separated by one week. A randomization scheme was generated using the website (randomization.com) to allocate participants to the experimental trials. Trials were marked as Control and PKU by the researcher. Each participant started first trial according to allocated trial randomization. On the morning of each experiment trial, participants reported to the

metabolic research unit between 8:00 and 9:00 after an overnight fast. Height, body mass, body fat, and resting metabolic rate (RMR) were measured. A cannula was introduced into an antecubital vein and after an interval of 10 min, a baseline blood sample was obtained. Subsequently, an appetite questionnaire was completed. Within 5 min, participants were then asked to consume 174 ml of either a PKU protein substitute drink (PKU Cooler20, white, Vitaflo® UK) (PKU trial) or skimmed milk drink enriched with a mixture of protein powder (Spectrum 12, 10 g and Iso 97 whey, 6 g) (Control trial). The drinks were isocaloric, weight matched and had a similar macronutrient composition (Table 1). PKU protein substitute provided 7.8 gm of sugar and 1.6 g of complex of CHO, while in Control drink all 8.4 g of CHO were sugars. Ninety minutes after drink consumption, the meal was served. The PKU meal comprised of protein free cheese sandwich (2 slices of bread Juvela®, 60 g; and 2.5 cheese slices Violife®, 50 g); mini crackers Vitaflo®, 20 g; chocolate cookies Juvela®, 10 g; and a glass of tap water. The Control meal comprised of a cheese sandwich of (2 slices of white bread Warburtons, 57 g; and 2 Lighter Mature cheese slices Tesco, 50 g); crackers Ritz®, 20 g; Everyday Value chocolate chip cookies Tesco, 10 g; and a glass of tap water. The meals were isocaloric and weight matched, but differed in macronutrient composition (Table 1). In Control trial lunch meal provided 22.6 grams of protein (18% of energy intake), which is close to average protein content of a typical lunch [20], while the meal based on PKU special low protein foods, protein content was negligible and consisted only of 0.31 grams (0.2% of energy intake). Participants were asked to consume the entire meal within 20 min. Appetite questionnaires were obtained and blood samples were collected at 30, 60 and 90 min post-drink, and at 120, 150, 180, 210, 240 and 300 min post meal, whilst metabolic rate was measured every 30 seconds for the duration of 20 min after each blood sample. After the completion of the last measurement, the participants were presented with an *ad libitum* buffet test meal, which is an established and reproducible method to assess under laboratory

conditions the spontaneous EI and macronutrient preferences [21]. Water was available throughout the trial, but intake was replicated in the second trial and consumption time was matched. During the two days before the first trial, participants weighed and recorded all foods and drinks consumed and were asked to replicate this intake during the two days preceding the second trial. In addition, for two days prior the experimental trials participants refrained from exercise and alcohol intake.

Appetite ratings

The validated Visual Analogue Scale (VAS) which consists of 100 mm lines was used to measure feelings of hunger, satiety, fullness, prospective food consumption and desire to eat. Participants were asked to place a vertical mark on the horizontal line at a point corresponding to their feelings at that time. The lines were anchored by most-positive (e.g. I am not hungry) and most-negative (e.g. never been hungrier) feeling words from the right to the left respectively.

Metabolic rate

Measurements of the rate of oxygen consumption ($\dot{V}O_2$) and rate of carbon dioxide production ($\dot{V}CO_2$), and rate of energy expenditure (EE) were conducted by means of computerised open-circuit ventilated-hood system (Oxycon Pro, Care Fusion, Germany). Each measurement was carried out for 20-25 minutes while participants were laying comfortably in the supine position. The data collected during first 5 minutes were discarded.

TEF was calculated as percentage increase in EE above RMR, and as increase in EE as percentage of energy intake. The cumulative TEF was calculated by multiplying the duration of the experimental trial (300 min) by the difference between the mean value of the postprandial metabolic rate and the metabolic rate measured at rest. An alcohol-burning validation test was

conducted every week and revealed averaged coefficient variation of 1.7. Volume and gas calibrations were performed prior to each measurement and were accepted if differences were $\leq \pm 1\%$.

Anthropometric and body composition measurements

Measurements were taken upon arrival in the fasted state while the participants wearing light cloths and without shoes at the metabolic research unit. All participants were measured in privacy. Height was measured to the nearest 0.5 cm using a stadiometer (Seca, Leicester, UK). Body mass was measured by a Tanita foot to foot scale (TANITA-TBF-310, Cranela, UK). The same scale was utilized to measure body composition by bioelectrical impedance analysis. The body mass index (BMI) was derived as weight (kg) divided by height (m) squared (kg/m^2).

Blood analysis

Venous blood samples were collected into EDTA vacuette tubes (Greiner Bio-One, Kremsmünster, Austria). After centrifugation (4°C, 3,000 rpm for 15 min) the plasma was collected and frozen at -80°C until further analysis. The blood samples used for the determination of GLP-1 and PYY were pipetted into eppendorf tubes containing Aprotinin (400 kIU activity per ml, SigmaeAldrich, UK) and centrifuged at 14,000 rpm for 4 min. Subsequently 300 µL of plasma was aliquoted into eppendorf tubes for the storage at -80°C. Hexokinase method (Randox Laboratories Ltd., Crumlin, UK) was used to measure glucose concentrations. ELISA kits were used to measure concentrations of plasma insulin (Mercodia AB, Uppsala, Sweden), active GLP-1 (Merck EMD Millipore, Millipore, Billerica, MO, USA) and total PYY (Merck EMD Millipore, Millipore, Billerica, MO, USA). CVs were <3% for the glucose, <4% for the insulin and, <8% for the GLP-1 and PYY assays.

Ad libitum buffet meals

Ad libitum buffet meals were composed of a variety of commonly used foods and included a starter, main dish, two side dishes and a sweet, providing energy of approximately three times what participants would normally consume (~5 MJ). The participants were advised to consume the meal until they reach comfortable fullness. The food was cut into smaller pieces to eliminate portion related cues. The offered and leftover foods were weighed by using an electronic kitchen scale (Salter Housewares Ltd., Tonbridge, U.K.). The participants were blinded to the actual purpose of the buffet meals and thus did not know that the intake of energy and macronutrients was measured.

Macronutrient and energy intake

The macronutrient and energy provided by drinks and meals, and EI during at *ad libitum* buffet was calculated using the dietary software Windiets 2010 (The Robert Gordon University, Aberdeen, Scotland, UK).

Statistical analyses

Data were assessed for normality of distribution using Anderson-Darling test. Data for the responses during the experimental trials were analysed using two-way repeated measures ANOVA, followed by post hoc Tukey test. Time averaged values for the post-drink (0-90 min), post-meal (90-300 min) and the entire period of the experiment trial (0-300 min), calculated as the time averaged areas under the variable versus time curve (AUC), and the EI of the buffet meal were compared by paired *t*-test. Statistical analyses were performed using Statistica (version 10.0; StatSoft, Inc., Tulsa, OK) and Minitab (version 17.3.1; Minitab, Inc., State College, PA).

3. Results

3.1. Participants

Of 30 eligible participants, 4 individuals declined to take part in the study because of time commitments, and thus 26 participants underwent randomization. Two participants were excluded, as prior to the second experimental trial they did not replicate their food intake while one participant dropped out due to having difficulty to ingest the PKU protein substitute drink. Thus, the study was completed by 23 participants, of which 11 were women with a mean (±SD) age of 22.9 ± 1.0 years, BMI of 21.3 ± 1.8 kg/m^2 and body fat mass of 13.0 ± 5.4 kg, and 12 men with age of 25.7 ± 6.7 years, BMI of 23.3 ± 2.7 kg/m^2 and body fat mass of 11.9 ± 5.5 kg.

3.2. Appetite ratings

Responses of appetite scores during the PKU and the Control trial are presented in Fig. 1. Responses of all appetite measures were not significantly different between the PKU and the Control trials (P>0.05, trial effects, two-way ANOVA). Time averaged appetite scores were also not significantly (P>0.05, paired t-tests) different between the PKU and the Control trials during both post-drink and post-meal periods (Table 2).

3.3. Appetite hormones, glucose, and insulin

Responses of plasma concentrations of GLP-1 and PYY were not significantly (P>0.05, trial effects, two-way ANOVA) different between the two trials (Fig. 2). Time averaged plasma concentrations of GLP-1 and PYY were also not significantly (P>0.05, paired t-tests) different between two trials while time averaged concentrations of insulin and glucose were significantly (P<0.05, paired t-tests) higher in the PKU trial than in the Control trial (Table 3). The differences in time averaged plasma concentrations of insulin and glucose were significant (P< 0.05, paired t-tests) for both post-drink and post-meal periods.

3.4. Metabolic rate and thermic effect of feeding

Resting metabolic rate (RMR) did not differ between trials (PKU, 4.14 ± 0.12 kJ; Control, 4.10 ± 0.13 kJ, P=0.4, paired t-test). Metabolic rate measured before and after drink and meal intake is illustrated in Fig. 3. Analysis of two-way repeated measures ANOVA showed that the postprandial responses of metabolic rate were significantly different between the two trials (P<0.05, trial effect), with the value at 90 minutes post-drink intake being significantly (P<0.05) higher and nearly all values of the post-meal period significantly (P<0.05) lower in the PKU trial than in the Control trial. Differences between the PKU and the Control trials for TEF, calculated as percentage increase in EE above RMR and the relative increase in EE (expressed as percentage of energy provided by the drink), were significant (P< 0.05, paired t-tests) only for the post-meal period (Table 4). The cumulative difference in energy expended above the RMR during the entire period of the experimental trials was 40.9 ± 15.8 kJ.

3.5. Fat and CHO oxidation

Data on the time average rates of fat and CHO oxidation and the time averaged respiratory exchange ratios (RER) are presented in Table 5 and the total amount of fat and CHO oxidized in Fig. 4. Following drink intake differences in the rate and the total amount of fat and CHO oxidized and the RER were not significantly (P>0.05, paired t-tests) different between the PKU and the Control trial. During the post-meal period and the entire period of the experimental trial, the rate and amount of fat oxidized was significantly (P<0.05, paired t-tests) lower and the amount of CHO oxidized significantly (P<0.05, paired t-tests) higher in the PKU than in the Control trial. Time averaged values of RER during the post-meal period and the entire period of the experimental trial were significantly lower (P<0.05, paired t-tests) in the Control than in the PKU trial.

3.6. Energy and macronutrient intake during ad libitum buffet dinner

During the *ad libitum* buffet dinner energy (PKU, 5.0 ± 0.3 MJ; Control, 5.3 ± 0.3 MJ), fat (PKU, 58 ± 4 g; Control, 62 ± 4 g), carbohydrate (PKU, 124 ± 9g; Control, 130. ± 9 g), and protein (PKU, 37 ± 5 g; Control, 38 ± 4 g,) intake were not significantly (P>0.05, paired *t*-tests) different between the PKU and the Control trials.

4. Discussion

This study reports for the first time that the consumption of a meal based on PKU special low protein foods, lacking in protein and composed of CHO and fat, has no detrimental impact on subjective appetite measures, appetite regulating gut hormones and subsequent EI during *ad libitum* buffet dinner, however it does diminish the TEF and reduces postprandial fat oxidation. Collectively, these data suggest that long-term adherence to a PKU dietary regimen may potentially contribute to the development of overweight and obesity. Although the trend of obesity and overweight in PKU is similar to that in general population [9-11], our data suggests that development of obesity in PKU patients may be related to wider spectrum of risk factors.

One of the study aims was to investigate whether meal based on PKU special low protein foods has a detrimental impact on appetite scores and plasma concentrations of PYY and GLP-1. Thus, study participants were investigated in a counter-balanced manner after the consumption of 2.0 MJ containing Control meal (18% of energy from protein, and 36 % and 47 % of energy from CHO and fat, respectively) and after an isocaloric PKU type meal lacking protein (54 % and 46 % energy from CHO and fat, respectively). Regardless of the differences in the macronutrient composition of the two meals, subjective appetite and satiety scores and plasma concentrations of PYY and GLP-1 during the post-meal period were not significantly

different between the PKU and the Control trials. In addition, EI during the subsequent *ad libitum* buffet style meal consisted of approximately 5 MJ and was also not significantly different between the two trials. Thus, the hypothesis that whole protein lacking and CHO and fat based PKU type meals detrimentally affect appetite and satiety regulation cannot be supported by our findings. This, when combined with other evidence [13-15], suggests that changes in satiety and appetite regulating hormones might be expected when the protein content of a meal becomes higher rather than lower in comparison to the protein intake provided by the habitual diet. Regardless of PKU meal based on special low protein foods having no impact on appetite hormones, the concentrations of GLP-1 after ingestion of PKU protein substitute drink tended to be higher than after milk based drink. This most likely coincided with greater increase in plasma concentrations of amino acids, which have been reported to be related to appetite hormones and satiety responses [22].

As expected, due to higher CHO content of the PKU lunch, plasma glucose and insulin glucose responses during post lunch period were significantly higher in the PKU than the Control trial. Plasma glucose and insulin glucose responses after drink intake were also significantly higher in the PKU than in the Control trial. This difference was found regardless the glycemic index of the PKU protein substitute drink being as low as 19% (value provided on request from the manufacturer). Therefore, significantly higher glucose and insulin concentrations in the PKU trial can be explained by protein substitute drink being composed of free amino acids and Control drink providing whole protein. It is known that ingestion of free amino acids in comparison to ingestion of equivalent amount of whole protein induces higher insulin [23] and glucose [24] responses. Thus, finding of enhanced postprandial responses of insulin and glucose found after both consumption of PKU protein substitute drink and lunch implies that long term PKU type dietary regimen may lead to the development of insulin resistance.

This is the first study investigating how a PKU type meal modifies TEF, a metabolic factor known to contribute to the regulation of energy balance and thus development of overweight and obesity [19]. In accordance with findings that TEF of separate nutrients is highest for protein [15-17], we found that during post-meal and the entire period of the experimental trial TEF was significantly lower in the PKU than in the Control trial, and that the cumulative difference in energy expended above RMR between trials lasting for 5 hours consisted of approximately 41 kJ. While this is a small difference and TEF varies within the range of 5–15% of total daily EE, long-term consumption of meals based on PKU medical foods can result in diminished daily EE and induce body weight gain of approximately 1 kg in a period of a single year [25]. Thus, unfavourable attenuation of TEF induced by PKU type dietary regimen may be among the physiological mechanisms explaining the risk of obesity in patients with PKU [9-11].

Previous evidence suggests that reduced fat oxidation promotes a positive fat balance and is one of the multifactorial origins of obesity [19]. Thus, we investigated how a PKU protein substitute and meal impact postprandial fat oxidation. We found that during the post-meal and the entire period of the experimental trial the amount of fat oxidized was significantly lower and the RER significantly higher in the PKU than in the Control trial. Diminished fat oxidation found in the PKU trial can be explained by the enhanced CHO content [15, 18] and therefore higher insulin response, and possibly lack of protein [18] in the PKU meal. Thus, regardless of the leading mechanism involved in reduced fat oxidation following the PKU meal, the obtained data suggest that the increased overweight and obesity reported in patients with PKU [9-11] may, at least in part, be related to the detrimental impact of PKU type foods on fat oxidation. Thus, future studies should investigate whether fat oxidation of the PKU population differs from that of matched controls and if so, how this affects energy balance and obesity in this group of patients.

Since the required amount of amino acids in a PKU type diet is obtained from consuming PKU protein substitutes [6, 7], the design of the experimental trial aimed to mimic a realistic scenario encountered in the daily life of patients with PKU and thus also involved consumption of the typical PKU protein substitute. The PKU protein substitute drink consisted of L-amino acids (free from PHE) while the Control drink was whole protein-enriched milk. The drinks were energy and volume matched and consumed 90 minutes prior to the intake of the corresponding meal. We found that appetite scores and plasma GLP-1 and PYY responses, as well as rate of fat oxidation and RER measured for 90 minutes after the PKU protein substitute drink, were not significantly different from the responses found after the intake of the Control drink. The TEF during the post-drink period was also not different between the PKU and the Control trials. However, at 90 minutes after drink intake, metabolic rate was significantly higher in the PKU than the Control trial. This enhancement in the metabolic rate was counterbalanced quickly after the consumption of the PKU type meal. Thus, when combined, findings of the post-drink and post-meal periods, imply that the PKU type dietary regimen may have adverse effect on energy balance due to the consumption of special low protein foods rather than the intake of the PKU protein substitute drink.

This study is not without limitations. Study participants were healthy men and women. We appreciate that the responses found in healthy individuals may not be applicable to patients with PKU. Therefore, future studies should investigate how measures considered in this study are impacted by meal based on special low protein foods consumed by patients with PKU. The relevance of the attenuated TEF and reduced postprandial fat oxidation for the enhanced prevalence of obesity reported in the PKU population [9-11] should be confirmed by future studies comparing how these factors are influenced by long-term adherence to meals based on PKU special low protein foods and how they differ between patients with PKU and healthy matched controls. It can be argued that the 90 minute interval used between drink and meal

intake is another limitation of this study as in clinical practice of PKU management dietitians advise protein substitute intake in very close proximity to the meal [26]. On the other hand, it has been reported that a reasonable proportion of patients with PKU fail to consume prescribed protein substitutes [5, 27, 28]. Future studies should investigate combined effect of both PKU protein substitute and meals based on special low protein foods on research outcomes of this study. The responses of gut hormones were measured in a subset of the volunteers since research previously conducted by us [29] and others [15, 18] has shown that using this number allowed identification of meaningful and statistically significant differences in plasma concentrations of PYY and GLP-1.

In conclusion, the findings of this study suggest that consumption of a meal composed of PKU special low protein foods has no detrimental impact on subjective appetite ratings and appetite hormones, yet produces a lower TEF and postprandial fat oxidation than an ordinary meal. These metabolic alterations may be amongst the factors which contribute to the origins of obesity in people with PKU on contemporary dietary management.

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Statement of authorship

DM, KG, HA, BC designed the study; HA, AMN, ME recruited participants, conducted experimental trials; HA, AMN performed plasma analyses, conducted statistical analyses; HA, DM drafted the original manuscript; KG, AMN, BC, ME contributed to the drafting of the manuscript and its revisions.

Conflict of interest statement

No authors have conflicts of interest to declare.

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Fig. 1. Mean ± SEM responses of hunger, satiety, fullness, desire to eat and prospective food consumption (PFC) scores in the PKU and the Control trials (n = 23). Analysis by two-way repeated measures ANOVA showed that for all appetite measures differences were not significantly different between two trials (P<0.05, trial effects). Statistical analyses based on time-averaged scores are given in Table 2.

Fig. 2. Mean ± SEM responses of plasma concentrations of glucagon-like peptide-1 (GLP-1), peptide YY (PYY) in the PKU and the Control trials (n = 12). Analysis by two-way repeated measures ANOVA showed that differences for both hormones were not significantly different between two trials (P>0.05, trial effects). Statistical analyses based on time-averaged concentrations are given in Table 3.

Fig. 3. Mean ± SEM responses of metabolic rate in the PKU and the Control trials (n = 23). Analysis by two-way repeated measures ANOVA showed that metabolic rate was significantly lower in the PKU than in the Control trial (P<0.05, trial effect); * P < 0.05, ** P < 0.03, *** P < 0.02, **** P < 0.001 (Tukey t-test). Statistical analyses for TEF calculate as % increase in EE above RMR and as increase in EE as % of EI in are given in Table 4.

Fig. 4. Mean ± SEM amount of fat and CHO oxidized during the post-drink (0-90 min) and post-meal (90-300 min) and the entire period (0-300 min) in the PKU and the Control trials (n = 23). * P < 0.005; ** P < 0.001 (paired t-test). Statistical analyses based on time-averaged rate of fat and CHO oxidation and time averaged value of respiratory exchange ratio are given in Table 5.

Table 1

Energy, fat, carbohydrate and protein provided by drinks and meals in the Control and the PKU trials.

	Drink		Meal	
	Control	PKU	Control	PKU
Energy (MJ)	0.51	0.52	2.0	2.0
Fat (g)	0.7	0.9	25.7	24.4
CHO (g)	9.4	9.2	44.5	63.7
Protein (g)	20	20	22.6	0.31
Energy from fat (%)	5.0	6.7	46.8	46.4
Energy from CHO (%)	30.3	29.4	35.8	53.8
Energy from protein (%)	64.5	63.8	18.0	0.2

Table 2

Time-averaged subjective appetite scores (mm) during post-drink (0-90 min), post-meal (90-300 min) and the entire period (0-300 min) in the Control and the PKU trial.

	Control	PKU	*P* value
Post-drink			
Hunger	58.6 ± 3.6	51.8 ± 3.5	0.12
Satiety	25.7 ± 2.5	31.7 ± 2.7	0.11
Fullness	23.2 ± 2.7	29.01 ± 3	0.17
Desire to eat	64.4 ± 3.4	63.2 ± 3.2	0.65
PFC	26.8 ± 2.7	28.4 ± 2.7	0.77
Post-meal			
Hunger	53.2 ± 3.5	53.9 ± 3.6	0.84
Satiety	49.8 ± 3.7	52.4 ± 3.4	0.43
Fullness	48.6 ± 3.5	51.4 ± 3.5	0.38
Desire to eat	58.8 ± 3.2	55.9 ± 3.2	0.43
PFC	48.9 ± 3.5	50.8 ± 3.5	0.26
Over 300 min			
Hunger	48.7 ± 2.9	48 ± 3.0	0.82
Satiety	40.1 ± 2.8	43 ± 2.4	0.27

Fullness	38.7 ± 2.8	41.7 ± 2.7	0.27
Desire to eat	53.8 ± 2.7	51.8 ± 2.6	0.46
PFC	39.8 ± 2.7	41.2 ± 2.7	0.36

All values are means ± SEM, n = 23

PFC, prospective food consumption

Table 3

Time-averaged concentrations of GLP-1, PYY, glucose and insulin for post-drink (0-90 min), post-meal (90-300 min) and the entire period (0-300min) in the Control and the PKU trials.

	Control	PKU	*P* value
GLP-1 (pmol/L)[a]			
Post-drink	4.12 ± 0.44	4.34 ± 0.46	0.09
Post-meal	5.03 ± 0.74	4.77 ± 0.52	0.64
Over 300 min	4.76 ± 0.65	4.64 ± 0.50	0.96
PYY (pg/mL)			
Post-drink	91.50 ± 17.4	93.00 ± 15.8	0.70
Post-meal	144.60 ± 16.8	133.3 ± 15.6	0.15
Over 300 min	128.7 ± 16.8	121.2 ± 15.4	0.22
Insulin (mU/L)			
Post-drink	12.70 ± 1.4	15.30 ± 2.18	0.04
Post-meal	18.60 ± 2.31	26.34 ± 3.41	0.01
Over 300 min	16.83 ± 2.01	23.03 ± 2.92	0.01
Glucose (mmol/L)			
Post-drink	4.22 ± 0.15	4.40 ± 0.12	0.02
Post-meal	4.77 ± 0.14	5.08 ± 0.22	0.02

Over 300 min	4.61 ± 0.14	4.88 ± 0.18	0.01

All values are means ± SEM, n = 12

[a] Statistical analysis conducted on log transformed values

GLP-1, glucagon-like peptide-1; PYY, peptide YY

Table 4

Thermic effect of feeding calculated as percentage (%) increase in EE above RMR and as increase in EE as percentage (%) of EI in the Control and the PKU trials.

	Control	PKU	*P* value
Increase above RMR (%)			
Post-drink	8.6 ± 1.1	10.1 ± 1.2	0.301
Post-meal	15.6 ± 1.2	10.3 ± 1.4	0.004
Over 300 min	13.2 ± 1.0	10.2 ± 1.5	0.056
Increase as % of EI			
Post-drink	6.1 ± 0.7	7.0 ± 0.7	0.35
Post-meal	6.6 ± 0.5	4.3 ± 0.5	0.001
Over 300 min	6.4 ± 0.4	4.8 ± 0.5	0.023

All values are means ± SEM, $n = 23$

Table 5

Time-averaged values of the rate of fat and carbohydrate oxidation, and respiratory exchange ratio (RER) during post-drink (0-90 min), post-meal (90-300 min), and the entire period (0-300 min) in the Control and the PKU trials.

	Control	PKU	*P* value
Fat oxidation (g/min)			
Post-drink	0.08 ± 0.00	0.08 ± 0.00	0.186
Post-meal	0.08 ± 0.00	0.07 ± 0.00	<0.001
Over 300 min	0.08 ± 0.00	0.07 ± 0.00	0.002
CHO oxidation (g/min)			
Post-drink	0.08 ± 0.00	0.10 ± 0.01	0.155
Post-meal	0.11 ± 0.00	0.13 ± 0.00	0.006
Over 300 min	0.10 ± 0.00	0.12 ± 0.00	0.013
RER			
Post-drink	0.79 ± 0.007	0.81 ± 0.010	0.072
Post-meal	0.81 ± 0.005	0.83 ± 0.008	0.002
Over 300 min	0.80 ± 0.005	0.82 ± 0.007	0.003

All values are means ± SEM, $n = 23$

References

[1] Blau N. Genetics of Phenylketonuria: Then and Now. Human Mutation. 2016;37:508-15.

[2] Hanley W. Phenylketonuria (PKU)-What Next? Mini-Review. J Genet Disor Genet Rep 2. 2013;2:2.

[3] Pimentel FB, Alves RC, Costa ASG, Torres D, Almeida MF, Oliveira MBPP. Phenylketonuria: Protein content and amino acids profile of dishes for phenylketonuric patients. The relevance of phenylalanine. Food Chemistry. 2014;149:144-50.

[4] MacDonald A, Gokmen-Ozel H, Daly A. Changing dietary practices in phenylketonuria. Turk J Pediatr. 2009;51:409-15.

[5] Schulz B, Bremer HJ. Nutrient intake and food consumption of adolescents and young adults with phenylketonuria. Acta Pædiatrica. 1995;84:743-8.

[6] Lammardo AM, Robert M, Rocha JC, Rijn M, Ahring K, Belanger-Quintana A. Main issues in micronutrient supplementation in phenylketonuria. Mol Genet Metab. 2013;110.

[7] Barretto JR, Silva LR, Leite ME, Boa-Sorte N, Pimentel H, Purificação AC, et al. Poor zinc and selenium status in phenylketonuric children and adolescents in Brazil. Nutrition Research. 2008;28:208-11.

[8] Pena MJ, Almeida MF, van Dam E, Ahring K, Bélanger-Quintana A, Dokoupil K, et al. Special low protein foods for phenylketonuria: availability in Europe and an examination of their nutritional profile. Orphanet Journal of Rare Diseases. 2015;10:1-6.

[9] Aldamiz-Echevarria L, Bueno MA, Couce ML, Lage S, Dalmau J, Vitoria I, et al. Anthropometric characteristics and nutrition in a cohort of PAH-deficient patients. Clin Nutr. 2014;33:702-17.

[10] Burrage LC, McConnell J, Haesler R, O'Riordan MA, Sutton VR, Kerr DS, et al. High prevalence of overweight and obesity in females with phenylketonuria. Molecular Genetics and Metabolism. 2012;107:43-8.

[11] Robertson LV, McStravick N, Ripley S, Weetch E, Donald S, Adam S, et al. Body mass index in adult patients with diet-treated phenylketonuria. J Hum Nutr Diet. 2013;26 Suppl 1:1-6.

[12] Rocha JC, MacDonald A, Trefz F. Is overweight an issue in phenylketonuria? Mol Genet Metab. 2013;110, Supplement:18-24.

[13] Bowen J, Noakes M, Clifton PM. Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. Journal of Clinical Endocrinology & Metabolism. 2006;91:2913-9.

[14] Belza A, Ritz C, Sørensen MQ, Holst JJ, Rehfeld JF, Astrup A. Contribution of gastroenteropancreatic appetite hormones to protein-induced satiety. The American Journal of Clinical Nutrition. 2013;97:980-9.

[15] Lejeune MP, Westerterp KR, Adam TC, Luscombe-Marsh ND, Westerterp-Plantenga MS. Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. The American Journal of Clinical Nutrition. 2006;83:89-94.

[16] Tappy L. Thermic effect of food and sympathetic nervous system activity in humans. Reproduction, nutrition, development. 1996;36:391-7.

[17] Westerterp-Plantenga MS. Protein intake and energy balance. Regulatory Peptides. 2008;149:67-9.

[18] Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. The American journal of clinical nutrition. 2003;77:91-100.

[19] Weinsier RL, Hunter GR, Heini AF, Goran MI, Sell SM. The etiology of obesity: relative contribution of metabolic factors, diet, and physical activity. The American Journal of Medicine. 1998;105:145-50.

[20] Millward DJ. The use of protein:energy ratios for defining protein requirements, allowances and dietary protein contents. Public Health Nutrition. 2013;16:763-8.

[21] Arvaniti K, Richard D, Tremblay A. Reproducibility of energy and macronutrient intake and related substrate oxidation rates in a buffet-type meal. Br J Nutr. 2000;83:489-95.

[22] Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, van Vught AJAH, Westerterp KR, Engelen MPKJ, et al. Dose-dependent satiating effect of whey relative to casein or soy. Physiology & Behavior. 2009;96:675-82.

[23] van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr. 2000;72:96-105.

[24] Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr. 2007;27:293-310.

[25] Lean ME, Malkova D. Altered gut and adipose tissue hormones in overweight and obese individuals: cause or consequence? Int J Obes (Lond). 2016;40:622-32.

[26] National Society for Phenylketonuria (NSPKU). Dietary Information for the Treatment of Phenylketonuria 2016/2017. Purley, UK2015.

[27] MacDonald A. Diet and compliance in phenylketonuria. Eur J Pediatr. 2000;159:S136-S41.

[28] MacDonald A, Harris G, Rylance G, Asplin D, Booth IW. Abnormal feeding behaviours in phenylketonuria. Journal of Human Nutrition and Dietetics. 1997;10:163-70.

[29] Fatima S, Gerasimidis K, Wright C, Tsiountsioura M, Arvanitidou E-I, Malkova D. Response of appetite and potential appetite regulators following intake of high energy nutritional supplements. Appetite. 2015;95:36-43.

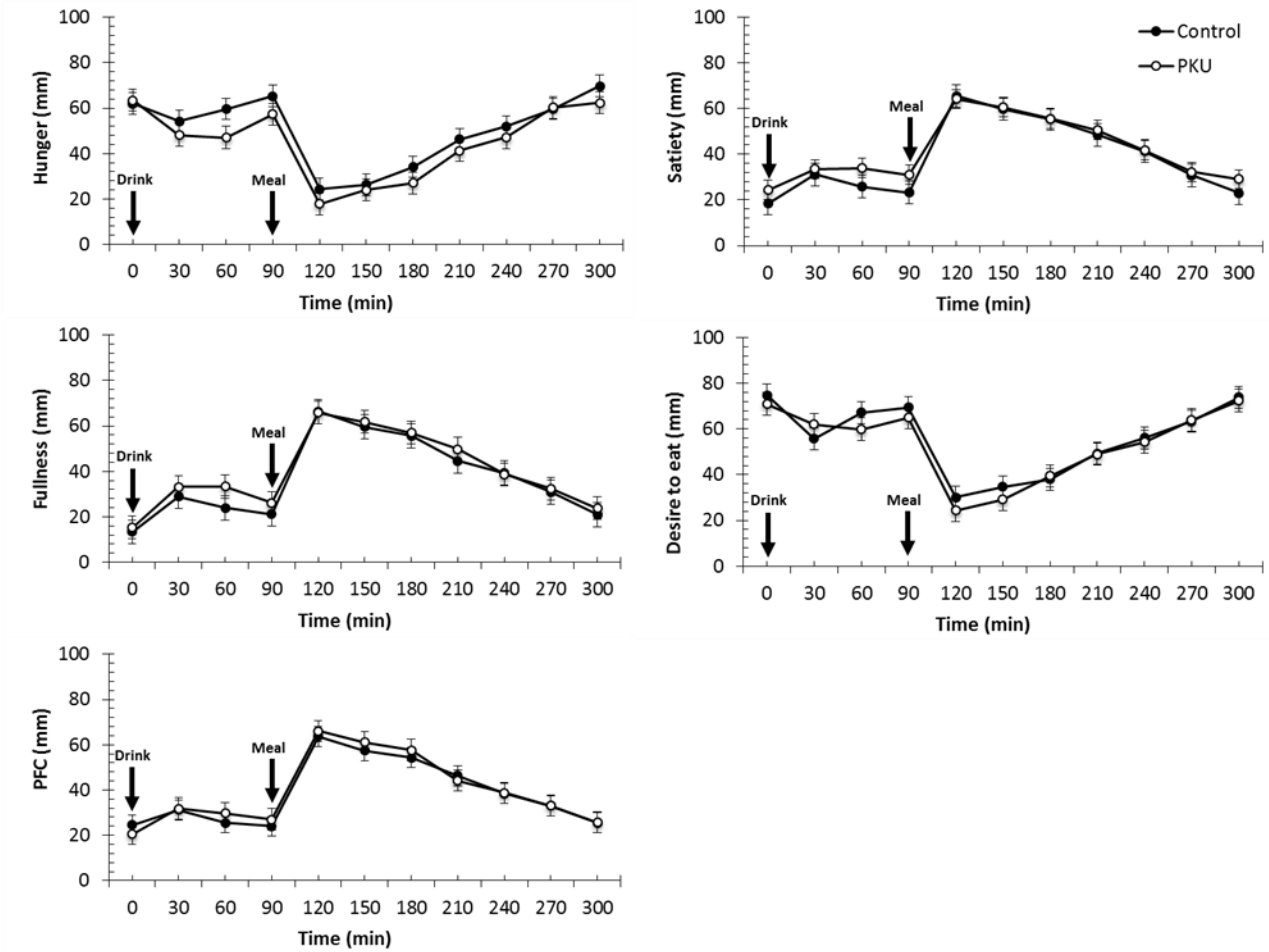
Hunger (mm)
Satiety (mm)
Fullness (mm)
Desire to eat (mm)
PFC (mm)
Drink
Meal
Time (min)
0
30
60
90
120
150
180
210
240
270
300
20
40
80
100
Control
PKU

Figure 1

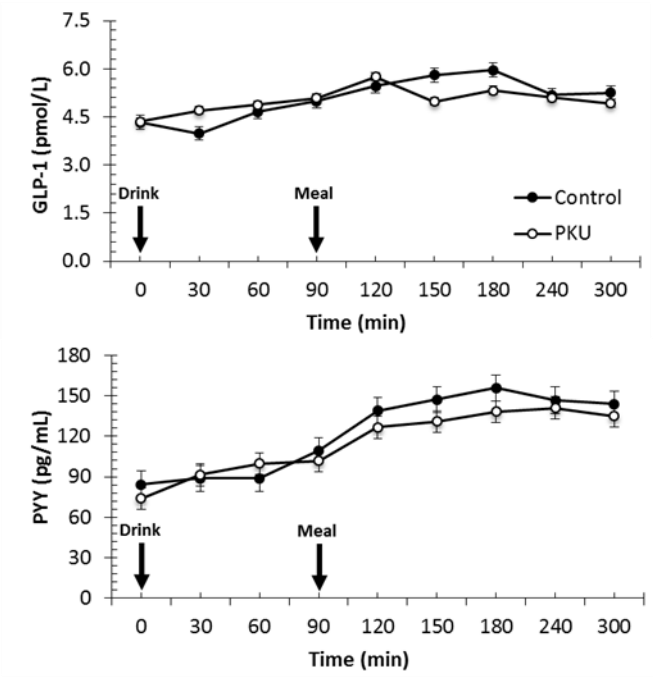
GLP-1 (pmol/L)
7.5
6.0
4.5
3.0
1.5
0.0
Drink
Meal
Control
PKU
0
30
60
90
120
150
180
240
300
Time (min)
PYY (pg/mL)
180
150
120
90
60
30
0
Drink
Meal
0
30
60
90
120
150
180
240
300
Time (min)

Figure 2

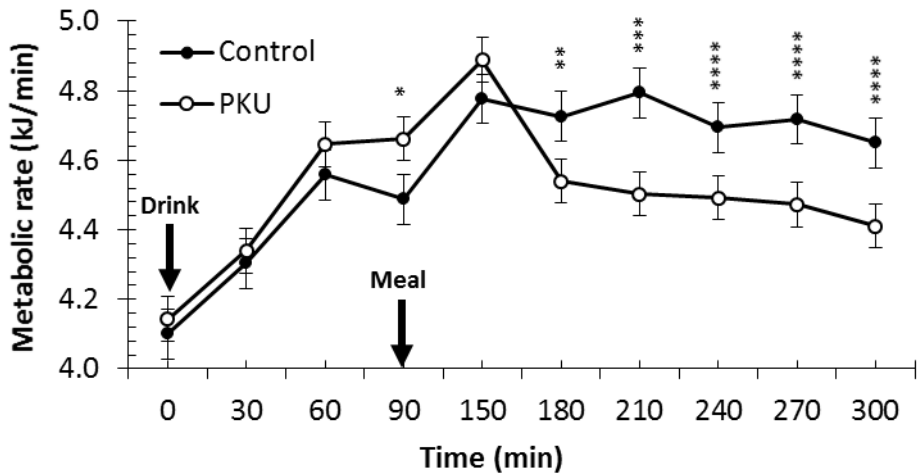
5.0
4.8
4.6
4.4
4.2
4.0
Metabolic rate (kJ/min)
Control
PKU
Drink
Meal
*
**
***
****
****
****
0
30
60
90
150
180
210
240
270
300
Time (min)

Figure 3

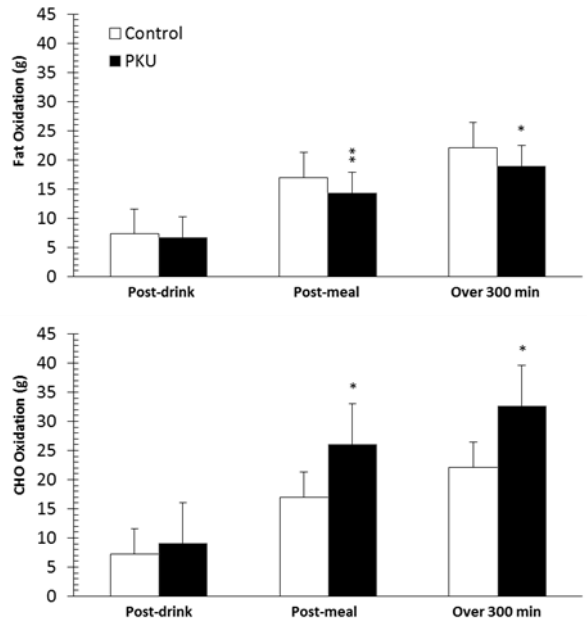
Control
PKU
Fat Oxidation (g)
45
40
35
30
25
20
15
10
5
0
Post-drink
Post-meal
Over 300 min
CHO Oxidation (g)
45
40
35
30
25
20
15
10
5
0
Post-drink
Post-meal
Over 300 min

Figure 4