Title: Mechanisms of obesity in children and adults with phenylketonuria on contemporary treatment

Nouf Alghamdi[1,2*], Hani Alfheeaid[1,3*], Barbara Cochrane[4], Sarah Adam[4], Peter Galloway[5], Alison Cozens[4], Tom Preston[6], Dalia Malkova[1], Konstantinos Gerasimidis[1]

[1] School of Medicine, Dentistry and Nursing, College of Medicine, Veterinary and Life Sciences, University of Glasgow, UK

[2] King Saud University, Riyadh, Kingdom of Saudi Arabia

[3] Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Kingdom of Saudi Arabia

[4] Department of Metabolic Medicine, Royal Hospital for Children, Queen Elizabeth University Hospital, Glasgow, UK

[5] Department of Metabolic Medicine, Glasgow Royal Infirmary Hospital, Glasgow, UK

[6] Scottish Universities Environmental Research Centre, Glasgow, UK

Corresponding Author and Guarantor:
Professor Konstantinos Gerasimidis
Professor of Clinical Nutrition
Human Nutrition
School of Medicine, Dentistry and Nursing
College of Medicine, Veterinary and Life Sciences
University of Glasgow
New Lister Building
Glasgow Royal Infirmary, 10 Alexander Parade, Glasgow G31 2ER
E-mail: Konstantinos.Gerasimidis@glasgow.ac.uk

*Shared first author.

Word counts: 1465

Number of tables: 1

Number of figures: 2

ABSTRACT

Background & Aims: Obesity prevalence in people with phenylketonuria (PKU) is comparable to that of the general population but the underlying aetiology remains unknown. To assess body composition, dietary intake, moderate physical activity duration (MPAD) and energy expenditure (MPAEE), resting metabolic rate (RMR), diet-induced thermogenesis (DIT), fasting and postprandial fat (FOx) and carbohydrate oxidation (CHOOx), in PKU people and healthy Controls.

Methods: Participants were PKU people (n=16) and healthy controls (n=15). Body composition was measured with stable isotopes using deuterium as tracer, dietary intake from 4-day food diaries, MPAD and MPAEE from 7-day activity counts measured by triaxial accelerometers, calibrated against individual rates of oxygen consumption and carbon dioxide production, RMR, DIT, FOx and CHOOx by indirect calorimetry.

Results: Body composition, DIT, FOx, CHOOx and RMR did not differ between the PKU and the Control groups. MPAD (PKU, 73±26 min/week; Control, 152±43 min/week) and MPAEE (PKU, 404±127 kcal/week; Control, 741±153 kcal/week) were lower ($P<0.05$) in the PKU than the Control group. Raised phenylalanine levels were inversely related with MPAD and MPAEE. Energy intake and energy provided by protein did not differ between the groups, while energy proportion obtained from carbohydrate was higher (PKU, 60±2 %; Control, 51±2%; $P<0.05$) and from fat lower (PKU, 24±2%; Control, 35±3%; $P<0.05$) in the PKU than in the Control group.

Conclusion: People with PKU spent less time and expend less energy in moderate physical activity and have a higher intake of energy from CHO which may be involved in the underlying mechanisms of obesity in PKU.

KEYWORDS Phenylketonuria, Body composition, Energy intake, Physical activity, Diet induced thermogenesis, Fat oxidation.

INTRODUCTION

Phenylketonuria (PKU) is a very rare disease associated with impaired metabolism of phenylalanine. Restriction of dietary protein to marginal levels of intake, and substitution of amino-acids with phenylalanine-free supplements is the only lifelong treatment of people with PKU.

Prevalence of obesity is becoming common is people with PKU(1). Disease and demographic characteristics have been associated with risk of obesity onset in PKU, including poor disease control and older age(1). However, the underlying mechanisms leading to obesity onset remain unknown and it is possible that different factors drive weight gain in people with PKU than in the general population. The diet of people with PKU is extensively modified; it is high in carbohydrate and very low in natural protein, with amino acid needs provided in the metabolic formula, which is high in protein, but free of phenylalanine(2).

To date, there are no published studies investigating the underlying aetiology of obesity in people with PKU using reference objective methodology. This study assessed body composition, dietary intake, duration (MPAD) and energy expenditure (MPAEE) of moderate intensity physical activity, resting metabolic rate (RMR), DIT, fasting and postprandial fat (FOx) and carbohydrate oxidation (CHOOx), in people with PKU using reference objective methodology.

MATERIAL & METHODS

Subjects

Children (10-15 years) and adults (16+ years) with PKU were recruited from the metabolic medicine clinics of the National Health Services of Greater Glasgow and Clyde, the largest tertiary service for PKU across Scotland. Classification to age groups was based on local law. Healthy participants were recruited as a Control group via advertisement in the local area. The study design and experimental procedures applied are summarised in Figure 1.

In patients with PKU, disease severity was defined based on phenylalanine levels at the time of new-born screening (classical PKU > 1200 μmol/L, mild-moderate < 1200 μmol/L) and using the UK Medical Research Council recommendations(3). Metabolic control was based on the proportion of raised measurements of blood phenylalanine the year prior to study enrolment. Visual analogue scales were completed by the hospital dietitians to assess participants' adherence to a) protein exchanges, b) adherence to amino-acid supplements and c) overall PKU control.

Participants provided written consent. The study was approved by the National Health Services-South West Health Research Authority with Research Ethic Committee (REC) reference ID: 16/SW/0288. The study was registered at ClinicalTrials.Gov database with identifying number NCT03309345.

Body composition assessment

Body composition was assessed using the stable isotope technique using deuterium as the tracer (Cambridge Isotope Laboratories, MA, USA) diluted in drinking water. Baseline and follow up saliva samples were analysed for measurements of total body water using Fourier Transform Infrared Spectrometry. Fat free mass hydration was calculated considering gender/age hydration constants(4). Fat free (FFMI) and fat mass (FMI) were corrected for height^2 to account for the variations in age and growth.

Indirect calorimetry in fasting and postprandial states

At fasting status, rate of oxygen ($\dot{V}O_2$) consumption and carbon dioxide production ($\dot{V}CO_2$) were measured using indirect calorimetry (Quark, COSMED, Italy). Participants then were provided with a PKU-type breakfast (e.g. low-protein bread, jam and low-protein cookies) containing 395 kcals for children and 490 kcals for adults. The Control group received a similar breakfast based on normal foods with calorific content and weight matched to that of the PKU group. Postprandial measurements of VO_2 and VCO_2 were performed for a period of 90 minutes. Fasting and postprandial respiratory exchange ratio (RER), FOx, CHOOx and RMR were calculated using the Frayn equations(5) and DIT as percentage increment in energy expenditure above RMR.

Incremental exercise test

Following postprandial measurements, participants walked on the treadmill at gradually increasing speed. Activity counts (ActiGraph GT3X+ accelerometer), heart rate (Soft Strap HRMonitor, Garmin Ltd), $\dot{V}O_2$ and $\dot{V}CO_2$ were measured when heart rate reached 80% of estimated maximum. Linear regressions of accelerometery counts with $\dot{V}O_2$ and $\dot{V}CO_2$ were established individually for each subject to estimate MPAEE.

Moderate intensity physical activity

To estimate MPAD and MPAEE, participants wore the accelerometers for seven consecutive days. Raw data (counts/min) were imported to ActiLife Software (v 6.13.2) and a cut-off of 1952-5724 was used to calculate the amount of counts corresponding to moderate physical activity(6). Using the coefficients produced during the incremental exercise test, $\dot{V}O_2$ and $\dot{V}CO_2$ rates were estimated and used to calculate weekly averaged rate of MPAEE**.**

Dietary assessment

Food diaries were collected over a period of four days, including a weekend day and analysed for energy and macronutrient intake using Windiets® 2010 (Robert Gordon University, Aberdeen, UK). Participants were instructed to record their food and drink intake using household measures. In the case of homemade dishes, the name of the recipe, ingredients, method of cooking and the number of servings the recipe made were recorded. Participants were asked not to make any changes to their usual dietary habits. Carers were asked to assist their children in completion of food diaries. Energy intake to RMR ratios of >2.40 and <1.35 were considered as diet over- and under-reporting (7).

Statistical analysis

Differences between two groups were tested using 2-sample t-test or Mann-Whitney U test. Relationships were explored with Spearman rank correlations.

RESULTS

Participant characteristics and body composition

Body composition, FOx, CHOOx and DIT and physical activity data are presented on 16 PKU and 15 Controls participants. There was no difference in demographics and BMI between groups (Table 1). People with PKU did not differ in FFMI, FMI and %fat compares with Control (Table 1). In people with PKU, adherence to amino-acid supplements (rho= -0.64, p= 0.007), natural protein exchanges (rho= -0.55, p= 0.02) and overall PKU diet (rho= -0.61, p= 0.01) were negatively related with FMI.

Fasting and postprandial metabolic rate and moderate-intensity physical activity

Measurements of resting metabolic rate did not differ between the PKU and Control groups (Table 1). DIT, fasting and postprandial FOx, CHOOx and RER were also not different between the two groups (Table 1). The MPAD of children (P= 0.002), adults (P= 0.05) and whole group (P= 0.009) was significantly shorter in people with PKU than Controls (Figure 2). Likewise, the MPAEE in

children (P= 0.04), adults (P= 0.03) and whole group (P= 0.02) was significantly lower in people with PKU than Controls (Figure 2). In people with PKU, low MPAD (rho=-0.63, p=0.020) and MPAEE (rho=-0.56, p=0.045) were related with a high percent of raised phenylalanine measurements, an estimate of poor metabolic control, the year before study enrolment.

Dietary assessment

Three dietary records were removed from analysis due to misreporting. Energy intake and the proportion of energy provided from sugars, proteins or amino-acid supplements in PKU, were not significantly different between the two groups. However, people with PKU consumed a higher proportion of energy from CHO (p=0.01) and from fat lower (p=0.001) than Controls (Table 1).

DISCUSSION

The current study found that people with PKU had a shorter MPAD and lower MPAEE than healthy Controls, whereas no significant differences were observed in RMR, DIT, FOx and energy intake between groups. These results suggest that diminished participation in moderate physical activity is potentially one of the contributing factors of positive energy balance and obesity in PKU. Additionally, people with PKU are unlikely to be prone to obesity due to an inherent anomaly with the way their body utilises energy substrates, at rest or following consumption of a meal.

MPAEE, the most variable component of energy expenditure(8) was lower in people with PKU than Controls, particularly in those with poor metabolic control. Low levels of engagement in moderate intensity exercise, may be associated with social isolation, depression and reduced quality of life usually reported in people with PKU(9). We noted that the weekly MPAD in PKU participants consisted of 105 min, which is well below current physical activity recommendations(10). Thus, enhancing physical activity might be a successful strategy to mitigate weight gain in people with PKU. To date, there are no published studies investigating physical activity energy expenditure and duration in people with PKU using reference objective methodology. Our finding that the RMR was not different between patients with PKU and healthy controls is in accordance with previous research(11, 12). Likewise the dietary intake profile of patients with PKU in the current study is similar to previous reports(2).

Previous preclinical research suggested that dietary management of PKU, which relies on high intake of CHO rich, low protein medical foods, may have detrimental effects on metabolic regulators to obesity, such as FOx and DIT (13). However, the current clinical study failed to confirm that. It is possible though that the long-term impact of a PKU diet on DIT and FOx, might be different from the acute impact of a single PKU type meal.

CONCLUSION

In conclusion, diminished participation in moderate intensity physical activity may be a cause of increased adiposity in people with PKU. The findings of the current study have significant implications for clinical practice and recommendations for obesity management and prevention in people with PKU.

FUNDING STATEMENT

NA received doctorate scholarship from the Government of Saudi Arabia, Royal Embassy of Saudi Arabia in London, UK.

CONFLICT OF INTEREST

KG has received research grants, acted as consultant and received speaker's fees from Nutricia-Danone, Nestle Health Science, Abbott, Baxter. The other authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION

KG, DM, BC, and TP conceived the study. PG and AC co-ordinated research in their clinical areas. NA, HA, BC, SA, PG, AC recruited participants. NA and HA conducted data collection. NA, HA and TP performed the field research activities, obtained measurements and analysed samples. NA, KG, and DM did the statistical analyses. KG, PG, AC interpreted the data. NA, DM and KG drafted the manuscript. All authors critically reviewed and helped with data interpretation and contributed to the final version of the manuscript. KG is guarantor of the study.

REFERENCES

1. Camatta GC, Kanufre VdC, Alves MRA, Soares RDL, Norton RdC, de Aguiar MJB, et al. Body fat percentage in adolescents with phenylketonuria and associated factors. Molecular Genetics and Metabolism Reports. 2020;23:100595.

2. Rocha JC, van Spronsen FJ, Almeida MF, Soares G, Quelhas D, Ramos E, et al. Dietary treatment in phenylketonuria does not lead to increased risk of obesity or metabolic syndrome. Mol Genet Metab. 2012;107(4):659-63.

3. MRC. Recommendations on the dietary management of PKU. Report of Medical Research Council Working Party on PKU. Arch Dis Child. 1993;68:426-7.

4. Lohman TG. Assessment of Body Composition in Children. 1989;1(1):19.

5. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol Respir Environ Exerc Physiol. 1983;55(2):628-34.

6. Freedson PS, Melanson E, Sirard J. Calibration of the Computer Science and Applications, Inc. accelerometer. Med Sci Sports Exerc. 1998;30(5):777-81.

7. Goldberg G, Black A, Jebb S, Cole T, Murgatroyd P, Coward W, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. European journal of clinical nutrition. 1991;45(12):569-81.

8. Westerterp KR. Alterations in energy balance with exercise. The American journal of clinical nutrition. 1998;68(4):970S-4S.

9. Clacy A, Sharman R, McGill J. Depression, anxiety, and stress in young adults with phenylketonuria: associations with biochemistry. Journal of Developmental & Behavioral Pediatrics. 2014;35(6):388-91.

10. Officers UCM. UK Chief Medical Officers' Physical Activity Guidelines 2019.

11. Allen JR, McCauley JC, Waters DL, O'Connor J, Roberts DC, Gaskin KJ. Resting energy expenditure in children with phenylketonuria. Am J Clin Nutr. 1995;62(4):797-801.

12. Quirk ME, Schmotzer BJ, Singh RH. Predictive equations underestimate resting energy expenditure in female adolescents with phenylketonuria. J Am Diet Assoc. 2010;110(6):922-5.

13. Alfheeaid H, Gerasimidis K, Nastase AM, Elhauge M, Cochrane B, Malkova D. Impact of phenylketonuria type meal on appetite, thermic effect of feeding and postprandial fat oxidation. Clin Nutr. 2018;37(3):851-7.

FIGURE LEGENDS

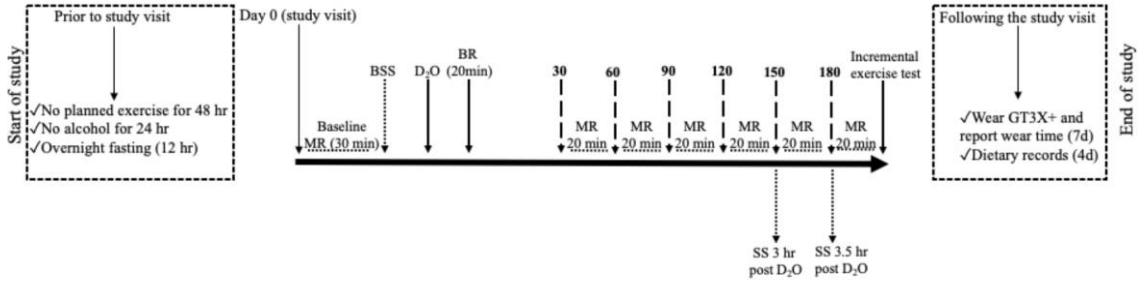

Figure 1. Schematic diagram of the study design.
MR, metabolic rate; BSS, baseline saliva sample; BR, breakfast; D_2O, deuterium oxide water; SS, saliva sample; GT3X+; ActiGraph, d; days; hr, hours; min, minutes

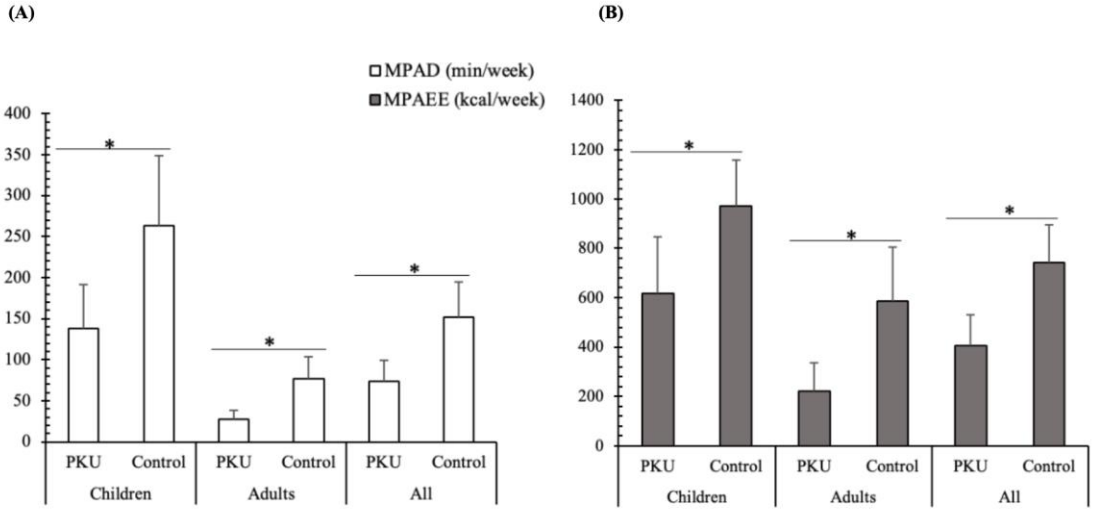

Figure 2. (A) moderate physical activity duration (MPAD min/week) and (B) moderate physical activity energy expenditure (MPAEE kcal/week) in patients with PKU (n= 16) and healthy Controls (n=15).

Values are mean ± SE.
* Significant difference ($P \leq 0.05$), between the PKU and Control group in children, adults and all participants.

TABLE

Table is submitted in separate file.