



Acute exercise in treated phenylketonuria patients: Physical activity and biochemical response



Priscila Nicolao Mazzola^{a,b}, Bruno Costa Teixeira^c, Gabriel Henrique Schirmbeck^c, Alvaro Reischak-Oliveira^c, Terry G.J. Derks^b, Francjan J. van Spronsen^b, Carlos Severo Dutra-Filho^{a,d}, Ida Vanessa Doederlein Schwartz^{e,f,*}

^a Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Ramiro Barcelos 2600 anexo, 90035-003, Porto Alegre, Brazil

^b Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands

^c Physical Education School, UFRGS, Rua Felizardo 750, 90690-200, Porto Alegre, Brazil

^d Departamento de Bioquímica, UFRGS, Rua Ramiro Barcelos 2600 anexo, 90035-003, Porto Alegre, Brazil

^e Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-003, Porto Alegre, Brazil

^f Department of Genetics, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2350, 90035-003, Porto Alegre, Brazil

ARTICLE INFO

Article history:

Received 5 September 2015

Received in revised form 11 October 2015

Accepted 11 October 2015

Available online 22 October 2015

Keywords:

Phenylketonuria

PKU

Aerobic exercise

Basal metabolic rate

Phenylalanine

Natural restricted diet

ABSTRACT

Background: In phenylketonuria, dietary treatment prevents most of the severe brain disease. However, patients have to follow a diet restricted in several natural components, what may cause decreased bone density and obesity. Exercise is known to improve both mental functioning and bone density also avoiding obesity, and could optimize aspects of central and peripheral outcome, regardless changes in phenylalanine (Phe) levels. However, the acute effects of exercise on metabolic parameters in phenylketonuria patients are unknown and thereby long-term adaptations are unclear. Therefore, this study aimed to evaluate patients' basal metabolic rate (BMR), and their acute response to an aerobic exercise session on plasma concentrations of Phe, tyrosine (Tyr), and branched-chain amino acids (BCAA), as well as metabolic and hormonal responses.

Methods: Five early- and four late diagnosed phenylketonuria patients aged 21 ± 4 years and 17 sex-, age-, and BMI-matched controls were evaluated for BMR, peak oxygen consumption (VO_{2peak}) and plasma amino acid, glucose, lipid profile and hormonal levels. At least one week later, participants performed a 30-min aerobic exercise session (intensities individually calculated using the VO_{2peak} results). Blood samples were collected in fasted state (moment 1, M1) and immediately after a small breakfast, which included the metabolic formula for patients but not for controls, and the exercise session (moment 2, M2).

Results: Phenylketonuria patients and controls showed similar BMR and physical capacities. At M1, patients presented higher Phe concentration and Phe/Tyr ratio; and lower levels of BCAA and total cholesterol than controls. Besides that, poorly controlled patients tended to stay slightly below the prescribed VO_2 during exercise. Both patients and controls showed increased levels of total cholesterol and LDL at M2 compared with M1. Only controls showed increased levels of Tyr, lactate, and HDL; and decreased Phe/Tyr ratio and glucose levels at M2 compared to values at M1.

Conclusions: Acute aerobic exercise followed by a Phe-restricted breakfast did not change Phe concentrations in treated phenylketonuria patients, but it was associated with decreased Phe/Tyr only in controls. Further studies are necessary to confirm our results in a higher number of patients.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations: BCAA, branched-chain amino acids; BMI, body mass index; BMR, basal metabolic rate; CTL, control; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N/A, not applicable; NS, non-significant; Phe, phenylalanine; PKU, phenylketonuria; RER, respiratory exchange ratio; Tyr, tyrosine; VCO_2 , carbon dioxide production; VO_2 , oxygen consumption; VO_{2peak} , peak oxygen consumption.

* Corresponding author at: Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-003, Porto Alegre, Brazil.

E-mail addresses: pkupriscilamazzola.com (P.N. Mazzola), brunoc100@hotmail.com (B.C. Teixeira), gschirmbeck@gmail.com (G.H. Schirmbeck), alvaro.oliveira@ufrgs.br (A. Reischak-Oliveira), t.g.j.derks@umcg.nl (T.G.J. Derks), f.j.van.spronsen@umcg.nl (F.J. van Spronsen), dutra@ufrgs.br (C.S. Dutra-Filho), ischwartz@hcpa.edu.br (I.V.D. Schwartz).

1. Introduction

Phenylketonuria (PKU, MIM 261600) is an autosomal recessive disease characterized by high levels of phenylalanine (Phe) in plasma and brain, due to the low activity of Phe hydroxylase (PAH, EC 1.14.16.1), which converts Phe into tyrosine (Tyr). Currently, patients are diagnosed by newborn screening programs and are treated with a Phe-restricted diet. Although mental retardation can be prevented with early diagnosis and dietary treatment, some peripheral and neurological problems remain. Regarding brain status, patients have shown non-optimal neuro-psychological outcome with decreased capacity especially in executive

functions [1,2], increased risk of depression [3,4], anxiety, and mood disturbances [5,6]. On the peripheral level, decreased bone density [7–9] and increased risk of overweight [10,11] are also reported in treated PKU patients. As yet, it is not clear whether these problems are due to the high blood Phe concentrations or to the dietary treatment restricting not only Phe, but also other important micronutrients. Moreover, a wide range of protein-rich foods are forbidden thus daily caloric needs are fulfilled with carbohydrates and lipids [10].

Exercise may represent a treatment strategy in PKU since it has been proven to enhance overall health in different populations. In healthy individuals, regular exercise improves mood and cognition [12], decreases depressive symptoms [13], decreases the risk of obesity [14], and improves bone density [15,16]. Moreover, physical training leads to better neurological outcomes in patients with neurodegenerative diseases [17,18], and mild cognitive impaired elderly [19]. Therefore, PKU patients might also benefit from the exercise-induced adaptations.

High plasma Phe along with the Phe-restricted diet may lead to different metabolic responses to exercise in continuously treated PKU, what could affect training adaptations [20]. Acutely, exercise increases the metabolic demand and protein turnover, which can affect amino acid levels [21]. In addition, different dietary composition can alter the metabolic response to exercise, leading to specific long-term adaptations [22]. So far, only the study by Grünert et al. [23] has reported the effects of exercise in PKU patients, suggesting aerobic exercise does not importantly affect peripheral Phe levels in these patients. However, that study was not controlled and had evaluated the pre- and post-exercise Phe and Tyr levels as secondary objectives and in a relatively fasted state.

Therefore, the aim of this study was to investigate the acute effects of an aerobic exercise in PKU patients regarding changes in metabolic parameters.

2. Methods

2.1. Study design

Participants performed two days of interventions (Fig. 1) at the Laboratory of Physical Exercise (LAPEX), Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil. This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The local Human Research Ethics Committee has approved the study (N.120292 HCPA-UFRGS), and all participants (or parent/legal tutor for patients younger than 18 years old) signed an informed consent term before starting the tests.

2.2. Participants

Inclusion criteria were: (a) confirmed diagnosis of classical PKU, (b) following treatment regularly in a PKU Center in the south of

Brazil, (c) aged >13 years, (d) not engaged in exercise training, and (e) mentally and physically able to perform exercise. From a total of 16 invited patients, five did not agree to participate due to travel issues and two because of personal reasons. Therefore, nine PKU patients from the Medical Genetics Service – Hospital de Clínicas de Porto Alegre, Brazil, were included. One patient did not follow the fasting requirement at day 1, so he was excluded from the exercise session results. Healthy non-PKU subjects were sex- age- and BMI-matched in an approximately 1:2 ratio to patients, and were not engaged in exercise training. The controls were invited through banners and advertisements in the University community.

Patients were classified as being early diagnosed (if the diagnosis was performed before the end of the first month of life) or late diagnosed (if the diagnosis was performed after the end of the first month of life), and were following treatment since diagnosis. Patients were also classified as “well controlled” if their current Phe levels at M1 were below 700 $\mu\text{mol/L}$, or as “poorly controlled” if these levels were equal or above 700 $\mu\text{mol/L}$. The treatment consisted of being on the Phe-restricted diet (low Phe intake along with the metabolic formula).

2.3. BMR test

The BMR was determined in a 10–12 h fasted state between 7.00 and 9.00 am at day 0 in order to assess daily basal caloric expenditure. The participants stayed in supine position for 30 min while their expiratory ventilation was analyzed by an automated open-circuit gas analysis system (MedGraphics Cardiorespiratory Diagnostic Systems, model CPX-D, and using the method Breath by Breath). The expired air fractions of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured every minute during the last 20 min of the test. The equation proposed by Weir [24], $[(3.9 \times \text{VO}_2) + (1.1 \times \text{VCO}_2)]$, was used to obtain the values in kcal/min, which were then transformed into kcal/kg/day. Respiratory exchange ratio (RER) was also calculated by the quotient between VCO_2 and VO_2 .

2.4. Standard breakfasts

All participants received a standard breakfast (day 0) after the BMR test and, at day 1, 30 min before the exercise session (Fig. 1). The PKU breakfast consisted of a banana, a rice cookie and 200 mL of water mixed with two tablespoons of the metabolic formula (PKU 2 Secunda, Milupa) and one tablespoon of crystal sugar. The controls also received a banana and a rice cookie, but a regular yogurt (200 mL) instead of the metabolic formula. Regarding nutritional facts, PKU patients received a breakfast of 198 kcal and 40 mg of Phe (67% of carbohydrates, 30% of protein – containing approximately 395 mg of tyrosine, 403 mg of isoleucine, 664 mg of leucine, and 470 mg of valine – and 3% of lipids), while controls received a 157 kcal meal containing 340 mg of Phe (80% of carbohydrates, 17% of proteins and 2% of lipids).

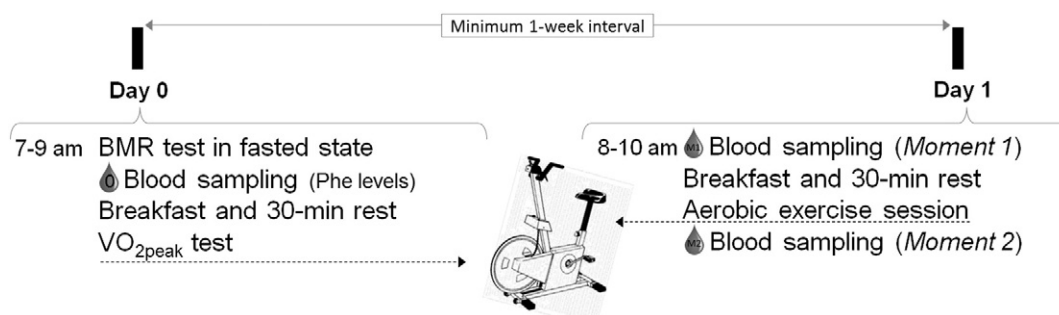


Fig. 1. Experimental design. In fasted state, participants went to the lab twice (at day 0 and day 1). In the day 0, participants performed the basal metabolic rate (BMR) test, followed by blood sampling for plasma phenylalanine (Phe) evaluation (0), breakfast and 30-min rest. Then the peak oxygen consumption ($\text{VO}_{2\text{peak}}$) test was performed. In the day 1, blood sampling was collected at moment 1 (M1), then participants received breakfast, waited 30-min in rest and performed the aerobic exercise session. Immediately after exercise (moment 2, M2), the last blood sample was collected.

2.5. Peak VO_2 (VO_{2peak}) test

VO_{2peak} (mL/kg/min) was determined by an incremental stationary cycling exercise test to the point of exhaustion [25,26], using the gas analyzer described to the BMR test. This test was performed at day 0 of evaluations, 30 min after the standard breakfast.

Regarding standard safety procedures, we monitored for headaches, dizziness, altered vision, heart rate and hematocrit levels during and after exercise bouts (maximum test and aerobic session). A stationary bicycle was used due to its safety even for those who were not used to exercise. A cardiologist followed all the procedures.

2.6. Acute exercise protocol

Using the same ventilatory analyzer described to the BMR and VO_{2peak} tests, all participants performed 30 min of cycling exercise at a prescribed VO_2 . The prescribed aerobic intensity was calculated for each participant representing the VO_2 value at 10% below the second ventilatory threshold, which was assessed by the VO_{2peak} test at least one week earlier. The one-week interval was chosen to avoid possible interferences of the maximum test on the submaximal exercise session, especially for sedentary individuals.

The VO_2 was tracked throughout the exercise bout and the participant was asked to keep his/her VO_2 within the target zone (prescribed $VO_2 \pm 2$ mL/kg/min). When the participant was below or above it, he/she was encouraged to either increase or decrease the load or speed to stay in his/her calculated aerobic zone. Values of prescribed and averaged actual VO_2 during the exercise session were compared and expressed as percentage of the prescribed VO_2 . Since executive function of early and late diagnosed patients has shown to be affected by current levels of Phe [2,27], the actual VO_2 values were analyzed with regard to Phe control in the group of patients.

2.7. Blood sampling

Blood collections were performed at day 0 after the BMR test in a 10–12 h fasted state (for evaluation of Phe levels only), at day 1 in a 10–12 h fasted condition, i.e. at moment 1 (M1), and immediately after the aerobic exercise bout, i.e. at moment 2 (M2) also having had the light breakfast. The number of samples varied in some evaluations because it was not possible to collect the same amount of blood from all participants.

2.8. Biochemical measurements

Quantitative analysis of Phe, Tyr, and branched-chain amino acid (BCAA, isoleucine, leucine, and valine) levels in plasma was carried out by HPLC with fluorescence detector [28] with an internal variation coefficient less than 3%.

Serum glucose, total cholesterol, HDL and triacylglycerol were determined by specific commercial kits for the automated analyzer Cobas C111. The LDL value was estimated by the difference of total cholesterol and HDL minus triacylglycerol divided by five.

Plasma adiponectin was evaluated using a commercial kit for human adiponectin (Invitrogen KHP0041) ELISA immunoassay. The catecholamines dopamine, noradrenaline and adrenaline were analyzed in plasma by HPLC with electrochemical detection.

2.9. Statistical analysis

Data normality was tested by Shapiro–Wilk test. Control and PKU groups were compared using the independent Student's *t*-test for basal and M1 analyses, and with two-sided paired *t*-test for comparisons between M1 versus M2 in each group. Correlations were performed using Spearman's correlation. Categorical variables were compared by Fisher's Exact Test. SPSS 22.0 was employed for all

statistical analyses and a $p < 0.002$ was considered statically significant after applying Bonferroni correction.

3. Results

3.1. Sample characterization

The data of the nine PKU patients and 17 controls are described in the Table 1. Five patients were early diagnosed and four patients were late diagnosed. At day 0, Patients' current Phe concentrations varied between 323 and 761 $\mu\text{mol/L}$.

3.1.1. BMR and VO_{2peak} tests

Two controls, but no PKU patient, experienced discomfort during the protocols. Concerning basal status, PKU patients and controls showed similar values of BMR and RER during the BMR test. In the same way, patients showed similar aerobic capacity and workload peak in the VO_{2peak} test in comparison to controls (Table 1).

3.2. Exercise session

3.2.1. Baseline values at M1

In rest and fasted state, patients showed higher Phe levels and Phe/Tyr ratio, and lower levels of BCAA and total cholesterol in comparison to controls (Table 2). Phe levels ranged between 583 and 1029 $\mu\text{mol/L}$ in the PKU group. Regarding Phe control at M1, five patients showed good control. All early-diagnosed patients had good control of Phe levels, while all late diagnosed patients had poor Phe control. Accordingly, patients showed positive correlation between the age at diagnosis and the Phe levels at M1 ($r = 0.97$; $p < 0.001$).

3.2.2. Ventilatory measurements during exercise

PKU patients and controls showed similar values of prescribed VO_2 (21 ± 6 versus 22 ± 4 mL/kg/min, respectively), and actual VO_2 during exercise (18 ± 5 versus 22 ± 4 mL/kg/min, respectively). Despite not statistically different, poorly controlled patients showed the lowest percentage of actual VO_2 during exercise in relation to the prescribed value (Fig. 2). Mean RER values during exercise were similar between PKU and controls (0.99 ± 0.09 versus 0.96 ± 0.05 , respectively).

3.2.3. Biochemical values at M2

Phe concentrations were not different between M1 and M2 in the PKU as well as in the control group. In the PKU group, Phe concentrations ranged between 490 and 984 $\mu\text{mol/L}$ at M2. Phe/Tyr ratio was lower at M2 than at M1 only in controls (Table 2). Total cholesterol, LDL levels were increased at M2 in both PKU patients and controls in comparison with M1 (Table 2). Only in controls, Tyr, lactate, and HDL levels were higher, while glucose levels were lower at M2 in comparison with values at M1. Levels of BCAA, triacylglycerol, dopamine, noradrenaline, adrenaline, and adiponectin were not modified between M1 and M2 in patients and controls.

Table 1
Clinical characteristics from control (CTL) and phenylketonuria (PKU) groups at day 0.

	CTL	PKU	p value
Gender (male:female)	12:5	7:2	NS
Age (years)	22 \pm 4 (17)	21 \pm 4 (9)	NS
BMI (kg/m ²)	23 \pm 2 (17)	24 \pm 3 (9)	NS
Phenylalanine ($\mu\text{mol/L}$)	57 \pm 14 (17)	562 \pm 141 (9)	<0.001
BMR (kcal/kg/day)	21 \pm 4 (17)	23 \pm 4 (9)	NS
RER during BMR	0.87 \pm 0.07 (17)	0.82 \pm 0.07 (9)	NS
VO_{2peak} (mL/kg/min)	31 \pm 6 (17)	28 \pm 8 (9)	NS
Workload peak (W)	216 \pm 49 (17)	203 \pm 31 (9)	NS

BMI, body mass index, BMR, basal metabolic rate, RER, respiratory exchange ratio, VO_{2peak} , peak oxygen consumption, NS, non-significant. Data for numeric variables are expressed as mean \pm SD (n). See methodology for details on the statistical analysis.

Table 2
Biochemical parameters at moments 1 (M1) and 2 (M2) in controls (CTL) and phenylketonuria (PKU) patients.

	M1		CTL M1 vs PKU M1		M2		CTL M1 vs CTL M2		PKU M1 vs PKU M2	
	CTL	PKU		p value	CTL	PKU		p value		p value
Phe ($\mu\text{mol/L}$)	81 \pm 24 (17)	773 \pm 190 (8)		<0.001	73 \pm 15 (17)	723 \pm 139 (8)		NS		NS
Tyr ($\mu\text{mol/L}$)	57 \pm 15 (17)	62 \pm 9 (8)		NS	79 \pm 20 (17)	90 \pm 24 (8)		<0.001		NS
Phe/Tyr ratio	1.4 \pm 0.2 (17)	12.4 \pm 2.4 (8)		<0.001	1.0 \pm 0.2 (17)	8.3 \pm 1.8 (8)		<0.001		NS
Tryptophan ($\mu\text{mol/L}$)	35 \pm 9 (17)	29 \pm 6 (8)		NS	35 \pm 8 (17)	36 \pm 12 (8)		NS		NS
BCAA ($\mu\text{mol/L}$)	456 \pm 86 (17)	332 \pm 50 (8)		0.001	403 \pm 63 (17)	478 \pm 132 (8)		NS		NS
Glucose (mg/dL)	89 \pm 8 (17)	83 \pm 7 (8)		NS	77 \pm 9 (17)	78 \pm 11 (8)		0.001		NS
Lactate (mg/dL)	1.3 \pm 0.5 (17)	1.3 \pm 0.3 (7)		NS	3.7 \pm 1.6 (17)	3.6 \pm 1.8 (7)		<0.001		NS
Triacylglycerol (mg/dL)	89 \pm 33 (17)	76 \pm 27 (8)		NS	100 \pm 39 (17)	77 \pm 27 (8)		NS		NS
Total cholesterol (mg/dL)	168 \pm 31 (17)	121 \pm 26 (8)		0.001	181 \pm 34 (17)	129 \pm 26 (8)		<0.001		<0.001
HDL (mg/dL)	54 \pm 12 (17)	42 \pm 6 (8)		NS	57 \pm 11 (17)	45 \pm 7 (8)		<0.001		NS
LDL (mg/dL)	97 \pm 28 (17)	63 \pm 19 (8)		NS	103 \pm 28 (17)	68 \pm 19 (8)		0.001		0.001
Dopamine (pg/mL)	56 \pm 15 (17)	58 \pm 17 (8)		NS	57 \pm 13 (17)	57 \pm 11 (8)		NS		NS
Noradrenaline (pg/mL)	228 \pm 77 (17)	220 \pm 52 (8)		NS	229 \pm 85 (17)	217 \pm 53 (8)		NS		NS
Adrenaline (pg/mL)	49 \pm 15 (17)	45 \pm 12 (8)		NS	52 \pm 15 (17)	43 \pm 11 (8)		NS		NS
Adiponectin (ng/mL)	28 \pm 6 (14)	37 \pm 8 (8)		NS	30 \pm 9 (14)	39 \pm 4 (7)		NS		NS

Phe, phenylalanine; Tyr, tyrosine; BCAA, branched chain amino acids (isoleucine, leucine, and valine); RER, respiratory exchange ratio; NS, non-significant; N/A, not applicable. Results are expressed as mean \pm SD (n), two-sided paired *t*-test. After applying Bonferroni correction, only results with $p < 0.002$ were considered significant (see methodology).

4. Discussion

To the best of our knowledge, this is the first study to evaluate a controlled aerobic exercise session in PKU patients and healthy individuals. For that, we analyzed patients' basal metabolic status compared to controls, as well as their immediate responses to an aerobic exercise session at the same relative intensity, which was calculated for each participant by a previous $\text{VO}_{2\text{peak}}$ test. Our data corroborate the study by Grünert et al. [23], where exercise did not change Phe concentrations acutely in PKU patients. Moreover, we showed that metabolic responses to exercise are similar between PKU patients and controls, despite different previous meal.

Our results were measured in a small number of patients of both genders immediately after a 30-min exercise session that was performed after a light breakfast. The small sample size is a limitation of the study, thus making comparisons between subgroups of patients very difficult. That possibly contributed to the fact that most analyses were not significant in the group of patients. To counteract that, we have included a greater number of controls, in a compatible ratio to male and female patients. Due to the nature of the disease, PKU patients and controls received distinct breakfasts, which varied in compositions, thereby possibly causing different biochemical responses to exercise

[22,29]. The different meals represent real dietary habits of PKU and non-PKU individuals, while studying the patients in fasting condition would have caused other biochemical responses that had to be prevented this way [23,30,31].

Regarding basal metabolic status, PKU patients showed similar BMR values in comparison to controls. Some studies have found increased basal metabolism in different diseases [32–35], although the mechanisms are not yet elucidated. Despite that, our results agree with the study by Allen et al. [36], where no differences in BMR were found between early diagnosed PKU children and matched controls.

In rest and fasting (M1), patients showed lower BCAA and total cholesterol levels in comparison with controls. Lower lipoprotein levels and disturbed amino acid concentrations have been already described in PKU children [37,38], being associated with the composition of the Phe-restricted diet [11].

PKU patients have been encouraged to exercise [11,39], although its efficacy is not evidence-based. The concern on low physical activity level for PKU patients has risen from data on bone density measurements [7–9], as well as the eminent weight gain associated to consuming Phe-free products, which are often rich in carbohydrates [10,11]. Obesity may become a spreading health issue of this era, and exercising regularly may also prevent overweight and its related disorders. Despite that, the PKU patients of the present study showed normal BMI and were so physically active as the controls, seen by the similar values observed on $\text{VO}_{2\text{peak}}$ and workload peak in the $\text{VO}_{2\text{peak}}$ test in both groups. However, the three patients who showed the highest Phe values at M1 (poorly controlled), stayed slightly below the prescribed VO_2 during the exercise in average. This result did not reach statistical significance probably due to the small sample of patients. However, high Phe levels impair executive functioning [2,27] that could, in turn, affect exercise performance. In this way, patients that showed bad Phe control before exercise seemed to have difficulties in keeping the prescribed VO_2 during cycling, even though being encouraged to do so.

Both patients and controls showed expected responses regarding higher total cholesterol and LDL levels after exercise, since exercise enhances the availability of energetic substrates [40,41]. Tyr levels were increased after exercise only in the control group. Grünert et al. [23] have shown that Tyr levels increase immediately after aerobic exercise in PKU patients. In that study, patients exercised during 20 min at night (three hours after a dinner) making difficult any comparison with our findings, since in our study patients exercised during 30 min at morning (shortly after a breakfast). Because of the different protein sources of the breakfasts, our patients may have absorbed L-amino acids from the metabolic formula faster than the controls had absorbed casein from the yogurt [42]. Therefore, the effects of the breakfast for

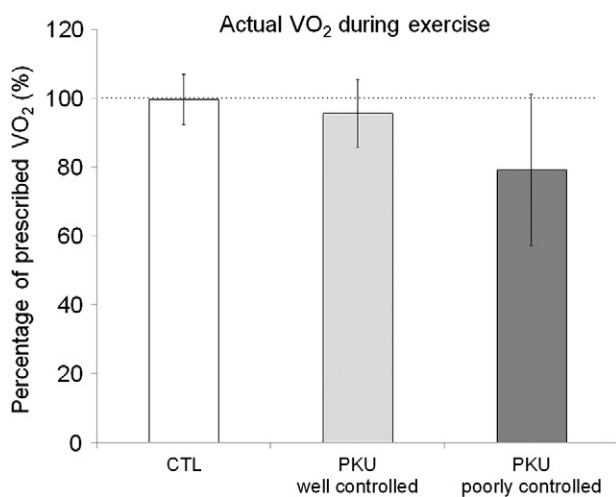


Fig. 2. Actual oxygen consumption (VO_2) during the 30-min exercise in controls (CTL) and well and poorly controlled phenylketonuria (PKU) patients. Data are expressed as mean \pm SD of percentage of prescribed VO_2 , $n = 16$ for controls, $n = 5$ and $n = 3$ for well and poorly controlled PKU subgroups, respectively.

patients might have been even more important with regard to amino acids levels at M2. Nevertheless, the aerobic exercise in combination with the amino acid-rich formula did not lead to unexpected metabolic responses in PKU patients. In addition, RER levels were similar between groups during basal condition and exercise. This result suggests that high Phe levels and different dietary composition did not modify substrate utilization in a small sample of treated patients with normal BMI.

5. Conclusions

Acute aerobic exercise followed by a Phe-restricted breakfast did not change Phe concentrations in treated PKU patients, but it is associated to lower Phe/Tyr ratio only in controls. Future studies are needed to confirm our results in a higher number of patients, as well as controlling for dietary intake before exercise.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PNM, BCT and GHS collected the data. PNM performed the statistical analyses and drafted the manuscript. All authors participated in the study design, contributed to the interpretation of the results and revised the manuscript.

Acknowledgments

This research project has been made possible thanks to a fellowship from PKU Academy under the auspices of EXCEMED – Excellence in Medical Education. Secondary financial support was provided by the “Fundo de Incentivo à Pesquisa e Eventos” (FIPE-HCPA, grant number 12-0292) and “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq, grant number 307540/2014-6).

References

- [1] K. VanZutphen, W. Packman, L. Sporri, M. Needham, C. Morgan, K. Weisiger, S. Packman, Executive functioning in children and adolescents with phenylketonuria, *Clin. Genet.* 72 (2007) 13–18.
- [2] S.C. Huijbregts, R. Gassio, J. Campistol, Executive functioning in context: relevance for treatment and monitoring of phenylketonuria, *Mol. Genet. Metab.* 110 (2013) S25–S30 (Suppl).
- [3] A. Clacy, R. Sharman, J. McGill, Depression, anxiety, and stress in young adults with phenylketonuria: associations with biochemistry, *J. Dev. Behav. Pediatr.* 35 (2014) 388–391.
- [4] R. Sharman, K. Sullivan, R.M. Young, J. McGill, Depressive symptoms in adolescents with early and continuously treated phenylketonuria: associations with phenylalanine and tyrosine levels, *Gene* 504 (2012) 288–291.
- [5] K. Anjema, M. van Rijn, P.H. Verkerk, J.G. Burgerhof, M.R. Heiner-Fokkema, F.J. van Spronsen, PKU: high plasma phenylalanine concentrations are associated with increased prevalence of mood swings, *Mol. Genet. Metab.* 104 (2011) 231–234.
- [6] A.E. ten Hoedt, L.M. de Sonnevile, B. Francois, N.M. ter Horst, M.C. Janssen, M.E. Rubio-Gozalbo, F.A. Wijburg, C.E. Hollak, A.M. Bosch, High phenylalanine levels directly affect mood and sustained attention in adults with phenylketonuria: a randomised, double-blind, placebo-controlled, crossover trial, *J. Inher. Metab. Dis.* 34 (2011) 165–171.
- [7] A. Miras, M.D. Boveda, M.R. Leis, A. Mera, L. Aldamiz-Echevarria, J.R. Fernandez-Lorenzo, J.M. Fraga, M.L. Couce, Risk factors for developing mineral bone disease in phenylketonuria patients, *Mol. Genet. Metab.* 108 (2013) 149–154.
- [8] A. Al-Qadreh, K.H. Schulpis, H. Athanasopoulou, C. Mengreli, A. Skarpalezou, I. Voskaki, Bone mineral status in children with phenylketonuria under treatment, *Acta Paediatr.* 87 (1998) 1162–1166.
- [9] M.J. de Groot, M. Hoeksma, M. van Rijn, R.H. Slart, F.J. van Spronsen, Relationships between lumbar bone mineral density and biochemical parameters in phenylketonuria patients, *Mol. Genet. Metab.* 105 (2012) 566–570.
- [10] A. MacDonald, J.C. Rocha, M. van Rijn, F. Feillet, Nutrition in phenylketonuria, *Mol. Genet. Metab.* 104 (2011) S10–S18 (Suppl).
- [11] J.C. Rocha, A. Macdonald, F. Trefz, Is overweight an issue in phenylketonuria? *Mol. Genet. Metab.* 110 (2013) S18–S24 (Suppl).
- [12] A.C. Deslandes, Exercise and mental health: what did we learn in the last 20 years? *Front. Psychol.* 5 (2014) 66.
- [13] A.L. Rebar, R. Stanton, D. Geard, C. Short, M.J. Duncan, C. Vandelanotte, A meta-meta-analysis of the effect of physical activity on depression and anxiety in non-clinical adult populations, *Health Psychol. Rev.* (2015) 1–78.
- [14] A. Wirth, M. Wabitsch, H. Hauner, The prevention and treatment of obesity, *Dtsch. Arztebl. Int.* 111 (2014) 705–713.
- [15] M.A. Stroppe, P. Nigh, M.I. Carter, N. Lin, J. Jiang, P.S. Hinton, Physical activity-associated bone loading during adolescence and young adulthood is positively associated with adult bone mineral density in men, *Am. J. Mens Health* (2014).
- [16] M. Tveit, B.E. Rosengren, J.A. Nilsson, M.K. Karlsson, Exercise in youth: high bone mass, large bone size, and low fracture risk in old age, *Scand. J. Med. Sci. Sports* (2014).
- [17] S.S. Hernandez, P.F. Sandreschi, F.C. Silva, B.A. Arancibia, R. da Silva, P.J. Gutierrez, A. Andrade, What are the benefits of exercise for Alzheimer's disease? A systematic review of past 10 years, *J. Aging Phys. Act.* (2014).
- [18] O. Oguh, A. Eisenstein, M. Kwasny, T. Simuni, Back to the basics: regular exercise matters in Parkinson's disease: results from the National Parkinson Foundation QIL registry study, *Parkinsonism Relat. Disord.* 20 (2014) 1221–1225.
- [19] C.M. Nascimento, J.R. Pereira, L.P. de Andrade, M. Garuffi, L.L. Talib, O.V. Forlenza, J.M. Cancela, M.R. Cominetti, F. Stella, Physical exercise in MCI elderly promotes reduction of pro-inflammatory cytokines and improvements on cognition and BDNF peripheral levels, *Curr. Alzheimer Res.* 11 (2014) 799–805.
- [20] S.P. Brown, Introduction to Exercise Science, Lippincott Williams & Wilkins, Baltimore, Maryland, USA, 2001.
- [21] C. Morris, C.O. Grada, M. Ryan, H.M. Roche, G. De Vito, M.J. Gibney, E.R. Gibney, L. Brennan, The relationship between aerobic fitness level and metabolic profiles in healthy adults, *Mol. Nutr. Food Res.* 57 (2013) 1246–1254.
- [22] G.A. Brooks, Mammalian fuel utilization during sustained exercise, *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 120 (1998) 89–107.
- [23] S.C. Grunert, C.M. Brichta, A. Krebs, H.W. Clement, R. Rau, C. Fleischhaker, K. Hennighausen, J.O. Sass, K.O. Schwab, Diurnal variation of phenylalanine and tyrosine concentrations in adult patients with phenylketonuria: subcutaneous microdialysis is no adequate tool for the determination of amino acid concentrations, *Nutr. J.* 12 (2013) 60.
- [24] J.B. Weir, New methods for calculating metabolic rate with special reference to protein metabolism, *J. Physiol.* 109 (1949) 1–9.
- [25] M.R. Boulay, P. Hamel, J.A. Simoneau, G. Lortie, D. Prud'homme, C. Bouchard, A test of aerobic capacity: description and reliability, *Can. J. Appl. Sport Sci.* 9 (1984) 122–126.
- [26] I. Dionne, S. Van Vugt, A. Tremblay, Postexercise macronutrient oxidation: a factor dependent on postexercise macronutrient intake, *Am. J. Clin. Nutr.* 69 (1999) 927–930.
- [27] R. Jahja, S.C. Huijbregts, L.M. de Sonnevile, J.J. van der Meere, F.J. van Spronsen, Neurocognitive evidence for revision of treatment targets and guidelines for phenylketonuria, *J. Pediatr.* 164 (2014) 895–899 e892.
- [28] M.H. Joseph, C.A. Marsden, Amino acids and small peptides, in: C.K. LIM (Ed.), *HPLC of Small Peptides* 1986, pp. 13–27 (Oxford).
- [29] E.F. Coyle, A.E. Jeukendrup, M.C. Oseto, B.J. Hodgkinson, T.W. Zderic, Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise, *Am. J. Physiol. Endocrinol. Metab.* 280 (2001) E391–E398.
- [30] F.J. van Spronsen, M. van Rijn, T. van Dijk, G.P. Smit, D.J. Reijngoud, R. Berger, H.S. Heymans, Plasma phenylalanine and tyrosine responses to different nutritional conditions (fasting/postprandial) in patients with phenylketonuria: effect of sample timing, *Pediatrics* 92 (1993) 570–573.
- [31] F.J. van Spronsen, T. van Dijk, G.P. Smit, M. van Rijn, D.J. Reijngoud, R. Berger, H.S. Heymans, Phenylketonuria: plasma phenylalanine responses to different distributions of the daily phenylalanine allowance over the day, *Pediatrics* 97 (1996) 839–844.
- [32] C. Bitz, S. Toubro, T.M. Larsen, H. Harder, K.L. Rennie, S.A. Jebb, A. Astrup, Increased 24-h energy expenditure in type 2 diabetes, *Diabetes Care* 27 (2004) 2416–2421.
- [33] T. Raj, G. D'Souza, M. Elia, A.V. Kurpad, Measurement of 24 h energy expenditure in male tuberculosis patients, *Indian J. Med. Res.* 124 (2006) 665–676.
- [34] G. Tarantino, M. Marra, F. Contaldo, F. Pasanisi, Basal metabolic rate in morbidly obese patients with non-alcoholic fatty liver disease, *Clin. Invest. Med.* 31 (2008) E24–E29.
- [35] D. Doneda, A.L. Lopes, A.R. Oliveira, C.B. Netto, C.C. Moulin, I.V. Schwartz, Gaucher disease type I: assessment of basal metabolic rate in patients from southern, Braz. *Blood Cells Mol. Dis.* 46 (2011) 42–46.
- [36] J.R. Allen, J.C. McCauley, D.L. Waters, J. O'Connor, D.C. Roberts, K.J. Gaskin, Resting energy expenditure in children with phenylketonuria, *Am. J. Clin. Nutr.* 62 (1995) 797–801.
- [37] K.H. Schulpis, I. Papassotiropoulos, S. Tsakiris, M. Vounatsou, G.P. Chrousos, Increased plasma adiponectin concentrations in poorly controlled patients with phenylketonuria normalize with a strict diet: evidence for catecholamine-mediated adiponectin regulation and a complex effect of phenylketonuria diet on atherogenesis risk factors, *Metabolism* 54 (2005) 1350–1355.
- [38] K.H. Schulpis, S. Tsakiris, G.A. Karikas, M. Moukas, P. Behrakis, Effect of diet on plasma total antioxidant status in phenylketonuric patients, *Eur. J. Clin. Nutr.* 57 (2003) 383–387.
- [39] A. Douglarakis, A. Skarpalezou, A. Theodosiadou, I. Monopolis, K. Schulpis, Body composition profile of young patients with phenylketonuria and mild hyperphenylalaninemia, *Int. J. Endocrinol. Metab.* 12 (2014) e16061.
- [40] R.S. Mazzeo, Catecholamine responses to acute and chronic exercise, *Med. Sci. Sports Exerc.* 23 (1991) 839–845.
- [41] T.D. Chinevere, R.D. Sawyer, A.R. Creer, R.K. Conlee, A.C. Parcell, Effects of L-tyrosine and carbohydrate ingestion on endurance exercise performance, *J. Appl. Physiol.* 93 (2002) 1590–1597.
- [42] S. Sindayikengera, W.S. Xia, Nutritional evaluation of caseins and whey proteins and their hydrolysates from protamex, *J. Zhejiang Univ. Sci. B* 7 (2006) 90–98.