UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
DEPARTAMENTO DE BIOQUÍMICA PROF. TUISKON DICK
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA

EXERCÍCIO FÍSICO NA FENILCETONÚRIA: AVALIAÇÃO DE MARCADORES METABÓLICOS EM PACIENTES E CAMUNDONGOS PAH^{enu2}

PRISCILA NICOLAO MAZZOLA

TESE DE DOUTORADO

Porto Alegre, Maio de 2017

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
DEPARTAMENTO DE BIOQUÍMICA PROF. TUISKON DICK
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA

EXERCÍCIO FÍSICO NA FENILCETONÚRIA: AVALIAÇÃO DE MARCADORES METABÓLICOS EM PACIENTES E CAMUNDONGOS PAH^{enu2}

PRISCILA NICOLAO MAZZOLA

Orientador: Prof. Carlos Severo Dutra Filho, MD, PhD
Coorientadora: Profa. Ida Vanessa Doederlein Schwartz, MD, PhD

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica como requisito parcial para a obtenção do título de Doutor na Universidade Federal do Rio Grande do Sul.

Porto Alegre, Maio de 2017

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Reitor: Prof. Dr. Rui Vicente Oppermann

Vice-Reitora: Profa. Dra. Jane Fraga Tutikian

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA

Coordenador: Prof. Dr. Diogo Onofre Gomes de Souza

Coordenadora Substituta: Profa. Dra. Angela Terezinha de Souza Wyse

Mazzola, Priscila Nicolao
Exercício físico na fenilcetonúria: avaliação de marcadores metabólicos em pacientes e camundongos PAHenu2 / Priscila Nicolao Mazzola. -- 2017.
83 f.

Orientador: Carlos Severo Dutra Filho.
Coorientadora: Ida Vanessa Doederlein Schwartz.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Porto Alegre, BR-RS, 2017.

1. Fenilcetonúria. 2. Exercício físico. 3. Fenilalanina. 4. Estresse oxidativo. 5. Metabolismo. I. Dutra Filho, Carlos Severo, orient. II. Schwartz, Ida Vanessa Doederlein, coorient. III. Título.

Este trabalho foi desenvolvido no Laboratório de Erros Inatos do Metabolismo (Laboratório 34D) do Departamento de Bioquímica, no Hospital de Clínicas de Porto Alegre (HCPA), ambos da Universidade Federal do Rio Grande do Sul, em Porto Alegre, e no Departamento de Neurobiologia Molecular da Universidade de Groningen, em Groningen, Holanda. Agradecemos, também, a FIPE-HCPA e a EXCEMED pelo apoio financeiro. Agradecimento especial a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), órgão financiador da bolsa de doutorado e de projeto de pesquisa para o desenvolvimento deste trabalho.

Dedico esta Tese às pessoas
com fenilcetonúria.

"Aprender é a única coisa de que a mente
nunca se cansa, nunca tem medo e
nunca se arrepende."

Leonardo da Vinci

Agradecimentos

Ao meu orientador, Prof. Dutra, pelo constante apoio e confiança;

À minha co-orientadora, Prof. Ida Schwartz, pelos conselhos e motivação;

Aos meus amigos de todas as partes do mundo, por me fazerem tão bem;

Aos meus colegas de laboratório por toda a ajuda e aprendizado;

Aos participantes das pesquisas contidas nesta tese, obrigada pela confiança e espero ter contribuído no campo da PKU;

Aos órgãos de fomento CAPES, EXCEMED, CNPq e FIPE-HCPA;

À minha família, que me permitiu voar alto e alcançar meus objetivos;

À minha família “postiça”, as Timmers, que estão sempre me encorajando nas dificuldades e comemorando conquistas comigo;

Ao Jotajota, que vem levando leve porém intensamente a vida comigo;

À própria vida, por me ter agraciado com tantas aventuras.

Índice

PARTE I

PARTE II

PARTE III

Parte I

RESUMO

A fenilcetonúria (PKU) é caracterizada por uma deficiente atividade da fenilalanina hidroxilase (PAH), que converte fenilalanina em tirosina. Consequentemente, pacientes fenilcetonúricos apresentam níveis aumentados de fenilalanina no sangue e em tecidos. A fenilalanina atinge níveis tóxicos no cérebro e, assim, pode levar a deficiência intelectual grave se não tratada. Hoje em dia, os pacientes fenilcetonúricos são diagnosticados através de programas de triagem neonatal e são colocados em tratamento imediatamente. O tratamento da PKU é baseado em uma dieta restrita em fenilalanina juntamente com uma mistura de aminoácidos, que visa reduzir a ingestão de fenilalanina ao mesmo tempo em que fornece todos os outros aminoácidos e nutrientes essenciais. Apesar de eficiente, o tratamento é extremamente difícil de seguir e, portanto, os pacientes ainda apresentam altos níveis de fenilalanina e seus problemas associados, tais como estresse oxidativo, distúrbios motores e cognitivos. Ainda, a dieta em si é tão restritiva que a completa adesão pode levar a problemas nutricionais como obesidade e perturbações hormonais. A fim de abordar estas questões na PKU, é necessário compreender os mecanismos pelos quais a fenilalanina perturba homeostasia, bem como propor novas estratégias de tratamento para a doença. Portanto, esta tese teve como objetivo verificar o estado metabólico basal e em exercício aeróbico em pacientes fenilcetonúricos, além de avaliar possíveis benefícios do treinamento físico em camundongos PAH^{enu2}. Para tanto, foram avaliados composição basal, estado nutricional, taxa metabólica basal, bem como a resposta ventilatória e bioquímica a uma sessão de exercício aeróbico em pacientes fenilcetonúricos em comparação com controles pareados, por bioimpedância elétrica e calorimetria indireta, respectivamente. Foram encontrados resultados similares entre pacientes e controles para todos os marcadores antropométricos e nutricionais, mostrando, assim, que os pacientes fenilcetonúricos têm a mesma composição corporal e perfil metabólico que controles. Além disso, os níveis de fenilalanina não se modificaram imediatamente após o exercício em comparação com a condição de repouso, demonstrando assim que o exercício aeróbico é seguro para pacientes fenilcetonúricos. No modelo animal de PKU, este estudo avaliou os efeitos crônicos do exercício aeróbico voluntário no cérebro de camundongos PAH^{enu2}. Apesar de correrem menores distâncias do que os controles, os camundongos PKU apresentaram melhoras em parâmetros de estresse oxidativo no cérebro, embora os níveis de fenilalanina no sangue e no cérebro permanecessem inalterados. Os animais PKU apresentaram habilidades motoras e de equilíbrio deficientes em comparação com os controles, enquanto o exercício não afetou esses parâmetros comportamentais. Adicionalmente, os aminoácidos gliconeogênicos e os relacionados com o ciclo da ureia foram encontrados em níveis inferiores no plasma dos animais PKU que se exercitaram em comparação com os sedentários. Por outro lado, os camundongos selvagens não apresentaram nenhuma alteração causada pelo exercício. Assim, os fenilcetonúricos não apresentam distúrbios em marcadores metabólicos tanto em repouso como durante exercício aeróbico. Por conseguinte, os fenilcetonúricos devem ser encorajados a praticar exercício para, possivelmente, beneficiarem-se das adaptações positivas geradas pelo treinamento físico. No entanto, o pequeno número de pacientes avaliados em nossos estudos destaca a necessidade de mais pesquisas para descrever o exercício mais adequado para pacientes com PKU.

Palavras-chave: Fenilcetonúria, hiperfenilalaninemia, exercício, índice de massa corporal, composição corporal, estado nutricional, estresse oxidativo

ABSTRACT

Phenylketonuria (PKU) is characterized by poor phenylalanine hydroxylase (PAH) activity, which acts converting phenylalanine into tyrosine. Consequently, PKU patients show increased levels of phenylalanine in the blood and tissues. Phenylalanine reaches toxic levels in the brain and therefore can lead to severe mental retardation, if untreated. Nowadays, PKU patients are diagnosed through newborn screening programs and are put on treatment immediately. PKU treatment is based on a phenylalanine-restricted diet along with an amino acid mixture, which aims to reduce the intake of phenylalanine while providing other essential amino acids and nutrients. Despite efficient, the treatment is extremely hard to follow so that PKU patients show high levels of phenylalanine and related issues such as oxidative stress, motor and cognitive disturbances. Moreover, the diet itself is so restrictive that adhering to it may lead to nutritional problems like obesity and hormonal disruptions. In order to tackle these issues in PKU, it is necessary to understand the mechanisms in which phenylalanine disturbs homeostasis as well as propose new treatment strategies for the disease. Therefore, this thesis aimed to evaluate metabolic markers in rest and exercise in PKU patients, as well as to verify the possible benefits of exercise in the brain of PAH^{enu2} mice. For that, we evaluated basal body composition, nutritional status, basal metabolic rate, as well as ventilatory and biochemical response to an aerobic exercise session in PKU patients and matched-controls using electrical bioimpedance analysis and indirect calorimetry, respectively. The groups did not differ for anthropometric and nutritional markers, thus showing that PKU patients have the same body and metabolic profiles as controls. Moreover, phenylalanine levels were not modified immediately after exercise in comparison to rest condition, thus proving that aerobic exercise is safe for PKU patients. In the animal model of PKU, we evaluated the effects of voluntary training in the brain of PAH^{enu2} mice. Despite running less distances than the controls, the PKU mice showed improved oxidative stress parameters although phenylalanine levels in the blood and in brain remained unchanged. PKU animals showed poor motor and balance skills than controls while exercise did not affect these behavioral markers. In addition, gluconeogenic and urea cycle-related amino acids were found in lower levels in the plasma of the exercised PKU animals in comparison to the sedentary PKU group. On the other hand, wild-type mice did not show any of those changes. Taken together, we conclude that PKU patients do not show disturbed metabolism in rest and during aerobic exercise. Therefore, PKU patients have to be encouraged to exercise in order to possibly benefit from long-term exercise-related adaptations. Nevertheless, the small number of patients evaluated in our studies highlights the need of further research to describe the most suitable exercise for PKU patients.

Keywords: Phenylketonuria, hyperphenylalaninemia, exercise, body mass index, body composition, nutritional status, oxidative stress

LISTA DE ABREVIATURAS

ADP – Pletismografia de ar deslocado, do inglês *air displacement plethysmography*

BCAA – Aminoácidos de cadeia ramificada, do inglês *branched-chain amino acids*

BCM – Massa celular corporal, do inglês *body cell mass*

BIA – Impedância bioelétrica, do inglês *bioelectrical impedance*

BMI – Índice de massa corporal, do inglês *body mass index*

CAT – Catalase

CTL – Controle

DXA – Absorciometria por duplo feixe de raio X, do inglês dual-energy X-ray absorptiometry

ECM – Massa extracelular, do inglês *extracellular mass*

Exe – Exercício

FFM – Massa livre de gordura, do inglês *fat-free mass*

FM – Massa de gordura, do inglês *fat mass*

GPx – Glutationa peroxidase

HPA – Hiperfenilalaninemia, do inglês *hyperphenylalaninemia*

N/A – Não se aplica

N/E – Não avaliado, do inglês *not evaluated*

PA – Ângulo fase, do inglês *phase angle*

PKU – Fenilcetonúria, do inglês Phenylketonuria

SAL – Salina

SD – Desvio-padrão, do inglês *standard deviation*

Sed – Sedentário

SOD – Superóxido dismutase

t-cholesterol – Colesterol total, do inglês *total cholesterol*

TBA-RS – Substâncias reativas ao ácido tiobarbitúrico, do inglês

TGA – Triacilgliceróis, do inglês *triacylglycerol*

TOBEC – Condução eléctrica corporal total, do inglês *total body electrical conductivity*

Tyr – Tirosina, do inglês *tyrosine*

VO_{2peak} – Pico de consumo de oxigênio

WT – Selvagem, do inglês *wild* type

1 INTRODUÇÃO

1.1 Breve História da Fenilcetonúria (PKU)

Em 1934, o médico bioquímico norueguês Asbjørn Følling foi procurado pela Sra. Egeland, uma mãe desesperada cujos filhos apresentavam deficiência intelectual grave (Folling 1994; Centerwall e Centerwall 2000). O quadro clínico daquelas crianças, a menina com seis e o menino com quatro anos de idade, não deixava dúvida do importante comprometimento neurológico da sua condição, porém nenhum diagnóstico havia sido dado até o momento. Após muita insistência, Dr. Følling resolveu coletar amostras de urina das crianças e as testar em seu laboratório. Após diversos testes, ele concluiu que as amostras dos irmãos apresentaram altos níveis de ácido fenilpirúvico, um composto derivado do aminoácido fenilalanina. Com isso, Dr. Følling chamou a condição de oligofrenia fenilpirúvica, que, anos mais tarde, foi rebatizada como fenilcetonúria (ou PKU na sigla em inglês de "phenylketonuria") por Lionel Penrose e Juda Hirsch Quastel (Penrose e Quastel 1937). Nos anos seguintes, outras pessoas com deficiência intelectual idiopático tiveram sua urina testada para a condição, comprovando, assim, mais casos da doença. Apesar do diagnóstico, nenhum tratamento estava disponível na época. Em torno de vinte anos mais tarde, Dr. Horst Bickel, um médico alemão, testou pela primeira vez uma dieta pobre em fenilalanina em uma criança com PKU (Bickel, Gerrard e Hickmans 1953). O teste foi um sucesso, apesar de contar apenas com uma paciente, uma menina inglesa de quatro anos. A menina, que apresentava problemas motores e de humor quando submetida a dieta normal, agiu como uma criança normal de sua idade quando sob dieta de baixa fenilalanina por apenas sete dias. Assim, em 1952

surgiu o primeiro tratamento da PKU, baseado em uma dieta restrita em fenilalanina. Anos mais tarde, essa dieta foi complementada com uma fórmula metabólica especial a fim de garantir a ingestão adequada de nutrientes (Anexo 4). Até hoje, essa restrição dietética é o principal tratamento disponível para PKU. Apesar de muito avanço ter ocorrido com estudos promissores inclusive utilizando terapias genéticas que poderão oferecer a cura para a doença, ainda há necessidade de estudar estratégias concomitantes para a dieta restrita e, assim, melhorar o desfecho clínico dos pacientes fenilcetonúricos (Al Hafid e Christodoulou 2015).

No Brasil, a pesquisa sobre PKU é bastante notória. O Departamento de Bioquímica Prof. Tuiskon Dick da UFRGS tem construído uma sólida bibliografia na área da doença. Assim, muitos estudos foram produzidos avaliando os mecanismos neurotóxicos da fenilalanina bem como novas possibilidades de tratamento (Bedin, et al. 2000, Bedin, et al. 2001; Sirtori et al. 2005; Sitta et al. 2006; Mazzola et al. 2011; Mazzola et al. 2013; Moraes et al. 2013; Moraes et al. 2014; Bortoluzzi et al. 2014; Preissler et al. 2016).

1.2 PKU

PKU (OMIM 261600) é o erro inato do metabolismo dos aminoácidos mais comum, apresentando uma frequência média de 1:12.000 nascidos vivos (Sarkissian, Gamez e Scriver 2009). A condição é causada por polimorfismos no gene codificador da enzima hepática fenilalanina hidroxilase (PAH, EC 1.14.16.1), que converte a fenilalanina em tirosina utilizando tetrahidrobiopterina (BH_4) como cofator. A capacidade da PAH em

desempenhar sua função em metabolizar fenilalanina depende da(s) mutação(ões) genética(s) encontrada(s) no DNA do paciente, que são refletidas pela tolerância na ingestão de fenilalanina. Desta forma, os pacientes são classificados como portadores de hiperfenilalaninemia, PKU leve ou PKU clássica, sendo a última considerada a forma mais grave da doença, em que a atividade da PAH é praticamente nula.

Em altas concentrações, a fenilalanina pode sofrer metabolismo alternativo, produzindo os metabólitos tóxicos fenilpiruvato, fenilacetato e fenilactato (Schuck et al. 2015). Esses metabólitos estão aumentados no cérebro do modelo animal da PKU (Sarkissian et al. 2000), bem como na urina dos pacientes (Michals e Matalon 1985). Por outro lado, especula-se que a própria fenilalanina é a maior responsável pelos distúrbios encontrados na PKU, uma vez que seus metabólitos não atingem níveis tóxicos como os do próprio aminoácido (Sarkissian et al. 2000, Hörster et al. 2006).

O diagnóstico da PKU deve ser realizado nos primeiros dias de vida através do “Teste do Pezinho”, em que uma amostra de sangue do calcanhar do recém-nascido é coletada para análise dos níveis de fenilalanina, entre outros marcadores. Esse teste de triagem neonatal foi elaborado em 1962 pelo médico bacteriologista Robert Guthrie para verificar doenças congênitas (Lesser 1963) e, a partir de 1992, tornou-se obrigatório em todo o território brasileiro (Lei Federal n° 8069). Assim que diagnosticada, a criança deve iniciar o tratamento padrão da doença, que consiste em uma dieta restrita em fenilalanina para a manutenção do seu nível sanguíneo dentro das concentrações subtóxicas, acompanhada de uma fórmula metabólica que contém todos os outros nutrientes necessários.

Quando em altos níveis no sangue, a fenilalanina atinge o cérebro em concentrações tóxicas, levando a distúrbios neurológicos parcialmente causados por estresse oxidativo (Surtees e Blau 2000). Muitos estudos têm relacionado altos níveis de fenilalanina com importantes danos ao sistema nervoso central, apesar dos mecanismos exatos não estarem totalmente elucidados (Blau, van Spronsen e Levy 2010; de Groot et al. 2013).

Atualmente, modelos animais geneticamente modificados são utilizados para o estudo da PKU. Entre eles, destaca-se a linhagem de camundongos C57BL/6 PAH^{enu2}. Nesses animais, a PAH não apresenta nenhuma atividade, o que os torna um modelo com características semelhantes a dos pacientes quanto aos altos níveis de fenilalanina no sangue e no cérebro, e seus consequentes distúrbios no sistema nervoso central (Bruinenberg et al. 2016).

1.3 Importância do Tratamento Dietético na PKU

Os pacientes fenilcetonúricos iniciam a dieta pobre em fenilalanina imediatamente após o diagnóstico a fim de prevenir o acúmulo de fenilalanina e, consequentemente, seus efeitos deletérios. Assim, com poucos dias de vida, o bebê diagnosticado com PKU recebe leite materno, que contém fenilalanina, e a fórmula metabólica especial de acordo com a prescrição de um nutricionista. As quantidades dos nutrientes são ajustadas de acordo com a tolerância à fenilalanina de cada paciente, que reflete a capacidade da enzima PAH em hidroxilar fenilalanina.

De fato, a dieta juntamente com a fórmula metabólica é efetiva na manutenção dos níveis de fenilalanina dentro de uma faixa tolerável, apesar de

nunca alcançarem valores de não portadores da doença (Blau, van Spronsen e Levy 2010). Porém, a dificuldade de manter uma dieta extremamente restritiva por toda a vida é um desafio (Pimentel et al. 2014). Desta forma, comumente pacientes perdem o controle da dieta especialmente quando chegam à adolescência e fase adulta (Vilaseca et al. 2010; Cleary et al. 2013). O correto controle da dieta e, consequentemente, dos níveis de fenilalanina na infância do paciente é fortemente influenciado pelo empenho dos pais ou tutores. Isso é de extrema importância, uma vez que o cérebro está em desenvolvimento e, assim, suscetível a danos graves e irreversíveis devido a exposição a altos níveis de fenilalanina. Porém, a manutenção dos níveis de fenilalanina nas fases seguintes da vida do paciente também é bastante importante, uma vez que altas concentrações de fenilalanina têm sido associadas com distúrbios cognitivos e do humor mesmo em pacientes diagnosticados e tratados precocemente (VanZutphen et al. 2007; Trefz et al. 2011; Huijbregts, Gassio e Campistol 2013; Jahja et al. 2014; Bilder et al. 2016). Os efeitos tóxicos da fenilalanina no cérebro adulto parecem ser reversíveis, o que evidencia a necessidade de estratégias concomitantes à difícil dieta no auxílio do controle dos níveis de fenilalanina, especialmente em pacientes adolescentes e adultos (Gassio et al. 2003; Gonzalez et al. 2011).

O tratamento da PKU ainda está longe de ser ideal e, no Brasil, a distribuição da fórmula metabólica apresenta falhas (Trevisan et al. 2015). Isso significa um difícil manejo da doença por parte dos pacientes e seus familiares, evidenciando que novas estratégias concomitantes a dieta são extremamente necessárias. Para tal, é importante conhecer as características metabólicas na PKU a fim de propor novos horizontes no manejo da doença.

1.4 Riscos Metabólicos na PKU

Tanto os altos níveis de fenilalanina quanto o próprio tratamento dietético restrito em fenilalanina podem proporcionar riscos à saúde dos pacientes fenilcetonúricos. Primeiramente, a dieta é tão restrita que, além da fenilalanina, restringe a captação de outros nutrientes e oligoelementos essenciais à manutenção da saúde (Mutze et al. 2012). Segundo, a dieta livre de fenilalanina possui baixo percentual de proteínas que é compensado pelo maior aporte de carboidratos e lipídeos para atingir as necessidades calóricas diárias dos pacientes, bem como proporcionar um sabor mais agradável aos alimentos especiais (Gokmen-Ozel et al. 2009). Assim, além de desequilibrar a disponibilidade de compostos importantes, o risco de desenvolver obesidade e suas doenças secundárias poderia estar aumentado em pacientes PKU.

O metabolismo corporal pode estar alterado na PKU devido a distúrbios na disponibilidade de nutrientes. Os altos níveis de fenilalanina perturbam a síntese de ácidos graxos insaturados de cadeia longa (Schulpis et al. 2004), hormônios e citocinas, como catecolaminas e adiponectina, respectivamente (Schulpis et al. 2005). Além disso, a restrição do consumo de produtos de origem animal leva a menor ingestão de micro e macronutrientes que são comumente encontrados nesses alimentos (Robert et al. 2013). Para suprir as necessidades nutricionais, a fórmula metabólica especial para a PKU contém todos esses nutrientes essenciais em quantidade adequada. Porém, esses nutrientes sintéticos não apresentam a mesma biodisponibilidade que compostos naturais (MacDonald et al. 2011). Nesse contexto, a qualidade do tratamento na PKU tem sido questionada quanto a déficits nutricionais (Camp et al. 2014) que causariam prejuízos no crescimento normal dos pacientes

(Arnold et al. 2002).

Distúrbios na composição corporal associados com sobrepeso e obesidade têm ganhado espaço na PKU (Rocha, Macdonald e Trefz 2013). Alguns autores encontraram valores de índice de massa corporal (IMC) em pacientes fenilcetonúricos acima dos encontrados para controles, sendo mais evidenciado no sexo feminino (Albersen et al. 2010; Burrage et al. 2012). Por outro lado, vários estudos não encontraram diferenças em composição corporal entre pacientes e controles (Allen et al. 1995; Huemer et al. 2007; Rocha et al. 2012; Rocha et al. 2013; Doulgeraki et al. 2014). Possivelmente, os diferentes resultados encontrados nos estudos citados acima podem representar um viés causado por amostras heterogêneas em relação à idade dos participantes. Além disso, é importante lembrar que tratar doenças secundárias à PKU, como a diabetes mellitus tipo II, resultaria em uma dieta praticamente impossível de ser seguida. Desta forma, conhecer os riscos envolvidos na PKU são imprescindíveis para uma melhor prescrição terapêutica e o estudo de novas estratégias intervencionais.

1.5 Exercício na PKU

O exercício representa um estresse físico que afeta todos os órgãos, estejam eles ativos ou não durante a prática desportiva. Devido a permeabilidade seletiva da barreira hematoencefálica, algumas substâncias são trocadas entre órgãos periféricos e centrais (Radak et al. 2007). Além disso, durante uma sessão de exercício, o aumento na demanda energética pode evidenciar alterações metabólicas que não são possíveis verificar em repouso (Brooks 1998). Por outro lado, o estresse causado pelo exercício pode

proporcionar adaptações em órgãos periféricos e centrais em longo prazo, especialmente quanto às capacidades antioxidantes no cérebro (Camiletti-Moiron et al. 2013).

O exercício aeróbico regular tem se mostrado eficaz na melhora do sistema redox e de neurotransmissores e, consequentemente, na saúde mental em indivíduos jovens (Radak et al. 2007), idosos (Goto et al. 2007) e em pacientes com doenças neurodegenerativas (Dibble, Addison e Papa 2009; Um et al. 2008). Durante o exercício, o aumento da demanda metabólica e, consequentemente, do consumo de oxigênio, aumenta a produção de radicais livres e outras espécies reativas. Assim, o cérebro é exposto a altas pressões de oxigênio e substâncias pró-oxidantes que atravessam a barreira hematoencefálica (Radak et al. 2007; Camiletti-Moiron et al. 2013). Desta forma, as capacidades antioxidantes do sistema nervoso central são incrementadas após recorrentes sessões de exercício, mediadas por aumento da atividade e expressão de enzimas antioxidantes (Halliwell 2011). Devido à adaptação mediada por exercício, o sistema redox é melhorado também em situação de repouso, levando à destoxificação de substâncias reativas que são normalmente formadas durante a respiração celular (Radak et al. 2007).

O exercício pode ser praticado em diferentes intensidades, causando adaptações específicas (Brooks 1998). Devido aos altos níveis de fenilalanina, bem como a composição da dieta especial, pacientes fenilcetonúricos podem apresentar respostas metabólicas em relação à utilização de substratos energéticos. Para avaliar essa hipótese, é importante conhecer o metabolismo basal e durante aumento de demanda metabólica (exercício). Um método utilizado para identificar a intensidade de exercício é a

avaliação das trocas gasosas por calorimetria indireta. Nessa análise ventilatória, é possível determinar o consumo de oxigênio (médio, de pico e máximo) e a produção de dióxido de carbono tanto no repouso como durante uma atividade física. Exercícios aeróbicos denotam a utilização de oxigênio durante determinada atividade. Assim, exercitar-se aerobicamente representa qualquer aumento da demanda metabólica que consegue ser mantido predominantemente por oxidação de lipídeos, uma vez que o oxigênio é utilizado na respiração celular ao final da cadeia respiratória promovida pela beta-oxidação e consequente oxidação dos carregadores de elétrons NADH e $FADH_2$ (Brooks 1998). Assim, quando a intensidade de exercício aumenta ou a musculatura em exercício não consegue manter o metabolismo aumentado às custas desse processo eficaz porém lento, outras reservas energéticas passam a ser utilizadas mais significativamente. A conversão de energia por meio de glicólise anaeróbica e degradação de fosfatocreatina leva à produção de ácido lático, que se dissocia em hidrogênio e lactato. Em consequência, a produção de dióxido de carbono por meio do tamponamento de íons de hidrogênio por bicarbonato aumenta consideravelmente ultrapassando os valores de consumo de oxigênio (Brooks 1998). Essa relação entre substratos utilizados por meio dos valores de consumo de oxigênio e produção de gás carbônico é dada pela variável ventilatória do quociente entre esses dois marcadores, conhecido como quociente respiratório. A dieta pode alterar a utilização de substratos energéticos durante exercício e, assim, alterar os valores de quociente respiratório (Dionne, Van Vugt e Tremblay 1999; Coyle et al. 2001). Igualmente, os altos níveis de fenilalanina e consequentes distúrbios nos níveis de catecolaminas e adipocinas circulantes podem alterar a utilização de substratos

energéticos (Brown 2001). Nesse contexto, é importante conhecer as respostas ventilatórias de pacientes fenilcetonúricos em repouso e exercício para melhor ilustrar o perfil metabólico nessa população.

2 OBJETIVOS

2.1 Objetivo Geral

Verificar o estado metabólico basal e em exercício aeróbico em pacientes fenilcetonúricos, bem como avaliar os efeitos do treinamento físico em um modelo animal da PKU.

2.2 Objetivos Específicos

a) Avaliar o estado nutricional e a composição corporal de pacientes fenilcetonúricos;
b) Avaliar o estado ventilatório e metabólico basal de pacientes fenilcetonúricos.
c) Avaliar o efeito agudo do exercício aeróbico no perfil metabólico e ventilatório em pacientes fenilcetonúricos;
d) Avaliar os efeitos crônicos do exercício voluntário sobre concentração de aminoácidos em sangue e cérebro e parâmetros de estresse oxidativo no cérebro em camundongos PKU.

Parte II

CAPÍTULO I

ANALYSIS OF BODY COMPOSITION AND NUTRITIONAL STATUS IN BRAZILIAN PHENYLKETONURIA PATIENTS

Artigo publicado

Mol Genet Metab Reports, 2016; 6:16–20
DOI: 10.1016/j.ymgmr.2015.12.003

Molecular Genetics and Metabolism Reports 6 (2016) 16–20

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Analysis of body composition and nutritional status in Brazilian phenylketonuria patients

Priscila Nicolao Mazzola [a,b], Tatiele Nalin [c,*], Kamila Castro [d], Margreet van Rijn [b], Terry G.J. Derks [b], Ingrid D.S. Perry [e], Alberto Scofano Mainieri [f], Ida Vanessa D. Schwartz [c,g,h]

[a] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul (UFRGS), Ramiro Barcelos 2600 anexo, 90035-003, Porto Alegre, Brazil
[b] Beatrix Children's Hospital, Section of Metabolic Diseases, University Medical Center Groningen, University of Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands
[c] Post-Graduation Program in Genetics and Molecular Biology, UFRGS, Bento Gonçalves 9500/43323M, PO Box 15053, Porto Alegre, Brazil
[d] Postgraduate Program in Pediatrics and Adolescent Health, UFRGS, Ramiro Barcelos 2400, 90035-003, Porto Alegre, Brazil
[e] Postgraduate Program in Collective Health, Health Unit, Universidade do Extremo Sul Catarinense, Universitária 1105, 88806-000 Criciúma, Brazil
[f] Department of Pediatrics, Hospital de Clínicas de Porto Alegre, Ramiro Barcelos 2400, 90035-003 Porto Alegre, Brazil
[g] Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-003 Porto Alegre, Brazil
[h] Department of Genetics, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2350, 90035-003 Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 20 November 2015
Received in revised form 15 December 2015
Accepted 15 December 2015
Available online xxxx

Keywords:
Inborn errors of metabolism
Phenylketonuria
Nutritional status
Body composition
Bioelectrical impedance

ABSTRACT

Background: Phenylketonuria (PKU) is characterized by phenylalanine (Phe) accumulation to toxic levels due to the low activity of phenylalanine-hydroxylase. PKU patients must follow a Phe-restricted diet, which may put them in risk of nutritional disturbances. Therefore, we aimed to characterize body composition parameters and nutritional status in Brazilian PKU patients also considering their metabolic control.
Methods: Twenty-seven treated PKU patients older than 5 years, and 27 age- and gender-matched controls, were analyzed for anthropometric features and body composition by bioelectrical impedance (BIA). Patients' metabolic control was assessed by historical Phe levels.
Results: There was no effect of PKU type, time of diagnosis, or metabolic control for any analyzed parameter. About 75% of patients and controls were eutrophic, according to their BMI values. There were no difference between groups regarding body composition and other BIA-derived parameters.
Conclusions: Brazilian PKU patients do not show differences in body composition and nutritional status in comparison with controls, regardless metabolic control. Although similar to controls, PKU patients may be in risk of disturbed nutritional and metabolic markers as seen for the general population.

1. Introduction

Phenylketonuria (PKU, OMIM: 261600) is an inherited metabolic disorder characterized by deficient (mild PKU) to null (classical PKU) activity of the hepatic enzyme phenylalanine (Phe) hydroxylase (PAH, EC 1.14.16.1), which converts Phe into tyrosine. PKU patients show high levels of Phe in the plasma and, consequently, in the brain [1]. Phe in high levels is toxic to the brain; therefore, untreated PKU patients show severe mental retardation. In order to avoid brain damage, patients must be early diagnosed and follow a lifelong Phe-restricted diet. This diet consists of low-Phe foods along with supplementation of an especial L-amino acid mixture [2]. Besides effective in lowering Phe levels, the Phe-restricted diet is hard to accomplish, so that patients may show high Phe levels in plasma reflecting poor dietary control [3–6].

Both high Phe levels and the dietary treatment may lead to nutritional deficiencies. First, increased levels of Phe have been related to disturbed synthesis of hormones and cytokines, such as catecholamines and adiponectin, thus affecting body metabolism [7,8]. Moreover, the diet restricts several sources of natural protein, thereby impairing the intake of essential micro- and macronutrients [9]. Finally, the L-amino acid mixture is composed by synthetic nutrients, which may compromise biological availability [4]. Because of those above-mentioned reasons, concern has been emerged on nutritional status [2], linear growth [10], body composition [11], and risk of overweight and obesity in PKU children [12,13]. Despite that, some studies did not find differences in the growth and body composition parameters in PKU patients compared with controls [14,15]. Therefore, perhaps the risk of disturbed

* Corresponding author.
E-mail addresses: pku@priscilamazzola.com (P.N. Mazzola), tatinalin@gmail.com (T. Nalin), kamilacastro@hotmail.com.br (K. Castro), m.van.rijn@umcg.nl (M. van Rijn), t.g.j.derks@umcg.nl (T.G.J. Derks), atputp@gmail.com (I.D.S. Perry), mainieri.alberto@gmail.com (A.S. Mainieri), ischwartz@hcpa.edu.br (I.V.D. Schwartz).

http://dx.doi.org/10.1016/j.ymgmr.2015.12.003

body composition in PKU just reflects the increased rates of nutritional imbalance as well as overweight and obesity seen in the general population and that varies from country to country. In addition, treatment management of PKU in Brazil needs special attention. In Brazil, the metabolic formula is supplied free of charge by the government, but the commercial low-Phe products are neither easily available nor typically reimbursed by insurance policies [5]. Besides that, the Neonatal Screening for PKU has been organized as a National Public Policy only since 2001, still existing many patients in the country diagnosed at a late age.

Bioelectrical impedance (BIA) represents a useful evaluation of overall body composition and nutritional status in PKU patients, in addition to the commonly analyzed body mass index (BMI). In this way, the BIA-derived data such as body fat mass (FM) and fat-free mass (FFM) proportion show more accurate values on real tissue mass than BMI estimations [16]. BIA analysis also provides an overview on metabolism and overall cellular integrity by the ratio between extracellular mass and body cell mass (ECM/BCM) and the phase angle (PA), respectively. These BIA-derived values are described as prognostic markers during hospitalization [17] and conditions like post-operative complications [18], kidney dysfunction [19], classical homocystinuria [20], and cancer [21]. In this way, abnormal values of ECM/BCM ratio and/or PA have been related to increased inflammatory processes [22]. Nutritional disturbances such as unbalanced polyunsaturated fatty acid intake [23] and obesity [24] have been related to increased inflammation. Because the PKU treatment seems to compromise the nutritional status in PKU patients, evaluating ECM/BCM ratio and PA can be of value to evaluate nutritional condition in PKU.

The current literature on body composition in PKU is still inconclusive. However, accurate measurements of peripheral markers in PKU patients are important to evaluate nutritional status which, in turn, can improve individual dietary management. Therefore, this study aimed to characterize body composition parameters and nutritional status in Brazilian PKU patients.

2. Methods

2.1. Participants

A cross-sectional study with PKU patients and age- and gender-matched healthy controls was conducted. Patients were recruited at the Medical Genetics Service from the Hospital de Clínicas de Porto Alegre (HCPA), Brazil. Controls were recruited in a routine follow-up at the Pediatric Service from the same hospital. Inclusion criteria included being aged 5 years or older and being able to lie quietly during the BIA test.

The study has been approved by the Research Ethics Committee of HCPA (protocol number 12-0115) and was conducted according to the Declaration of Helsinki guidelines. All subjects or their parents/caregivers signed an informed consent form.

2.2. Patients' characterization

PKU type was defined as mild- or classical PKU according to patients' Phe levels at diagnosis if levels were between 600–1200 μmol/L and >1200 μmol/L, respectively. Time of diagnosis was defined as early when patients were diagnosed before 60 days of life, and late when diagnosed at 60 days or older. Metabolic control was assessed by the median of historical serum Phe concentrations measured in the previous 12 months, with a minimum of three measurements in that period. Thus, patients were classified as having good metabolic control if those Phe levels were ≤360 μmol/L and ≤600 μmol/L for patients aged ≤12 and >12 years, respectively; otherwise, patients were classified as having poor metabolic control. All patients were following treatment since diagnosis, and it consisted of having a low-Phe diet and the L-amino acid mixture. No patient was on tetrahydrobiopterin (BH_4) treatment before or during the study.

2.3. Anthropometric measurements

Height was measured with a wall-mounted stadiometer (Harpenden, Holtain®, Crymych, UK) to the nearest 0.1 cm and weight was obtained using a digital platform scale with a resolution of 0.1 kg (Toledo®, Model 2096PP/2, São Paulo, Brazil), while participants were barefoot and wearing lightweight clothing. BMI was calculated by the quotient between weight (kg) and squared height (m^2), and classified into underweight, normal weight, overweight, or obese according to WHO 2009 [25].

2.4. BIA analysis

Measurements of body composition such as FM, FFM, ECM/BCM ratio, and PA were performed using a BIA device (Biodynamics 450® version 5.1, Biodynamics Corporation, Seattle, WA, USA) and Resting ECG tab electrodes (Conmed Corporation, Utica, NY, USA) according to previously described standards [26]. Briefly, the participants laid in supine position with arms and legs stretched out and kept from touching the body by non-conductor foam objects to prevent from adduction or crossing of the limbs, which would shorten the electrical circuit and reduce the impedance values. One pair of electrodes was placed on the right wrist and hand and the other on the right ankle and foot of the participant.

2.5. Statistical analysis

The Statistical Package for Social Sciences 19.0 (SPSS® Inc., Chicago, IL) was used. Data were described using absolute and relative frequencies. Continuous variables were expressed as mean ± standard deviation (SD). Unpaired and paired Student's *t*-tests were used to compare means of independent variables and to compare means between patients and controls, respectively. Effects of the co-factors were tested by ANOVA. The level of significance was set at 5%.

Table 1
Clinical characteristics of the phenylketonuria (PKU) patients.

Patient	Age (years)	Gender	Time of diagnosis[a]	PKU type[a]	Treatment adherence[a]
#1	6	Male	Late	Mild	Poor
#2	7	Female	Late	Classical	Poor
#3	11	Female	Late	Mild	Poor
#4	11	Female	Early	Mild	Good
#5	11	Male	Early	Classical	Poor
#6	11	Female	Late	Mild	Poor
#7	11	Male	Early	Mild	Poor
#8	11	Female	Early	Mild	Poor
#9	11	Female	Late	Classical	Poor
#10	12	Male	Early	Classical	Poor
#11	12	Male	Early	Mild	Good
#12	12	Female	Late	Mild	Poor
#13	12	Male	Late	Mild	Poor
#14	13	Female	Early	Classical	Poor
#15	13	Female	Late	Mild	Good
#16	14	Male	Late	Classical	Good
#17	14	Female	Early	Classical	Good
#18	14	Female	Late	Mild	Good
#19	15	Male	Late	Classical	Good
#20	16	Female	Late	Classical	Good
#21	16	Male	Late	Mild	Good
#22	16	Male	Early	Classical	Good
#23	17	Male	Early	Classical	Poor
#24	19	Female	Late	Mild	Good
#25	22	Male	Early	Classical	Good
#26	22	Male	Late	Mild	Good
#27	25	Male	Late	Classical	Poor

[a] See the text for details on classification.

Table 2
Anthropometrics and bioelectrical impedance parameters of phenylketonuria (PKU) patients and controls.

	PKU	Controls	p value
Weight (kg)	47.93 ± 15.99	51.52 ± 18.15	NS
Height (m)	1.52 ± 0.14	1.54 ± 0.17	NS
BMI (kg/m^2)	20 ± 4	21 ± 4	NS
BMI classification[a] (n)			NS
Underweight	1 (4%)	0 (0%)	
Normal weight	20 (74%)	18 (67%)	
Overweight	4 (15%)	6 (22%)	
Obese	2 (7%)	3 (11%)	
Fat mass (%)	20 ± 7	22 ± 9	NS
Free-fat mass (%)	80 ± 7	78 ± 9	NS
ECM/BCM ratio	1.05 ± 0.08	1.04 ± 0.10	NS
Phase angle (°)	6.22 ± 0.86	6.33 ± 1.05	NS

BMI, body mass index; ECM/BCM, extracellular mass/body cell mass; NS, non-significant. Fat mass and fat-free mass are expressed as percentage of total body weight. Data are expressed as mean ± SD or frequency (percentage); n = 27/group.
[a] According to WHO 2009. See the text for details on statistics.

3. Results

Each group of patients and controls had 27 participants (14 males and 13 females); patients originated from 26 nonrelated families. Concerning PKU type, 14 (52%) patients were mild PKU and 13 (48%) were classical PKU (Table 1). Eleven (41%) patients were early diagnosed and 16 (59%) patients were late diagnosed. Current Phe levels ranged between 102 and 1660 μmol/L while historical Phe levels ranged between 258 and 1482 μmol/L. Regarding treatment adherence, 13 (48%) patients had good metabolic control and 14 (51%) patients had poor metabolic control. The cofactors type of PKU, time of diagnosis, and metabolic control did not affect the anthropometric and BIA-derived measurements; thereby all patients were grouped in a single PKU group for further analyses.

No differences were found between PKU patients and controls regarding body composition, including the BIA-derived parameters FM, FFM, ECM/BCM ratio, and PA (Table 2). Three patients and three controls were below the cutoff values for PA (−3.2%, −14.3%, −17.4%, and −2.4%, −2.8%, −4.4%, respectively) according to Bosy-Westphal et al. [27] and Barbosa-Silva and Barros [18].

4. Discussion

This is the first study evaluating the body composition of Brazilian PKU patients, which represent a heterogeneous cohort of patients that differs from those from Europe and USA in terms of diagnosis, treatment, and ethnicity. First, the proportion of late-diagnosed patients in Brazil is still high, since the mandatory Neonatal Screening Program was implemented in 2001 in Brazil. Moreover, Brazilian PKU patients, although having access to the L-amino acid mixture, do not have access to protein-enriched low-Phe food, a fact that could compromise their nutritional status. Finally, Brazil is essentially a mixed country with several ethnic backgrounds. In this way, patients with different PKU types, time of diagnosis, and treatment adherence were included in our study, therefore these factors were also taken into account in the analysis of our results. Even though BIA analysis gives more accurate values on body composition than overall measurements such as BMI [16], our study did not find differences between patients and controls regarding anthropometric features evaluated by both methods. Therefore, our main findings pointed out that patients and controls were similar in body composition parameters, despite PKU type, time of diagnosis, and metabolic control. To the best of our knowledge, this is the first study that evaluated several markers on body composition in a heterogeneous group of PKU patients using BIA analysis.

Overweight has been pointed out as an issue for PKU patients, although this is not a consensus in the literature (Table 3). This disagreement in the literature may be caused by a lack of control for the puberty status of patients, since most studies, including ours, evaluate pre- and post-pubertal patients in a single group. In this way, some studies have found that PKU patients, especially females, show higher BMI and fat mass than controls [11,12]. On the other hand, several authors have not found differences in body composition between patients and age-matched controls [14,15,28–30] as well as comparing patients' outcomes to reference values [31–34]. In our study, the majority of patients

Table 3
Review of literature on studies regarding body composition in early treated phenylketonuria (PKU) patients.

Authors/location	Controlled	Number (n)/age	Type of PKU	Method	Body fat mass	Body fat-free mass	ECM/BCM	Phase angle
Das et al. (2014) Germany [31]	No[a]	51 27 ± 7 (range 16–44)	Classical	BIA	Normal	Normal	Normal	Normal
Doulgeraki et al. (2014) Greece [28]	Yes	80 10 ± 3 (range 5–18)	Mild, classical	DXA	No difference	No difference	N/E	N/E
Rocha et al. (2013); Rocha et al. (2012) Portugal [15,30]	Yes	89 14 ± 7	HPA, mild, classical	BIA	No difference	No difference	N/E	No difference
Douglas et al. (2013) USA [34]	No[a]	59 (range 10–19)	Classical	ADP	Normal	N/E	N/E	N/E
Adamczyk et al. (2010) Poland [33]	No[a]	45 14 ± 5	Classical	DXA	Normal	Normal	N/E	N/E
Albersen et al. (2010) The Netherlands [11]	Yes	20 median 10 (range 6–16)	Classical	ADP	Higher in patients	N/E	N/E	N/E
Huemer et al. (2007) Austria [14]	Yes	34 8.7 ± 3.9 (range 0.2–15)	Classical	TOBEC	No difference	No difference	N/E	N/E
Dobbelaere et al. (2003) France [32]	No[a]	20 4.5 ± 1.6 (range 0.7–7)	Classical	BIA	Normal	Normal	N/E	N/E
Allen et al. (1995) Australia [29]	Yes	30 10 ± 3	Classical	Skinfold-thickness	No difference	No difference	N/E	N/E

ECM/BCM, extracellular mass/body cell mass ratio; HPA, hyperphenylalaninemia; DXA, dual energy X-ray absorptiometry; BIA, bioelectrical impedance; ADP, air displacement plethysmography; TOBEC, total body electrical conductivity; N/E, not evaluated. Values of age are shown as mean ± SD if not stated otherwise.
[a] Non-controlled study that compared the results from patients with reference values.

and controls showed to be eutrophic regarding their BMI values. Moreover, no differences in body composition were found between patients and controls. Although not at higher risk of developing obesity, PKU patients may show the same risk of having disturbed body composition as the general population. Then, it is important to highlight that Brazil is undergoing an epidemiological transition associated with demographic and nutritional changes, which has led to high prevalence of obesity especially for the age group and geographic area covered by our study [35,36].

BIA analysis provides a range of data that assesses overall nutritional status such as relative active tissue (ECM/BCM ratio) and cellular integrity (PA), which can be of interest to PKU patients. In our study, values of ECM/BCM ratio and PA were similar between patients and controls. In agreement with our results, Rocha et al. [15] have found similar PA and body cell mass index values between Portuguese PKU patients and controls. In other pathologic conditions, ECM/BCM ratio has positively correlated with risk of death in dialysis patients [19], while PA has been negatively affected by nutritional imbalances, thus used as a prognostic factor in patients with homocystinuria [20] and cancer [21]. Both ECM/BCM ratio and PA values have been related to increased inflammation [17–19,21], which are seen in several pathological processes including in PKU patients [37,38]. Because nutritional imbalances have shown to underlie increased inflammation [23,24], the Phe-restricted diet could represent a threat to the overall health of patients. In addition, Brazilian PKU patients do not have adequate insurance coverage for commercial low-Phe products [2,5,6], which compromises the adherence to the dietary treatment. Despite that, the PKU patients of our study were not in higher risk of nutritional disturbances than the general population.

5. Conclusions

Overall, our results pointed to similar values of body composition and nutritional parameters between PKU patients and controls. Moreover, the analyzed values were not affected by the PKU type, time of diagnosis, or metabolic control.

Abbreviations

ADP	air displacement plethysmography
BCM	body cell mass
BIA	bioelectrical impedance
BMI	body mass index
DXA	dual energy X-ray absorptiometry
ECM	extracellular mass
FFM	fat-free mass
FM	fat mass
HPA	hyperphenylalaninemia
N/E	not evaluated
NS	non-significant
PA	phase angle
PAH	phenylalanine hydroxylase
Phe	phenylalanine
PKU	phenylketonuria
TOBEC	total body electrical conductivity

Competing interests

The authors declare that they have no competing interests relevant for this manuscript. In addition, in the last 5 years, TGJD had received speaker's fees from Recordati Rare Diseases, Danone Nutricia and Vitaflo, and research fees from Sigma Tau and Vitaflo.

Authors' contributions

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

Acknowledgments

We thank Taika I. Brandorff and Petra M. G. Brusschers for their contribution in collecting the data. This study received financial support from FIPE/HCPA (Research Incentive Fund — Hospital de Clínicas de Porto Alegre, grant number 12-0115) and the UMC Groningen (Mandema Stipend to TGJD, grant number Man-081219-802).

References

[1] C.R. Scriver, S. Kaufman, Hyperphenylalaninemia: phenylalanine hydroxylase deficiency, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Walle (Eds.), The Metabolic & Molecular Inherited Disease, McGraw-Hill, New York 2001, pp. 1667–1724.
[2] K.M. Camp, M.A. Parisi, P.B. Acosta, G.T. Berry, D.A. Bilder, N. Blau, O.A. Bodamer, J.P. Brosco, C.S. Brown, A.B. Burlina, B.K. Burton, C.S. Chang, P.M. Coates, A.C. Cunningham, S.F. Dobrowolski, J.H. Ferguson, T.D. Franklin, D.M. Frazier, D.K. Grange, C.L. Greene, S.C. Groft, C.O. Harding, R.R. Howell, K.L. Huntington, H.D. Hyatt-Knorr, I.P. Jevaji, H.L. Levy, U. Lichter-Konecki, M.L. Lindegren, M.A. Lloyd-Puryear, K. Matalon, A. MacDonald, M.L. McPheeters, J.J. Mitchell, S. Mofidi, K.D. Moseley, C.M. Mueller, A.E. Mulberg, L.S. Nerurkar, B.N. Ogata, A.R. Pariser, S. Prasad, G. Pridjian, S.A. Rasmussen, U.M. Reddy, F.J. Rohr, R.H. Singh, S.M. Sirrs, S.E. Stremer, D.A. Tagle, S.M. Thompson, T.K. Urv, J.R. Utz, F. van Spronsen, J. Vockley, S.E. Waisbren, L.S. Weglicki, D.A. White, C.B. Whitley, B.S. Wilfond, S. Yannicelli, J.M. Young, Phenylketonuria Scientific Review Conference: state of the science and future research needs, Mol. Genet. Metab. 112 (2014) 87–122.
[3] M.A. Vilaseca, N. Lambruschini, L. Gomez-Lopez, A. Gutierrez, E. Fuste, R. Gassio, R. Artuch, J. Campistol, Quality of dietary control in phenylketonuric patients and its relationship with general intelligence, Nutr. Hosp. 25 (2010) 60–66.
[4] A. MacDonald, J.C. Rocha, M. van Rijn, F. Feillet, Nutrition in phenylketonuria, Mol. Genet. Metab. 104 (2011) S10–S18 (Suppl).
[5] L.M. Trevisan, T. Nalin, T. Tonon, L.M. Veiga, P. Vargas, B.C. Krug, P.G. Leivas, I.V. Schwartz, Access to treatment for phenylketonuria by judicial means in Rio Grande do Sul, Braz. Cienc. Saude Colet. 20 (2015) 1607–1616.
[6] T.A. Vieira, T. Nalin, B.C. Krug, C.M. Bittar, C.B.O. Netto, I.V.D. Schwartz, Adherence to treatment of phenylketonuria: a study in southern Brazilian patients, J. Inborn Errors Metab. Screen. 3 (2015) 1–7.
[7] K.H. Schulpis, I. Papassotiriou, M. Vounatsou, G.A. Karikas, S. Tsakiris, G.P. Chrousos, Morning preprandial plasma ghrelin and catecholamine concentrations in patients with phenylketonuria and normal controls: evidence for catecholamine-mediated ghrelin regulation, J. Clin. Endocrinol. Metab. 89 (2004) 3983–3987.
[8] K.H. Schulpis, I. Papassotiriou, 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.
[9] J.C. Rocha, A. Macdonald, F. Trefz, Is overweight an issue in phenylketonuria? Mol. Genet. Metab. 110 (Suppl) (2013) S18–S24.
[10] G.L. Arnold, C.J. Vladutiu, R.S. Kirby, E.M. Blakely, J.M. Deluca, Protein insufficiency and linear growth restriction in phenylketonuria, J. Pediatr. 141 (2002) 243–246.
[11] M. Albersen, M. Bonthuis, N.M. de Roos, D.A. van den Hurk, E. Carbasius Weber, M.M. Hendriks, M.G. de Sain-van der Velden, T.J. de Koning, G. Visser, Whole body composition analysis by the BodPod air-displacement plethysmography method in children with phenylketonuria shows a higher body fat percentage, J. Inherit. Metab. Dis. 33 (Suppl. 3) (2010) S283–S288.
[12] L.C. Burrage, J. McConnell, R. Haesler, M.A. O'Riordan, V.R. Sutton, D.S. Kerr, S.E. McCandless, High prevalence of overweight and obesity in females with phenylketonuria, Mol. Genet. Metab. 107 (2012) 43–48.
[13] L.V. Robertson, N. McStravick, S. Ripley, E. Weetch, S. Donald, S. Adam, A. Micciche, S. Boocock, A. MacDonald, Body mass index in adult patients with diet-treated phenylketonuria, J. Hum. Nutr. Diet. 26 (Suppl. 1) (2013) 1–6.
[14] M. Huemer, C. Huemer, D. Moslinger, D. Huter, S. Stockler-Ipsiroglu, Growth and body composition in children with classical phenylketonuria: results in 34 patients and review of the literature, J. Inherit. Metab. Dis. 30 (2007) 694–699.
[15] J.C. Rocha, F.J. van Spronsen, M.F. Almeida, E. Ramos, J.T. Guimaraes, N. Borges, Early dietary treated patients with phenylketonuria can achieve normal growth and body composition, Mol. Genet. Metab. 110 (2013) S40–S43 (Suppl).
[16] R. Roubenoff, G.E. Dallal, P.W. Wilson, Predicting body fatness: the body mass index vs estimation by bioelectrical impedance, Am. J. Public Health 85 (1995) 726–728.
[17] U.G. Kyle, E.P. Soundar, L. Genton, C. Pichard, Can phase angle determined by bioelectrical impedance analysis assess nutritional risk? A comparison between healthy and hospitalized subjects, Clin. Nutr. 31 (2012) 875–881.
[18] M.C. Barbosa-Silva, A.J. Barros, Bioelectric impedance and individual characteristics as prognostic factors for post-operative complications, Clin. Nutr. 24 (2005) 830–838.
[19] M.M. Avram, P.A. Fein, C. Borawski, J. Chattopadhyay, B. Matza, Extracellular mass/body cell mass ratio is an independent predictor of survival in peritoneal dialysis patients, Kidney Int. Suppl. (2010) S37–S40.

[20] S. Poloni, I.D. Schweigert Perry, V. D'Almeida, I.V. Schwartz, Does phase angle correlate with hyperhomocysteinemia? A study of patients with classical homocystinuria, Clin. Nutr. 32 (2013) 479–480.
[21] S.I. Paiva, L.R. Borges, D. Halpern-Silveira, M.C. Assuncao, A.J. Barros, M.C. Gonzalez, Standardized phase angle from bioelectrical impedance analysis as prognostic factor for survival in patients with cancer, Support. Care Cancer 19 (2010) 187–192.
[22] U.G. Kyle, C.P. Earthman, C. Pichard, J.A. Coss-Bu, Body composition during growth in children: limitations and perspectives of bioelectrical impedance analysis, Eur. J. Clin. Nutr. (2015).
[23] A.P. Simopoulos, The importance of the ratio of omega-6/omega-3 essential fatty acids, Biomed. Pharmacother. (Biomed. Pharmacother.), 56 (2002) 365–379.
[24] M.F. Gregor, G.S. Hotamisligil, Inflammatory mechanisms in obesity, Annu. Rev. Immunol. 29 (2011) 415–445.
[25] WHO, WHO AnthroPlus for personal computers Manual: software for assessing growth of the world's children and adolescents, World Health Organization (WHO), Geneva, Switzerland, 2009.
[26] U.G. Kyle, I. Bosaeus, A.D. De Lorenzo, P. Deurenberg, M. Elia, J.M. Gomez, B.L. Heitmann, L. Kent-Smith, J.C. Melchior, M. Pirlich, H. Scharfetter, A.M. Schols, C. Pichard, Composition of the E.W.G., Bioelectrical impedance analysis—part I: review of principles and methods, Clin. Nutr. 23 (2004) 1226–1243.
[27] A. Bosy-Westphal, S. Danielzik, R.P. Dorhofer, W. Later, S. Wiese, M.J. Muller, Phase angle from bioelectrical impedance analysis: population reference values by age, sex, and body mass index JPEN, J. Parenter. Enter. Nutr. 30 (2006) 309–316.
[28] A. Doulgeraki, 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.
[29] 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.
[30] J.C. Rocha, F.J. van Spronsen, M.F. Almeida, G. Soares, D. Quelhas, E. Ramos, J.T. Guimaraes, N. Borges, Dietary treatment in phenylketonuria does not lead to increased risk of obesity or metabolic syndrome, Mol. Genet. Metab. 107 (2012) 659–663.
[31] A.M. Das, K. Goedecke, U. Meyer, N. Kanzelmeyer, S. Koch, S. Illsinger, T. Lucke, H. Hartmann, K. Lange, H. Lanfermann, L. Hoy, X.Q. Ding, Dietary habits and metabolic control in adolescents and young adults with phenylketonuria: self-imposed protein restriction may be harmful, JIMD Rep. 13 (2014) 149–158.
[32] D. Dobbelaere, L. Michaud, A. Debrabander, S. Vanderbecken, F. Gottrand, D. Turck, J.P. Farriaux, Evaluation of nutritional status and pathophysiology of growth retardation in patients with phenylketonuria, J. Inherit. Metab. Dis. 26 (2003) 1–11.
[33] P. Adamczyk, A. Morawiec-Knysak, P. Pludowski, B. Banaszak, J. Karpe, W. Pluskiewicz, Bone metabolism and the muscle–bone relationship in children, adolescents and young adults with phenylketonuria, J. Bone Miner. Metab. 29 (2011) 236–244.
[34] T.D. Douglas, M.J. Kennedy, M.E. Quirk, S.H. Yi, R.H. Singh, Accuracy of six anthropometric skinfold formulas versus air displacement plethysmography for estimating percent body fat in female adolescents with phenylketonuria, JIMD Rep. 10 (2013) 23–31.
[35] B.M. Popkin, The nutrition transition and its health implications in lower-income countries, Public Health Nutr. 1 (1998) 5–21.
[36] N.L. Fleischer, A.V. Diez Roux, M. Alazraqui, H. Spinelli, F. De Maio, Socioeconomic gradients in chronic disease risk factors in middle-income countries: evidence of effect modification by urbanicity in Argentina, Am. J. Public Health 101 (2011) 294–301.
[37] M. Gunduz, S. Cakar, P. Kuyum, B. Makay, N. Arslan, Comparison of atherogenic risk factors among poorly controlled and well-controlled adolescent phenylketonuria patients, Cardiol. Young (2015) 1–8.
[38] M. Deon, A. Sitta, J.L. Faverzani, G.B. Guerreiro, B. Donida, D.P. Marchetti, C.P. Mescka, G.S. Ribas, A.S. Coitinho, M. Wajner, C.R. Vargas, Urinary biomarkers of oxidative stress and plasmatic inflammatory profile in phenylketonuric treated patients, Int. J. Dev. Neurosci. 47 (2015) 259–265.

CAPÍTULO II

ACUTE EXERCISE IN TREATED PHENYLKETONURIA PATIENTS: PHYSICAL ACTIVITY AND BIOCHEMICAL RESPONSE

Artigo publicado

Mol Genet Metab Reports 2015; 5:55–59
DOI: 10.1016/j.ymgmr.2015.10.003

Molecular Genetics and Metabolism Reports 5 (2015) 55–59

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

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 xxxx

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.

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 neuropsychological outcome with decreased capacity especially in executive

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: pku@priscilamazzola.com (P.N. Mazzola), brunoct100@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).

http://dx.doi.org/10.1016/j.ymgmr.2015.10.003

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 μmol/L, or as "poorly controlled" if these levels were equal or above 700 μmol/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], $[(3.9 \times VO_2) + (1.1 \times VCO_2)]$, 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).

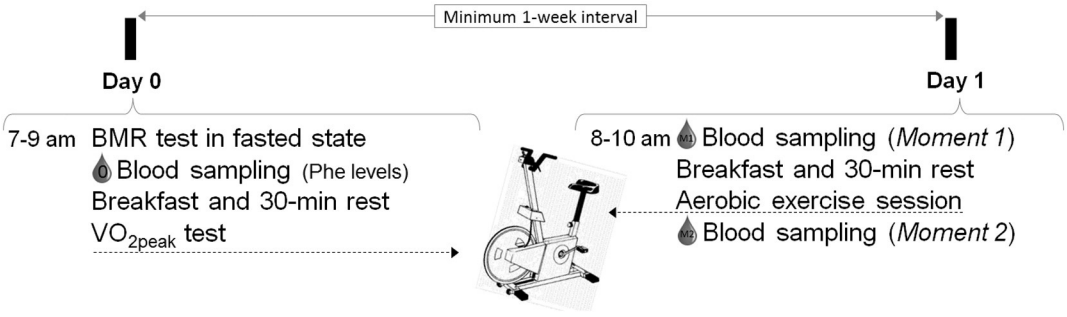

Fig. 1. Experimental design. In fasted state, participants went to the lab twice (at day 0 and day 1). In the day 0, participants performed the basal metabolic rate (BMR) test, followed by blood sampling for plasma phenylalanine (Phe) evaluation (0), breakfast and 30-min rest. Then the peak oxygen consumption (VO_{2peak}) 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 μmol/L.

3.1.1. BMR and VO2peak 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 μmol/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 μmol/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 ± 4 (17)	21 ± 4 (9)	NS
BMI (kg/m^2)	23 ± 2 (17)	24 ± 3 (9)	NS
Phenylalanine (μmol/L)	57 ± 14 (17)	562 ± 141 (9)	<0.001
BMR (kcal/kg/day)	21 ± 4 (17)	23 ± 4 (9)	NS
RER during BMR	0.87 ± 0.07 (17)	0.82 ± 0.07 (9)	NS
VO_{2peak} (mL/kg/min)	31 ± 6 (17)	28 ± 8 (9)	NS
Workload peak (W)	216 ± 49 (17)	203 ± 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 ± 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 (μmol/L)	81 ± 24	(17)	773 ± 190	(8)	<0.001	73 ± 15	(17)	723 ± 139	(8)	NS	NS
Tyr (μmol/L)	57 ± 15	(17)	62 ± 9	(8)	NS	79 ± 20	(17)	90 ± 24	(8)	<0.001	NS
Phe/Tyr ratio	1.4 ± 0.2	(17)	12.4 ± 2.4	(8)	<0.001	1.0 ± 0.2	(17)	8.3 ± 1.8	(8)	<0.001	NS
Tryptophan (μmol/L)	35 ± 9	(17)	29 ± 6	(8)	NS	35 ± 8	(17)	36 ± 12	(8)	NS	NS
BCAA (μmol/L)	456 ± 86	(17)	332 ± 50	(8)	0.001	403 ± 63	(17)	478 ± 132	(8)	NS	NS
Glucose (mg/dL)	89 ± 8	(17)	83 ± 7	(8)	NS	77 ± 9	(17)	78 ± 11	(8)	0.001	NS
Lactate (mg/dL)	1.3 ± 0.5	(17)	1.3 ± 0.3	(7)	NS	3.7 ± 1.6	(17)	3.6 ± 1.8	(7)	<0.001	NS
Triacylglycerol (mg/dL)	89 ± 33	(17)	76 ± 27	(8)	NS	100 ± 39	(17)	77 ± 27	(8)	NS	NS
Total cholesterol (mg/dL)	168 ± 31	(17)	121 ± 26	(8)	0.001	181 ± 34	(17)	129 ± 26	(8)	<0.001	<0.001
HDL (mg/dL)	54 ± 12	(17)	42 ± 6	(8)	NS	57 ± 11	(17)	45 ± 7	(8)	<0.001	NS
LDL (mg/dL)	97 ± 28	(17)	63 ± 19	(8)	NS	103 ± 28	(17)	68 ± 19	(8)	0.001	0.001
Dopamine (pg/mL)	56 ± 15	(17)	58 ± 17	(8)	NS	57 ± 13	(17)	57 ± 11	(8)	NS	NS
Noradrenaline (pg/mL)	228 ± 77	(17)	220 ± 52	(8)	NS	229 ± 85	(17)	217 ± 53	(8)	NS	NS
Adrenaline (pg/mL)	49 ± 15	(17)	45 ± 12	(8)	NS	52 ± 15	(17)	43 ± 11	(8)	NS	NS
Adiponectin (ng/mL)	28 ± 6	(14)	37 ± 8	(8)	NS	30 ± 9	(14)	39 ± 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 ± 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 VO_{2peak} 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].

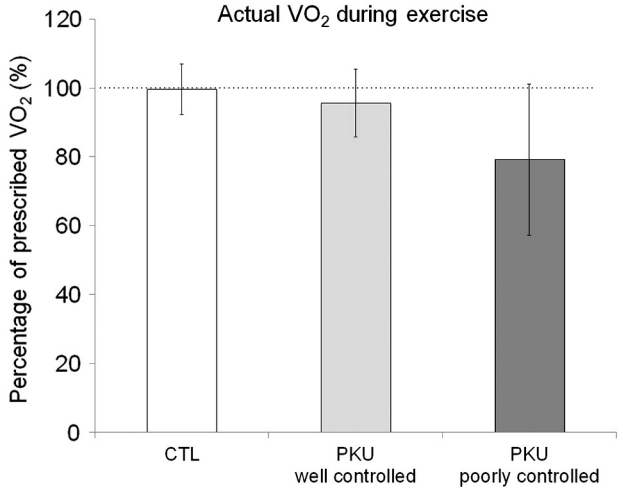

Fig. 2. Actual oxygen consumption (VO_2) during the 30-min exercise in controls (CTL) and well and poorly controlled phenylketonuria (PKU) patients. Data are expressed as mean ± SD of percentage of prescribed VO_2, n = 16 for controls, n = 5 and n = 3 for well and poorly controlled PKU subgroups, respectively.

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 VO_{2peak} and workload peak in the VO_{2peak} 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 availably 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

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. (2011).
[6] A.E. ten Hoedt, L.M. de Sonneville, 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. Inherit. 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 phenylketonuric 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. Strope, 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. Gutierres, 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 QII 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. Rauh, 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 Sonneville, 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. Papassotiriou, 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. Doulgeraki, 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.

CAPÍTULO III

VOLUNTARY EXERCISE PREVENTS OXIDATIVE STRESS IN THE BRAIN OF PHENYLKETONURIA MICE

Artigo publicado

JIMD Reports 2016; 27:69–77
DOI: 10.1007/8904_2015_498

JIMD Reports
DOI 10.1007/8904_2015_498

RESEARCH REPORT

Voluntary Exercise Prevents Oxidative Stress in the Brain of Phenylketonuria Mice

Priscila Nicolao Mazzola • Vibeke Bruinenberg • Karen Anjema • Danique van Vliet • Carlos Severo Dutra-Filho • Francjan J. van Spronsen • Eddy A. van der Zee

Received: 27 May 2015 / Revised: 14 September 2015 / Accepted: 15 September 2015

Abstract *Background*: High phenylalanine levels in phenylketonuria (PKU) have been associated with brain oxidative stress and amino acid imbalance. Exercise has been shown to improve brain function in hyperphenylalaninemia and neurodegenerative diseases. This study aimed to verify the effects of exercise on coordination and balance, plasma and brain amino acid levels, and brain oxidative stress markers in PKU mice.

Methods: Twenty wild-type (WT) and 20 PAH^{enu2} (PKU) C57BL/6 mice were placed in cages with (exercise, Exe) or without (sedentary, Sed) running wheels during 53 days. At day 43, a balance beam test was performed. Plasma and brain were collected for analyses of amino acid levels and the oxidative stress parameters superoxide dismutase (SOD) activity, sulfhydryl and reduced glutathione (GSH) contents, total radical-trapping antioxidant potential (TRAP), and total antioxidant reactivity (TAR).

Results: SedPKU showed poor coordination ($p < 0.001$) and balance ($p < 0.001$), higher plasma and brain phenylalanine ($p < 0.001$), and increased brain oxidative stress ($p < 0.05$) in comparison to SedWT. ExePKU animals ran less than ExeWT ($p = 0.018$). Although no improvement was seen in motor coordination and balance, exercise in PKU restored SOD, sulfhydryl content, and TRAP levels to controls. TAR levels were increased in ExePKU in comparison to SedPKU ($p = 0.012$). Exercise decreased plasma and brain glucogenic amino acids in ExePKU, but did not change plasma and brain phenylalanine in both WT and PKU.

Conclusions: Exercise prevents oxidative stress in the brain of PKU mice without modifying phenylalanine levels. Hence, exercise positively affects the brain, demonstrating its value as an intervention to improve brain quality in PKU.

Communicated by: Nenad Blau, PhD

Competing interests: None declared

Electronic supplementary material: The online version of this chapter (doi:10.1007/8904_2015_498) contains supplementary material, which is available to authorized users.

P.N. Mazzola (✉) · V. Bruinenberg · E.A. van der Zee
Department of Molecular Neurobiology, Groningen Institute for Evolutionary Life Sciences (GELIFES) – University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
e-mail: pku@priscilamazzola.com

P.N. Mazzola · K. Anjema · D. van Vliet · F.J. van Spronsen
Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

P.N. Mazzola · C.S. Dutra-Filho
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

Abbreviations

BCAA	Branched-chain amino acid
Exe	Exercise
GSH	Reduced glutathione
PAH	Phenylalanine hydroxylase
Phe	Phenylalanine
PKU	Phenylketonuria
Sed	Sedentary
SOD	Superoxide dismutase
TAR	Total antioxidant reactivity
TRAP	Total radical-trapping antioxidant potential
WT	Wild type

Introduction

Phenylketonuria (PKU, MIM 261600) is characterized by accumulation of phenylalanine (Phe) to toxic levels due to absent activity of Phe hydroxylase (PAH, EC 1.14.16.1).

High Phe concentration can impair brain function even in early-treated PKU patients, who have shown poor cognitive function (Gonzalez et al. 2011; Weglage et al. 2013; Jahja et al. 2014). Although the mechanisms are not yet fully understood, oxidative stress and brain amino acid imbalance caused by high Phe levels are speculated to underlie the impaired clinical outcomes. At the biochemical level, high blood Phe disturbs the concentration of other large neutral amino acids in the brain (de Groot et al. 2010; Martynyuk et al. 2010). Moreover, high Phe levels have been related to oxidative stress in blood from patients (Sierra et al. 1998; van Bakel et al. 2000; Schulpis et al. 2005; Sitta et al. 2009a, b; Sanayama et al. 2011), in the brain of animal models of the disease (Ercal et al. 2002; Moraes et al. 2014) and in in vitro experiments (Hagen et al. 2002; Sitta et al. 2009b; Fernandes et al. 2010; Moraes et al. 2010).

PKU treatment is based on a Phe-restricted diet, which aims to prevent high Phe concentrations in blood and tissues (Surtees and Blau 2000). Although efficient in lowering Phe levels, this diet is extremely hard to follow (Vilaseca et al. 2010). Therefore, other treatment strategies are still needed for PKU in order to improve patients' clinical and biochemical outcomes. In this way, exercise could be a concomitant treatment in PKU. Exercising regularly can lead to peripheral and central adaptations such as strengthening brain antioxidant capacity (Elokda and Nielsen 2007; Radak et al. 2007; Tsou et al. 2015) and improving dopaminergic and serotoninergic systems (Stroth et al. 2010; Wipfli et al. 2011; Chang et al. 2012; Lin and Kuo 2013). Additionally, aerobic exercise has improved cognition in elderly individuals (Kirk-Sanchez and McGough 2014) and in patients with neurodegenerative diseases (Petzinger et al. 2013; Radak et al. 2010). Moreover, in rats chemically subjected to hyperphenylalaninemia, regular exercise improved the brain antioxidant system (Mazzola et al. 2011). However, little is known about the effects of exercise in PKU and whether it can be beneficial for patients. Therefore, this study aimed to determine the effects of voluntary exercise on behavioral and biochemical parameters in a genetic mouse model of PKU, by the means of motor coordination and balance performance, plasma and brain amino acid concentrations, and brain oxidative stress parameters.

Methods

Animals

All the experimental procedures were approved by the Animal Welfare Committee of the University of Groningen, the Netherlands. A total of 40 adult (4 months old) C57Bl/6 homozygous (−/−) PAH^{enu2} (PKU) and (+/+) PAH^{enu2} (wild type, WT) female mice were used in this experiment. WT and PKU animals were individually housed and randomly assigned to sedentary (Sed) or exercise (Exe) groups. Mice were given water and regular chow ad libitum, while kept in a 12:12-h light–dark regime and weighed weekly.

Voluntary Exercise

Mice from ExeWT and ExePKU had free access to a running wheel placed in their home cage throughout the experiment, i.e., 4 days of acclimatization plus 53 days of voluntary training. Daily running wheel activity was calculated as described before (Mulder et al. 2014).

Balance Beam Test

The balance beam is a sensorimotor integration test, which focuses on hind limb functioning (Carter et al. 1999; Soderling et al. 2003). The apparatus consisted of a 50-mm wide beam with a "safe cage" placed at the end of it. Animals performed nonconsecutive four trials (5, 10, 40, and 100 cm) on the beam, which were recorded. The number of hind limb steps and slips was counted in the 100-cm trial using the video files.

Tissue Preparation

Animals were sacrificed by cervical dislocation. Blood was centrifuged at 1,500 × *g* for 10 min and then plasma was harvested and stored at −80°C. The total brain was immediately frozen in liquid nitrogen. Shortly before analysis, brain tissue was grinded in liquid nitrogen and then divided into weighed aliquots. Later, the aliquots were homogenized in specific buffers as required for each technique and sonified (30 s per sample at 11–12 W). The brain homogenates were then centrifuged at 1,000 × *g* for 10 min at 4°C, and the supernatant was used for the biochemical measurements.

Plasma and Brain Amino Acid Levels

Brain homogenates were prepared using phosphate-buffered saline (pH 7.4) at a 1:4 weight to volume ratio (mg/μL). Plasma and brain amino acid concentrations were determined using HPLC coupled to derivatization with ninhydrin, according to the manufacturer's protocol (Pharmacia Biotech, Cambridge, UK).

Oxidative Stress Parameters

Cerebral tissue was homogenized in 50 mM Tris–HCl buffer containing 1 mM EDTA (pH 8.2) at a 1:10 (w/v)

ratio. All measurements were normalized by protein concentration using albumin as standard (Lowry et al. 1951).

Superoxide Dismutase (SOD) Activity Assay

This assay is based on the capacity of pyrogallol to autoxidize and on the ability of SOD to inhibit this reaction (Marklund 1985). Therefore, SOD activity can be indirectly assayed spectrophotometrically at 420 nm by comparing the samples' values with a standard curve. These data are expressed as percentage of control (%SedWT).

Sulfhydryl Content

5,5′dithiobis(2-nitrobenzoic acid) (DTNB) color reagent is reduced by thiols, thus generating a yellow derivative (TNB) which can be spectrophotometrically read at 412 nm (Aksenov and Markesbery 2001). Oxidation of free thiol groups in proteins leads to the formation of disulfide bonds, which will not react with DTNB. Therefore, the sulfhydryl content is inversely correlated to oxidative damage to proteins. The results are expressed as nmol TNB/mg protein.

Reduced Glutathione (GSH) Content

This method is based on the reaction of GSH with the fluorophore *ortho*-phthalaldehyde (Browne and Armstrong 1998). Briefly, metaphosphoric acid was used to deproteinize samples, which were then centrifuged at 1,000 × *g* for 10 min. Then, sodium phosphate buffer at pH 8.0 and *ortho*-phthalaldehyde 1 mg/mL solution were added to the samples' supernatants. After standing in the dark for 15 min, the fluorescence of this mixture was measured at excitation 350 nm and emission 420 nm. A calibration curve was made with a commercial GSH solution, and the results were expressed as μmol GSH/mg protein.

Total Radical-Trapping Antioxidant Potential (TRAP) and Total Antioxidant Reactivity (TAR)

TRAP and TAR were determined by measuring the chemiluminescence intensity of luminol induced by ABAP thermolysis (free radical source) in a scintillation counter (Evelson et al. 2001). After adding 3 mL of 10 mM ABAP and 10 μL of 5.6 mM luminol to scintillation vials, the initial light intensity was obtained. Ten microliters of 160 μM Trolox or 30 μL of sample was added to assess antioxidant content. At this point, the luminescence intensity is practically abolished. The consumption of active antioxidants present in samples results in the return of the luminescence (TRAP). For each sample, the time required to return of luminescence (TAR) was compared to that obtained by employing Trolox under identical experimental conditions. Values were calculated as Trolox equivalents and were represented as nmol Trolox/mg protein.

Statistical Analysis

The statistical analyses were performed with the Pearson's correlation coefficient, independent Student's *t*-test, or two-way ANOVA followed by the Tukey post hoc test for multiple comparisons and repeated measures ANOVA for longitudinal analyses. The SPSS was used and $p < 0.05$ was considered to be statistically significant. Number of animals per group varied due to technical sampling problems or due to exclusion of outliers (values that were two or more SDs away from the group mean).

Results

In order to evaluate the effects of voluntary exercise in PKU mice, we performed a study in which WT and PKU animals had free access to running wheels (Exe groups) and compared these to animals that did not have the apparatus in their home cages (Sed groups). As shown in Fig. 1, ExePKU group ran significantly less than did the ExeWT group (6,064 ± 1,937 m/day and 10,627 ± 4,868 m/day, respectively, $p = 0.018$), and the effect of the genotype was significant ($p = 0.027$). Exercise did not modify body weight ($p = 0.758$), and both PKU groups were lighter than WT groups throughout the experiment ($p = 0.001$) (Fig. 1).

PKU animals from both Sed and Exe groups showed poor performance in the balance beam test as compared to WT mice, as shown in Table 1. When crossing the beam, both PKU groups had a higher number of steps ($p < 0.001$) and slips ($p < 0.001$) in comparison to SedWT, representing deficits in motor coordination and balance, respectively. Neither Exe group differed from the Sed groups, therefore showing no exercise effect for this task.

As shown before (Ney et al. 2008; Solverson et al. 2012; Sawin et al. 2014), Phe levels in plasma and brain of SedPKU mice were higher in comparison to respective levels in SedWT group ($p < 0.001$). Exercise did not modify Phe levels when comparing each Exe group to its Sed control (Fig. 1). A comparison of plasma values between PKU groups showed that exercise decreased levels of alanine ($p = 0.038$), citrulline ($p = 0.002$), glutamine ($p = 0.040$), glycine ($p = 0.006$), ornithine ($p = 0.008$), and proline ($p = 0.028$) (Fig. 2). Furthermore, exercise reduced brain amino acid levels in ExePKU compared to SedPKU for histidine ($p = 0.036$), isoleucine ($p = 0.011$),

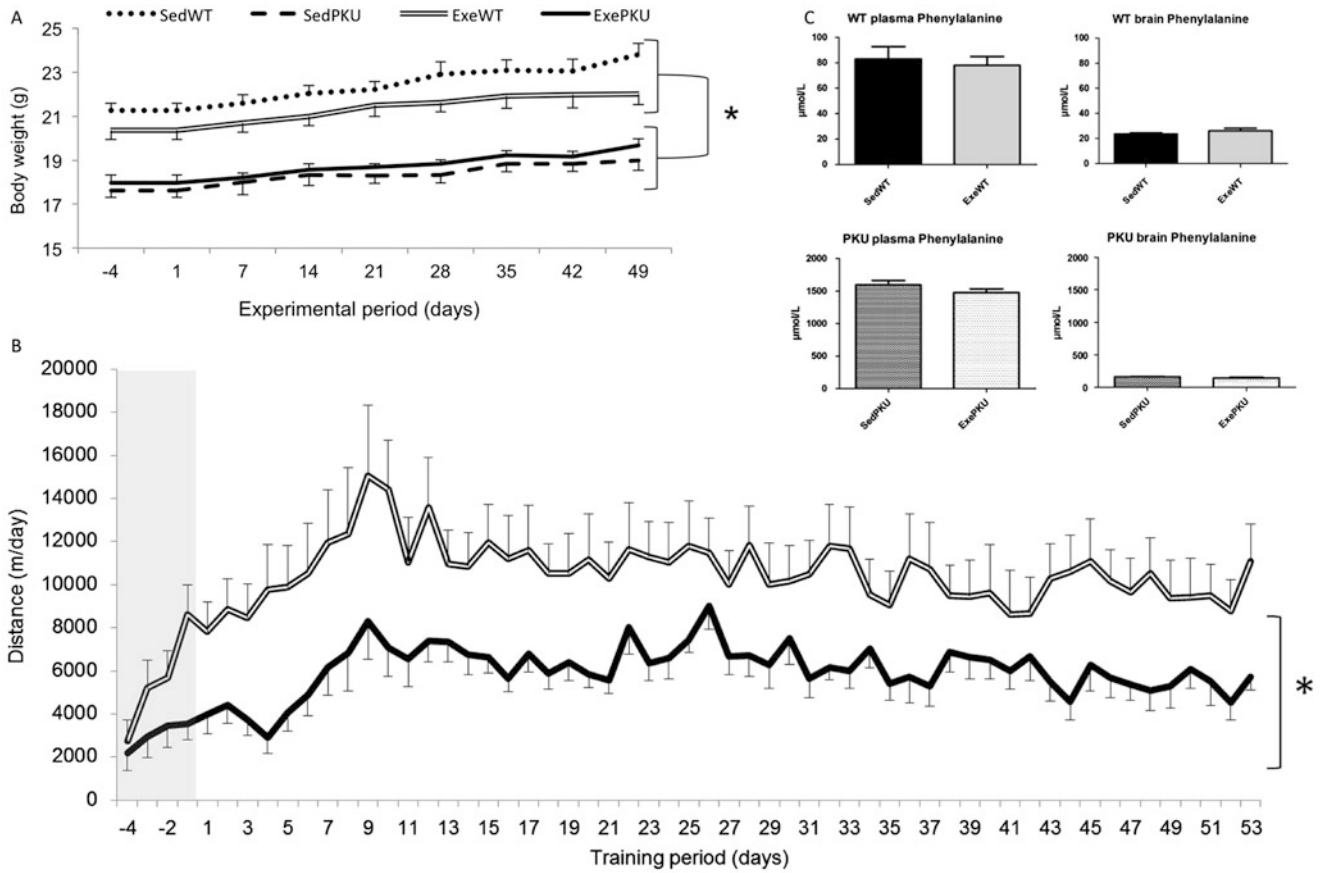

Fig. 1 (**a**) Body weight from sedentary (Sed) and exercise (Exe) wild-type (WT) and PAH^{enu2} (PKU) mice, (**b**) daily running wheel activity ExeWT and ExePKU mice during the 4-day acclimatization period (*gray area*) followed by 53 days of training and (**c**) phenylalanine levels in the plasma and brain of Sed and Exe WT and PKU. Data are shown as mean ± SEM ($n = 10$/group, except for plasma phenylalanine in SedPKU where $n = 8$). *$p < 0.05$, repeated measures ANOVA

Table 1 Balance beam test outcomes

	SedWT	SedPKU	ExeWT	ExePKU
Time (s)	19 ± 9	22 ± 9	11 ± 5	17 ± 5
Number of steps	40 ± 3	54 ± 7*	39 ± 6	50 ± 7*
Number of slips	8 ± 5	29 ± 10*	9 ± 8	27 ± 11*

Results are expressed as mean ± SD ($n = 10$/group)
*$p < 0.001$, compared to SedWT (Tukey post hoc)

methionine ($p = 0.005$), proline ($p = 0.005$), and valine ($p = 0.010$), and a positive correlation ($r = 0.817$; $p = 0.004$) between brain proline levels and distance run was found only for ExePKU (Fig. 2). No effects of exercise were found on plasma and brain amino acid levels for WT mice (Supplemental Table 1).

Regarding oxidative stress in the brain, the SedPKU group showed lower superoxide dismutase activity ($p = 0.029$), sulfhydryl ($p = 0.043$), GSH ($p = 0.007$), and TRAP ($p = 0.003$) in comparison to the SedWT group (Fig. 3). Exercise prevented those changes, so the ExePKU group reached levels similar to SedWT for SOD, sulfhydryl, TRAP and also tended to restore GSH levels ($p = 0.049$). Furthermore, exercise led to higher levels of TAR only for the ExePKU group in comparison to the SedPKU group ($p = 0.012$), although TAR levels were not lower in SedPKU in comparison to SedWT. No exercise effect was found for WT animals (ExeWT group).

Discussion

To the best of our knowledge, this study was the first to evaluate the long-term effects of voluntary exercise in a genetic mouse model of PKU. The protocol used was voluntary exercise by animals having free access to running wheels in their home cages. Therefore, Exe animals could run whenever and for as long as they desired. PKU mice, although running less than controls, improved brain oxidative stress markers, while no changes in plasma and blood Phe levels were found.

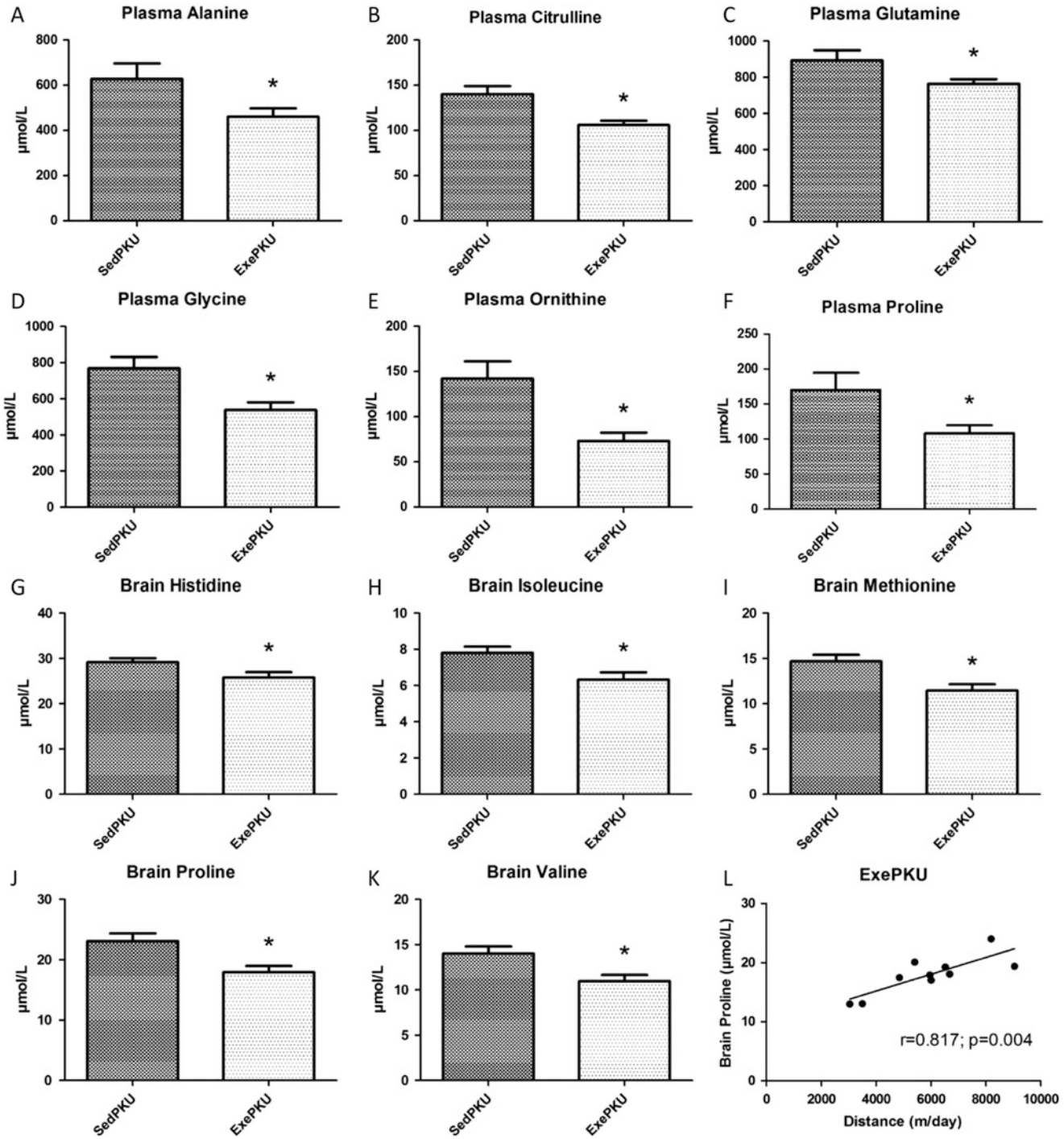

Fig. 2 Plasma amino acid levels of (**a**) alanine, (**b**) citrulline, (**c**) glutamine, (**d**) glycine, (**e**) ornithine, and (**f**) proline and brain amino acid levels of (**g**) histidine, (**h**) isoleucine, (**i**) methionine, (**j**) proline, (**k**) valine, and (**l**) correlation between brain proline levels and distance ran in sedentary (Sed) and exercise (Exe) PAH^{enu2} (PKU) mice. Results are expressed as mean ± SEM ($n = 10$/group, except for plasma levels in SedPKU where $n = 8$). *$p < 0.05$

The lower running wheel activity of the ExePKU group might be caused by their specific motor problems and/or early fatigue. Both SedPKU and ExePKU groups showed worse performance in the balance beam task than WT mice. As various brain regions are affected by Phe toxicity (Qin and Smith 2007; Fernandes et al. 2010), the balance beam findings indicate possible motor cortex and cerebellum impairments in this PKU mouse model. Voluntary training has been effective in improving motor performance in the rotarod test in healthy mice (Clark et al. 2008). However,

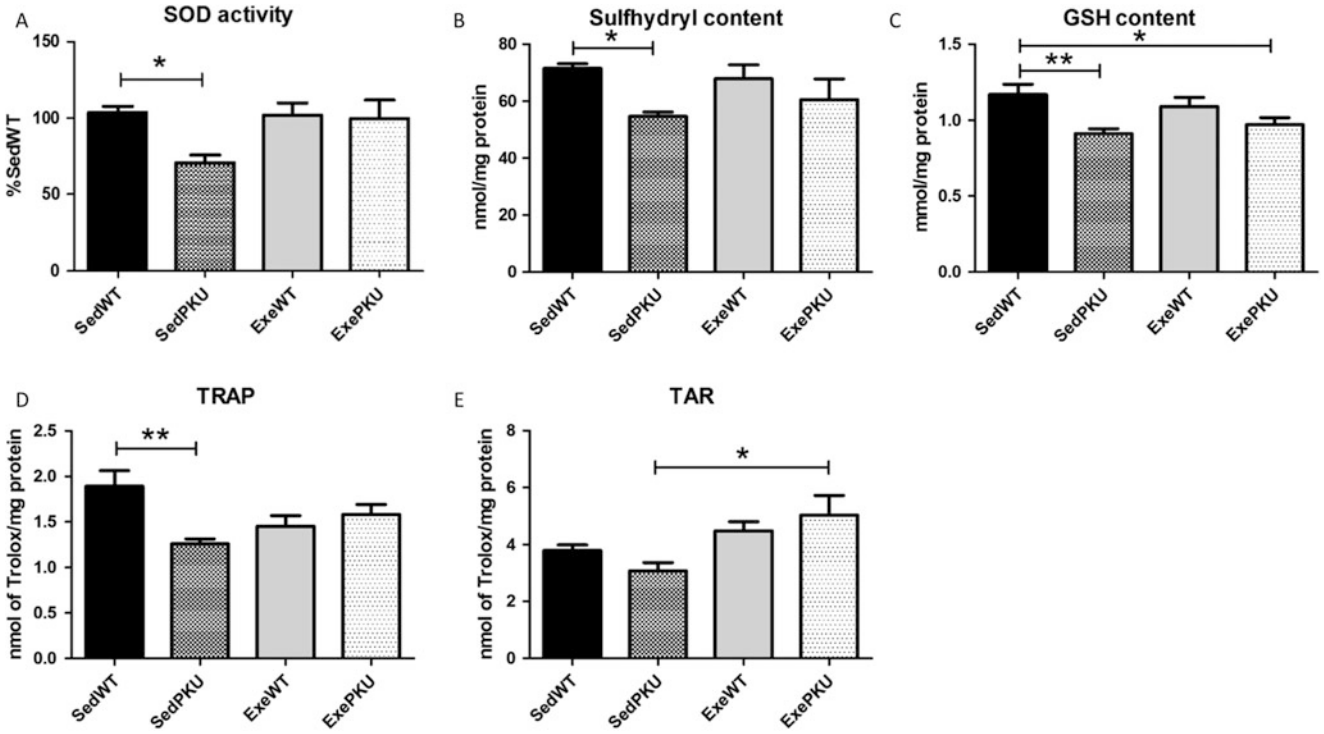

Fig. 3 Oxidative stress parameters (**a**) superoxide dismutase (SOD) activity, (**b**) sulfhydryl and (**c**) reduced glutathione (GSH) contents, (**d**) total radical-trapping antioxidant potential (TRAP), and (**e**) total antioxidant reactivity (TAR) in the brain of sedentary (Sed) and exercise (Exe) wild-type (WT) and PAH^{enu2} (PKU) mice. Results are expressed as mean ± SEM (n = 8–10/group). *$p < 0.05$ and **$p < 0.01$

the exercise used in the present study did not improve balance beam outcomes in both PKU and WT groups. In this way, perhaps this exercise protocol was not able to address those skills specifically necessary for crossing a narrow beam. On the other hand, the exercise used in the present study decreased plasma glucogenic amino acid levels only in the PKU mice. Skeletal muscles produce alanine to get rid of nitrogen groups during amino acid catabolism, thus preventing accumulation of ammonia to toxic levels (Graham and MacLean 1998). The lower plasma levels of alanine, glutamate precursors (glutamine and proline), and urea cycle intermediates (ornithine and citrulline) in ExePKU may indicate that trained PKU mice did not have an efficient nitrogen buffering mechanism. Supporting this hypothesis, supplementations of ornithine, citrulline, and glutamine have shown to postpone fatigue by decreasing blood ammonia in rodents (Meneguello et al. 2003; Takeda et al. 2011; Kim and Kim 2013). In this way, the PKU mice in the present study might have run less than WT due to fatigue, besides motor problems.

Exercise decreased brain levels of glucogenic amino acids, including branched-chain amino acids (BCAA) that were not changed in plasma. Lower availability of brain large neutral amino acids in PKU has been related to the clinical problems caused by high Phe levels, and this can be explained by the a critical imbalance in the competition to cross the blood–brain barrier when Phe is relatively more concentrated (de Groot et al. 2010). However, in the present study, among the amino acids that were decreased in plasma of ExePKU mice, proline was the only amino acid also decreased in the brain in comparison to SedPKU. Moreover, as concentrations of Phe and other large neutral amino acids did not change in the brain due to exercise, competition between aromatic amino acids and BCAA might not have hampered BCAA uptake in ExePKU in comparison to SedPKU. This observation could indicate an increased amino acid metabolism to yield energy, as the brain has high activity of the key enzymes especially for BCAA catabolism (Piscopo et al. 2011). Furthermore, brain proline levels show a positive correlation with the daily distance run only for PKU mice ($r = 0.817$; $p = 0.004$); therefore, the amount of running, and hence the amount of training, might have influenced this result.

The PKU mouse model used in this study showed oxidative stress in the brain by lower levels of SOD activity, sulfhydryl, and GSH contents and TRAP levels, which was mostly prevented by the voluntary exercise.

Solverson et al. (2012) have found metabolic stress in the same strain of PKU mice (C57BL/6). As the brain is the most affected organ in PKU, our results corroborate the already stated hypothesis that oxidative stress is involved in the pathophysiology of the disease (Ribas et al. 2011). While exercise did not change any oxidative stress parameter in the control (WT) mice, the PKU group benefitted from exercising. ExePKU had SOD, sulfhydryl content and TRAP restored to control levels, and increased TAR in comparison to SedPKU. In this study, PKU animals that voluntarily exercised showed similar oxidative stress parameters to those of controls, thus preventing the impairments caused by PKU without changing brain Phe levels. Previous research on voluntary wheel running has shown enhancement of antioxidant enzymatic activity in arteries of old mice thus preventing age-related oxidative stress (Durrant et al. 2009). Furthermore, exercise has been shown to prevent oxidative stress in the brain of animal models of neurodegenerative diseases (Ang et al. 2010; Souza et al. 2013) as well as in hyperphenylalaninemia (Mazzola et al. 2011). In the same way, exercise improved brain oxidative stress parameters in PKU animals in the present study.

The focus of PKU treatment strategies should not only be on reducing Phe but also on enhancing central parameters that are impaired by high Phe levels (van Spronsen et al. 2009; van Vliet et al. 2015). In this way, the intermittent stress caused by exercise might be able to overcome high Phe issues and improve PKU outcomes. Moreover, even though PKU animals showed less physical activity than WT mice, only the PKU group showed changes in amino acid and oxidative stress levels. Therefore, PKU mice were more responsive to exercise effects. Future studies might evaluate the effects of exercise when introduced at early ages as well as in male mice, therefore shedding light in the importance of physical activity in this population. Although therapeutic strategies in PKU primarily address high Phe-related problems, the beneficial effects of exercise on other PKU-related problems as shown here should open new avenues in combined treatment strategies.

Conclusions

Exercise decreased levels of glucogenic amino acids in plasma and brain of PKU mice, but did not improve motor coordination or balance. Voluntary exercise training prevented oxidative stress in the brain of PKU mice without changing Phe levels in the plasma or brain. Therefore, exercise may be a concomitant strategy for PKU patients to improve brain redox status and hence brain biochemistry and function.

Acknowledgments This research project has been made possible thanks to a fellowship from PKU Academy under the auspices of EXCEMED, Excellence in Medical Education, the Abel Tasman Talent Program from the University Medical Center Groningen and the University of Groningen. We thank Pim de Blaauw for the amino acid analyses and Wanda Douwenga and Jan Keijser for their technical support.

Concise 1: Sentence Take-Home Message

Voluntary training improved brain oxidative stress and reduced brain and plasma glucogenic amino acids in phenylketonuria mice without changing phenylalanine levels.

Compliance with Ethics Guidelines

Conflict of Interest

Priscila Nicolao Mazzola, Vibeke Bruinenberg, Karen Anjema, Danique van Vliet, Carlos Severo Dutra-Filho, Francjan J. van Spronsen, and Eddy A. van der Zee declare that they have no conflict of interest.

Animal Rights

All institutional and national guidelines for the care and use of laboratory animals were followed.

Details of the Contribution of Individual Authors

Priscila Nicolao Mazzola, Vibeke Bruinenberg, Karen Anjema, and Danique van Vliet collected the data. Priscila Nicolao Mazzola 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.

References

Aksenov MY, Markesbery WR (2001) Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett 302:141–145

Ang ET, Tai YK, Lo SQ, Seet R, Soong TW (2010) Neurodegenerative diseases: exercising toward neurogenesis and neuroregeneration. Front Aging Neurosci 2:25

Browne RW, Armstrong D (1998) Reduced glutathione and glutathione disulfide. Methods Mol Biol 108:347–352

Carter RJ, Lione LA, Humby T et al (1999) Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci 19:3248–3257

Chang YK, Liu S, Yu HH, Lee YH (2012) Effect of acute exercise on executive function in children with attention deficit hyperactivity disorder. Arch Clin Neuropsychol 27:225–237

Clark PJ, Brzezinska WJ, Thomas MW, Ryzhenko NA, Toshkov SA, Rhodes JS (2008) Intact neurogenesis is required for benefits of exercise on spatial memory but not motor performance or contextual fear conditioning in C57BL/6J mice. Neuroscience 155:1048–1058

de Groot MJ, Hoeksma M, Blau N, Reijngoud DJ, van Spronsen FJ (2010) Pathogenesis of cognitive dysfunction in phenylketonuria: review of hypotheses. Mol Genet Metab 99(Suppl 1):S86–S89

Durrant JR, Seals DR, Connell ML et al (2009) Voluntary wheel running restores endothelial function in conduit arteries of old mice: direct evidence for reduced oxidative stress, increased superoxide dismutase activity and down-regulation of NADPH oxidase. J Physiol 587:3271–3285

Elokda AS, Nielsen DH (2007) Effects of exercise training on the glutathione antioxidant system. Eur J Cardiovasc Prev Rehabil 14:630–637

Ercal N, Aykin-Burns N, Gurer-Orhan H, McDonald JD (2002) Oxidative stress in a phenylketonuria animal model. Free Radic Biol Med 32:906–911

Evelson P, Travacio M, Repetto M, Escobar J, Llesuy S, Lissi EA (2001) Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. Arch Biochem Biophys 388:261–266

Fernandes CG, Leipnitz G, Seminotti B et al (2010) Experimental evidence that phenylalanine provokes oxidative stress in hippocampus and cerebral cortex of developing rats. Cell Mol Neurobiol 30:317–326

Gonzalez MJ, Gutierrez AP, Gassio R, Fuste ME, Vilaseca MA, Campistol J (2011) Neurological complications and behavioral problems in patients with phenylketonuria in a follow-up unit. Mol Genet Metab 104(Suppl):S73–S79

Graham TE, MacLean DA (1998) Ammonia and amino acid metabolism in skeletal muscle: human, rodent and canine models. Med Sci Sports Exerc 30:34–46

Hagen MEK, Pederzolli CD, Sgaravatti AM et al (2002) Experimental hyperphenylalaninemia provokes oxidative stress in rat brain. Biochim Biophys Acta 1586:344–352

Jahja R, Huijbregts SC, de Sonneville LM, van der Meere JJ, van Spronsen FJ (2014) Neurocognitive evidence for revision of treatment targets and guidelines for phenylketonuria. J Pediatr 164:895.e2–899.e2

Kim DI, Kim KS (2013) Walnut extract exhibits anti-fatigue action via improvement of exercise tolerance in mice. Lab Anim Res 29:190–195

Kirk-Sanchez NJ, McGough EL (2014) Physical exercise and cognitive performance in the elderly: current perspectives. Clin Interv Aging 9:51–62

Lin TW, Kuo YM (2013) Exercise benefits brain function: the monoamine connection. Brain Sci 3:39–53

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Marklund SL (1985) Pyrogallol autoxidation. In: Greenwald RA (ed) Handbook of methods for oxygen radical research. CRC, Boca Raton, pp 243–247

Martynyuk AE, van Spronsen FJ, Van der Zee EA (2010) Animal models of brain dysfunction in phenylketonuria. Mol Genet Metab 99(Suppl 1):S100–S105

Mazzola PN, Terra M, Rosa AP et al (2011) Regular exercise prevents oxidative stress in the brain of hyperphenylalaninemic rats. Metab Brain Dis 26:291–297

Meneguello MO, Mendonca JR, Lancha AH Jr, Costa Rosa LF (2003) Effect of arginine, ornithine and citrulline supplementation upon performance and metabolism of trained rats. Cell Biochem Funct 21:85–91

Moraes TB, Zanin F, da Rosa A et al (2010) Lipoic acid prevents oxidative stress in vitro and in vivo by an acute hyperphenylalaninemia chemically-induced in rat brain. J Neurol Sci 292:89–95

Moraes TB, Dalazen GR, Jacques CE, de Freitas RS, Rosa AP, Dutra-Filho CS (2014) Glutathione metabolism enzymes in brain and liver of hyperphenylalaninemic rats and the effect of lipoic acid treatment. Metab Brain Dis 29:609–615

Mulder CK, Papantoniou C, Gerkema MP, Van Der Zee EA (2014) Neither the SCN nor the adrenals are required for circadian time-place learning in mice. Chronobiol Int 31:1075–1092

Ney DM, Hull AK, van Calcar SC, Liu X, Etzel MR (2008) Dietary glycomacropeptide supports growth and reduces the concentrations of phenylalanine in plasma and brain in a murine model of phenylketonuria. J Nutr 138:316–322

Petzinger GM, Fisher BE, McEwen S, Beeler JA, Walsh JP, Jakowec MW (2013) Exercise-enhanced neuroplasticity targeting motor and cognitive circuitry in Parkinson's disease. Lancet Neurol 12:716–726

Piscopo P, Crestini A, Adduci A et al (2011) Altered oxidative stress profile in the cortex of mice fed an enriched branched-chain amino acids diet: possible link with amyotrophic lateral sclerosis? J Neurosci Res 89:1276–1283

Qin M, Smith CB (2007) Regionally selective decreases in cerebral glucose metabolism in a mouse model of phenylketonuria. J Inherit Metab Dis 30:318–325

Radak Z, Kumagai S, Taylor AW, Naito H, Goto S (2007) Effects of exercise on brain function: role of free radicals. Appl Physiol Nutr Metab 32:942–946

Radak Z, Hart N, Sarga L et al (2010) Exercise plays a preventive role against Alzheimer's disease. J Alzheimers Dis 20:777–783

Ribas GS, Sitta A, Wajner M, Vargas CR (2011) Oxidative stress in phenylketonuria: what is the evidence? Cell Mol Neurobiol 31:653–662

Sanayama Y, Nagasaka H, Takayanagi M et al (2011) Experimental evidence that phenylalanine is strongly associated to oxidative stress in adolescents and adults with phenylketonuria. Mol Genet Metab 103:220–225

Sawin EA, Murali SG, Ney DM (2014) Differential effects of low-phenylalanine protein sources on brain neurotransmitters and behavior in C57Bl/6-Pah(enu2) mice. Mol Genet Metab 111:452–461

Schulpis KH, Tsakiris S, Traeger-Synodinos J, Papassotiriou I (2005) Low total antioxidant status is implicated with high 8-hydroxy-2-deoxyguanosine serum concentrations in phenylketonuria. Clin Biochem 38:239–242

Sierra C, Vilaseca MA, Moyano D et al (1998) Antioxidant status in hyperphenylalaninemia. Clin Chim Acta 276:1–9

Sitta A, Barschak AG, Deon M et al (2009a) L-carnitine blood levels and oxidative stress in treated phenylketonuric patients. Cell Mol Neurobiol 29:211–218

Sitta A, Manfredini V, Biasi L et al (2009b) Evidence that DNA damage is associated to phenylalanine blood levels in leukocytes from phenylketonuric patients. Mutat Res 679:13–16

Soderling SH, Langeberg LK, Soderling JA et al (2003) Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. Proc Natl Acad Sci U S A 100:1723–1728

Solverson P, Murali SG, Brinkman AS et al (2012) Glycomacropeptide, a low-phenylalanine protein isolated from cheese whey, supports growth and attenuates metabolic stress in the murine model of phenylketonuria. Am J Physiol Endocrinol Metab 302: E885–E895

Souza LC, Filho CB, Goes AT et al (2013) Neuroprotective effect of physical exercise in a mouse model of Alzheimer's disease

induced by beta-amyloid(1)(-)(4)(0) peptide. Neurotox Res 24:148–163

Stroth S, Reinhardt RK, Thone J et al (2010) Impact of aerobic exercise training on cognitive functions and affect associated to the COMT polymorphism in young adults. Neurobiol Learn Mem 94:364–372

Surtees R, Blau N (2000) The neurochemistry of phenylketonuria. Eur J Pediatr 159(Suppl 2):S109–S113

Takeda K, Machida M, Kohara A, Omi N, Takemasa T (2011) Effects of citrulline supplementation on fatigue and exercise performance in mice. J Nutr Sci Vitaminol 57:246–250

Tsou YH, Shih CT, Ching CH et al (2015) Treadmill exercise activates Nrf2 antioxidant system to protect the nigrostriatal dopaminergic neurons from MPP+ toxicity. Exp Neurol 263:50–62

van Bakel MM, Printzen G, Wermuth B, Wiesmann UN (2000) Antioxidant and thyroid hormone status in selenium-deficient phenylketonuric and hyperphenylalaninemic patients. Am J Clin Nutr 72:976–981

van Spronsen FJ, Hoeksma M, Reijngoud DJ (2009) Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? J Inherit Metab Dis 32:46–51

van Vliet D, Anjema K, Jahja R et al (2015) BH4 treatment in BH4-responsive PKU patients: preliminary data on blood prolactin concentrations suggest increased cerebral dopamine concentrations. Mol Genet Metab 114:29–33

Vilaseca MA, Lambruschini N, Gomez-Lopez L et al (2010) Quality of dietary control in phenylketonuric patients and its relationship with general intelligence. Nutr Hosp 25:60–66

Weglage J, Fromm J, van Teeffelen-Heithoff A et al (2013) Neurocognitive functioning in adults with phenylketonuria: results of a long term study. Mol Genet Metab 110(Suppl): S44–S48

Wipfli B, Landers D, Nagoshi C, Ringenbach S (2011) An examination of serotonin and psychological variables in the relationship between exercise and mental health. Scand J Med Sci Sports 21:474–481

Parte III

3 DISCUSSÃO

Essa tese reuniu estudos em diferentes modelos e abordagens que verificaram o estado metabólico basal e em exercício físico em pacientes fenilcetonúricos, a fim de propor o exercício como nova abordagem terapêutica no manejo da doença. Assim, um modelo animal geneticamente modificado foi utilizado para análise do efeito do exercício no cérebro desses roedores. Apesar de pacientes fenilcetonúricos serem estimulados a aumentar níveis de atividade física (Rocha, Macdonald e Trefz 2013; Doulgeraki et al. 2014), pouco tem sido examinado sobre o assunto. Portanto, essa tese traz novos dados científicos que embasarão futuras prescrições e investigações sobre metabolismo e exercício na PKU.

O tratamento da PKU tem sido aprimorado nos últimos anos, porém novas estratégias concomitantes ainda são necessárias (Al Hafid e Christodoulou 2015). Se não tradado, o recém-nascido com PKU desenvolve dano cerebral irreversível, uma vez que altos níveis de fenilalanina impactam o cérebro em desenvolvimento. Por outro lado, mesmo pacientes diagnosticados precocemente podem sofrer com os efeitos dos altos níveis de fenilalanina na idade adulta. Cessar ou negligenciar o controle da dieta na fase adulta resulta em déficits em marcadores cognitivos e em órgãos periféricos, tais como função executiva e densidade mineral óssea, respectivamente (Miras et al. 2013; Jahja et al. 2014). Assim, novas estratégias concomitantes à dieta restrita devem ser estudadas. Nesse contexto, o exercício poderia ser utilizado por essa população, uma vez que essa estratégia é amplamente comprovada como benéfica para a população em geral em termos de saúde física e mental. A fim de descrever o estado nutricional e metabólico dos pacientes

fenilcetonúricos, nossos estudos focaram em análises basais e, então, em resposta ao exercício aeróbico.

Os pacientes avaliados em nosso estudo (Capítulo I) não apresentaram discrepâncias em relação à massa e composição corporal bem como estado nutricional em comparação a controles pareados. Alguns autores encontraram um risco elevado de sobrepeso e obesidade em pacientes fenilcetonúricos (Albersen et al. 2010; Burrage et al. 2012) possivelmente devido à composição da dieta pobre em fenilalanina e, assim, com maior aporte de carboidratos e lipídeos (MacDonald et al. 2011). Por outro lado, muitos autores não encontraram essas diferenças entre pacientes e controles (Allen et al. 1995; Huemer et al. 2007; Rocha et al. 2012; Rocha et al. 2013; Doulgeraki et al. 2014). Um dos motivos da falta de consenso no assunto pode ser a característica das amostras estudadas. Apesar de terem a mesma idade, as amostras são normalmente compostas por pacientes e controles em diferentes fases de crescimento e desenvolvimento puberal que não são levadas em consideração na análise dos dados. Com a mesma limitação, uma vez que o nosso estudo foi composto por uma amostra de sujeitos entre 12 e 25 anos de idade, nossos resultados mostraram que pacientes e controles não apresentam diferenças entre composição corporal e estado nutricional. Apesar disso, a Região Sul do Brasil, onde o estudo foi conduzido, vem mostrando aumentos nos índices de sobrepeso e obesidade (Popkin 1998; Fleischer et al. 2011), sendo importante salientar estratégias que previnam a obesidade nessa população.

No estado basal, nossos resultados mostraram que pacientes e controles apresentam valores similares de marcadores ventilatórios e

metabólicos (Capítulo II). Apesar de estudos anteriores terem mostrado que pacientes fenilcetonúricos apresentam discrepâncias nos valores séricos de adrenalina, noradrenalina, grelina e leptina (Schulpis, Papakonstantinou e Tzamouranis 2000; Schulpis et al. 2005; Schulpis et al. 2004), entre outros fatores que poderiam afetar o metabolismo (Schuck et al. 2015), os pacientes do nosso estudo não tiveram esses valores alterados. Além disso, os valores ventilatórios analisados, especialmente o quociente respiratório, foram similares aos controles, demonstrando uma utilização de energia compatível com os controles apesar dos altos níveis de fenilalanina e da própria dieta especial. Contudo, salientamos que o pequeno número de pacientes avaliados (n=9) e o distinto café da manhã recebido por pacientes e controles podem ter afetado os resultados encontrados (ver Anexo 4 para composição da fórmula metabólica). Assim, estudos futuros são necessários para validar nossos achados.

Ainda em relação à resposta aguda a exercício aeróbico, nossos achados foram similares entre pacientes e controles (Capítulo II). É importante notar que os níveis de fenilalanina não se alteraram logo após o exercício em comparação com os valores de repouso de cada paciente. Nossos resultados corroboram o estudo de Grunert et al. (2013), que, como objetivo secundário, avaliou oito pacientes submetidos a uma sessão de exercício não relativizado quanto a intensidade e duração. Em indivíduos saudáveis treinados aerobicamente, Morris et al. (2013) encontraram diminuição de aminoácidos plasmáticos, incluindo fenilalanina, em repouso devido à prática regular de exercício (efeito crônico). Essas alterações crônicas causadas por treinamento são explicadas pelo aumento na demanda de energia através do recrutamento

de diferentes substratos, aumentando também a gliconeogênese a partir de aminoácidos. Embora os aminoácidos de cadeia ramificada (BCAA: valina, isoleucina e leucina) sejam transaminados e, portanto, sujeitos à oxidação, os aminoácidos aromáticos tais como fenilalanina podem ser utilizados como fonte energética, além de serem recrutados para anabolismo muscular (Berg, Tymoczko e Stryer 2002). Além disso, aumento ou diminuição nas concentrações dos outros aminoácidos neutros no sangue pode significar, respectivamente, vantagem ou desvantagem competitiva na barreira hematoencefálica, onde todos utilizam as mesmas proteínas transportadoras para chegar ao cérebro (Surtees e Blau 2000). Ao analisarmos as respostas metabólicas frente ao exercício no nosso estudo, tanto os dados ventilatórios quanto os bioquímicos foram semelhantes entre pacientes fenilcetonúricos e controles. Desta forma, o Capítulo II apresenta a evidência científica de que o exercício aeróbico é seguro para pacientes fenilcetonúricos, ao menos nas condições realizadas nesse estudo. Esse foi o primeiro relato de dados ventilatórios de pacientes fenilcetonúricos; porém, os resultados finais podem ter sido influenciados pela pequena amostra de pacientes bem como pelo café da manhã recebido antes da sessão de exercício. Assim, pesquisas futuras poderão confirmar nossos achados em um número maior de pacientes, também relacionando com a dieta.

Em relação aos efeitos crônicos do exercício sobre o sistema nervoso central, avaliamos o cérebro de camundongos geneticamente modificados (PAH^{enu2}) que não expressam a enzima PAH e, portanto, apresentam altos níveis de fenilalanina (Capítulo III). Sem alterar as concentrações de fenilalanina tanto em sangue como no cérebro, o treinamento

voluntário em roda melhorou parâmetros de estresse oxidativo no cérebro desses camundongos PKU (Capítulo III). Esses resultados corroboram nosso estudo precedente, em que exercício forçado (esteira) preveniu estresse oxidativo em cérebro de ratos submetidos quimicamente à hiperfenilalaninemia (Mazzola et al. 2011). A melhora nos marcadores de estresse oxidativo no cérebro pode resultar em uma melhor função cognitiva, independentemente das mudanças dos níveis de fenilalanina. As adaptações causadas pelo exercício no sistema antioxidante cerebral são benéficas ao tratamento da PKU, uma vez que o estresse oxidativo é considerado um problema importante causado por altos níveis de fenilalanina (Preissler et al. 2016; Moraes et al. 2014; Mazzola et al. 2013; Moraes et al. 2013; Rocha e Martins 2012; Sitta et al. 2006; Sirtori et al. 2005). De fato, os camundongos PAH^{enu2} sedentários apresentaram aumento em parâmetros que indicam estresse oxidativo em relação aos selvagens também sedentários.

Adicionalmente, o exercício regular modificou os níveis de alguns aminoácidos no sangue e no cérebro dos camundongos PKU em comparação com o grupo PKU que não exercitou-se, o que pode ter levado a fadiga precoce nesses animais. O protocolo de exercício utilizado foi de acesso livre a uma roda durante toda a duração do estudo para os grupos exercício. Portanto, os camundongos puderam exercitar-se quando e o quanto queriam. Assim, verificamos que os camundongos PKU percorreram, em média, distâncias menores que os animais selvagens. A princípio, o distúrbio motor relacionado com os altos níveis de fenilalanina já conhecido (Surtees e Blau 2000), corroborado pelo pior desempenho no teste de trave de equilíbrio (*balance beam test*) no nosso estudo, poderia explicar a menor atividade dos

camundongos PKU. Por outro lado, as menores concentrações de aminoácidos no sangue, especialmente dos aminoácidos gliconeogênicos e dos relacionados ao ciclo da ureia, poderiam representar uma maior mobilização de aminoácidos como fonte bioenergética, juntamente a um sistema de tamponamento de nitrogênio falho nos camundongos PKU treinados. Dessa forma, o grupo PKU teria percorrido menores distâncias do que os selvagens devido à maior exposição de amônia gerada no processo de gliconeogênese. Infelizmente, não avaliamos os níveis de amônia ou ureia nesses animais, nem conhecemos outro estudo em camundongos PAH^{enu2} que o tenha feito, Nesse sentido, a suplementação de aminoácidos relacionados com o ciclo da ureia, como ornitina, citrulina e glutamina, mostra-se eficaz em retardar a fadiga ao exercício devido à diminuição dos níveis de amônia no sangue de roedores (Meneguello et al. 2003; Takeda et al. 2011; Kim e Kim 2013). Esses resultados podem demonstrar desequilíbrios no metabolismo de substratos energéticos na PKU, especialmente combinado à dieta especial e sua aderência por pacientes.

Toda prescrição terapêutica deve ser baseada em evidências. Porém, pacientes fenilcetonúricos têm sido encorajados a se exercitar mesmo com pouca comprovação científica de seus benefícios (Rocha, Macdonald e Trefz 2013; Doulgeraki et al. 2014). O exercício aeróbico parece não alterar os níveis de fenilalanina no sangue de pacientes fenilcetonúricos (agudamente) (Grunert et al. 2013), (Capítulo II), assim como no sangue ou no cérebro de modelo animal (cronicamente) (Capítulo III). Areces et al. (2015) avaliaram dano muscular e alterações nos níveis de aminoácidos causados por provas de *ironman* em triatletas. Nessa situação de exercício extenuante em indivíduos altamente treinados, os atletas apresentaram níveis séricos de creatina quinase

aumentados após a prova em relação ao repouso, significando dano muscular causado pelo exercício, além de alteração nas concentrações de aminoácidos circulantes. Enquanto os níveis da maioria dos aminoácidos diminuíram (principalmente os BCAA) após a prova, os níveis de fenilalanina aumentaram 7% em média, porém não estatisticamente. Como os pacientes fenilcetonúricos apresentam altos níveis de fenilalanina, talvez essas pequenas mudanças causadas pelo catabolismo muscular representem um risco que, em pessoas saudáveis, não alcança significância estatística. Além disso, a redução de outros aminoácidos neutros combinada com o aumento da fenilalanina no sangue representa um potencial aumento de captação de fenilalanina para o cérebro (de Groot et al. 2013). Deste modo, salienta-se a importância da realização de mais estudos sobre os efeitos do exercício em diferentes abordagens e intensidades na PKU.

4 CONCLUSÕES

Essa tese concluiu que pacientes fenilcetonúricos não apresentam discrepâncias em parâmetros metabólicos e que o exercício aeróbico pode ser uma estratégia terapêutica concomitante à dieta restrita, como detalhado abaixo.

a) Pacientes fenilcetonúricos não estão em maior risco metabólico, pois apresentam estado nutricional e composição corporal similares à população em geral (Capítulo I);
b) Pacientes fenilcetonúricos não apresentam discrepâncias em valores ventilatórios e metabólicos em repouso (Capítulo II);
c) Agudamente, exercício aeróbico não altera os níveis plasmáticos de fenilalanina ou acarreta alterações inesperadas em pacientes fenilcetonúricos (Capítulo II);
d) Cronicamente, exercício aeróbico voluntário melhora as defesas antioxidantes no cérebro de camundongos fenilcetonúricos mesmo sem alterar as concentrações de fenilalanina, porém diminuindo os níveis de aminoácidos gliconeogênicos em sangue e cérebro (Capítulo III).

Portanto, pacientes fenilcetonúricos devem ser encorajados a praticar exercício físico para, possivelmente, usufruir adaptações positivas geradas pelo treinamento físico. Devido ao pequeno número amostral de participantes em nossos estudos, futuras investigações são ainda necessárias para a prescrição de exercício a pacientes fenilcetonúricos.

5 PERSPECTIVAS

a) Avaliar o efeito de exercício em combinação a dietas específicas que podem afetar o metabolismo;
b) Analisar diferentes intensidades de exercício;
c) Analisar adaptações relacionadas a exercício regular em pacientes fenilcetonúricos.

6 REFERÊNCIAS BIBLIOGRÁFICAS

Al Hafid, N., Christodoulou, J. (2015) Phenylketonuria: a review of current and future treatments. Transl Pediatr 4(4): 304-317.

Albersen, M., Bonthuis, M., de Roos, N. M., van den Hurk, D. A., Carbasius Weber, E., Hendriks, M. M., de Sain-van der Velden, M. G., de Koning, T. J. Visser, G. (2010) Whole body composition analysis by the BodPod air-displacement plethysmography method in children with phenylketonuria shows a higher body fat percentage. J Inherit Metab Dis 33 Suppl 3: S283-288.

Allen, J. R., McCauley, J. C., Waters, D. L., O'Connor, J., Roberts, D. C., Gaskin, K. J. (1995) Resting energy expenditure in children with phenylketonuria. Am J Clin Nutr 62(4): 797-801.

Areces, F., Gonzalez-Millan, C., Salinero, J. J., Abian-Vicen, J., Lara, B., Gallo-Salazar, C., Ruiz-Vicente, D., Del Coso, J. (2015) Changes in Serum Free Amino Acids and Muscle Fatigue Experienced during a Half-Ironman Triathlon. PLoS One 10(9): e0138376.

Arnold, G. L., Vladutiu, C. J., Kirby, R. S., Blakely, E. M., Deluca, J. M. (2002) Protein insufficiency and linear growth restriction in phenylketonuria. J Pediatr 141(2): 243-246.

Bedin, M., Estrella, C. H., Duarte, D. V., Ponzi, D., Dutra-Filho, C. S., Wyse, A. T., Wajner, M. and Wannmacher, C. M. (2000) Platelet Na+, K+-ATPase activity as a possible peripheral marker for the neurotoxic

effects of phenylalanine in phenylketonuria. Metab Brain Dis 15(2): 115-121.

Bedin, M., Estrella, C. H., Ponzi, D., Duarte, D. V., Dutra-Filho, C. S., Wyse, A. T., Wajner, M. and Wannmacher, C. M. (2001) Reduced Na(+), K(+)-ATPase activity in erythrocyte membranes from patients with phenylketonuria. Pediatr Res 50(1): 56-60.

Berg, J. M., Tymoczko, J. L., Stryer, L. (2002) Protein Turnover and Amino Acid Catabolism. Biochemistry. W. H. Freeman. New York.

Bickel, H., Gerrard, J., Hickmans, E. M. (1953) Influence of phenylalanine intake on phenylketonuria. Lancet 265(6790): 812-813.

Bilder, D. A., Noel, J. K., Baker, E. R., Irish, W., Chen, Y., Merilainen, M. J., Prasad, S., Winslow, B. J. (2016) Systematic Review and Meta-Analysis of Neuropsychiatric Symptoms and Executive Functioning in Adults With Phenylketonuria. Dev Neuropsychol 41(4): 1-16.

Blau, N., van Spronsen, F. J., Levy, H. L. (2010) Phenylketonuria. Lancet 376(9750): 1417-1427.

Bortoluzzi, V. T., de Franceschi, I. D., Rieger, E. and Wannmacher, C. M. (2014) Co-administration of creatine plus pyruvate prevents the effects of phenylalanine administration to female rats during pregnancy and lactation on enzymes activity of energy metabolism in cerebral cortex and hippocampus of the offspring. Neurochem Res 39(8): 1594-1602.

Brooks, G. A. (1998) Mammalian fuel utilization during sustained exercise. Comp Biochem Physiol B Biochem Mol Biol 120(1): 89-107.

Brown, S. P. (2001). Introduction to Exercise Science. Baltimore, Maryland, USA, Lippincott Williams & Wilkins.

Bruinenberg, V. M., van der Goot, E., van Vliet, D., de Groot, M. J., Mazzola, P. N., Heiner-Fokkema, M. R., van Faassen, M., van Spronsen, F. J. and van der Zee, E. A. (2016) The Behavioral Consequence of Phenylketonuria in Mice Depends on the Genetic Background. Front Behav Neurosci 10: 233.

Burrage, L. C., McConnell, J., Haesler, R., O'Riordan, M. A., Sutton, V. R., Kerr, D. S., McCandless, S. E. (2012) High prevalence of overweight and obesity in females with phenylketonuria. Mol Genet Metab 107(1-2): 43-48.

Camiletti-Moiron, D., Aparicio, V. A., Aranda, P., Radak, Z. (2013) Does exercise reduce brain oxidative stress? A systematic review. Scand J Med Sci Sports 23(4): e202-212.

Camp, K. M., Parisi, M. A., Acosta, P. B., Berry, G. T., Bilder, D. A., Blau, N., Bodamer, O. A., Brosco, J. P., Brown, C. S., Burlina, A. B., Burton, B. K., Chang, C. S., Coates, P. M., Cunningham, A. C., Dobrowolski, S. F., Ferguson, J. H., Franklin, T. D., Frazier, D. M., Grange, D. K., Greene, C. L., Groft, S. C., Harding, C. O., Howell, R. R., Huntington, K. L., Hyatt-Knorr, H. D., Jevaji, I. P., Levy, H. L., Lichter-Konecki, U., Lindegren, M. L., Lloyd-Puryear, M. A., Matalon, K., MacDonald, A.,

McPheeters, M. L., Mitchell, J. J., Mofidi, S., Moseley, K. D., Mueller, C. M., Mulberg, A. E., Nerurkar, L. S., Ogata, B. N., Pariser, A. R., Prasad, S., Pridjian, G., Rasmussen, S. A., Reddy, U. M., Rohr, F. J., Singh, R. H., Sirrs, S. M., Stremer, S. E., Tagle, D. A., Thompson, S. M., Urv, T. K., Utz, J. R., van Spronsen, F., Vockley, J., Waisbren, S. E., Weglicki, L. S., White, D. A., Whitley, C. B., Wilfond, B. S., Yannicelli, S., e Young, J. M. (2014) Phenylketonuria Scientific Review Conference: state of the science and future research needs. Mol Genet Metab 112(2): 87-122.

Centerwall, S. A., Centerwall, W. R. (2000) The discovery of phenylketonuria: the story of a young couple, two retarded children, and a scientist. Pediatrics 105(1): 89-103.

Cleary, M., Trefz, F., Muntau, A. C., Feillet, F., van Spronsen, F. J., Burlina, A., Belanger-Quintana, A., Gizewska, M., Gasteyger, C., Bettiol, E., Blau, N., MacDonald, A. (2013) Fluctuations in phenylalanine concentrations in phenylketonuria: a review of possible relationships with outcomes. Mol Genet Metab 110(4): 418-423.

Coyle, E. F., Jeukendrup, A. E., Oseto, M. C., Hodgkinson, B. J., Zderic, T. W. (2001) Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise. Am J Physiol Endocrinol Metab 280(3): E391-398.

de Groot, M. J., Hoeksma, M., Reijngoud, D. J., de Valk, H. W., Paans, A. M., Sauer, P. J., van Spronsen, F. J. (2013) Phenylketonuria: reduced

tyrosine brain influx relates to reduced cerebral protein synthesis. Orphanet J Rare Dis 8: 133.

Dibble, L. E., Addison, O., Papa, E. (2009) The effects of exercise on balance in persons with Parkinson's disease: a systematic review across the disability spectrum. J Neurol Phys Ther 33(1): 14-26.

Dionne, I., Van Vugt, S., Tremblay, A. (1999) Postexercise macronutrient oxidation: a factor dependent on postexercise macronutrient intake. Am J Clin Nutr 69(5): 927-930.

Doulgeraki, A., Skarpalezou, A., Theodosiadou, A., Monopolis, I., Schulpis, K. (2014) Body composition profile of young patients with phenylketonuria and mild hyperphenylalaninemia. Int J Endocrinol Metab 12(3): e16061.

Estatuto da Criança e do Adolescente (13 de Julho de 1990). Lei Federal inciso III do Artigo 10 da Lei n 8069, Brasil.

Fleischer, N. L., Diez Roux, A. V., Alazraqui, M., Spinelli, H., De Maio, F. (2011) Socioeconomic gradients in chronic disease risk factors in middle-income countries: evidence of effect modification by urbanicity in Argentina. Am J Public Health 101(2): 294-301.

Folling, I. (1994) The discovery of phenylketonuria. Acta Paediatr Suppl 407: 4-10.

Gassio, R., Campistol, J., Vilaseca, M. A., Lambruschini, N., Cambra, F. J., Fuste, E. (2003) Do adult patients with phenylketonuria improve their

quality of life after introduction/resumption of a phenylalanine-restricted diet? Acta Paediatr 92(12): 1474-1478.

Gokmen-Ozel, H., MacDonald, A., Daly, A., Hall, K., Ryder, L., Chakrapani, A. (2009) Long-term efficacy of 'ready-to-drink' protein substitute in phenylketonuria. J Hum Nutr Diet 22(5): 422-427.

Gonzalez, M. J., Gutierrez, A. P., Gassio, R., Fuste, M. E., Vilaseca, M. A., Campistol, J. (2011) Neurological complications and behavioral problems in patients with phenylketonuria in a follow-up unit. Mol Genet Metab 104 Suppl: S73-79.

Goto, S., Naito, H., Kaneko, T., Chung, H. Y., Radak, Z. (2007) Hormetic effects of regular exercise in aging: correlation with oxidative stress. Appl Physiol Nutr Metab 32(5): 948-953.

Grunert, S. C., Brichta, C. M., Krebs, A., Clement, H. W., Rauh, R., Fleischhaker, C., Hennighausen, K., Sass, J. O., Schwab, K. O. (2013) 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: 60.

Halliwell, B. (2011) Free radicals and antioxidants - quo vadis? Trends Pharmacol Sci 32(3): 125-130.

Huemer, M., Huemer, C., Moslinger, D., Huter, D., Stockler-Ipsiroglu, S. (2007) Growth and body composition in children with classical phenylketonuria:

results in 34 patients and review of the literature. J Inherit Metab Dis 30(5): 694-699.

Huijbregts, S. C., Gassio, R., Campistol, J. (2013) Executive functioning in context: Relevance for treatment and monitoring of phenylketonuria. Mol Genet Metab 110 Suppl: S25-30.

Jahja, R., Huijbregts, S. C., de Sonneville, L. M., van der Meere, J. J., van Spronsen, F. J. (2014) Neurocognitive evidence for revision of treatment targets and guidelines for phenylketonuria. J Pediatr 164(4): 895-899 e892.

Kim, D. I., Kim, K. S. (2013) Walnut extract exhibits anti-fatigue action via improvement of exercise tolerance in mice. Lab Anim Res 29(4): 190-195.

Lesser, A. J. (1963) Phenylketonuria and the Guthrie Test. Pediatrics 32: 940.

MacDonald, A., Rocha, J. C., van Rijn, M., Feillet, F. (2011) Nutrition in phenylketonuria. Mol Genet Metab 104 Suppl: S10-18.

Mazzola, P. N., Karikas, G. A., Schulpis, K. H. and Dutra-Filho, C. S. (2013) Antioxidant treatment strategies for hyperphenylalaninemia. Metab Brain Dis 28(4): 541-550.

Mazzola, P. N., Terra, M., Rosa, A. P., Mescka, C. P., Moraes, T. B., Piccoli, B., Jacques, C. E., Dalazen, G., Cortes, M. X., Coelho, J., Dutra-Filho, C. S. (2011) Regular exercise prevents oxidative stress in the brain of hyperphenylalaninemic rats. Metab Brain Dis 26(4): 291-297.

Meneguello, M. O., Mendonca, J. R., Lancha, A. H., Jr., Costa Rosa, L. F. (2003) Effect of arginine, ornithine and citrulline supplementation upon performance and metabolism of trained rats. Cell Biochem Funct 21(1): 85-91.

Miras, A., Boveda, M. D., Leis, M. R., Mera, A., Aldamiz-Echevarria, L., Fernandez-Lorenzo, J. R., Fraga, J. M., Couce, M. L. (2013) Risk factors for developing mineral bone disease in phenylketonuric patients. Mol Genet Metab 108(3): 149-154.

Moraes, T. B., Dalazen, G. R., Jacques, C. E., de Freitas, R. S., Rosa, A. P. and Dutra-Filho, C. S. (2014) Glutathione metabolism enzymes in brain and liver of hyperphenylalaninemic rats and the effect of lipoic acid treatment. Metab Brain Dis 29(3): 609-615.

Moraes, T. B., Jacques, C. E., Rosa, A. P., Dalazen, G. R., Terra, M., Coelho, J. G. and Dutra-Filho, C. S. (2013) Role of catalase and superoxide dismutase activities on oxidative stress in the brain of a phenylketonuria animal model and the effect of lipoic acid. Cell Mol Neurobiol 33(2): 253-260.

Morris, C., Grada, C. O., Ryan, M., Roche, H. M., De Vito, G., Gibney, M. J., Gibney, E. R., Brennan, L. (2013) The relationship between aerobic fitness level and metabolic profiles in healthy adults. Mol Nutr Food Res 57(7): 1246-1254.

Mutze, U., Beblo, S., Kortz, L., Matthies, C., Koletzko, B., Bruegel, M., Rohde, C., Thiery, J., Kiess, W., Ceglarek, U. (2012) Metabolomics of dietary

Fatty Acid restriction in patients with phenylketonuria. PLoS One 7(8): e43021.

Penrose, L., Quastel, J. H. (1937) Metabolic studies in phenylketonuria. Biochem J 31(2): 266-274.

Pimentel, F. B., Alves, R. C., Oliva-Teles, M. T., Costa, A. S., Fernandes, T. J., Almeida, M. F., Torres, D., Delerue-Matos, C., Oliveira, M. B. (2014) Targeting specific nutrient deficiencies in protein-restricted diets: some practical facts in PKU dietary management. Food Funct 5(12): 3151-3159.

Popkin, B. M. (1998) The nutrition transition and its health implications in lower-income countries. Public Health Nutr 1(1): 5-21.

Preissler, T., Bristot, I. J., Costa, B. M., Fernandes, E. K., Rieger, E., Bortoluzzi, V. T., de Franceschi, I. D., Dutra-Filho, C. S., Moreira, J. C. and Wannmacher, C. M. (2016) Phenylalanine induces oxidative stress and decreases the viability of rat astrocytes: possible relevance for the pathophysiology of neurodegeneration in phenylketonuria. Metab Brain Dis 31(3): 529-537.

Radak, Z., Kumagai, S., Taylor, A. W., Naito, H., Goto, S. (2007) Effects of exercise on brain function: role of free radicals. Appl Physiol Nutr Metab 32(5): 942-946.

Robert, M., Rocha, J. C., van Rijn, M., Ahring, K., Belanger-Quintana, A., Macdonald, A., Dokoupil, K., Gokmen Ozel, H., Lammardo, A. M.,

Goyens, P., Feillet, F. (2013) Micronutrient status in phenylketonuria. Mol Genet Metab 110 Suppl: S6-S17.

Rocha, J. C. e Martins, M. J. (2012) Oxidative stress in phenylketonuria: future directions. J Inherit Metab Dis 35(3): 381-398.

Rocha, J. C., Macdonald, A., Trefz, F. (2013) Is overweight an issue in phenylketonuria? Mol Genet Metab 110 Suppl: S18-24.

Rocha, J. C., van Spronsen, F. J., Almeida, M. F., Ramos, E., Guimaraes, J. T., Borges, N. (2013) Early dietary treated patients with phenylketonuria can achieve normal growth and body composition. Mol Genet Metab 110 Suppl: S40-43.

Rocha, J. C., van Spronsen, F. J., Almeida, M. F., Soares, G., Quelhas, D., Ramos, E., Guimaraes, J. T., Borges, N. (2012) Dietary treatment in phenylketonuria does not lead to increased risk of obesity or metabolic syndrome. Mol Genet Metab 107(4): 659-663.

Sarkissian, C. N., Gamez, A., Scriver, C. R. (2009) What we know that could influence future treatment of phenylketonuria. J Inherit Metab Dis 32(1): 3-9.

Sarkissian, C. N., Scriver, C. R. and Mamer, O. A. (2000) Measurement of phenyllactate, phenylacetate, and phenylpyruvate by negative ion chemical ionization-gas chromatography/mass spectrometry in brain of mouse genetic models of phenylketonuria and non-phenylketonuria hyperphenylalaninemia. Anal Biochem 280(2): 242-249.

Schuck, P. F., Malgarin, F., Cararo, J. H., Cardoso, F., Streck, E. L. and Ferreira, G. C. (2015) Phenylketonuria Pathophysiology: on the Role of Metabolic Alterations. Aging Dis 6(5): 390-399.

Schulpis, K. H., Papakonstantinou, E. D., Tzamouranis, J. (2000) Plasma leptin concentrations in phenylketonuric patients. Horm Res 53(1): 32-35.

Schulpis, K. H., Papassotiriou, I., Tsakiris, S., Vounatsou, M., Chrousos, G. P. (2005) 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(10): 1350-1355.

Schulpis, K. H., Papassotiriou, I., Vounatsou, M., Karikas, G. A., Tsakiris, S., Chrousos, G. P. (2004) Morning preprandial plasma ghrelin and catecholamine concentrations in patients with phenylketonuria and normal controls: evidence for catecholamine-mediated ghrelin regulation. J Clin Endocrinol Metab 89(8): 3983-3987.

Sirtori, L. R., Dutra-Filho, C. S., Fitarelli, D., Sitta, A., Haeser, A., Barschak, A. G., Wajner, M., Coelho, D. M., Llesuy, S., Bello-Klein, A., Giugliani, R., Deon, M. and Vargas, C. R. (2005) Oxidative stress in patients with phenylketonuria. Biochim Biophys Acta 1740(1): 68-73.

Sitta, A., Barschak, A. G., Deon, M., Terroso, T., Pires, R., Giugliani, R., Dutra-Filho, C. S., Wajner, M. and Vargas, C. R. (2006) Investigation of

oxidative stress parameters in treated phenylketonuric patients. Metab Brain Dis 21(4): 287-296.

Surtees, R., Blau, N. (2000) The neurochemistry of phenylketonuria. Eur J Pediatr 159 Suppl 2: S109-113.

Takeda, K., Machida, M., Kohara, A., Omi, N., Takemasa, T. (2011) Effects of citrulline supplementation on fatigue and exercise performance in mice. J Nutr Sci Vitaminol (Tokyo) 57(3): 246-250.

Trefz, F., Maillot, F., Motzfeldt, K., Schwarz, M. (2011) Adult phenylketonuria outcome and management. Mol Genet Metab 104 Suppl: S26-30.

Trevisan, L. M., Nalin, T., Tonon, T., Veiga, L. M., Vargas, P., Krug, B. C., Leivas, P. G., Schwartz, I. V. (2015) Access to treatment for phenylketonuria by judicial means in Rio Grande do Sul, Brazil. Cien Saude Colet 20(5): 1607-1616.

Um, H. S., Kang, E. B., Leem, Y. H., Cho, I. H., Yang, C. H., Chae, K. R., Hwang, D. Y., Cho, J. Y. (2008) Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. Int J Mol Med 22(4): 529-539.

VanZutphen, K., Packman, W., Sporri, L., Needham, M., Morgan, C., Weisiger, K., Packman, S. (2007) Executive functioning in children and adolescents with phenylketonuria. Clin Genet 72(1): 13-18.

Vilaseca, M. A., Lambruschini, N., Gomez-Lopez, L., Gutierrez, A., Fuste, E., Gassio, R., Artuch, R., Campistol, J. (2010) Quality of dietary control in phenylketonuric patients and its relationship with general intelligence. Nutr Hosp 25(1): 60-66.

7 ANEXOS

Anexo 1 – Pareceres do Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO CIENTÍFICA

A Comissão Científica do Hospital de Clínicas de Porto Alegre analisou o projeto:

Projeto: 120115
Data da Versão do Projeto:

Pesquisadores:
IDA VANESSA DOEDERLEIN SCHWARTZ
TATIELE NALIN

Título: AVALIAÇÃO DO ESTADO NUTRICIONAL E ANÁLISE DO ÂNGULO DE FASE A PARTIR DA BIOIMPEDÂNCIA ELÉTRICA EM PACIENTES COM FENILCETONÚRIA ACOMPANHADOS NO SERVIÇO DE GENÉTICA MÉDICA DO HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG)

Porto Alegre, 26 de abril de 2012.

Profª Nadine Clausell
Coordenadora GPPG

HOSPITAL DE CLÍNICAS DE PORTO ALEGRE - HCPA / UFRGS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito agudo do exercício sobre parâmetros metabólicos, hormonais e de estresse oxidativo em pacientes fenilcetonúricos

Pesquisador: Ida Vanessa Doederlein Schwartz

Área Temática:

Versão: 5

CAAE: 06101512.1.0000.5327

Instituição Proponente: Hospital de Clínicas de Porto Alegre - HCPA / UFRGS

Patrocinador Principal: Financiamento Próprio
Conselho Nacional de Desenvolvimento Científico e Tecnologico ((CNPq))
Hospital de Clínicas de Porto Alegre - HCPA / UFRGS

DADOS DO PARECER

Número do Parecer: 227.193

Data da Relatoria: 20/03/2013

Apresentação do Projeto:

Em 14/03/2013 os pesquisadores adicionaram emenda ao projeto.

A emenda prevê a inserção de novas intervenções nos participantes, conforme abaixo relacionado:

(1) Três participantes de cada grupo (pacientes e controles) deverão ir novamente ao laboratório em jejum de 12 h, onde irão fazer uma coleta de 2 mL de sangue, receber o café da manhã já padronizado e, após aguardar 1 h em repouso, realizar última coleta de 2 mL de sangue. Para o grupo de pacientes, esse protocolo poderá ser realizado no mesmo dia de consulta ao ambulatório.

(2) Será aplicado um novo Termo de Consentimento Livre e Esclarecido (TCLE) explicando esta nova coleta de dados, e mencionando as duas coletas de sangue de 2 mL cada.

Objetivo da Pesquisa:

Apreciação da emenda e do TCLE pelo Comitê de Ética em Pesquisa (CEP) do Hospital de Clínicas de Porto Alegre (HCPA).

Avaliação dos Riscos e Benefícios:

Não se aplica.

Comentários e Considerações sobre a Pesquisa:

Não se aplica.

Endereço: Rua Ramiro Barcelos 2.350 sala 2227 F
Bairro: Bom Fim **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (513)359--7640 **Fax:** (513)359--7640 **E-mail:** cephcpa@hcpa.ufrgs.br

Considerações sobre os Termos de apresentação obrigatória:

O TCLE apresentado contempla os requisitos necessários.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

A emenda e o TCLE encontra-se em condições de aprovação.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Emenda aprovada versão 14/03/2013 e TCLE aprovado versão 14/03/2013.

PORTO ALEGRE, 22 de Março de 2013

Assinador por:
José Roberto Goldim
(Coordenador)

Endereço: Rua Ramiro Barcelos 2.350 sala 2227 F
Bairro: Bom Fim **CEP:** 90.035-903
UF: RS **Município:** PORTO ALEGRE
Telefone: (513)359--7640 **Fax:** (513)359--7640 **E-mail:** cephcpa@hcpa.ufrgs.br

Anexo 2 – Ficha de Registro do Participante

Nome (iniciais): ( ) PKU ( ) Controle

Data de Nascimento: Sexo: ( ) Feminino ( ) Masculino

Estado Civil: Naturalidade:

Endereço:

Celular: e-mail:

Idade do Diagnóstico: Tipo de HPA:

Número de dosagens realizadas no último ano e resultados:

Data	Fenilalanina (mg/dL)	Observação

Mediana de fenilalanina plasmática no último ano:

Data/valor do último nível de fenilalanina:

Dieta atualmente prescrita:

Usa algum tipo de medicamento: ( ) Não

( ) Sim. Quais?

_____

_____

_____

É gestante: ( ) Não ( ) Sim

Data de realização do teste:

Peso: Altura:

Resultados:

TMB =

$VO_{2máx}$ =

Lactato =

Perfil lipídico

Ácidos graxos livres =

TG =

Colesterol=

HDL =

LDL =

Estresse oxidativo

CAT =

SOD =

GPx =

G6PD =

TBA-RS =

TRAP =

TAR =

Carbonilas =

Hormônios

Leptina =

Adiponectina =

Grelina =

Aminoácidos:

Anexo 3 – Termo de Consentimento para pacientes e controles

Termo de Consentimento LIVRE E ESCLARECIDO - Paciente

Projeto: **Efeito agudo do exercício sobre parâmetros metabólicos, hormonais e de estresse oxidativo em pacientes fenilcetonúricos**

Pesquisador responsável**: Dra. Ida Vanessa D. Schwartz. Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre.** Rua Ramiro Barcelos, 2350. Porto Alegre-RS. Tel: 51-3359 8011.

Paciente: _____

Prezado paciente,

Você participou do estudo que visa avaliar os efeitos do exercício na fenilcetonúria recentemente, e está sendo convidado a participar da sua segunda etapa. Esta nova etapa visa avaliar o efeito do café da manhã, que inclui a fórmula metabólica sem fenilalanina, sobre parâmetros bioquímicos. O maior objetivo da pesquisa é avaliar os efeitos de uma sessão de exercício aeróbico em pacientes fenilcetonúricos adultos em relação a parâmetros que são positivamente alterados pelo treinamento físico, e comparar esta resposta a indivíduos não portadores de PKU. Esse estudo será realizado em pacientes fenilcetonúricos em tratamento no Ambulatório de Distúrbios Metabólicos do Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre (SGM-HCPA), e pessoas não portadoras de PKU, que tenham no mínimo 14 anos de idade, e que, no caso do sexo feminino, não sejam gestantes.

Caso você decida participar dessa etapa da pesquisa, será necessário que você (paciente) permaneça uma manhã no HCPA, que poderá ser o mesmo dia da sua consulta, fazendo avaliações com duração de 1 hora e meia. No início da manhã, por volta das 8.00 h, você deverá chegar ao HCPA em jejum, e irá realizar uma coleta de 2 mL de sangue. Após isso, você receberá o mesmo café da manhã da primeira etapa da pesquisa (de acordo com a sua tolerância à fenilalanina e juntamente à fórmula metabólica). Depois disso, você deverá

aguardar por 1 h em repouso. Por fim, haverá a última coleta de 2 mL de sangue.

Os resultados destas análises sanguíneas serão comunicados a você assim que estiverem disponíveis (imagina-se que no máximo até três meses após a realização do teste), juntamente com os resultados da etapa anterior da pesquisa.

RISCOS E DESCONFORTOS

Os riscos e desconfortos causados pela coleta de sangue são semelhantes aos riscos envolvidos na coleta de sangue para exames laboratoriais de rotina (manchas roxas e dor no local da coleta). O desconforto e os riscos associados a estas avaliações serão minimizados pela realização da coleta por profissional treinado.

CUSTOS

Não haverá compensação financeira pela sua participação no estudo.

AUTORIZAÇÕES

Você (paciente) autorizou:

1) A coleta de 2 mL de sangue em jejum, e 2 mL após a refeição?

( ) Sim

( ) Não

2) Se você permitir, o material coletado que restar após a realização dos exames previstos neste estudo, serão armazenados por cinco anos e poderão ser utilizados, neste período, em estudos aprovados eticamente pelos órgãos ou comissões responsáveis sobre esse mesmo tema. Em relação ao armazenamento e utilização de algum material (sangue) que tenha restado após a realização dos exames previstos neste estudo, você declara que autorizou:

( ) que este material poderá ser armazenado por cinco anos e poderá vir a ser utilizado em estudos futuros aprovados eticamente pelos órgãos ou comissões responsáveis sobre esse mesmo tema, desde que você revise e assine o termo de consentimento de tais estudos futuros. Após cinco anos, este material será obrigatoriamente descartado.
( ) que este material não poderá ser armazenado por cinco anos e não poderá vir a ser utilizado em estudos futuros aprovados eticamente pelos órgãos ou comissões responsáveis. O material coletado deverá ser utilizado somente neste estudo, e o material que sobrar não deverá ser armazenado, sendo obrigatoriamente descartado.

Cabe salientar que esse estudo talvez não traga benefícios para você, mas que pode ajudar a melhorar, futuramente, o tratamento dos pacientes com fenilcetonúria e hiperfenilalaninemias.

DÚVIDAS

Se você tiver alguma dúvida em relação à pesquisa, deve contatar a Dra. Ida Vanessa D. Schwartz (Fone: 51- 99017418 ou 3359-8011), no Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre, ou contatar o Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre pelo telefone: 51-33598304.

RECUSA OU DESCONTINUAÇÃO NA PARTICIPAÇÃO DO ESTUDO

Sua participação no estudo é voluntária. Se você decidir não participar do estudo, isto não afetará em nada o seu tratamento no seu hospital. A sua participação pode também ser interrompida a qualquer momento por você mesmo(a). Em qualquer caso, você não será penalizado(a).

CONFIDENCIALIDADE DAS INFORMAÇÕES

As informações dessa pesquisa serão mantidas em sigilo, sendo apenas utilizadas de forma científica e anônima, em relatos especializados, com autorização prévia. Caso alguma informação derivada desse estudo for importante a você, todo esforço será realizado para informá-lo.

Pelo presente termo, você declara que foi informado(a), de forma clara e detalhada, sobre a presente pesquisa, e que teve suas dúvidas esclarecidas por _____. Declara ter sido esclarecido que não receberá nenhuma remuneração financeira pela participação no estudo. Declara que foi informado da garantia de receber resposta ou esclarecimento sobre a pesquisa a ser realizada, bem como da liberdade de não participar do estudo e da possibilidade de desistir, em qualquer momento, da participação. Além disso, declara que assinou duas vias deste consentimento, e que uma ficou em seu poder.

Data: ___/___/_____

Assinatura do paciente: _____

Eu expliquei a _____ os objetivos, riscos, benefícios e procedimentos necessários para esta pesquisa, e entreguei cópia deste termo de consentimento para o mesmo.

Data: ____/_____/_____

Nome:_____

Se o paciente é menor de 18 anos:

Assinatura do responsável: _____

Obs.: *O presente documento, baseado no item IV das diretrizes e normas regulamentadoras para pesquisa em saúde, do Conselho Nacional de Saúde (Resolução 196/96), será assinada em duas vias, de igual valor, ficando uma via em poder do voluntário ou de seu responsável legal e outra com o pesquisador responsável.*

Termo de Consentimento LIVRE E ESCLARECIDO - Controle

Projeto: **Efeito agudo do exercício sobre parâmetros metabólicos, hormonais e de estresse oxidativo em pacientes fenilcetonúricos**

Pesquisador responsável**: Dra. Ida Vanessa D. Schwartz. Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre.** Rua Ramiro Barcelos, 2350. Porto Alegre-RS. Tel: 51-3359 8011.

Paciente: _____

Prezado participante,

Você participou do estudo que visa avaliar os efeitos do exercício na fenilcetonúria recentemente, e está sendo convidado a participar da sua segunda etapa. Esta nova etapa visa avaliar o efeito do café da manhã sobre parâmetros bioquímicos. O maior objetivo da pesquisa é avaliar os efeitos de uma sessão de exercício aeróbico em pacientes fenilcetonúricos adultos em relação a parâmetros que são positivamente alterados pelo treinamento físico, e comparar esta resposta a indivíduos não portadores de PKU. Esse estudo será realizado em pacientes fenilcetonúricos em tratamento no Ambulatório de Distúrbios Metabólicos do Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre (SGM-HCPA), e pessoas não portadoras de PKU, que tenham no mínimo 14 anos de idade, e que, no caso do sexo feminino, não sejam gestantes.

Caso você decida participar dessa etapa da pesquisa, será necessário que você permaneça uma manhã no HCPA, fazendo avaliações com duração de 1 hora e meia. No início da manhã, por volta das 8.00 h, você deverá chegar ao HCPA em jejum, e irá realizar uma coleta de 2 mL de sangue. Após isso, você receberá o mesmo café da manhã da primeira etapa da pesquisa. Depois disso, você deverá aguardar por 1 h em repouso. Por fim, haverá a última coleta de 2 mL de sangue.

Os resultados destas análises sanguíneas serão comunicados a você assim que estiverem disponíveis (imagina-se que no máximo até três meses após a realização do teste), juntamente com os resultados da etapa anterior da pesquisa.

RISCOS E DESCONFORTOS

Os riscos e desconfortos causados pela coleta de sangue são semelhantes aos riscos envolvidos na coleta de sangue para exames laboratoriais de rotina (manchas roxas e dor no local da coleta). O desconforto e os riscos associados a estas avaliações serão minimizados pela realização da coleta por profissional treinado.

CUSTOS

Não haverá compensação financeira pela sua participação no estudo.

AUTORIZAÇÕES

Você autorizou:

1) A coleta de 2 mL de sangue em jejum, e 2 mL após a refeição?

( ) Sim

( ) Não

2) Se você permitir, o material coletado que restar após a realização dos exames previstos neste estudo, serão armazenados por cinco anos e poderão ser utilizados, neste período, em estudos aprovados eticamente pelos órgãos ou comissões responsáveis sobre esse mesmo tema. Em relação ao armazenamento e utilização de algum material (sangue) que tenha restado após a realização dos exames previstos neste estudo, você declara que autorizou:

( ) que este material poderá ser armazenado por cinco anos e poderá vir a ser utilizado em estudos futuros aprovados eticamente pelos órgãos ou comissões

responsáveis sobre esse mesmo tema, desde que você revise e assine o termo de consentimento de tais estudos futuros. Após cinco anos, este material será obrigatoriamente descartado.

( ) que este material não poderá ser armazenado por cinco anos e não poderá vir a ser utilizado em estudos futuros aprovados eticamente pelos órgãos ou comissões responsáveis. O material coletado deverá ser utilizado somente neste estudo, e o material que sobrar não deverá ser armazenado, sendo obrigatoriamente descartado.

Cabe salientar que esse estudo talvez não traga benefícios para você, mas que pode ajudar a melhorar, futuramente, o tratamento dos pacientes com fenilcetonúria e hiperfenilalaninemias.

DÚVIDAS

Se você tiver alguma dúvida em relação à pesquisa, deve contatar a Dra. Ida Vanessa D. Schwartz (Fone: 51- 99017418 ou 3359-8011), no Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre, ou contatar o Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre pelo telefone: 51-33598304.

RECUSA OU DESCONTINUAÇÃO NA PARTICIPAÇÃO DO ESTUDO

Sua participação no estudo é voluntária. Se você decidir não participar do estudo, isto não afetará em nada o seu tratamento no seu hospital. A sua participação pode também ser interrompida a qualquer momento por você mesmo(a). Em qualquer caso, você não será penalizado(a).

CONFIDENCIALIDADE DAS INFORMAÇÕES

As informações dessa pesquisa serão mantidas em sigilo, sendo apenas utilizadas de forma científica e anônima, em relatos especializados, com autorização prévia. Caso alguma informação derivada desse estudo for importante a você, todo esforço será realizado para informá-lo.

Pelo presente termo, você declara que foi informado(a), de forma clara e detalhada, sobre a presente pesquisa, e que teve suas dúvidas esclarecidas por _____. Declara ter sido

esclarecido que não receberá nenhuma remuneração financeira pela participação no estudo. Declara que foi informado da garantia de receber resposta ou esclarecimento sobre a pesquisa a ser realizada, bem como da liberdade de não participar do estudo e da possibilidade de desistir, em qualquer momento, da participação. Além disso, declara que assinou duas vias deste consentimento, e que uma ficou em seu poder.

Data: ___/___/_____

Assinatura do participante: _____

Eu expliquei a _____ os objetivos, riscos, benefícios e procedimentos necessários para esta pesquisa, e entreguei cópia deste termo de consentimento para o mesmo.

Data: ____/_____/_____

Nome:_____

Se o paciente é menor de18 anos:

Assinatura do responsável: _____

Obs.: *O presente documento, baseado no item IV das diretrizes e normas regulamentadoras para pesquisa em saúde, do Conselho Nacional de Saúde (Resolução 196/96), será assinada em duas vias, de igual valor, ficando uma via em poder do voluntário ou de seu responsável legal e outra com o pesquisador responsável.*

Anexo 4 – Composição da fórmula metabólica dos pacientes fenilcetonúricos (Milupa 2-secunda)

Informação nutricional

Conteúdo médio por 100 g		pku 2- secunda
Energia	kJ	1306
kcal		307
Proteína*	g	70
Aminoácidos	g	84
dos quais		
Arginina	g	2,8
Cistina	g	1,9
Histidina	g	1,9
Isoleucina	g	4,8
Leucina	g	7,9
Lisina	g	5,6
Metionina	g	1,9
Treonina	g	3,8
Triptofano	g	1,4
Tirosina	g	4,7
Valina	g	5,6
Alanina	g	3,4
Ácido aspártico	g	8,0
Ácido glutâmico	g	16,8
Glicina	g	1,9
Prolina	g	7,6
Serina	g	4,2
Carnitina	mg	150
Lipídeos	g	0
Carboidratos	g	6,8

** **Conversão:** 1 g Proteína = 1,2 g Amino ácidos = 17 kJ = 4 kcal*

Conteúdo médio por 100 g		pku 2- secunda
Minerais		
Sódio	mg	<3
Potássio	mg	1400
Cálcio	mg	1680
Magnésio	mg	350
Fósforo	mg	990
Cloreto	mg	<1
Ferro	mg	20
Elementos traço		
Zinco	mg	20
Cobre	mg	1,4
Iodo	µg	230
Manganês	mg	2,8
Cromo	µg	42
Fluoreto	mg	1,4
Molibdênio	µg	90
Selênio	µg	60
Vitaminas		
Vitamina A	mg	1,07
Vitamina D_3	µg	11
Vitamina E	mg	15,0
Vitamina K_1	µg	51
Vitamina B_1		1,8
Vitamina B_2	mg	2,2
Vitamina B_6	mg	2,5
Niacina	mg	13,1
Ácido fólico	µg	294
Biotina	µg	70
Vitamina B_{12}	µg	3,5
Ácido pantoténico	mg	8,2
Vitamina C	mg	105
Colina	mg	630
Mio-Inositol	mg	315

Ingredientes

L-aminoácidos sem fenilalanina

Anexo 5 – Parecer do Comitê de Ética em Pesquisa Animal da Universidade de Groningen, Holanda (traduzido e original)

Overview: Decision on Subproject

Date 21-6-2013

Description

1/ Condition
You have mentioned in the application a number of staff in the investigation. As stated previously by Danique van Vliet, a DAP account is needed before they can be added to the project. Also, Karen Anjema and Priscila Mazzola are not yet included in the DAP. We ask you to take care that these people apply for a DAP account. You can then at the basic application request for the people on this project.
Yours sincerely, on behalf of the DEC-RUG,
Dr. Jochum Prop

Decision [4] **approved** + condition

Validity [5] 2 years

Animals	**Species**		**Inbred**	**Estimated number for the entire subproject**	**Required number of animals**	**Allocated animals**
	Mice	C57Bl/J6	[1] Inbred	100-250	62	62 mice
	Mice	C57Bl/6-PAH(enu2)	[1] Inbred	100-250	59	59 mice

Original:

Overzicht: Besluiten deelproject

Datum 21-6-2013

Omschrijving 1/ **Voorwaarde**
U noemt in de aanvraag een aantal medewerkers aan het onderzoek. Zoals eerder aangegeven heeft Danique van Vliet een account in DAP nodig voordat ze aan het project kan worden toegevoegd. Ook Karen Anjema en Priscila Mazzola zijn nog niet in DAP opgenomen. We vragen u om er voor zorg te dragen dat deze personen een DAP-account aanvragen. U kunt daarna bij de basisaanvraag een verzoek indienen deze personen aan dit project te koppelen.

Met vriendelijke groeten, namens de DEC-RUG,

Dr. Jochum Prop

Besluit [4] akkoord + voorwaarden

Geldigheidsduur besluit [5] 2 jaar

Diersoorten projecten	**Diersoort stam**		**Inteelt**	**Geschat aantal voor hele deelproject**	**Benodigd aantal dieren**	**Toegewezen aantal dieren**
	Muizen	C57Bl/J6	[1] Inteelt	100-250	62	62 muis
	Muizen	C57Bl/6-PAH(enu2)	[1] Inteelt	100-250	59	59 muis