Classical homocystinuria, is it safe to exercise?

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ABSTRACT

Background

Cystationine β-synthase (CBS) deficiency is a genetic disorder characterized by severe hyperhomocysteinemia and thrombotic complications. In healthy individuals, physical exercise may result in a transient increase in plasma total homocysteine (tHcy) raising the possibility that exercise might be detrimental in CBS deficiency. Our main objective was to determine plasma tHcy kinetics in response to physical exercise in homocystinuria patients.

Methods

Six adult patients (2 males, 4 females) with homocystinuria and 6 age- and gender-matched controls completed a 30-min aerobic exercise of moderate-intensity with fixed power output (50 W for women and 100 W for men). Blood samples were drawn before, immediately, 180 min and 24 h after exercise. tHcy levels were determined by standard procedures; substrate oxidation and energy expenditure were measured using indirect calorimetry.

Results

Acute exercise was well tolerated and safe in patients and controls. During the exercise bout, heart rate and energy expenditure increased equally in both groups. tHcy levels were higher in patients compared to controls at all time points ($p < 0.05$). There was no significant effect of exercise on tHcy levels at any time point ($p = 0.36$). Although two patients with partial pyridoxine responsiveness presented higher homocysteine responses, their highest value remained below 55 µmol/l.

Conclusions

Overall metabolic responses to acute exercise were similar between homocystinuria patients and controls; specifically, exercise did not significantly change tHcy concentrations. Moderate physical exercise was well tolerated without any adverse event in our cohort of patients. Further studies are needed to identify the effects of different intensities and modes of exercise in larger cohorts of CBS patients with different levels of pyridoxine responsiveness.

1. Introduction

Cystathionine beta-synthase (CBS) deficiency, also known as classical homocystinuria (OMIM# 236200), is a rare inherited autosomal recessive disorder affecting methionine transsulfuration. The characteristic biochemical pattern consists of severely elevated plasma homocysteine (Hcy) and methionine and low-normal to reduced cysteine [1]. Hcy is a non-proteogenic amino acid produced in the liver from dietary methionine. CBS, a pyridoxine (vitamin B_6)-dependent enzyme, catalyzes the condensation of homocysteine with serine to form cystathionine, a precursor of cysteine. CBS deficiency impairs homocysteine conversion leading to plasma hyperhomocysteinemia [2]. CBS

deficiency occurs worldwide with variable prevalence across countries depending on ethnicity and a wide range of age at presentation. Clinical symptoms are variable affecting the ocular, musculoskeletal, vascular and central nervous systems [3]. Thromboembolism is the major cause of early death and morbidity [2]. Patients with pyridoxine-responsiveness have usually a milder phenotype [4,5].

In the general population, plasma Hcy is associated with an increased risk of venous and arterial thrombosis, cardiovascular and all-cause mortality [6–8]. Although plasma concentrations in this population are 10-folds lower than in untreated CBS deficient patients, these phenomena have been explained by impairment of vascular endothelial function [9,10], increase in oxidative stress, and impaired peripheral vasomotor response [11]. Thus, Hcy has been proposed as an independent cardiovascular risk factor [6,7,12,13], where each increase of 5 µmol/l in plasma homocysteine level increases the risk of coronary heart disease (CHD) events by approximately 20% independently from other CHD risk factors [14]. While regular exercise is recommended in individuals with CHD risk factors [15], the impact of physical activity on Hcy remains unclear. Some studies show a transient increase of Hcy during and/or after acute exercise in healthy subjects, other show no clear relationship between Hcy kinetics and exercise [16–18].

Although these findings suggesting that physical exercise results in increased plasma Hcy in the general population are controversial, they raise an important question in our clinics: could a bout of exercise be detrimental to patients with homocystinuria? There is currently no information on the effect of acute exercise on total Hcy levels in CBS deficient individuals. Consequently, recommendations are lacking in regards to exercise safety, duration, intensity and frequency in this specific population. The aim of this study was to 1) assess whether one acute bout of moderate-intensity exercise would increase Hcy in CBS deficient patients compared to healthy controls, 2) ascertain exercise safety and tolerance in CBS deficient patients and 3) evaluate metabolic adaptations using indirect calorimetry and plasma amino acid measurements.

2. Methods

2.1. Subjects and study design

Patients were recruited from the outpatient clinic of the Adult Metabolic Clinic, Division of genetic medicine at the Lausanne University Hospital (CHUV). To be included they had to be over 18 years of age with genetically confirmed CBS deficiency. For each patient, an age- and gender-matched healthy control was recruited. Exclusion criteria for all participants comprised any clinically unstable condition, any contraindication to moderate exercise, blood sampling or a recent participation in another clinical study. Patients were also excluded if their total plasmatic homocysteine (tHcy) concentration at the screening visit was above 100 µmol/l and above 20 µmol/l for controls. The study design consisted in one screening visit followed after inclusion by a pre−/post-acute exercise intervention comparison consisting in two visits (Fig. S1). Patients were required to continue their regular treatment and diet throughout the duration of the study.

The study protocol was approved by the ethics committee of the Canton of Vaud (Protocol# 2018-02400). All patients provided written informed consent for participation in this study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. This trial was registered at the U.S Clinical Trials Registry as NCT04021732.

2.2. Screening visit (visit 1)

Participants were invited to the screening visit after an overnight fast at their habitual outpatient clinic. After signing the informed consent, the visit comprised the medical interview, clinical examination and blood sampling to assess tHcy, vitamin B9 and B12. After estimating daily caloric requirements based on the Harris-Benedict equation, a trained dietician implemented an individualized isoenergetic diet including 1.2 g/kg/day of proteins. Participants were instructed to follow this diet for 3 days, starting 24 h before visit 2. To evaluate energy and protein intake, a 5 days food record was requested starting 3 days before visit 2. Participants were also instructed to refrain from any structured exercise for 7 days prior to visit 2 and abstain from consuming caffeine, tobacco and any drugs.

2.3. Exercise visit (visit 2) and follow up (visit 3)

On the day of visit 2, participants arrived to the clinical physiology lab (*Plateau Technique de Physiologie Humaine)* at 8:00 AM following a 12-h overnight fast. Upon arrival, weight was measured on a calibrated medical digital scale (Seca, Hamburg, Germany) in light clothes without shoes. Vital signs were collected after 10 min resting in the supine position. Compliance with the dietary and behavioral instructions described above was assessed and participants were asked to rest in bed without any stimuli for 30 min. This was followed by the resting metabolic evaluation with indirect calorimetry as specified below. A venous catheter was inserted into a forearm vein for repeated blood sampling. The pre-exercise blood sample was drawn (T0) and was followed by the 30-min exercise protocol described below. Immediately after the exercise bout, a blood sample was collected (T1). While recovering in the supine position under close surveillance a third blood sample was collected at +180 min since the start of the exercise bout (T2). After this time point, the participant was fed and discharged.

On the following day, participants referred to the outpatient clinic after an overnight fast. A clinical interview was performed to assess potential complications and all dietary records were collected. A last blood sample was taken at exactly +24 h since the start of the acute exercise bout (T3). A follow up call was performed 1-week post-exercise for a final check-up.

2.4. Resting metabolic rate and heart rate

Indirect calorimetry was performed using an open canopy system (Quark, Cosmed, Rome, Italy). Subjects were placed under the canopy while resting in bed in a thermally neutral environment. This was combined with resting heart rate (HR) recording using an integrated fingertip pulse meter (ipod, Nonin Medical Inc., Plymouth, MN). Subjects were told to close their eyes and try to sleep, refrain from fidgeting, and not to perform any type of activity (i.e., watching TV or reading). The duration of the test was between 30 min to assure a minimal steady state of 20 min with the first 10 min of data discarded. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were recorded every 10 s (Omnia software, Cosmed) and used for the computation of respiratory exchange ratio (RER=VCO_2/VO_2) also known as respiratory quotient at rest. Resting energy expenditure (EE) was calculated using Weir's equation: REE = [(3.9 × VO2) + (1.1 × VCO2)] [19]. Systemic rate of fat oxidation (Fat-ox) and carbohydrate oxidation (CHO-ox) were computed as previously described [20]. Protein oxidation rates were not measured based on the assumption that the amount of protein oxidized are quantitatively negligible compared to glucose and fatty acid oxidation [21].

2.5. Exercise protocol

The exercise test consisted of a constant 30-min submaximal effort on a calibrated electronically braked cycle ergometer (Excalibur; Lode B.V., Groningen, The Netherlands). For safety reasons, given that no previous data exist on the effect of acute exercise in CBS patient's, we decided to avoid maximal exercise testing and chose to use a single-stage fixed intensity submaximal exercise test with power output based on gender and fitness status as suggested from the American College of Sports

Medicine guidelines for exercise testing [15]. Given that all patients and controls were non-athletic, the chosen submaximal fixed power outputs were 100 W for men and 50 W for women. VCO_2 and VO_2 were measured breath-by-breath by indirect calorimetry (CPET, Cosmed, Rome, Italy) using fitted facemasks. Metabolic computations included exercise EE, RER, Fat-ox and CHO-ox as described above. Metabolic equivalents (MET) were used to compute relative exercise intensity taking into account each individual's VO_2 and body weight [15]. HR was measured continuously with a wireless monitor (ANT+, Garmin Canada Inc., Cochrane, Canada). Rate of perceived exertion (RPE) was ascertained each 5 min with the Borg scale [22]. Both HR and RER were closely monitored to remain within the limits of moderate intensity exercise defined as a HR range between 55 and 70% of the computed maximal HR and a RER < 1.1. Participants were instructed to share any discomfort experienced during the exercise test. Exercise would have been discontinued in case of HR above 85% of maximal HR, RER > 1.1, chest discomfort, breath shortness, sudden fatigue, nauseas or lightheadedness. None of the patients or controls required an early termination, thus all volunteers completed the exercise protocol.

2.6. Biological analysis and genotyping

Total plasma homocysteine (tHcy) was measured on a Zorbax Eclipse XDB-C18 column (Agilent) on an Acquity UPLC™ system (Waters, Milford, USA). Free plasma amino acids were measured by LCMS using an Acquity UPLC H-Class system (Waters, USA). Molecular studies of CBS gene were done by Next Generation Sequencing (NGS; Ion S5, Themofisher) and results confirmed by bidirectional fluorescent direct sequencing (Sanger Sequencing; 3500 Genetic Analyzer, Thermofisher).

2.7. Data management and statistical analyses

Unless specified, data are expressed as mean ± SEM. Statistical analyses and figures were done using Prism version 8 for MacOs (GraphPad Software LLC, San Diego, CA). Comparisons between groups were tested with the non-parametric Kolmogorov-Smirnov test. Repeated measures two-way ANOVA with the Geisser-Greenhouse correction were used to assess tHcy and amino acids differences across groups (2 levels for the between factor) and time points (4 levels for the within factor). Post hoc analyses were performed using the Tukey's HSD method to control for multiple comparisons. Repeated measures two-way ANOVA with the Geisser-Greenhouse correction were used to assess metabolic adaptations between groups (2 levels for the between factor) and two conditions (at rest and during exercise, 2 levels for the within factor). Post hoc analyses were performed using the Sidak correction for multiple comparisons. Associations were assessed using Spearman's rank-order correlation. Two tailed tests were used for all analyses. The statistical significance level was set at 0.05.

3. Results

3.1. Subjects characteristics

Six patients with confirmed homocystinuria were recruited in this study, 4 females and 2 males. Genetic diagnostics, screening visit tHcy, classification of pyridoxine (B6) responsiveness according to the European network and registry for homocystinurias and methylation defects (*E*-HOD registry) [23] and clinical presentation are indicated in Table 1. Two patients (P1 and P5) were B6 partial responders while the other patients were extreme (P2) or full responders (P3, P4 and P6).

All patients were on daily oral vitamin B6 (doses ranging from 40 to 300 mg/day) and vitamin B9 (0.4 to 7.5 mg/day). B6 partial responders (P1 and P5) were also treated with oral Betaine (respectively 12 and 8 g/day) as well as methionine-free L-amino acid mixture (respectively 120 and 90 g/day, containing 84 and 63 g of protein) and a methionine-restricted diet.

Per design, age and gender were not different between patients and controls. Patients had on average higher tHcy than controls (Table 1).

3.2. Kinetic of plasma homocysteine with exercise

At each time point, tHcy levels were higher in patients compared to controls with non-parametric comparisons (Fig. 1A; all $p < 0.05$). When analyzing the two groups across time points (2×4 repeated measures ANOVA), having the disease accounted for 28.3% of the total variance after adjusting for the matched design ($p = 0.07$). There was no significant effect of time ($p = 0.36$) and no interaction effect ($p = 0.45$). Individual responses to the acute bout of exercise are presented in Fig. 1B. Although the time point with a larger modification from T0 was T2, as witnessed by the larger Y axis scale in Fig. 1D, no significant differences were evidenced at any of the time points. Strong associations were present between T0 and all post exercise points (Fig. 1G–I). The strength of these correlations was explained by the patient's results.

Important individual tHcy variations were observed for the two patients with partial B6 responsiveness (P1 and P5). P1 tHcy went from 36.4 μmol/l at T0, to 38.6 at T1, 50.5 at T2 and 35 at T3. P5 tHcy went from 13.3 μmol/l at T0, to 18 at T1, 51.6 at T2 and finally 20.6 at T3. Thus for these two patients, percent changes between the lower and the higher tHcy concentrations were respectively 39% and 288% (Fig. 1F). Importantly at each time point, tHcy level remained close to the target limit for treated patients (< 50 μmol/l).

Another interesting observation was made for a fully vitamin B6 responsive patient (P3) who had a screening visit tHcy of 35.3 μmol/l. This patient's tHcy levels were high to start with and remained in the same range across the four time points (111.7, 114.7, 115.5 and 119.2 μmol/l). Very probably, would the experimenters not be blinded at that time of the day, the exercise test would have been cancelled. Indeed, for methodological reasons although each sample was processed and centrifuged immediately after each blood drawn; chromatography was done overnight and the results were available on the next day. A posteriori, no explanation was obtained to explain such an elevated tHcy in this patient who was followed with frequent visits afterwards.

3.3. Tolerance to exercise and metabolic adaptations

RPE was similar in patients and controls (Fig. 2A). Exercise intensity was within the submaximal target and similar in both groups as witnessed by MET (Fig. 2B). HR and EE increased with exercise equally in both groups (Fig. 2C–E). Respiratory exchange ratios reflected expected adaptations for submaximal exercise intensity without significant differences between groups (Fig. 2F). From rest to moderate exercise, the contribution of energy source shifted as expected with a reduction of energy from fat oxidation. Both groups responded to the exercise stimulus but with a higher difference in patients than controls explaining the significant interaction (Fig. 2G).

Plasmatic concentrations of the different amino acids involved in CBS and creatine metabolism (ornithine, glycine, arginine and methionine) as well as branched-chain amino acids (leucine, isoleucine and valine) were not significantly different across groups or time points (supplementary Table S1). This was comparable to a previous study with healthy volunteers [24].

When analyzing the percent change differences in methionine between baseline and all post exercise time points, we observed that two patients were explaining the large variability in this group, these were the two patients with partial B6 responsiveness (P1 and P5). As an illustration of this point, if we take the difference between T0 and T3, which is the larger percent change in methionine, this was on average $-3.428 \pm 8.188\%$ in patients and $-8.629 \pm 2.111\%$ in controls ($p = 0.56$). All participants had values ranging from -3.846 to -21.849%, while the change for P1 was of $+3.625\%$ and P5 of $+32.653\%$.

Protein intake, including formula and medical food, was significantly lower in patients than controls when expressed relative to body weight

Table 1
Subject inclusion criteria and clinical presentation.

ID	Group	Gene variant	Gender	Age (years)	Plasma tHcy (μmol/l)	Plasma vitamin B9 (nmol/l)[a]	Plasma vitamin B12 (pmol/l)	B6 responsive	Vitamin therapy (B6, B9, B12)	BP >140/90 mmHg	Epilepsy	Cognitive delay	CV event	Eye event	Bone event	Amino acid mixture
P1	CBS	c.1058C>T hom	M	21	74.6	45.4	588	PR	Y	N	N	Y	N	Y	Y	Y
P2	CBS	c.341C>T hom	F	26	9	45.4	426	ER	Y	N	N	N	N	N	Y	N
P3	CBS	c.502G>T/ c.770C>T	F	25	35.3	45.4	1476	FR	Y	N	N	N	Y	Y	Y	N
P4	CBS	c.502G>T/ c.770C>T	F	28	18.1	45.4	616	FR	Y	N	N	N	N	N	Y	N
P5	CBS	c.19dup/ c.805˙807del	M	26	28.9	45.4	1091	PR	Y	N	N	Y	N	Y	Y	Y
P6	CBS	c.833T>C hom	F	50	21.2	35.5	469	FR	Y	N	Y	N	Y	N	N	N
Median			4F/ 2M	26.0	25.05	45.40	602.0		6Y	6N	1Y/5N	2Y/4N	2Y/4N	3Y/3N	5Y/1N	2Y/4N
Mean (±SD)				29.3 (10.4)	31.18 (23.11)	43.75 (4.04)	777.7 (416.4)									
C7	Ctrl		M	22	9.8	20.4	438	NA	N	N	N	N	N	N	N	N
C8	Ctrl		F	25	9.5	16	271	NA	N	N	N	N	N	N	N	N
C9	Ctrl		F	23	7.8	20.7	262	NA	N	N	N	N	N	N	N	N
C10	Ctrl		F	32	14.4	7.3	389	NA	N	N	N	N	N	N	N	N
C11	Ctrl		M	25	11.7	18.5	347	NA	N	N	N	N	N	N	N	N
C12	Ctrl		F	49	12.4	14.5	778	NA	N	N	N	N	N	N	N	N
Median			4F/ 2M	25.0	10.75	17.25	368.0		6N	6N	6N	6N	6N	6N	6N	6N
Mean (±SD)				29.3 (10.3)	10.93 (2.36)	16.23 (5.01)	414.2 (190.7)									
Group comparison exact p-value				0.896	0.026	0.002	0.143									

Abbreviations: CBS, cystathionine-β synthase; Ctrl: control; CV: cardio-vascular; del: deletion; dup: duplication; ER: extreme B6 responsiveness; F: female; hom: homozygous; FR: full B6 responsiveness; M: man; mmHg: millimeter of mercury; N: no; NA: not applicable; PR: partial B6 responsiveness, tHcy: total plasmatic homocysteine, Y: yes.

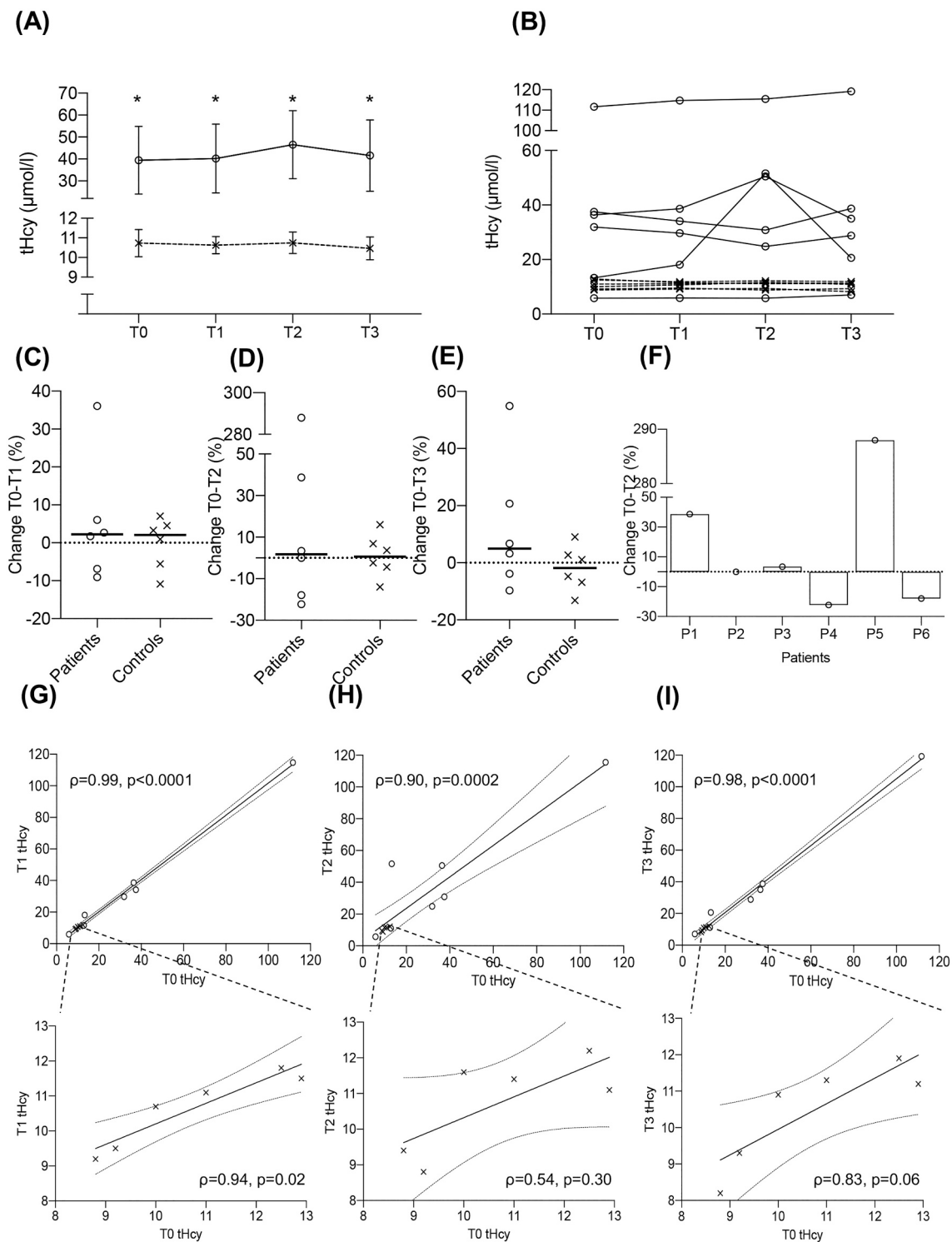[a] For vitamin B9, measurement upper limit was >45.4 pmol/l.

Fig. 1. Total homocysteine response to acute exercise.
(A) Group tHcy kinetics across time points (mean and SEM). (B) Individual tHcy across time points. (C-E) Percent change from baseline. Each group median is represented by a straight line. (F) Patients (P#) variability at the maximal change. (G-I) Correlations between baseline and each time point across all subjects. Magnifications correspond to the control group. ρ is the Spearman's correlation coefficient. For all panels, open circles are patients, crosses are controls, *$p < 0.05$. tHcy is total homocysteine.

(0.95 g/d/kg and 1.30 g/d/kg of body weight respectively) (Fig. 3A–B). Overall energy intake was similar in both groups with lower recorded daily consumptions compared to the nutritional requirements (Fig. 3C–D).

4. Discussion

To the best of our knowledge, this is the first study to evaluate exercise safety in CBS patients. To do so, we analyzed patient's tHcy response to a controlled moderate-intensity aerobic exercise bout with a 24-h follow up. We observed 1) that exercise did not change significantly
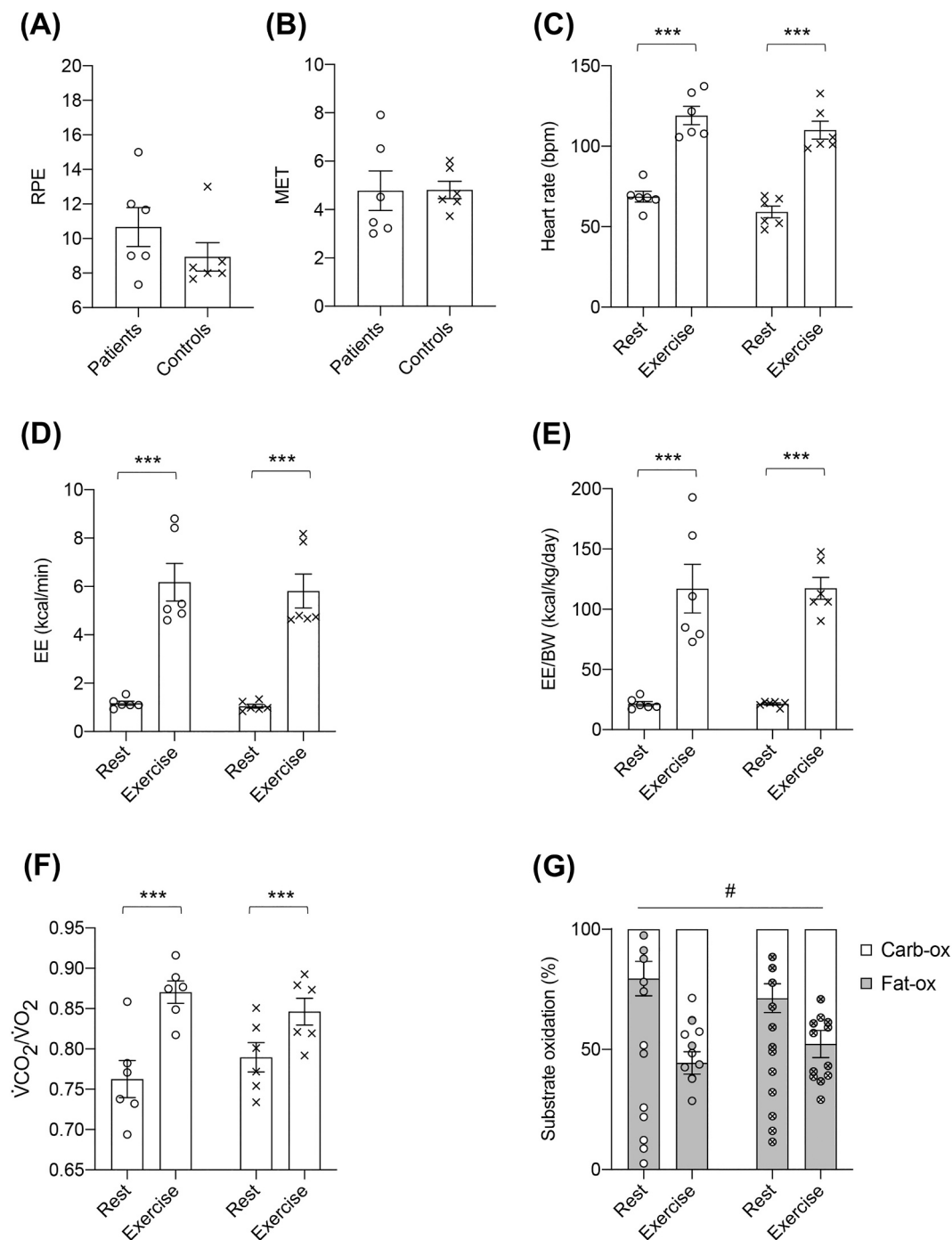
Fig. 2. Exercise tolerance and metabolic adaptations.

(A) Mean rate of perceived exercise (RPE) during the exercise bout. (B) Mean metabolic equivalents (MET) during the exercise bout. (C) Mean heart rate at rest and during the exercise bout. (D-E) Energy expenditure (EE) in absolute units and relative to body weight (BW) at rest and during the exercise bout. (F) Respiratory exchange ratios at rest and during the exercise bout. (G) Relative fat and carbohydrate (Carb) oxidation at rest and during the exercise bout. Open circles are patients, crosses (A-F) or crossed circles (G) are controls. Bars are mean, error bars are SEM, #significant interaction, *significant effect of time, 1 symbol $p < 0.05$, 3 symbols $p < 0.0001$.

tHcy concentrations in CBS patients and controls matched by age and gender, 2) that one bout of 30 min exercise was well tolerated and safe in CBS patients and 3) that metabolic responses to exercise were similar between CBS patients and controls. However, our results point to a potential specificity of two patients with partial B6 responsiveness in which the response to exercise seemed different from the other CBS patients with full responsiveness.

An important factor to consider is that we did not perform a maximal

exercise testing in each subject to allow the prescription of relative exercise intensity to each person's physical condition. Indeed, this was not possible given that no previous data was available to ascertain safety in these patients. Nevertheless, the results of energy expenditure and metabolic equivalents measured during the acute exercise bout prove that with the chosen fixed submaximal power outputs, similar exercise intensities were reached in both groups and across genders.

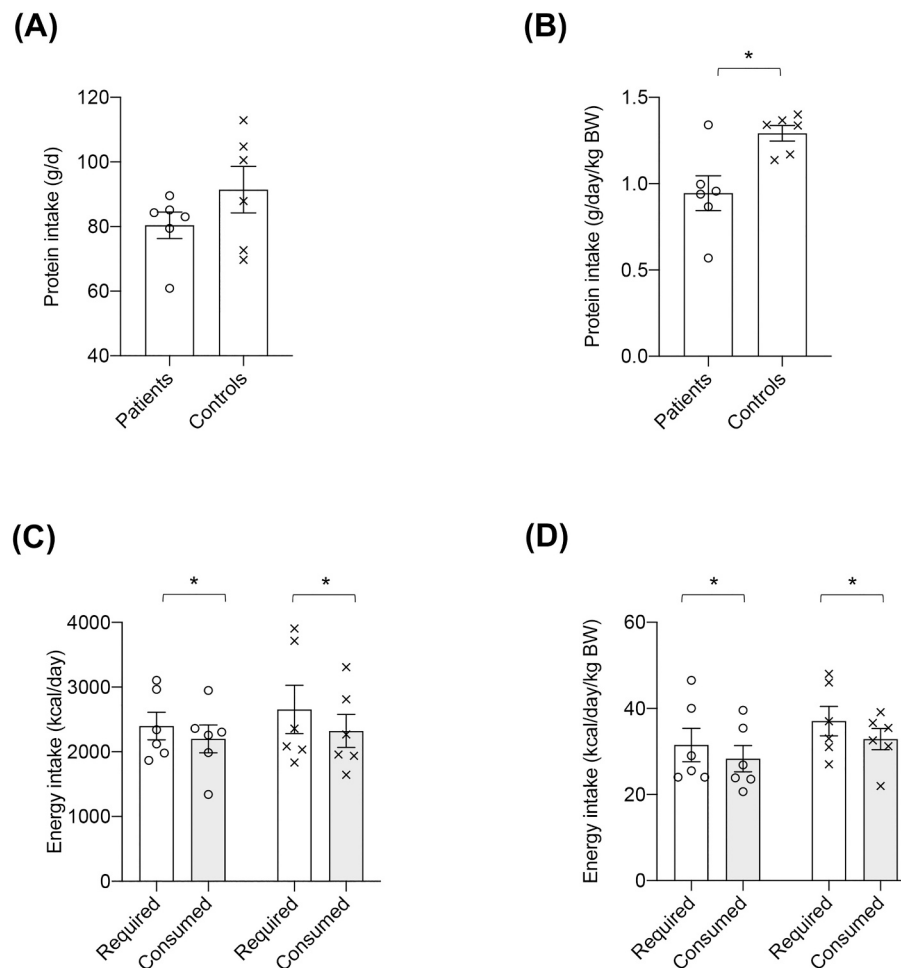Our results were measured in all CBS patients followed in the

Fig. 3. Nutritional adherence measured during the 3-days preceding the exercise test.
(A–B) Total protein intake (natural protein and methionine-free L-amino acid mixture) in absolute units and relative to body weight. (C—D) Energy intake requirements and consumption in absolute units and relative to body weight. Circles are patients, crosses are controls. Bars are mean, error bars are SEM, *$p < 0.05$.

Division of Genetic Medicine who fulfilled the inclusion criteria. The small sample size is a limitation in this study, thus making comparisons between subgroups of patients not possible. Importantly our data suggests individual variations and also the fact that the magnitude of change in tHcy after exercise does not depend on baseline values since the patient with the highest tHcy showed almost no variation after exercise and the patient that showed the greatest variations was within target concentrations of tHcy.

The genotype of patient 1 is known to be associated with partial B6 responsiveness [25], while that of patient 5 suggests complete loss of function and thus probably also associated with limited response to B6. Indeed, patient 1 and patient 5 were the ones with the highest increase in tHcy elevation from baseline to 180 min post exercise. Further, these two patients were the only ones presenting positive methionine percent changes post-exercise. This raises the question of a genotype-phenotype relationship with regard to the effect of exercise on Hcy levels as well as the mechanisms driving this effect.

The exact mechanism by which acute exercise affects Hcy concentrations is unknown. Findings in intervention studies remain controversial with works showing increases in Hcy and others showing decreases or no effects, in different study populations or exercise protocols [18,24,26–28]. Among the hypotheses explored to explain the impact of acute exercise on Hcy, previous authors have suggested an accelerated protein catabolism [29], increases in methionine leading to an increased methionine transsulfuration [30], an increased demand for creatine synthesis which requires methylated substrates [31], a higher

production of free radicals influencing thiol-disulfide exchange and thiol redox reactions [32] and finally an increased cellular export of the reduced form of Hcy which is exported to the extracellular space when the remethylation and transsulfuration pathways are inefficient or saturated [27]. In our study methionine levels, branched-chain amino acids and also amino acids involved in the pathways of creatine (arginine, ornithine and glycine) remained unchanged after 30 min of moderate intensity aerobic exercise.

In the current clinical management guidelines for CBS patients, there are no recommendations in terms of habitual physical activity and exercise prescription [1,2]. Given the multitude of epidemiological studies in the general population pointing to the relationship between levels of Hcy and cardiovascular events [6,7,12], as well as to the increase of tHcy after acute exercise [16–18], more studies are needed to understand the long-term effects of exercise as well as different exercise intensities and modes in CBS patients.

In addition to homocysteinemia, CBS patients may present with other medical complications, such as body habitus (i.e., dolichosteomelia), osteoporosis, various bone deformities and/or ophthalmological complications (i.e., ectopia lentis) [1]. Furthermore, for patients with history of hypercoagulability, a special attention should be brought to the possible transient effect of exercise in increasing the hypercoagulable state. As for all individuals with disabilities or specific health conditions, exercise prescription for CBS patient's should be planned individually, addressing (1) the patient's phenotype, compliance with treatment, and usual homocysteine levels, and (2) the type and intensity

of exercise, particularly those sports that may increase intravascular pressure, the risk of traumatic lens dislocation and/or fracture.

5. Conclusion

In summary, our study shows that an acute bout of moderate aerobic exercise was safe and well tolerated in our cohort of CBS patients and had no significant impact on tHcy concentrations. Future studies are needed to confirm exercise safety and tolerance in a larger number of patients as well as to evaluate the impact of gender, B6-responsiveness, genotype and underlying treatments in tHcy response to exercise. We hope that these observations will confirm that physical activity can be encouraged in CBS patients and considered in further CBS guidelines.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2021.100746.

Author's contributions

FA, CT and ASF contributed to the conception and design of the study. ATT, GVW, FA and CT implemented and/or performed the experiments. RP planed and analyzed all dietary records. ATT, GVW, FA and CT organized the data, performed the statistical analyses, and created the figures. OB and BCX supervised laboratory analysis. ATT, FA and CT wrote the first draft. All authors contributed to the manuscript revision, read and approved the submitted version. FA and CT are the guarantors for the article who accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

Availability of data and material statement

All data was generated during the study and is available upon request.

Declaration of competing interest

The authors declare no potential conflict of interest.

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