



**Muscular contraction frequency does not affect plasma
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 sedentary males**

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Abstract

Acute exercise seems to increase total plasma homocysteine (tHcy). That is related to cardiovascular risk, therefore it is important to understand the determinants of its response to all types of exercise. The aim of this study was to examine the impact of cycling at two different rates of muscle contraction on the complete tHcy kinetics. Eight young sedentary males were required to complete two isocaloric (400 kcal) acute exercise trials at 50% $\text{VO}_{2\text{peak}}$ on separate occasions at 50 or 80 revolutions per minute (rpm). Blood samples were drawn at different points before (pre4 and pre0 h), during (exer10, exer20, exer30, exer45, and exer60 min), and after exercise (post0 and post19 h). Dietary and lifestyle factors were controlled during the research. Maximum tHcy occurred during exercise, for both conditions (50 rpm: $11.4 \pm 2.7 \mu\text{mol/L}$; 80 rpm: $10.8 \pm 3.2 \mu\text{mol/L}$). From this point onwards tHcy declined until the cessation of exercise and continued descending below pre exercise values at 19h post exercise ($p < 0.05$). No hyperhomocysteinemia were observed at any sampling point in both trials. In conclusion, the different muscular contraction frequency during exercise has no impact on tHcy during an acute bout of exercise in sedentary individuals, when at least 400 kcal are spent during exercise and the nutritional status for folate, B_{12} , and B_6 is adequate. This information is relevant to further inform healthy exercise prescription, not only in terms of duration and intensity of exercise, but also taking into account frequency of contraction.

Keywords: Homocysteine kinetics; Exercise prescription; frequency of contraction.

Introduction

Homocysteine (tHcy) is a non-protein sulphur-containing amino acid whose elevated plasma concentration constitutes an independent risk of cardiovascular disease (CVD), via endothelial dysfunction, oxidative stress mechanisms and inflammatory vascular processes (Mangoni and Woodman 2011; Moat 2008; Refsum et al. 1998; Veeranna et al. 2011). tHcy is strongly influenced by genetic and lifestyle factors such as diet, especially vitamins B₁₂, B₆ and folic acid (Chrysoshoou et al. 2004). However, the impact of exercise on tHcy remains unclear. A recent review (Maroto-Sánchez et al. 2016) identifies that acute exercise induces increases in tHcy. However, no consensus exists regarding chronic exercise due to a large variety of exercise interventions, with different intensities, duration and mode of exercise. Deminice et al. (2016), in a meta-analysis, also reported that acute exercise increases plasma Hcy concentration independent of duration or intensity of the exercise performed and, at the same time, regular resistance training can decrease plasma Hcy concentration, though this was not observed after aerobic exercise training.

We have previously reported that acute exercise at high and low intensity in healthy sedentary individuals causes a transient increase of plasma tHcy proportional to the intensity of exercise, but not hyperhomocysteinemia (Iglesias-Gutiérrez et al. 2012). According to the American Heart Association (AHA) advisory statement (Malinow et al. 1999) normal homocysteine concentrations range from 5-15 µmol/L. Deminice et al. (2016) also provided evidence that the elevation of tHcy as a consequence of an acute bout of exercise does not cause hyperhomocysteinemia. However, we have previously shown a 26% increase in tHcy (Iglesias-Gutiérrez et al. 2012), which could have physiological or clinical relevance.

The underlying mechanism remains unclear, although our previous data suggest that it could be related to increased energy expenditure (Iglesias-Gutiérrez et al. 2012), since the maximal tHcy concentration occurred during exercise. At the same time, we have also showed that it could be related to changes in renal function in response to exercise (Venta et al. 2009). In this sense, increased tHcy is not as a direct result of exercise, but could be an indirect consequence of the whole body homeostatic perturbation in response to exercise.

Plasma concentration of homocysteine is related to cardiovascular risk and exercise is one of the interventions most widely recommended to reduce CVD risk. On the other hand, an important proportion of European citizens (59%) never exercises or seldom do so (Eurobarometer, 2014). Therefore, it is important to understand the determinants of the tHcy response to exercise with the aim of optimizing exercise prescription for the prevention of CVD, especially in sedentary people. These recommendations should not be expressed only in terms of duration and intensity of exercise; since some authors have described that the frequency of contraction or the velocity of shortening of skeletal muscle can also affect energy expenditure and substrate utilization (Kang et al. 2004) and, in consequence, could also influence the plasma concentration of tHcy.

Therefore, the aim of this study was to assess the effect of frequency of muscle contraction in tHcy kinetics. In this sense, we examined the impact of cycling at two different rates/frequencies of muscle contraction (50 and 80 rpm), on the complete tHcy kinetics in sedentary individuals, when dietary and lifestyle factors were controlled.

Subjects and methods

Ethics statement

All experimental procedures were approved by the Dublin City University Research Ethics Committee in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Subjects

Participants were recruited using flyers posted on campus and e-mail sent to the student and staff mailing lists. Prior to participation, each volunteer underwent a thorough medical screening to determine eligibility. Eight young males (19-32 yr) who were healthy, and had been physically inactive for at least 6 months, took part in the study.

Anthropometry and aerobic capacity assessment

On their first visit, the participants had their body composition assessed and their peak oxygen uptake ($\text{VO}_{2\text{peak}}$) determined at the Metabolic Physiology Research Unit.

Height and body mass (BM) were measured using a combined medical scale (model 778, Seca Ltd, Hamburg, Germany; precision 0.1 cm for height and 0.1 kg for weight) and the body mass index (BMI) was calculated. Body density was calculated by the method of Jackson and Pollock (1978), based on the sum of seven skinfolds (triceps, subscapular, mid-axillary, pectoral, suprailiac, abdominal, thigh) measured with Harpenden skinfold calipers (Holtain Ltd., Crosswell, Crymych, Pembrokeshire, UK). Percentage body fat (%BF) was calculated using the equation of Siri (1961). $\text{VO}_{2\text{peak}}$ was determined by indirect calorimetry (Vmax 29C, SensorMedics, Yorba Linda, CA, USA) using an incremental protocol on an electronically braked stationary cycle ergometer (Ergoline 900, SensorMedics). Subjects began cycling at 100 W for five minutes to warm-up and the power output was increased by 50 W every two minutes thereafter until volitional fatigue. Oxygen uptake was considered to have peaked if two of the following criteria were met: (i) a levelling off of VO_2 with increasing power output (increase of less than $2 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), (ii) a HR within 10 beats of the age

predicted HR_{max} ($220 \text{ bpm} - \text{age in years}$), (iii) a RER greater than 1.10. Between four and seven days later, each participant returned to the laboratory to perform sub-maximal exercise to verify the power outputs required to elicit 50% VO_{2peak} . The power output required to elicit a given percentage of VO_{2peak} was estimated based on the linear relationship between oxygen uptake (y-axis) and power output (x-axis). For a given percentage of VO_{2peak} , the corresponding power output was estimated by solving for x using the linear function, $y=mx+c$, where y is VO_2 , x is power output, m is the slope of the relationship between VO_2 and power output and c is the y-axis intercept. Subjects cycled for 10 min at a number of different power outputs, starting at 15 W below the predicted power output and adjusted thereafter until the correct percentage of VO_{2peak} was maintained in steady state. This trial took approximately 30-40 minutes.

Experimental design

In a randomized crossover design (Figure 1), subjects were required to exercise at the workload that corresponds to 50% of their VO_{2peak} (determined from the maximal test) at either 50 or 80 revolutions per minute (rpm) on an electronically braked stationary cycle ergometer (Ergoline 900, SensorMedics). Each exercise bout was separated by 7 days and required the participants to expend 400 kcal, determined by indirect calorimetry monitored on a minute-by-minute basis and calculated using the equations of Weir et al (1949). The duration of both exercise trials to expend 400 kcal were not significantly different (50rpm: $58.5 \pm 3.1 \text{ min}$; 80rpm: $52.6 \pm 3.0 \text{ min}$)

Dietary control

Participants were asked to keep a one-day food diary on the day prior to the first experimental trial and asked to repeat the content and pattern of dietary intake on the day preceding the second experimental trial. They were asked to abstain from caffeine and alcohol consumption for 24 h prior to testing, and none of them reported the use of

vitamin supplements. Dietary records were analyzed using a nutrient analysis program (WISP, Tinuviel Software, UK) (Food Standards Agency 2002).

The dietary intake during each experimental trial was standardized in terms of food type and macronutrient composition, and individualized for each participant in terms of total energy content. The individual energy requirements were calculated using the Harris-Benedict equation (Harris and Benedict 1919), multiplied by a physical activity factor (1.4), and with 400 kcal added to account for the exercise trial (Durnin 1996). Three meals, each with 30% of predicted total energy expenditure, were provided, with the remaining 10% energy supplied with an evening snack. In accordance with current Recommended Dietary Allowances (RDA) (FSAI 1999) and Acceptable Macronutrient Distribution Range (AMDR) (Food and Nutrition Board 2005), these meals were designed to provide 45-65%, 20-35%, and 10-35% of total energy intake from carbohydrate (CHO), fat and protein sources, respectively, as well as an adequate intake of folate, vitamin B12, and vitamin B6.

Experimental protocol

For the experimental trials subjects reported to the laboratory after an overnight fast and had a blood sample taken (pre4 point). Subjects then consumed a standardized breakfast and remained in the laboratory for 4 h, at which point they started the exercise bout. Immediately before exercise another blood sample was taken (pre0). Participants then began cycling on a stationary ergometer (cadence at 50 or 80 rpm) and continued until 400 kcal were expended. If participants deviated from the designated cadence by more than 5RPM they were encouraged by the experimenters to adopt the correct cadence, which was easily accommodated at sub-maximal exercise. During exercise blood samples were drawn every 10-15 min (exer10, exer20, and exer30...) until exhaustion, via catheter placed in the antecubital vein. Sampling points were the same for both

exercise trials, though a smaller number of subjects reached exer50 (n=5 for 50rpm; n=2 for 80rpm) and exer60 (n=2 for 50rpm; n=1 for 80 rpm) as they had already expended 400kcal. The total volume of blood taken during the trial was less than 40 ml. Another blood sample was drawn immediately at the end of exercise (post0). After 3 hours, subjects were provided with a standard meal, after which they were free to leave the laboratory. Another meal and snack were provided to eat later that evening, and water intake was permitted to their satisfaction. No other food or beverages were allowed. The following morning subjects returned to the laboratory at the same time as the previous day, after an overnight fast, for a final blood sample taken at 19 h after the cessation of exercise (post19).

Blood samples (4 ml) were collected in vacutainers (No Additive (Z), Becton Dickinson, Franklin Lakes, NJ), kept at room temperature for 20 min, and then centrifuged at 3000 rpm for 15 min at 4°C. The serum was stored at -80°C for later analysis.

Biochemical determinations

tHcy were determined by HPLC using a commercially available kit (Chromsystems Instruments & Chemicals GmbH, Munich, Germany) and fluorescent detection, where a derivatization process of the sample takes place. Once the sample is prepared, 50 µl are injected into the HPLC and fluorescence is measured at 385 nm excitation and 515 nm emission. Folate and vitamin B₁₂ serum concentrations were measured using an ELECSYS system (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) based on an electrochemiluminescence immunoassay (ECLIA).

Kinetic parameters

Area under the curve (AUC), maximum concentration (C_{max}) and time to maximum concentration (T_{max}), of tHcy, folate and vitamin B₁₂ in serum were calculated by a

noncompartmental method, assuming apparent first-order kinetic in the terminal phase of elimination, evidenced by linearity of a semi-log plot. Pharmacokinetic parameters have been obtained by means of an Excel spreadsheet (Aguilar-Ros et al. 2014).

Statistical analysis

Normality of variables was tested using Shapiro Wilk's test. In light of the results obtained, descriptive values are presented as mean \pm standard deviation or median (interquartile range).

T-Student test for related samples was performed to analyze differences between contraction frequencies at the same sampling points, as well as between sampling points at the same contraction frequencies, for the variables considered. In order to check the relationship among the different variables at each time point, a full correlation analysis was made by using Pearson correlation coefficient.

The level of significance was set at $p < 0.05$ for all analyses; Descriptive and analytical statistical analyses were performed using IBM® SPSS® Statistics, version 20.0 (Somers, NY).

Results

Anthropometric characteristics (height, body mass, BMI, % body fat) and aerobic capacity (VO_{2peak}) of volunteers are presented in Table 1, showing a profile of young, non-obese, sedentary males, with low aerobic capacity.

The graphic representation of the serum clearance kinetics of tHcy is shown in Figure 2. No statistical differences in tHcy were found at baseline (pre4 and pre0) between 50 rpm and 80 rpm. tHcy response to 50 and 80 rpm was similar and no statistically significant differences were observed at any sampling point between trials. An increase in tHcy was observed in both cases, reaching C_{max} ($11.4 \pm 2.7 \mu\text{mol/L}$ for 50 rpm and

10.8 ± 3.2 µmol/L for 80 rpm) after 23 and 14 min, respectively. This represents a 16.3% and 10.2% increase. No statistically significant differences were found for C_{max} and T_{max} at 50 vs. 80 rpm. From this point onwards tHcy progressively decreased and continued after the cessation of exercise. At post19, tHcy was not different from pre4 but was significant lower than pre0 value in 80 rpm exercise. At 50 rpm of exercise, tHcy significantly decreased in post19 point with respect to pre4 point. Surprisingly, the increase observed in tHcy during 50 rpm was slightly higher. No values of hyperhomocysteinemia (tHcy > 15 µmol/L) (Selhub 1999; Welch and Loscalzo 1998) were observed at any sampling point in both exercise trials.

Table 2 shows data relative to area under curve (AUC) between 0 and 30 minutes of exercise (coincident duration time in all exercises) together with C_{max} and T_{max}. Table 2 also shows differences between the tHcy concentration at points post0-pre0 (representative of an acute bout of exercise) and difference between points post19-pre4 (representative of recovery post exercise). There were no between trial differences in these parameters except a significantly lower tHcy at 80rpm at post0-pre0.

Table 3 shows the serum concentration of folate and vitamin B12 at every sample point. Serum folate concentration increased after the exercise (post0 and post19) with respect to the other points and this change is significant in the 80rpm exercise. We also observed a higher C_{max} and T_{max} at 80 rpm exercise compared with 50 rpm exercise (Table 4). There were significant differences between trials at pre4 and post 19 for folate and at post 19 for vitamin B₁₂. In both vitamins the higher concentration was found in the 80 rpm exercise. The serum concentration of the vitamins were adequate at every sampling point (folate: >3.4 nmol/L (>1.5 ng/ml); vitamin B12: >120 pmol/L (>162 pg/ml) (Joubert and Manore 2008; Brito et al. 2012).

The correlation analysis among the serum concentration of tHcy and the different vitamins analyzed showed a large variability and no relevant tendency was observed.

Energy, macronutrient, folate, vitamin B₁₂, and vitamin B₆ intake during the experimental trials, compared to the RDA and AMDR, are shown in Table 5. The dietary intake of the volunteers was in accordance with the proposed targets for energy, macronutrients, and vitamins.

Discussion

We examined the impact of exercise at two different rates of muscle contraction (high and low cycle cadence: 80 and 50 rpm) on the complete tHcy kinetics in an energy expenditure and intensity-matched experiment, carried out in sedentary individuals with low aerobic capacity, controlling dietary and lifestyle factors.

tHcy response to cycling at 50% VO_{2peak} at 50 and 80 rpm was similar and no statistically significant differences were observed at any sampling point between trials. Therefore, no effect of frequency of contraction on tHcy kinetics was observed when the exercise was carried out at the same intensity and energy expenditure. An increase in tHcy during exercise was observed in both trials, reaching C_{max} during exercise, although hyperhomocysteinemia was not observed at any time point. tHcy showed a modest reduction 19h post exercise in both conditions suggesting a positive effect of exercise in the recovery period.

Souza-Silva and Gonçalves da Mota (2014) in a recent revision asserted that it would be prudent to discern the types of exercise that reduce homocysteine levels and therefore potentially reduce the risk of blood vessel damage. According to these authors, and others (Maroto-Sánchez et al. 2016), homocysteine was lower in patients with the greatest amount of daily physical activity.

Several studies have reported increased homocysteine levels immediately after an acute bout of exercise. Deminice et al. (2016), in a recent meta-analysis, showed that this elevation is independent of exercise intensity, although changes appear to be more sensitive to long-term exercise of low to moderate intensity than short-term exercise of high intensity, i. e. increased homocysteine induced by exercise was significantly associated with volume of exercise. On the other hand, the aerobic exercise training analysis did not demonstrate a significant impact on homocysteine levels in the blood, whereas resistance training was found to decrease plasma tHcy.

Taking together our results and those of other authors aforementioned, we could deduce that the type and volume of exercise could influence the concentration of tHcy rather than the intensity or frequency of contraction.

Kinetic studies that include multiple sampling points both pre, during and post exercise regarding Hcy metabolism such as those reported here are recommended in order to clarify the mechanisms through which tHcy is impacted by exercise (Deminice et al. 2016). Our studies are unique in providing a complete Hcy kinetics perspective pre, during and post exercise. Our previous study showed a transitory increase in tHcy levels during exercise and a recovery of basal levels 19 hours after the exercise (Iglesias-Gutiérrez et al. 2012). Also, in the present study, tHcy was significantly lower at post19 compared with pre4, indicating a recovery of tHcy within 19 hours of exercise. Understanding this response could be relevant in terms of further informing healthy exercise prescription, since this could mean that acute exercise, even performed by sedentary people, would not have the deleterious effects previously reported by several authors. This could also elucidate the mechanism through which reduced circulating Hcy is observed in those who are more physically active (Maroto-Sánchez et al. 2016). Therefore, the 24 hour kinetic profile investigated in our studies indicate a post exercise

recovery in response to exercise of varying intensity and contraction frequency, with no negative effects on tHcy as independent risk factor for CVD. Deminice et al. (2016) support the view that increased tHcy during acute exercise might not be deemed a risk factor of cardiovascular events mediated by hyperhomocysteinemia. Le Goff et al. (2011) also informed that isokinetic exercise leads to the release of CVD biomarkers in the blood, but these do not exceed healthy reference values in sedentary subjects. Maximal concentric isokinetic exercise does not, therefore, lead to an increased risk of cardiovascular pathologies. Understanding the mechanisms of changes in these markers after one session of exercise is essential for specifying the exact cardio protective benefits of long term exercise (Markovitch et al. 2008).

Folates and other B-vitamins, are essential to maintain safe homocysteine concentrations. An inverse relationship between tHcy and serum folate has been described, but only when the nutritional status for folic acid was adequate (González-González et al. 2005; Úbeda et al. 2011; Varela-Moreiras et al. 2007). Despite the optimal folate and vitamin B₁₂ status of the volunteers that took part in our study, an increase in tHcy was observed during exercise. So, in light of our results, adequate folate and vitamin B₁₂ intake could be particularly important for people that are less active, and who are exposed to transient increases in tHcy during exercise. This may be particularly pertinent for exercise at high cycling cadence, since we observed a significant increase in both vitamins immediately post- and 19 hours after exercise, indicating the high demand of these vitamins in this type of exercise.

Consequently, acute exercise, even of high intensity and high cycling cadence, has no negative effect on tHcy as an independent risk factor for CVD, when at least 400 kcal are spent during exercise and the nutritional status for folates and vitamin B₁₂ was adequate. These results inform the response of a risk factor for CVD to acute exercise in

sedentary people and are relevant for public health recommendations and exercise prescription. Therefore, attention should be given to identifying the optimal combination of exercise intensity, type and volume needed to induce favorable adaptations in the most time efficient manner, considering that a single bout of acute exercise does not increase tHcy over cardiovascular risk values in sedentary people.

Strengths and limitations

The strengths of our study are the meticulous experimental protocol and the strict control and characterization of the volunteers, particularly of their dietary intake. Furthermore, sequential blood samples were collected during exercise, which provides a unique perspective of the kinetics of the parameters assessed in response to acute exercise. Finally, the randomized crossover design of this study enabled each subject to be his own control, reducing the variability and strengthening the statistical analysis.

Some limitations should also be noted. First, the strict inclusion criteria and the invasive nature of the study, limited the possibility of recruiting a larger number of subjects. However, as mentioned before, in the repeated-measures design of the present study, the same 8 subjects participated in two different acute exercise bouts (at 50 and 80 rpm) and provided at least 7 samples before, during and after each event, which led us to analyse more than 100 samples. Second, generalization of results is limited by the characteristics of the study subjects: young, physically inactive males, without cardiovascular risk factors. Thus, the conclusions have been written accordingly. Finally, although the aim of this study focused on tHcy, the analysis of other cardiometabolic risk factors could have also been of interest. However, considering the plethora of cardiometabolic risk factors, the parameters selected could be biased by subjective selection. Furthermore, considering that each parameter could show different

plasma appearance-clearance kinetics, the experimental protocol designed could not account for all this heterogeneous response.

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Tables and figures

Table 1. Characteristics of the subjects (n=8).

	Mean	SD
Height (m)	1.82	0.06
Body mass (kg)	81.0	7.3
BMI (kg·m ⁻²)	24.5	2.5
Sum of 7 skinfolds (mm) ^a	100.9	39.8
%BF ^b	13.4	5.9
VO _{2peak} (ml·kg ⁻¹ ·min ⁻¹)	37.4	5.9

^aSum of 7 skinfolds: triceps, pectoralis, subscapular, abdominal, mid-axillary, suprailiac, and thigh. ^bBody density was calculated by the Jackson and Pollock (1978) equation and %BF was estimated using the Siri (1961) equation.

SD: standard deviation; %BF: Percentage of body fat VO_{2peak}: Peak oxygen uptake.

Table 2. Characteristic kinetic parameters (Area under the curve, AUC, Maximum concentration, Cmax and Time to maximum concentration, Tmax) and differences between points post0 and pre0 and post 19 and pre4 of tHcy in different types of exercise.

Hcy	50rpm	80rpm	p
AUC (0-30 min)	304.6 ± 83.5	345.7 ± 107.6	n.s.
Cmax (μmol/L)	11.4 ± 2.7	10.8 ± 3.2	n.s.
Tmax (min)	22.8 ± 16	13.8 ± 10,6	n.s.
Dif (final-pre0)	-1.33	-2.80*	*p<0.05 vs 50 rpm
Dif (post19-basal)	-0.85	-0.40	n.s.

Data are presented as means and standard deviation

Table 3. Serum folate and vitamin B₁₂ concentration before, during, and after two isocaloric exercise trials at low and high frequencies of contraction (50 rpm and 80 rpm) in sedentary volunteers (n=8).

	Folate (ng/ml)		Vitamin B12 (pg/ml)	
	50rpm	80rpm	50rpm	80rpm
pre4	7.1 ± 2.8	9.2 ± 6.3*	437.3 ± 207.8	429.0 ± 229.7
pre0	9.5 ± 3.6 ^a	10.5 ± 4.3	433.6 ± 156.7	367.0 ± 113.9
exer10	8.9 ± 1.9	10.1 ± 3.3	411.0 ± 189.9	384.0 ± 166.2
exer20	8.1 ± 1.3	10.9 ± 2.8	363.8 ± 70.8	387.4 ± 124.4
exer40	8.6 ± 1.0	10.2 ± 2.8	342.8 ± 58.5	388.4 ± 190.0
exer50	8.2 ± 2.4		431.3 ± 210.4	
exer60	8.2 ± 1.3			
post0	10.3 ± 2.6	16.8 ± 6.1 ^b	441.2 ± 183.7	630.4 ± 299.3 ^{bt}
post19	9.5 ± 3.3	19.5 ± 7.0 ^{*c}	392.8 ± 141.5 ^d	801.1 ± 249.9 ^{*cde}

Data are presented as means and standard deviation. Significant differences (p<0.05) from: *between the two trials in the same sampling point; ^apre0 vs. pre4; ^bpost0 vs. exer40; ^cpost19 vs. pre4; ^dpost 19 vs. pre0; ^epost19 vs. post0; ^fpost0 vs. pre0. Pre4: blood sample 4 hours before exercise; pre0: blood sample immediately before exercise; exer40: blood sample 40 minutes during exercise; post0: blood sample immediately after exercise; post19: blood sample 19 hours after exercise.

Table 4. Characteristic kinetic parameters (Area under the curve, AUC, Maximum concentration, C_{max} and Time to maximum concentration, T_{max}) of Folate in different types of exercise.

	Folate			Vitamin B12		
	50rpm	80rpm	p	50rpm	80rpm	p
AUC (0-30 min)	295.1 ± 90.6	296.9 ± 88.2	n.s.	118020 ± 3043	11780 ± 4075	n.s.
C _{max} (ng/mL)	11.6 ± 4.3	20.2 ± 9.7*	*p<0.05 vs 50 rpm	468.7 ± 198.6	640.0 ± 288.5	n.s.
T _{max} (min)	20.2 ± 16.3	51.4 ± 22.7*	*p<0.05 vs 50 rpm	37.1 ± 24.3	50.0 ± 19.1	n.s.

Data are presented as means and standard deviation

Table 5. Nutritional intake of volunteers (n=8) during the experimental trial days, target energy intake, Recommended Dietary Allowances (RDA) and Acceptable Macronutrient Distribution Range (AMDR).

	Intake	Intake targets ^a
Energy (kcal)	2869 ± 171	3123
Carbohydrates (%E)	67.3 ± 2.9	45-65
Lipids (%E)	19.1 ± 3.5	20-35
Proteins (%E)	13.6 ± 13.6	10-35
Folate (µg)	646.0 (177.0-683.0)	300
Vitamin B ₁₂ (µg)	22.7 (3.2-25.7)	1.4
Vitamin B ₆ (mg)	6.2 (1.8-6.5)	1.5

Data are presented as Median and standard deviation or Median (Interquartile range).

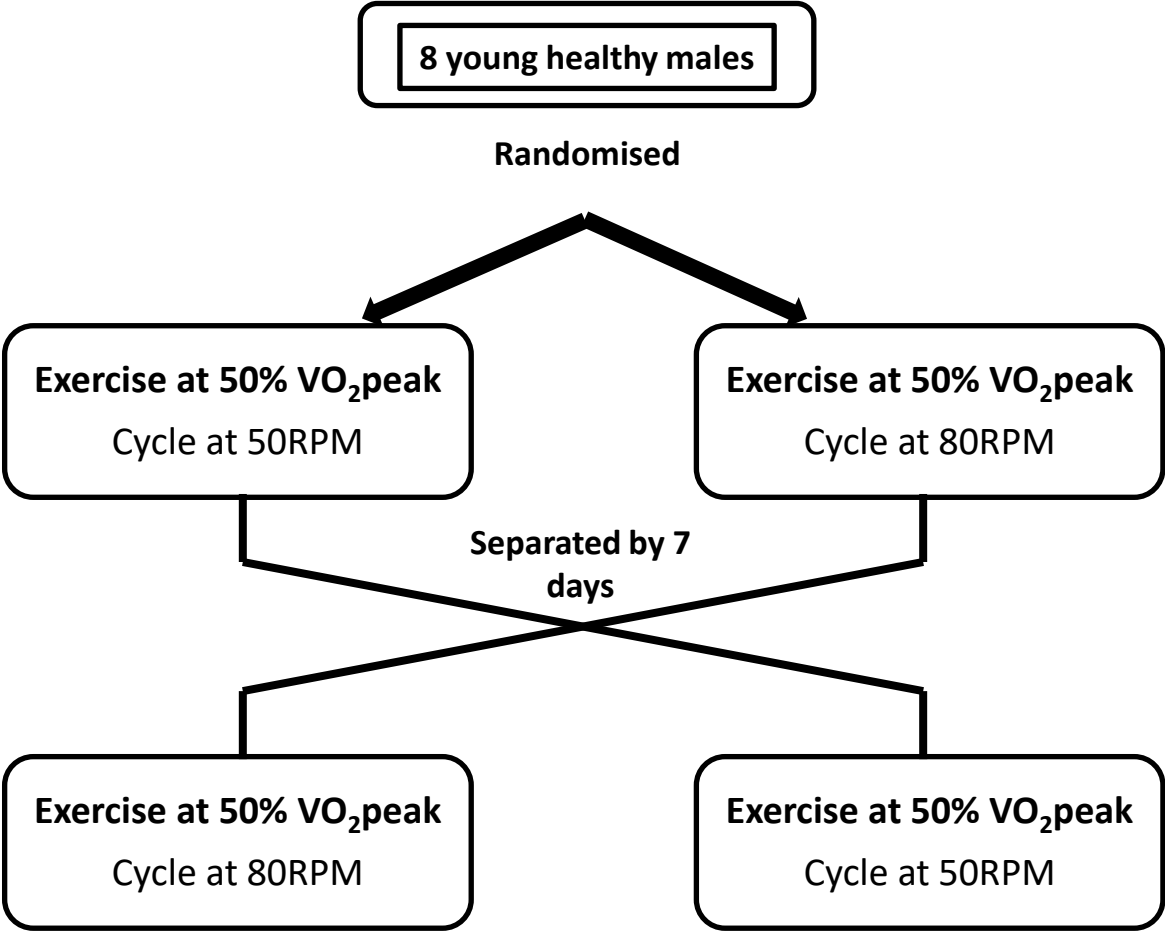
^a Intake targets: For Energy intake the energy requirements were calculated using the Harris-Benedict equation, multiplied by a physical activity factor (1.4), and with 400 kcal added to account for the energy expenditure during the exercise trial. For Macronutrients and vitamins, the Acceptable Macronutrient Distribution Range (AMDR) and the Recommended Dietary Allowances (RDA) were used, respectively.

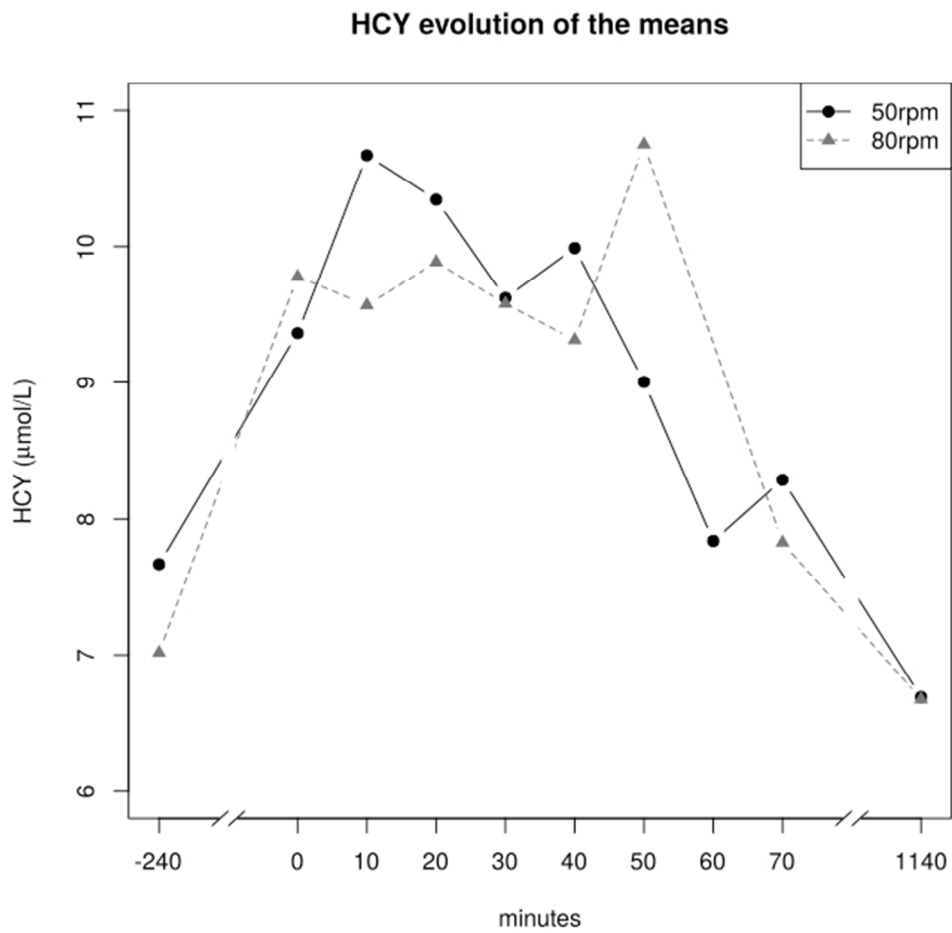
%E: percent of energy intake

Figure 1. Experimental design**Figure 2. Serum homocysteine kinetics at two exercise trials.**

Solid lines and dots in low cycle cadence (50 rpm) exercise and dashed line and triangles in high cycle cadence (80 rpm) exercise. Data are presented as means and standard deviation. No statistically significant differences were observed between values obtained at the same sampling points in both trials.*Significant differences ($p < 0.05$) in high cycle cadence (80 rpm) exercise between pre0 vs. pre4, post19. &Significant differences ($p < 0.05$) in low cycle cadence (50 rpm) exercise between pre4 vs. pre0 and post19; pre0 vs. exer20 and post19; post0 vs. post 19.

Pre4: blood sample 4 hours before exercise; pre0: blood sample immediately before exercise; exer20: blood sample 20 minutes during exercise; post0: blood sample immediately after exercise; post19: blood sample 19 hours after exercise.





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