

The Effects of Muscle Mass on Homocyst(e)ine Levels in Plasma and Urine

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ABSTRACT

Int J Exerc Sci 5(1) : 26-38, 2012. The present study was designed to examine the relationship between homocyst(e)ine (H[e]) levels and muscle mass. Two experimental groups each of 24 Caucasian males, one consisting of higher-muscle mass subjects (HMM) and the other of lower-muscle mass subjects (LMM) participated in this study. Muscle mass was estimated from 24-hour urine collections of creatinine (Crt). Muscle mass was 40.3 ± 15.9 kg in HMM and 37.2 ± 11.4 kg in LMM ($P = 0.002$). Mean plasma H(e) levels in HMM were 10.29 ± 2.9 nmol/mL, and in LMM were 10.02 ± 2.4 nmol/L (Not significant, [NS]). Urinary H(e) levels (UH[e]) were 9.95 ± 4.3 nmol/mL and 9.22 ± 2.9 nmol/mL for HMM and LMM, respectively (NS). Plasma H(e) levels correlated well with UH(e) (HMM: $r = 0.58$, $P = 0.009$; LMM: $r = 0.66$, $P = 0.004$). Muscle mass and was not correlated to either plasma H(e) or UH(e). However, in HMM trends were identified for body mass to be correlated with UH(e) ($r = 0.39$, $P = 0.10$) and UCrt ($r = 0.41$, $P = 0.08$). Surprisingly, in HMM plasma and UCrt were only weakly correlated ($r = 0.44$, $P = 0.06$). Our results do not support a causal relationship between the amount of muscle mass and H(e) levels in plasma or urine.

KEY WORDS: Creatinine, homocysteine, estrogen, gender-differences, skeletal muscle

INTRODUCTION

Today, atherosclerotic cardiovascular disease (CVD) is representing a leading cause of mortality in the United States (37) and many European countries (33). The risk factors are broadly the same for both genders: cigarette smoking, inactivity, high LDL cholesterol levels, hypertension, and

diabetes (33, 37, 43, 48). One predictor of CVD that has received much attention since the 1990s is homocyst(e)ine (8, 42, 47). Homocyst(e)ine is an amino acid that is produced during the conversion of dietary protein into energy, a process which seems to damage the arteries (25, 40, 44). Homocyst(e)ine levels are higher in males than in females, and it has been speculated

that this might contribute to the overall higher incidence of CVD in men, when compared to women (3, 25, 26). Exactly why this gender-specific difference in homocyst(e)ine concentrations exists has long been the subject of considerable debate.

Brattström et al. (6) have hypothesized that such sex-differences could be attributable to muscle mass. It is known that approximately 98% of creatinine is derived from creatine formed in the muscle, and that the amount of creatinine released is determined by muscle mass (38). The formation of creatine (from which creatinine proceeds) depends on the methyl donation by S-adenosylmethionine, a process which leads to the formation of homocysteine (2, 40). Mudd and Poole (32) and Brattström et al. (6), consequently, were able to demonstrate positive correlations between levels of homocyst(e)ine and plasma creatinine. These findings led these authors to opine that the homocysteine concentration too would be expected to reflect muscle mass as much as the creatinine concentration does, and speculated that this might be the reason why men will generally have higher concentrations of both creatinine and homocysteine (3, 13).

However, the actual relationship between homocysteine and muscle mass itself has not been investigated sufficiently. The few studies that have looked into the relationship between both, have compared males and females hence having been confounded by many important factors such as particularly sex steroids and menstrual cycle fluctuations (10, 13). It therefore remains somewhat surprising that

to date, to the best of our knowledge, no study has attempted to assess muscle mass effects on homocysteine levels in same-sex groups in order to exclude this major confounder represented by an estrogen- *vs.* a testosterone-rich environment. Thus, it is the purpose of the present study to investigate the possible role of muscle mass on plasma and urine homocyst(e)ine and creatinine levels in same-gender subjects (males). Our hypothesis is that differences in H(e) levels might be attributed to differences in muscle mass. In addition, we also wanted to see if muscle mass might affect the clearance of H(e).

METHODS

Participants

All procedures of this study were conducted in compliance with institutional ethics procedures and expectations described in the Declaration of Helsinki. Following institutional ethics approval, subjects provided informed consent after the purpose and content of the experiments were carefully explained. Two different subject groups each consisting of 24 Caucasian males, between 18-45 years, volunteered to participate in this study. Based upon effect sizes calculated from meta-analysis of other studies that have measured homocyst(e)ine in subjects of similar ages (26, 36, 42), the minimal required sample size in each group in order to maintain a statistical power of 0.8, was determined as 23. Subjects were assigned to either the higher (HMM) or the lower muscle mass group (LMM) based on the consideration that those subjects regularly engaging in weightlifting and resistance training would likely have more muscle mass than those only engaging in aerobic

running. To avoid subjects with more muscle mass being also heavier and therefore introducing an overall higher body mass as a confounder, we elected that subjects should be matched for body mass. HMM subjects engaged in weightlifting for at least 4 days per week for more than a year, whilst not engaging in regular aerobic exercise. Subjects in this experimental group all were members of a police riot squad, whose professional training included regular weightlifting. Inclusion criteria for the second experimental group, which consisted exclusively of LMM subjects, required being involved in regular (at least 4 times per week) aerobic training and not lifting weights. LMM subjects were selected from the local community. Levels of physical activity of the subjects were obtained through interview questions but not experimentally verified.

None of the subjects in either group, as far as known, suffered from any disease that might interfere with H(e) metabolism. Subjects were not controlled for caloric intake, but the following restrictions on dietary supplements were enforced. In particular, subjects were not allowed to take (or have taken within the previous two months) any vitamin B or folate supplements (these lower H(e) levels), or protein or creatine monohydrate supplements (these would interfere with and artificially increase urine creatinine concentrations). Relevant personal details (anamnesis, training history) were collected using a questionnaire which the subjects completed in the presence of an exercise physiologist familiar with the questionnaire's contents and aims. Individuals with a family history of CVD, or who had self-reported hypertension,

hypercholesterolemia, or diabetes were excluded from the study.

Experimental design

The study consisted of a single trial during which data were collected on the subjects' body composition to allow calculation of muscle mass, and to correlate these values with H(e) concentrations in plasma and urine. HMM subjects were given instructions on how to collect 24h-urine, and they were provided with 5-liter containers during a subject group meeting at their Bedford Police Station; LMM subjects were provided with the same during individual meetings prior to scheduling their experimental lab visits. Subjects were also reminded to refrain from taking vitamin B, folate, protein or creatine monohydrate dietary supplements, and were told to stay sufficiently hydrated when completing 24-hour urine collections and before reporting to the lab for bioimpedance body composition measurements. After having explained instructions and addressed eventual questions, subjects were told to schedule a visit to the lab facility between 10-12 am, during which they provided a fasting single venous blood sample. At that occasion they also delivered their containers with 24-hour urine collections. Subjects had been instructed to not exercise prior to blood, urine and bioimpedance body composition data collection. Exclusion criteria were: having taken dietary supplements, illness, or showing signs of dehydration, such as, for example, 24-hour urine collections <1,000 mL or containing <400 mg creatinine, as recommended by Flegg & Lakatta (11).

Anthropometrical measurements

For both groups height, body mass, and age were recorded. In addition, body composition was measured using a bio-electrical impedance analyzer (Biostat 1500, Bodystat Ltd., Douglas Isle of Man, British Isles). This method sends a minimal current through the body and measures its impedance. Recommendations to subjects before and during bioimpedance analysis included abstinence from caffeine and remaining sufficiently hydrated. Lean body mass (LBM) was calculated by subtracting fat tissue mass from total body mass. Muscle mass was estimated from creatinine excretion as described later in this paper.

Blood collection and analysis

Subjects had to provide a single 10-mL sample of venous blood obtained from an antecubital vein using a disposable syringe. The sample was immediately transferred to pre-cooled 5-mL di-potassium ethylenediamine tetra-acetate (EDTA) polypropylene tubes. These tubes were then centrifuged for 5 minutes at 3,000 g. The plasma was pipetted into 1.5 mL Eppendorf cups and immediately frozen at -80°C until analysis.

Blood samples were analyzed for plasma total homocyst(e)ine (H[e]), which is the sum of homocysteine and the homocysteinyI moieties of the disulfides homocystine and cysteine-homocystine, whether free or bound to proteins (25, 26, 44). All H(e) analyses were performed at, or according to procedures identical to those established by Malinow et al. (27). This is an automated method based on high-pressure liquid chromatography (HPLC) with electrochemical detection, as

previously described in detail (27). The within-assay coefficient of variation (cv_i) of the method described above was 2.0% and the between-assay coefficient of variation (cv_b) below 7.0%.

Plasma samples were also analyzed for creatinine. Therefore we used a commercially available kit from Sigma (St. Louis, MO) for quantitative colorimetric analysis. This method was based on the color change that occurs when alkaline picrate is exposed to creatinine (Jaffé reaction) (5).

Urine collection and analysis

Subjects provided 24-hour samples of urine for analysis of urinary homocyst(e)ine (UH[e]) and creatinine (UCrt). Urine was collected in previously distributed 5-L plastic containers (Richardsons, Leicester, UK or similar), which were kept refrigerated during use. The container was then transported to the lab, the urine volume (UVol) recorded, mixed and two 30-mL aliquots frozen and stored at -80 °C until analysis. UH(e) and UCrt were measured in 30 mL aliquots according to methods similar to those described above for plasma concentrations.

UCrt excretion was used to predict muscle mass. The 24-hour creatinine excretion is an easily measurable and validated index of total muscle mass (38, 46). Because it has been demonstrated that per 18.5 kg of muscle, one gram of creatinine is excreted (7, 17), muscle mass can be estimated by the formula:

Muscle mass in kg= 1.85 × mg creatinine excreted per day: body mass

HOMOCYST(E)INE AND MUSCLE MASS

Despite prior validation of estimating body mass on urinary creatinine excretion (11, 12, 17, 38, 46), one limitation is that creatinine will also be derived from ingested muscle such as red meat, which means that ideally a meat-free diet for approximately 3 days prior to the testing should be required. However, because our estimations used 24-hour samples of urine the possible effect of meals is attenuated (18). Therefore, we chose not to apply the fasting method.

Calculation of renal clearance

Because we measured H(e) and creatinine in both plasma and urine, it allows us to calculate clearance rates and renal function (39). The excretion rate of creatinine (EXR_{Crt}) and homocyst(e)ine (EXR_{He}) were estimated as follows:

$$\text{EXR}_{\text{Crt}} = \text{UCrt} \times \text{UVol}$$

$$\text{EXR}_{\text{He}} = \text{UH(e)} \times \text{UVol}$$

The clearance rates of creatinine (CR_{Crt}) and homocyst(e)ine (CR_{He}) were computed according to the knowledge that the excretion of a substance equals the urinary excretion of the substance divided by the plasma concentration of the substance (39):

$$\text{CR}_{\text{Crt}} = \frac{\text{EXR}_{\text{Crt}}}{\text{Crt}} = \frac{(\text{UCrt} \times \text{UVol})}{\text{Crt}}$$

$$\text{CR}_{\text{He}} = \frac{\text{EXR}_{\text{He}}}{\text{H(e)}} = \frac{(\text{UH(e)} \times \text{UVol})}{\text{H(e)}}$$

This, then also allows estimation of tubular resorption of creatinine (TRES_{Crt}) and homocyst(e)ine (TRES_{He}) (39):

$$\text{TRES}_{\text{Crt}} = (\text{CR}_{\text{Crt}} \times \text{Crt}) - (\text{UCrt} \times \text{UVol})$$

$$\text{TRES}_{\text{He}} = (\text{CR}_{\text{He}} \times \text{He}) - (\text{UH(e)} \times \text{UVol})$$

Statistical analysis

Normal probability plot showed that data sets were normally distributed. Student's

Table 1: Anthropometrical characteristics of the participants (high-muscle mass subjects *vs.* low-muscle mass subjects). Values represent means \pm SD (N= 24, in each group).

Variable	High-Muscle Mass Group	Low-Muscle Mass Group	P-value
Age (yr)	32.4 \pm 2.5	31.8 \pm 2.4	NS
Height (cm)	181.1 \pm 2.6	186.2 \pm 4.2	0.01
Body mass (kg)	86.2 \pm 11.0	85.7 \pm 9.8	NS
Body fat (%)	17.2 \pm 4.0	14.8 \pm 2.6	0.03
Lean body mass (kg)	72.1 \pm 2.9	73.1 \pm 3.5	NS
Muscle mass (kg)	40.3 \pm 15.9	37.2 \pm 11.4	0.02
BMI	26.7 \pm 2.8	24.6 \pm 2.6	0.03

two-sample t-test was anticipated to be appropriate. The t-test remains robust even with moderate departures from normality. In a recently completed study using similar outcome variables, the distribution of the levels of H(e) showed no radical departures from normality. If the dispersion of the data was strongly related to the mean, the t-test was applied to the log-transformed data. The Bonferroni correction (dividing alpha by the number of comparisons) was considered but rejected for a number of reasons, such as groups compared not being identical on all variables and because of a reluctance to inflate type II errors in order to decrease type I errors. To support the conclusions based on the t-test, the corresponding rank tests were also carried out.

Pearson's correlation coefficient was used to assess the relationship between H(e) and indicators of muscle mass, being lean body mass and creatinine concentrations, respectively. The α -level was set *a priori* at 0.05. Data analysis was completed using the Statistical Package for the Social Sciences, versions 13-17 for MS-Windows (SPSS Inc., Chicago, IL). Results are presented as means \pm SD.

RESULTS

Body composition and estimation of muscle mass

Twenty-four male subjects in each group successfully completed this trial. Their descriptive values are mentioned in Table 1. Mean body mass was 86.2 ± 11.0 kg in HMM subjects and 85.7 ± 9.8 kg in LMM subjects (not significant, [NS]). Although the HMM subjects had a mean BMI of 26.7 ± 2.8 their mean percentage of body fat was

$17.2 \pm 4.0\%$ and their muscle mass 40.3 ± 15.9 kg, whereas the LMM group had a mean BMI of 24.6 ± 2.6 ($P=0.03$), body fat percentage of 14.8 ± 2.6 ($P= 0.03$), and a muscle mass of 37.2 ± 11.4 kg ($P= 0.02$).

Homocyst(e)ine and creatinine values

Mean plasma homocyst(e)ine levels were 10.29 ± 2.9 nmol/mL in HMM and 10.02 ± 2.4 nmol/mL in LMM (NS) (standard laboratory values: 5-15.9 nmol/mL) (Figure 1). However, one HMM subject had elevated values at 16.90 nmol/mL. Urinary H(e) values averaged 9.95 ± 4.3 nmol/mL for HMM and 9.22 ± 2.9 nmol/mL for LMM (NS), respectively.

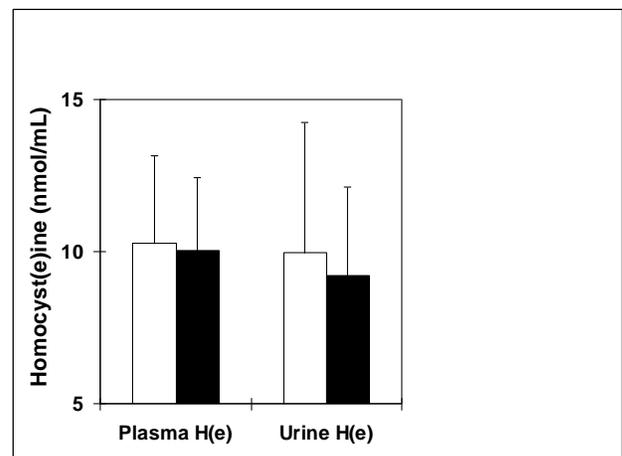


Figure 1. Plasma and urine homocyst(e)ine levels in high-muscle mass (white bars) vs. low-muscle mass (black bars) males. Values are means \pm SD. *Significantly different $P<0.05$.

Creatinine concentrations in plasma were for HMM: 1.3 ± 0.2 mg/dL and for LMM: 1.2 ± 0.2 mg/dL (NS; normal: 0.9-1.4 mg/dL), and urinary creatinine concentrations expressed per metric unit were 124.8 ± 49.7 mg/dL for HMM and 103.1 ± 32.4 mg/dL ($P= 0.03$) for LMM; Total UCrt excretion per day for LMM was $2,264 \pm 542$ mg/d and $1,729 \pm 334$ mg/d for

HMM (normal: 1,100-2,800 mg/d) (Figure 2).

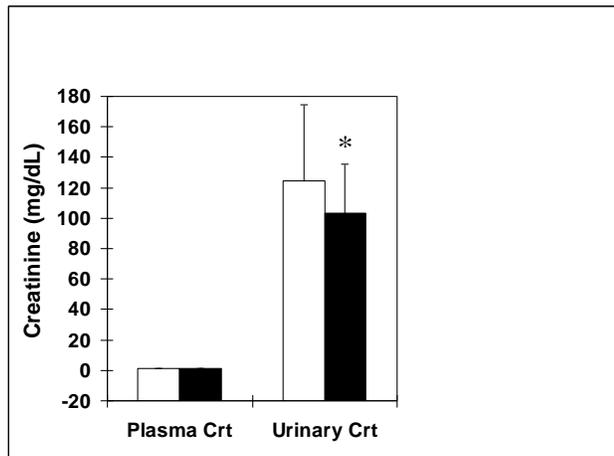


Figure 2. Plasma and urine creatinine levels in weight-trained or high-muscle mass (white bars) vs. low-muscle mass (black bars) males. Values are means \pm SD. *Significantly different $P < 0.05$.

Correlations between homocyst(e)ine, creatinine, and body composition

Plasma H(e) correlated moderately with

urine H(e) levels in both subject groups (HMM: $r = 0.44$, $P = 0.03$; LMM: $r = 0.54$, $P = 0.01$). Plasma creatinine in HMM, however, did not significantly correlate with urinary creatinine. No significant correlations were found between plasma H(e) and creatinine in either group, whereas the urinary equivalents of both parameters were significantly correlated (HMM: $r = 0.64$, $P = 0.001$; LMM: $r = 0.67$, $P = 0.001$). Plasma H(e) levels in either group were not significantly correlated with BMI (HMM: $r = 0.50$; LMM: $r = 0.38$), body mass (HMM: $r = 0.35$; LMM: $r = 0.29$), muscle (HMM: $r = 0.02$; LMM: $r = 0.34$) or body fat (HMM: $r = 0.26$; LMM: $r = 0.28$). Both urinary H(e) and creatinine levels showed trends towards weak correlations with body mass ($r = 0.39$, $P = 0.10$ & $r = 0.41$, $P = 0.08$, respectively), but only in the HMM subjects.

Table 2: Overview of the contribution of renal function to H(e) and creatinine concentrations in high-muscle mass subjects vs. low-muscle mass subjects. Values represent means \pm SD (N= 24, in each group).

Parameter	High-Muscle Mass Group	Low-Muscle Mass Group	P-value
Excretion Volume			
Homocyst(e)ine ($\mu\text{mol/d}$)	18.1 \pm 4.7	15.6 \pm 3.1	NS
Creatinine (g/d)	2.3 \pm 0.5	1.7 \pm 0.3*	0.03
Clearance Rate			
Homocyst(e)ine (mL/min)	1.2 \pm 1.1	1.1 \pm 0.8	NS
Creatinine (mL/min)	120.9 \pm 21.7	100.1 \pm 16.2	NS
Tubular Reabsorption			
Homocyst(e)ine (nmol/min)	<0.1	<0.1	NS
Creatinine (mg/min)	1.4 \pm 0.3	1.1 \pm 0.2	NS

*Significantly different LMM vs. LMM

Renal clearance and tubular resorption

Data of renal function are summarized in Table 2. The mean EXR_{Cr} was 2.3 ± 0.5 g/d or 26.3 ± 5.0 mg \cdot kg⁻¹ \cdot day⁻¹ in HMM and 1.7 ± 0.3 g/d or 20.2 ± 3.4 mg \cdot kg⁻¹ \cdot day⁻¹ in LMM ($P= 0.03$). CR_{Cr} values were 120.9 ± 21.7 mL/min and 100.1 ± 16.2 mL/min in HMM and LMM, respectively (NS), which was within normal laboratory ranges (90-135 mL/min). The mean $EXR_{H(e)}$ and $CR_{H(e)}$ values were 18.1 ± 4.7 μ mol/d and 1.2 ± 1.1 mL/min, respectively in HMM, and 15.6 ± 3.1 μ mol/d and 1.1 ± 0.8 mL/min, respectively in LMM (NS). No significant group differences for $TRES_{He}$ values were found.

DISCUSSION

With the exception of two HMM individuals who showed moderate hyperhomocyst(e)inemia' (between 16-30 nmol/mL), all subjects had mean H(e) values which were within normal ranges and well in agreement with typical concentrations in healthy males reported by other researchers (6, 26, 43).

H(e) is traditionally determined in plasma, but can also be measured in urine. This is not generally performed because of the difficulties with obtaining 24-hour collections of urine. In the present study we found that urinary H(e) concentrations correlated well with those in plasma. This finding is in agreement with existing literature. For example, Brattström et al. (6) found significant correlations between urine and plasma H(e) correlations of $r= 0.51$ in athero-thrombotic patients and $r= 0.42$ in controls (both, $P<0.001$), which is similar to what we found.

Our initial hypothesis was that differences in H(e) levels might be attributed to differences in muscle mass. In this study, muscle mass was estimated based on urinary creatinine excretion. The total daily volume of excreted creatinine in urine was higher in the HMM than in the LMM, which is in line with expectations. Our further findings, in particular our observation of H(e) in either plasma or urine not being significantly correlated with muscle mass, body mass, or percentage of body fat, does not lend support to our hypothesis. It is therefore highly unlikely that muscle mass would represent an important factor in determining H(e) levels.

Renal clearance is the volume of blood that is cleared of a particular substance per unit of time (39). It provides an approximation of glomerular filtration rate (GFR) and of renal function. GFR can theoretically be estimated in measuring the clearance of any chemical substance on the condition that it has a stable level in blood, and that it is freely filtered but not reabsorbed or secreted by the kidney. Tubular reabsorption then is the process by which solutes and water are removed from tubular fluid and transported back into the blood (39). In the present study, data for renal clearance rates and tubular resorption of both creatinine and H(e) were within normal ranges. The absence of any significant differences for these parameters between the two subject groups suggests that our subjects had a clinically normal renal function, and that differences in muscle mass do not affect clearance of either H(e) or creatinine. We also note that our data suggest that tubular resorption of H(e) is almost nonexistent. This finding is in agreement with Arnadottir & Hultberg

(2) and with Wollesen et al. (49), who concluded that H(e) is ultrafiltrated in glomeruli, almost completely absorbed in tubuli, and degraded in kidney tissue by transmethylation and transsulfuration, and that healthy humans typically return as much H(e) to the circulation as is ultrafiltrated in glomeruli.

It has been suggested before that 75% of H(e) production takes place in direct conjunction with the formation of creatine and creatinine (1, 32), but literature data are inconsistent. Some authors have found positive correlations between plasma creatinine and H(e) in patients with CHD and stroke (6, 20, 24), while others have not (49). Brattström and colleagues (6) have previously demonstrated that only in male and female patients with CVD they were able to perceive such a correlation between plasma creatinine and H(e), but not in their healthy control subgroups. If muscle mass would have been a major mediating factor of H(e), one would expect to find such a correlation in healthy subjects too.

With the exception of anthropometric differences (mostly as a consequence of subjects being selected into either HMM or LMM groups), total creatinine excretion and urinary creatinine levels, no significant differences were found in metabolic parameters between the HMM and LMM subject groups. The absence of differences in H(e) as far as related to muscle mass seems to support the conclusions of Fukagawa et al. (13), and is also in agreement with Mora and colleagues (30). Fukagawa et al. (13) investigated the effect of remethylation of homocysteine in the liver in determining H(e) levels. During the process of remethylation homocysteine is

salvaged by acquisition of a methyl group from N^5 -methyl-tetrahydrofolate in a vitamin B-12-dependent pathway or from betaine. Using ANCOVA, these authors adjusted remethylation for fat-free mass and muscle mass, respectively, and noted that only adjustment for fat-free mass attenuated gender-differences in H(e). In other words, fat-free mass, but not muscle mass, appeared to affect remethylation. Thus, similar to us, their findings too were unable to confirm the amount of muscle mass as a critical determinant in mediating H(e) levels as had been suggested previously (2, 6, 10, 32).

Another consideration that is hard to join with the muscle mass/homocysteine hypothesis is that muscle mass typically decreases with advancing age, whereas homocysteine levels tend to increase. Certainly, there are other confounders involved in the process of aging, but it nevertheless adds even more arguments against than in support of the muscle mass-homocysteine hypothesis.

Because neither we, nor Fukagawa et al. (13), nor Mora et al. (30) have been able to confirm a muscle mass/homocysteine relationship, and since we were not even able to find a significant correlation between creatinine and H(e) in every subject, other explanations should be considered. Creatinine also represents an index of renal function, and its circulating levels and metabolism are influenced by renal and liver disease (12, 34). Potentially, it may be or may have been kidney function rather than muscle mass which was reflected in the relationship creatinine/H(e) which Brattström and colleagues (6) observed in patients with CVD. Based on

increased H(e) levels found in haemodialysis patients and chronic kidney disease patients (19, 23), it has, indeed, been argued that H(e) levels would depend on the level of renal function (23, 24, 26).

Since muscle mass, contrary to our starting hypothesis, does not appear to be a determinant in H(e) levels in same-gender subjects, it is unlikely it would be in causing male *vs.* female differences in H(e) levels. The results from Fukagawa et al. (13) obtained in male *vs.* female subjects lend credence to this assumption. The most obvious confounder to present a challenge to the muscle/H(e) hypothesis when comparing homocysteine levels across gender, clearly are the major differences in sex steroid hormones (29). An abundant number of studies has now been able to demonstrate that H(e) concentrations are negatively correlated with estradiol levels (4, 9, 31, 41, 45, 50). Moreover, H(e) levels significantly decrease following four months of treatment with ethinyl estradiol and the anti-androgen cyproterone acetate in the male-to-female transsexuals, whereas they significantly went up in the female-to-male transsexuals H(e) levels after two weeks of treatment with testosterone (16). Sex steroids may also play a role in same-gender differences in H(e) levels related to different types of physical activity. For example, animal experiments have shown that testosterone significantly alters the formation and utilization of S-adenosylmethionine, a key factor in the synthesis of H(e) (28).

The authors recognize that today other perhaps more reliable techniques, such as notably CAT-scan, Magnetic Resonance Imaging (MRI), or Dual X-ray-Energy

Absorptiometry (DEXA) (22), have become the method of choice for estimating muscle mass. However, this was not yet so at the time we commenced the present study, which took much longer to complete than expected due the difficulties in recruiting a sufficient number of LMM subjects weight-matched for all HMM subjects. One reason for these difficulties was that aerobic athletes on the average are lighter than athletes or subjects who only engage in weight-training making it difficult to weight-match subjects from both groups.

In the present study we did not make use of a methionine-loading test (1) mainly because of evidence suggesting that this method in people with the so-called 'thermolabile' alternative form of one of the converting enzymes (N^5, N^{10} -methylenetetrahydrofolate reductase might lead to considerable errors (15). The authors are aware of potential additional confounders, such as age, temperature, pH (low pH causes increased formation of creatinine), and an approximately 15% daily intra-individual variation in creatinine excretion (14, 21, 35). In addition, inter-individual differences in H(e) levels may be caused by the rate of remethylation in the liver. In remethylation, homocysteine is salvaged by acquisition of a methyl group from N5-methyl-tetrahydrofolate in a vitamin B-12-dependent pathway or from betaine. Other limitations are that, if one individual at the same time has a 20% higher creatinine production rate and clearance rate when compared to another individual, the creatinine levels of both individuals may very well be similar though they may reflect a thoroughly different muscle mass.

The results obtained from the present study suggest that: (a) our data do not support the existence of a causal relationship between the amount of muscle mass and H(e) levels in plasma or urine in healthy male volunteers; (b) plasma and urinary H(e) levels correlate well.

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