Homocysteine, cysteine, and body composition in the Hordaland Homocysteine Study: does cysteine link amino acid and lipid metabolism?[1–3]

Amany K Elshorbagy, Eha Nurk, Clara Gram Gjesdal, Grethe S Tell, Per M Ueland, Ottar Nygård, Aage Tverdal, Stein E Vollset, and Helga Refsum

ABSTRACT

Background: The lean phenotype of cystathionine β-synthase–deficient homocystinuria and the positive association of plasma total cysteine (tCys) with body mass index (BMI) suggest that total homocysteine (tHcy) and tCys are associated with body composition.

Objectives: We aimed to study associations of tCys and tHcy with body composition in the general population.

Design: Using data from 7038 Hordaland Homocysteine Study participants, we fitted regression models and dose-response curves of tCys and tHcy with BMI. In 5179 participants, we investigated associations of tCys and tHcy with fat mass and lean mass and examined whether changes in these aminothiols predicted body composition 6 y later.

Results: tCys showed positive associations with BMI (partial $r = 0.28$, $P < 0.001$), and fat mass (partial $r = 0.25$, $P < 0.001$), independent of diet, exercise, and plasma lipids. Women in the highest tCys quintile had fat mass 9 kg (95% CI: 8, 10 kg; $P < 0.001$) greater than that of women in the lowest quintile. The corresponding values for men were 6 kg (95% CI: 5, 7 kg; $P < 0.001$; $P < 0.001$ in both sexes, ANOVA across quintiles). The rise in tCys over 6 y was associated with greater fat mass at follow-up ($P < 0.001$), but there was no effect on lean mass. tHcy was not associated with lean mass, and it became significantly inversely associated with BMI and fat mass only after adjustment for tCys. The association between tHcy and lean mass was not significant.

Conclusions: tCys concentrations show a strong positive association with BMI, mediated through fat mass. The link between cysteine and lipid metabolism deserves further investigation. *Am J Clin Nutr* 2008;88:738–46.

INTRODUCTION

Cystathionine β-synthase (CBS) deficiency is the most common cause of homocystinuria (1). Normally, homocysteine, produced in *S*-adenosylmethionine–dependent transmethylation reactions, is either remethylated to methionine or irreversibly metabolized to cysteine by the action of CBS and cystathionase enzymes (2). CBS deficiency therefore leads not only to upstream accumulation of homocysteine and methionine but also to reduced synthesis of cystathionine and cysteine (3).

The characteristic phenotype of the disorder includes vascular, neurologic, ocular, and skeletal abnormalities (1). Common skeletal abnormalities include marfanoid features, dolichostenomelia (unusually long, thin extremities), osteoporosis, and arachnodactyly (1, 4). In addition, patients are reported to have a thin build (1), low subcutaneous fat (5), and low body mass index (BMI; in kg/m^2), as calculated from Brenton et al (4).). The cause of skeletal changes is unknown. Impaired cross-linking of collagen as a result of hyperhomocysteinemia has been suggested, but the evidence is insufficient (6). Notably, skeletal changes have not been reported in patients with inborn errors of homocysteine remethylation, who also have severe hyperhomocysteinemia but who synthesize normal amounts of cysteine (7). Despite this, the role of decreased cysteine synthesis in the pathophysiology of skeletal changes in CBS deficiency has received little attention.

Plasma total cysteine (tCys) has been shown to be strongly related to BMI in the Hordaland Homocysteine Study (HHS) (8). Moreover, changes in tCys over a 6-y period were positively associated with changes in BMI (9). Although the tCys-BMI association was conventionally interpreted as indicating that

[1] From the Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, United Kingdom (AKE and HR); the Institute of Basic Medical Sciences, Department of Nutrition, University of Oslo, Oslo, Norway (EN and HR); the Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway (CGG, GST, and SEV); the Department of Rheumatology, Haukeland University Hospital, Bergen, Bergen, Norway (CGG); the Section of Pharmacology, Institute of Medicine, University of Bergen, and Haukeland University Hospital, Bergen, Norway (PMU); the Section of Cardiology, Institute of Medicine, University of Bergen, and Haukeland University Hospital, Bergen, Norway (ON); and the Norwegian Institute of Public Health, Oslo, Norway (AT and SEV).

[2] Supported by the Advanced Research Programme of Norway, The Norwegian Research Council, The Johan Throne Holst Foundation for Nutrition Research (University of Oslo), the Foundation to Promote Research into Functional B12 Deficiency (Norway), and an Egyptian Government scholarship (to AKE). None of the funding bodies were involved in the study design; collection, analysis, or interpretation of the data; or preparation of the manuscript.

[3] Reprints not available. Address correspondence to AK Elshorbagy, Le Gros Clarke Building, Department of Physiology, Anatomy and Genetics, Oxford University, Oxford, United Kingdom. E-mail: amany.elshorbagy@dpag.ox.ac.uk.

Received December 6, 2007.

Accepted for publication May 22, 2008.

BMI was a determinant of tCys, the evidence from homocystinuria due to CBS deficiency (1, 4, 5) suggests that the reverse may be true. In CBS-deficient subjects, the pathologic condition most likely proceeds from the primary abnormal concentrations of the aminothiols, including low tCys, to the skeletal manifestations and the thin phenotype.

Whereas it is possible that elevated plasma total homocysteine (tHcy) may also determine body composition, evidence on an association of tHcy with BMI in the general population has been conflicting. Most cross-sectional studies report weak-positive (10–12) or no (13) associations between BMI and tHcy, but clinical trials have found weight loss to be associated with an elevation of tHcy (14–16), even without a lowering of serum folate or vitamin B-12 (14). Similarly inconsistent associations have been observed between tHcy and total-body (TB) lean mass (17, 18) and fat mass (18, 19).

In the present study, we raised the hypothesis that tHcy, tCys, or both are associated with body composition in the general population. Using data from the HHS, we examined the relations between tCys, tHcy, and BMI in 7038 subjects and between these aminothiols and TB lean mass and fat mass in 5179 subjects.

SUBJECTS AND METHODS

Study population

The first HHS (HHS-I) was conducted in 1992 and 1993 on 18 043 middle-aged (40–42-y-old) or elderly (65–67-y-old) residents of Hordaland county in western Norway. From 1997 through 1999, a follow-up study (HHS-II) of participants living in Bergen and its surroundings was conducted as part of the Hordaland Health Study. In HHS-II, 9187 of the HHS-I subjects were invited to return, of whom 7074 (77%) attended: 3341 elderly subjects (then aged 71–73 y) and 3733 middle-aged subjects (then aged 47–48 y) (20).

This study is based on data from HHS-I and HHS-II. For 7038 subjects, information was available on BMI, plasma tHcy, tCys, and total cysteinylglycine (tCysGly) in both HHS I and II. We examined the cross-sectional association between tHcy, tCys, and BMI by using data from HHS-II. The relation of tHcy and tCys with TB lean mass and TB fat mass was examined in 5179 HHS-II participants in whom fat mass and lean mass were measured. We also investigated the association of changes in tCys and tHcy over the 6-y period with BMI, lean mass, and fat mass at follow-up.

For 3516 of the HHS-II participants (including 2894 elderly), nonfasting plasma concentrations of cystathionine and methionine were measured. Body composition data were available for 2696 of these participants (including 2083 elderly), whereas BMI data were available for all. Using these data, we tested whether the associations of tCys with body composition remained robust after adjustment for variations in cystathionine and methionine. Because the measurements of these aminothiols were nonfasting, and because these measurements are known to vary with food intake (21), analyses involving cystathionine and methionine were adjusted for time since last meal.

All subjects provided written informed consent. Study protocols for HHS-I and HHS-II were approved by the Regional Committee for Medical Research Ethics Ethical Committee of Western Norway, whose directives are based on the Helsinki Declaration.

Study variables

BMI, body composition, and blood pressure

Height and weight were measured while subjects were wearing light clothing, and BMI was calculated. Seated arterial blood pressure was measured 3 times in each subject; the average of the second and third measurements was used.

Lean mass and fat mass were measured by using dual-energy X-ray absorptiometry (DXA) (22), which is based on the different attenuation of photons by different body tissues. Transmission of X-rays at 2 energy levels allows the derivation of TB bone mineral mass, lean mass, and fat mass. Measurements were done on a stationary fan-beam densitometer using EXPERT-XL software (version 1.72–1.9; Lunar Corporation, Madison, WI). The CVs for lean mass and fat mass were 1.3% and 1.9%, respectively.

Lifestyle and dietary data

Self-administered questionnaires provided information on diet (23) and lifestyle. Nutrient intakes were calculated by using KOSTBEREGNINGSSYSTEM software (version 3.2; Department of Nutrition, University of Oslo, Oslo, Norway). Physical activity included 2 variables indicating heavy or light physical activity in the past year, and each variable comprised 4 categories: *1*) none, *2*) ≤1 h/wk, *3*) 2–3 h/wk, and *4*) >4 h/wk. Smoking and coffee consumption were used as continuous variables comprising the number of cigarettes smoked per day or the cups of coffee consumed per day.

Biochemical measurements

Nonfasting plasma samples were collected in EDTA-containing tubes for analyses of tCys, tHcy, tCysGly, folate, and vitamin B-12 as described previously (24). Plasma tHcy, tCys, and tCysGly were analyzed by using HPLC with fluorescence detection. The intraassay CV was <4% (25). Liquid chromatography–tandem mass spectroscopy was used for analyzing methionine and cystathionine as described previously (26). Plasma folate and vitamin B-12 were measured by using microbiological assays (27, 28). Serum HDL cholesterol (from HHS II only), triacylglycerol, and total cholesterol were measured at the Department of Clinical Chemistry (Ullevål Hospital, Oslo, Norway) by using enzymatic methods. Creatinine (from HHS II only) was measured in stored plasma by using a modification of a liquid chromatography–tandem mass spectroscopy described previously (29).

Statistical analysis

Despite statistically significant tCys × sex interactions for BMI and fat mass (stronger in women than men) and statistically significant tHcy × age interactions for BMI (stronger in younger than in older subjects), stratified analysis showed only modest differences in the patterns and strengths of these associations between middle-aged and elderly men and women. Unless otherwise stated, we therefore combined the 4 age-sex groups with adjustment for age and sex.

One-way ANOVA and chi-square tests with Bonferroni correction were used to determine significant differences between groups, and simple correlations were assessed by using Spearman's rank correlation coefficient. Skewed variables were log-transformed before analysis. Multiple linear regression models were used to assess the role of tCys and tHcy as determinants of

The American Journal of Clinical Nutrition

TABLE 1
Selected characteristics of the study population at follow-up[1]

		47–48 y old		70–73 y old	
	Subjects	Men (n = 1659)	Women (n = 2058)	Men (n = 1466)	Women (n = 1855)
	n				
BMI (in kg/m^2)	7038	26.1 (26.0, 26.3)[2]	24.8 (24.7, 25.0)[3]	25.9 (25.8, 26.1)	26.2 (26.0, 26.5)[4]
Obese, BMI > 30 (%)		11.2	9.6	8.8	17.5[4,5]
Underweight, BMI < 18.5 (%)		0.2	1.3[3]	0.5	2.1[5]
Lean mass (kg)	5179	59.8 (59.5, 60.2)	40.3 (40.1, 40.5)[3]	55.1 (54.7, 55.4)[3]	37.6 (37.4, 37.9)[4,5]
Fat mass (kg)	5179	20.6 (20.1, 21.1)	24.5 (24.0, 24.9)[3]	21.2 (20.7, 21.8)	27.0 (26.4, 27.5)[4,5]
Ratio of fat mass to lean mass	5179	0.34 (0.34, 0.35)	0.60 (0.59, 0.61)[3]	0.38 (0.38, 0.39)[3]	0.71 (0.70, 0.73)[4,5]
Total cysteine (μmol/L)	7038	282 (281, 284)	266 (265, 268)[3]	318 (317, 320)[3]	322 (320, 323)[4,5]
Total homocysteine (μmol/L)[6]	7038	10.4 (10.3, 10.6)	8.8 (8.7, 8.9)[3]	12.5 (12.3, 12.7)[3]	11.1 (11.0, 11.3)[4,5]
Vitamin B-12 (pmol/L)[6]	7030	353 (348, 358)	359 (353, 364)	340 (333, 348)[3]	359 (352, 366)[5]
Folate (nmol/L)[6]	7015	6.5 (6.4, 6.7)	7.3 (7.1, 7.4)[3]	6.4 (6.2, 6.6)	7.7 (7.4, 7.9)[4,5]
Creatinine (μmol/L)[6]	7029	79 (78, 79)	64 (63, 64)[3]	84 (83, 85)[3]	67 (67, 68)[4,5]

[1] Differences between the 4 age-sex groups were first tested by chi-square test or ANOVA ($P < 0.001$ for all variables) followed by group-wise comparisons with Bonferroni adjustment.
[2] $\bar{x}$; 95% CI in parentheses (all such values).
[3] $P < 0.05$ compared with middle-aged men.
[4] $P < 0.05$ compared with middle-aged women.
[5] $P < 0.05$ compared with elderly men.
[6] Using log-transformed data.

BMI, lean mass, and fat mass. Adjustments were made for variables associated with tCys (8) or tHcy (20) that are potentially related to body build and for related metabolites including cystathionine, methionine, and tCysGly.

To show nonlinear relations, dose-response curves were constructed to show the estimated difference in tCys by tHcy and in BMI, lean mass, and fat mass by tCys and tHcy. We used Gaussian generalized additive regression models, as implemented in S-PLUS for WINDOWS software (version 6.2; Insightful Corporation, Seattle, WA). On the y-axis, the model generates a reference value of zero that approximately corresponds to the value of BMI, lean mass, or fat mass associated with the mean tCys or tHcy for all subjects. Various models with different covariates are specified in the figure legends. Corresponding P values were obtained from multiple linear regression analyses.

To assess the effect of changes in tCys or tHcy over 6 y as predictors of BMI, fat mass, or lean mass at follow-up, multiple linear regression models were used. BMI, fat mass, or lean mass at follow-up was used as the dependent variable, whereas changes in tHcy or tCys over 6 y were represented in the models as indicator variables, denoting membership to 1 of the 5 quintiles for changes in tCys or tHcy. Thus, each regression coefficient estimated the difference in BMI, fat mass, or lean mass between the lowest quintile (reference) and the other 4 quintiles of changes in tHcy or tCys.

All statistical analyses, except dose-response curves, were performed by using SPSS for WINDOWS software (version 12.0; SPSS Institute, Chicago. IL). Tests of significance were 2-tailed, and $P < 0.05$ was considered significant.

RESULTS

Characteristics of the study population

Selected population characteristics are shown in **Table 1**. BMI was significantly ($P < 0.001$) higher in middle-aged men than in women, although men had significantly ($P < 0.001$) lower fat mass than did women. The ratio of fat mass to lean mass differed significantly ($P < 0.001$) among the 4 age-sex groups, increasing from middle-aged men to elderly men to middle-aged women to elderly women. With the use of Spearman correlations, lean mass and fat mass were positively associated ($r_s = 0.28$, $P < 0.001$ in men; $r_s = 0.37$, $P < 0.001$ in women). The correlation of directly measured body weight with the sum of body-composition elements as obtained by DXA (lean mass + fat mass + bone mineral content) was almost perfect ($r_s = 0.98$). Mean plasma tCys, tHcy, and creatinine were significantly ($P < 0.001$) higher in the elderly than in the middle-aged and significantly ($P < 0.05$) higher in men than in women of the same age group.

Relation of total homocysteine and total cysteine

Linear regression analysis showed a significant positive association between tHcy as a determinant variable and tCys as dependent variable, after adjustment for age and sex (partial $r = 0.37$, $P < 0.001$; **Figure 1**A), and this association was unchanged by adjustment for folate, vitamin B-12, creatinine, fat mass, and lean mass (data not shown). From low to high tHcy concentrations, tCys differed by ≈60 μmol/L, although, toward the higher tHcy values, tCys concentrations leveled off. Change in tHcy over 6 y was linearly associated with change in tCys in the same direction (partial $r = 0.32$, $P < 0.001$; Figure 1B), with adjustment for age and sex. Because of this strong relation between tHcy and tCys, the linear regression analyses were always performed both with and without reciprocal adjustment for tCys and tHcy.

Total cysteine and indexes of body mass

tCys and BMI

The association between tCys and BMI, after control for age and sex, was linear, highly significant (partial $r = 0.28$, $P < 0.001$; **Figure 2**A), and not affected by adjustments for tHcy (partial $r = 0.29$, $P < 0.001$ for tCys) or plasma creatinine, lipids

The American Journal of Clinical Nutrition

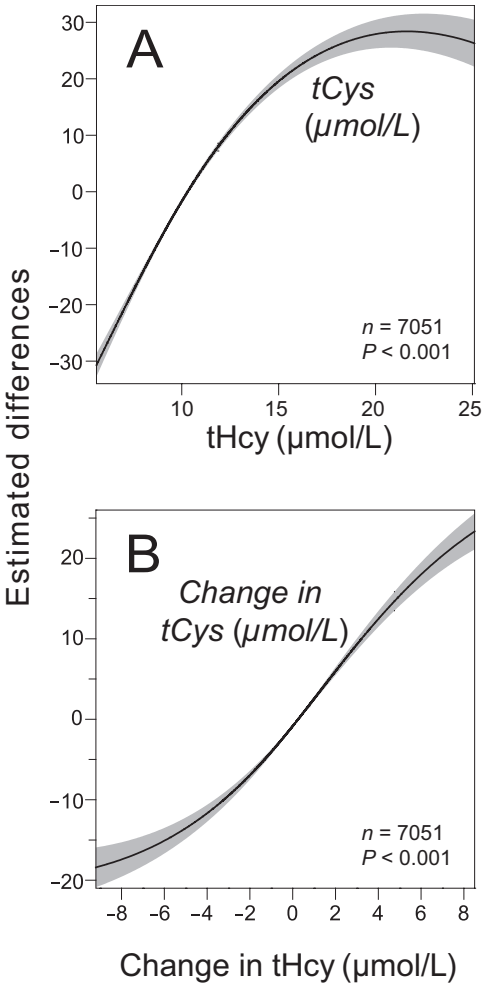

FIGURE 1. Dose-response curves (solid lines) with 95% CIs (shaded areas) for associations of plasma total homocysteine (tHcy) with plasma total cysteine (tCys) and of change in tHcy during 6 y with change in tCys. Curves were fitted by Gaussian generalized additive regression models and were adjusted for age and sex. *P* values were obtained by corresponding linear regression analyses. The lowest and highest 1 percentiles of independent variables are not shown.

(ie, total cholesterol, triacylglycerol, and HDL), coffee intake, or systolic blood pressure (partial $r = 0.26$, $P < 0.001$ for tCys). In a model fully adjusted for age, sex, tHcy, creatinine, lifestyle factors (eg, coffee intake, smoking, and physical activity), nutritional intake (ie, total energy, protein, and fat intakes), and serum lipids, tCys was the strongest determinant of BMI (partial $r = 0.23$, $P < 0.001$).

Total cysteine and body composition

There was no effect of tCys on lean mass when fat mass was taken into account, and the dose-response curves were essentially horizontal with (partial $r = 0.02$, $P = 0.10$) and without ($r = 0.02$, $P = 0.20$; Figure 2B) adjustment for tHcy. There was a strong positive linear association between tCys and fat mass, after control for age, sex, and lean mass (partial $r = 0.26$, $P < 0.001$; Figure 2C). This association was largely unaffected by adjustment for tHcy, plasma creatinine, total cholesterol, triacylglycerol, HDL, coffee intake, and systolic blood pressure (partial $r = 0.25$, $P < 0.001$ for tCys). In this latter model, tCys was the strongest plasma determinant of fat mass, followed by HDL (partial $r = -0.21$, $P < 0.001$) and triacylglycerol (partial $r = 0.1$, $P < 0.001$). In a fully adjusted model, including age, sex, lean mass, and lifestyle (eg, coffee consumption, smoking, and physical activity), nutritional (ie, total energy, protein, and fat intakes), and plasma (ie, creatinine, lipids, and tHcy) variables, tCys was second only to sex and lean mass as a determinant of fat mass (partial $r = 0.21$, $P < 0.001$ for tCys).

Lean mass, fat mass, and anthropometric measures by quintiles of tCys in men and women are shown in **Table 2**. Women in the highest quintile of tCys had an average weight, fat mass, and waist circumference that were 11 kg (95% CI: 10, 12 kg), 9 kg (8, 10 kg), and 9 cm (8, 10 cm), respectively, higher than those

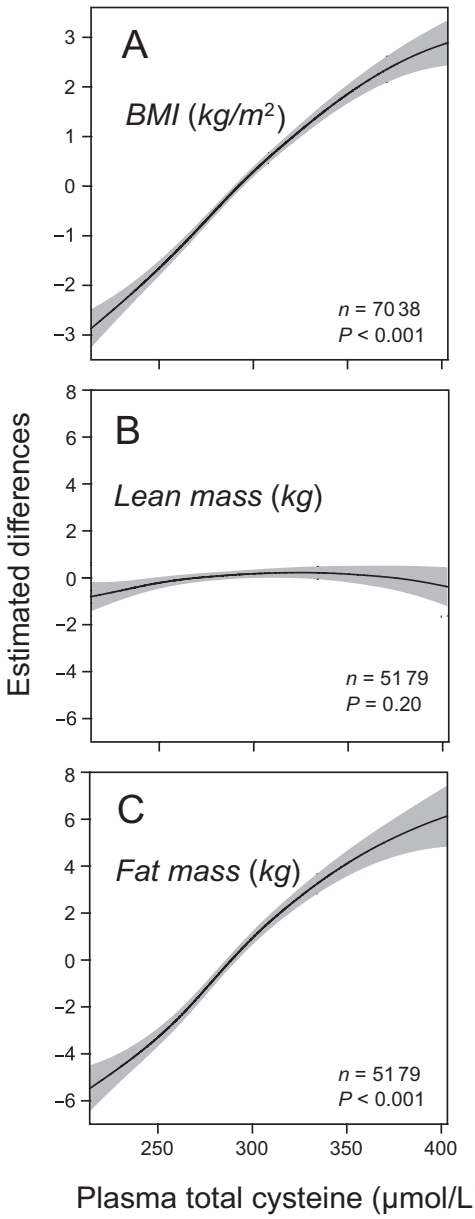

FIGURE 2. Association of plasma total cysteine with BMI, total-body lean mass, and total-body fat mass. All graphs were obtained by Gaussian generalized additive regression models and were adjusted for age and sex. Additional adjustments include fat mass (B) and lean mass (C). *P* values were obtained by corresponding linear regression analyses. The lowest and highest 1 percentiles of independent variable are not shown.

The American Journal of Clinical Nutrition

TABLE 2
Body composition and anthropometric variables by quintile (Q) of total cysteine (tCys)[1]

	Quintiles of tCys				
	Q1	Q2	Q3	Q4	Q5
Men					
Height (cm)	176 (176, 177)	177 (177, 178)	177 (177, 178)	177 (177, 178)	177 (177, 178)
Weight (kg)	78 (77, 79)	80 (79, 81)[2]	82 (81, 84)[3]	84 (83, 85)[3]	85 (84, 86)[3]
Fat mass (kg)	18 (17, 19)	19 (18, 20)	21 (20, 22)[3]	22 (22, 23)[3]	24 (23, 25)[3]
Lean mass (kg)	57 (56, 57)	58 (57, 58)	58 (57, 59)[2]	58 (57, 59)[2]	58 (57, 59)[2]
Waist circumference (cm)	91 (90, 92)	92 (91, 93)	94 (94, 96)[3]	96 (95, 97)[3]	97 (96, 98)[3]
Hip circumference (cm)	99 (99, 99)	100 (100, 101)[2]	101 (101, 102)[3]	102 (101, 102)[3]	102 (102, 103)[3]
Waist-hip ratio	0.92 (0.91, 0.92)	0.92 (0.91, 0.92)	0.93 (0.93, 0.94)[3]	0.94 (0.94, 0.95)[3]	0.94 (0.94, 0.95)[3]
Women					
Height (cm)	163 (163, 164)	164 (164, 165)[2]	163 (163, 164)	163 (163, 164)	164 (164, 165)[2]
Weight (kg)	63 (63, 64)	67 (66, 68)[3]	67 (66, 67)[3]	70 (69, 71)[3]	74 (73, 75)[3]
Fat mass (kg)	22 (21, 22)	24 (23, 25)[3]	24 (23, 25)[3]	27 (26, 28)[3]	30 (29, 31)[3]
Lean mass (kg)	38 (38, 39)	39 (39, 40)[3]	39 (39, 39)[2]	40 (39, 40)[3]	40 (40, 41)[3]
Waist circumference (cm)	78 (77, 79)	81 (80, 81)[3]	81 (80, 81)[3]	84 (83, 85)[3]	87 (86, 88)[3]
Hip circumference (cm)	98 (97, 98)	100 (99, 100)[3]	100 (99, 101)[3]	101 (101, 102)[3]	105 (104, 105)[3]
Waist-hip ratio	0.80 (0.79, 0.80)	0.81 (0.80, 0.81)[2]	0.81 (0.81, 0.82)[2]	0.83 (0.82, 0.83)[3]	0.83 (0.83, 0.84)[3]

[1] All values are $\bar{x}$; 95% CI in parentheses. Quintiles are age group– and sex-specific. Significance of difference between quintiles was first tested by ANOVA ($P < 0.001$ for all variables except height in men) followed by group-wise comparisons with Bonferroni adjustment using the lowest quintile as reference.
[2] $P < 0.05$ compared with first quintile.
[3] $P < 0.001$ compared with first quintile.

in women in the lowest quintile. In men, the corresponding values were 7 kg (6, 8 kg), 6 kg (5, 7 kg), and 6 cm (5, 7 cm), respectively ($P < 0.001$ across quintiles in men and women, ANOVA). Waist and hip circumferences and waist/hip ratio also increased significantly across tCys quintiles. In contrast, height showed only a minor fluctuation of up to 1 cm between the tCys quintiles, and there was no apparent trend.

Change in tCys over a 6-y follow-up period was associated with significant differences in BMI and fat mass, with a negligible effect on lean mass. Estimated differences in BMI, fat mass, and lean mass at follow-up by quintiles of change in tCys compared with first quintile, after adjustment for various covariates, are shown in **Table 3**. The group of participants with the highest increase in tCys had fat mass at follow-up that was >2 kg greater

TABLE 3
Estimated differences in BMI, lean mass, and fat mass at follow-up by quintile (Q) of change in total cysteine (tCys) and total homocysteine (tHcy) over 6 y[1]

	Estimated difference					
	BMI (kg/m^2)		Lean-mass (kg)		Fat-mass (kg)	
Quintiles of mean change in tCys						
Q1 (7% decrease; reference category)						
Q2 (1% increase)	0.15	0.20	0.1	0.1	0.7	1.0
Q3 (6% increase)	0.24	0.32	0.0	0.0	1.4	1.3
Q4 (12% increase)	0.38	0.44	0.2	0.0	1.8	2.0
Q5 (23% increase)	0.53	0.57	0.0	0.1	2.1	2.2
P for trend	<0.0001	<0.0001	0.87	0.68	<0.0001	<0.0001
Quintiles of mean change in tHcy						
Q1 (24% decrease; reference category)						
Q2 (9% decrease)	0.02	−0.15	0.2	0.2	0.2	−0.3
Q3 (1% increase)	−0.06	−0.33	0.3	0.1	0.1	−0.6
Q4 (11% increase)	0.03	−0.24	0.5	0.7	0.1	−0.8
Q5 (35% increase)	−0.09	−0.48	0.1	0.0	0.0	−1.2
P for trend	0.26	<0.0001	0.87	0.76	0.87	<0.0001

[1] The models were calculated by linear regression and estimated the difference in mean BMI, fat mass, and lean mass between each quintile and the reference quintile (lowest quintile) of change in tCys or tHcy. Models 1 (left-hand column under each heading) and 2 (right-hand column under each heading) were used for tCys; models 3 (left-hand column under each heading) and 4 (right-hand column under each heading) were used for tHcy. Model 1: adjusted for age, sex, baseline BMI, baseline tCys, fat mass in case of lean mass, and lean mass in case of fat mass. Model 2: adjusted for all model 1 variables + changes in plasma total cholesterol and triacylglycerol, change in smoking habits, and systolic blood pressure + plasma creatinine and physical activity at follow-up. Model 3: adjusted for age, sex, baseline BMI, baseline tHcy, fat mass in case of lean mass, and lean mass in case of fat mass. Model 4: adjusted for all model 3 variables + changes in plasma concentrations of tCys, vitamin B-12, folate, triacylglycerol, and cholesterol; change in smoking habits; and plasma creatinine and physical activity at follow-up.

The American Journal of Clinical Nutrition

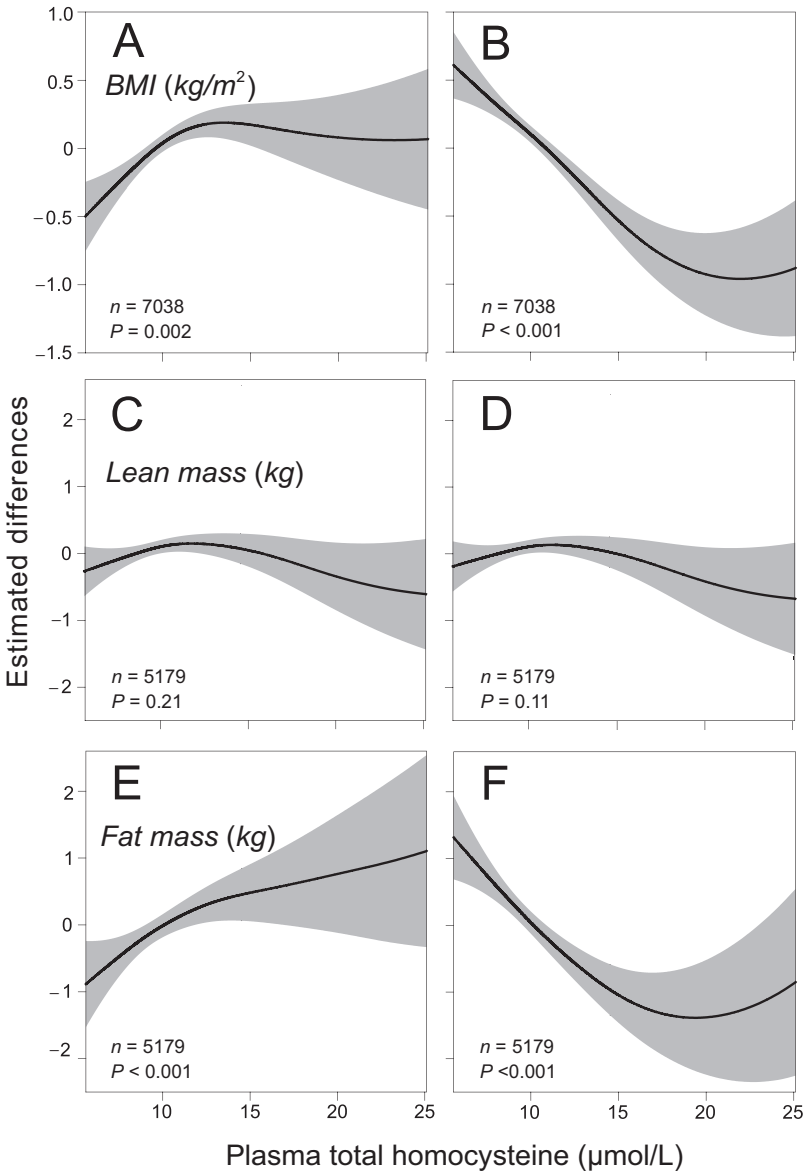

FIGURE 3. Association of plasma total homocysteine with BMI, lean mass, and fat mass. All graphs were obtained by Gaussian generalized additive regression models and were adjusted for age and sex. Additional adjustments include plasma total cysteine (B), fat mass (C), fat mass and total cysteine (D), lean mass (E), and lean mass and total cysteine (F). *P* values were obtained by corresponding linear regression analyses. The lowest and highest 1 percentiles of independent variables are not shown.

than that in the reference category (P for trend < 0.001), with adjustment for baseline tCys.

Total homocysteine and indexes of body mass

Total homocysteine and BMI

After control only for age and sex, linear regression analysis showed a weak positive association between tHcy and BMI (partial $r = 0.04$, $P = 0.002$; **Figure 3**A), which weakened after adjustment for creatinine (partial $r = 0.03$, $P = 0.039$). With adjustment for tCys, a significant negative association of tHcy and BMI was found (partial $r = -0.08$, $P < 0.001$; Figure 3B). This association was strengthened by adjustment for plasma concentration of B vitamins (folate and vitamin B-12) as well as lifestyle factors (ie, smoking, physical activity, and coffee consumption), nutritional factors (ie, total energy, protein, and fat intakes) and serum lipids (partial $r = -0.13$, $P < 0.001$).

Total homocysteine and body composition

The associations of tHcy as an independent variable with fat mass or lean mass as the outcome in various models were weak. A nonsignificant trend toward an inverse association between tHcy and lean mass, with adjustment for age, sex, and fat mass (Figure 3C,D), reached statistical significance only when after adjustment for creatinine (partial $r = -0.03$, $P = 0.026$). However, with further adjustment for tCys, B vitamins, lifestyle factors (ie, smoking, coffee consumption, and physical activity), and nutritional intakes (ie, total energy, protein, and fat intakes),

The American Journal of Clinical Nutrition

the association of tHcy and lean mass was not significant (partial $r = -0.03$, $P = 0.085$).

The tHcy-fat mass association in the simple linear regression model after adjustment for age, sex, and lean mass was positive (partial $r = 0.05$, $P < 0.001$; Figure 3E) and became inverse after control for tCys (partial $r = -0.05$, $P < 0.001$; Figure 3F). Further adjustment for lifestyle variables (ie, smoking, coffee intake, and physical activity), nutritional factors (ie, total energy, protein, and fat intakes), serum lipids, and plasma B vitamins strengthened this inverse tHcy-fat mass association (partial $r = -0.1$, $P < 0.001$).

There was no significant association between change in tHcy over a 6-y follow-up period and BMI, lean mass, and fat mass at follow-up (Table 3). After the inclusion of additional covariates in the models, the associations with BMI and fat mass turned inverse and became statistically significant. The covariate reversing the direction of the association in the fully adjusted models was tCys, which is consistent with its strong correlations both with tHcy and with BMI and fat mass (Figures 1 and 2).

Other plasma sulfur amino acids

We investigated whether plasma concentrations of other aminothiols involved in the cysteine metabolic pathway could explain the strong association of tCys with fat mass. A multiple linear regression model examined the roles of tCys, tHcy, cystathionine, methionine, and tCysGly as predictors of fat mass after adjustment for age, sex, lean mass, and time since last meal. tCys remained the strongest aminothiol determinant (partial $r = 0.25$, $P < 0.001$), followed by cystathionine (partial $r = 0.1$, $P < 0.001$). Methionine and tHcy showed weak inverse associations with fat mass (partial $r = -0.04$, $P = 0.038$, and partial $r = -0.05$, $P = 0.011$ respectively), whereas tCysGly showed no significant association (partial $r = 0.03$, $P = 0.161$). With further adjustment for plasma lipids, only tCys (partial $r = 0.24$, $P < 0.001$), cystathionine (partial $r = 0.05$, $P = 0.011$), and tHcy (partial $r = -0.06$, $P = 0.002$) remained significantly associated with fat mass.

DISCUSSION

Homocystinuria due to CBS deficiency is characterized by extremely elevated tHcy concentrations combined with low tCys concentrations (1), and it leads to a thin phenotype (1, 4, 5). On the basis of data from the HHS presented here and previously (8, 9), we suggest that more modest variations in these 2 amino acids, particularly tCys, could have an effect on body composition in the general population.

Total cysteine and indexes of body mass

Unlike homocysteine, cysteine is proteinogenic, with a key role in maintaining protein structure and folding (2) and varied functions in cell growth and survival (30). The sulfur amino acid content of diet correlates strongly with food conversion efficiency (g body wt gain/g food intake) (31). Yet, in the present study, tCys showed no association with lean mass but a strong positive association with fat mass. As a determinant of fat mass, tCys was even stronger than serum lipids such as triacylglycerol, HDL, and total cholesterol. Thus, our data suggest that decreased tCys may explain the thinness (1), low BMI (4), and decreased subcutaneous fat (5) seen in patients with homocystinuria due to CBS deficiency. In view of the positive influence of fat mass on bone mineral density (32), it is conceivable that the decreased fat mass may also contribute to osteoporosis in these patients (1). More important, our data raise the possibility that high tCys or a related factor is causally related to body fat and obesity in the general population.

The transsulfuration pathway is important in meeting metabolic needs for cysteine (2)—and hence the low plasma tCys concentrations seen in CBS deficiency (1). Diverse evidence suggests an impairment of lipid metabolism in CBS deficiency (33). Besides the reported low subcutaneous fat (5), there is often a notable absence of arterial lipid deposition, despite extensive vascular pathology (1), fatty liver (1, 5), and decreased plasma concentrations of triacylglycerol and total cholesterol (34). Methionine and cystathionine concentrations also are altered in CBS deficiency (26). However, our results do not suggest a major role for these amino acids in body composition, although this may need to be confirmed by using fasting samples.

A literature search for mechanisms associating tCys with lipid metabolism retrieved no conclusive explanation but several potential links, which we will discuss briefly. One possibility is that high tCys in obese persons is merely a marker for reduced oxidation of cysteine to taurine and hence taurine deficiency, which is believed to result in obesity (35).

Cysteine is also the precursor of glutathione, the excess of which is cleaved by gamma- glutamyl transferase (GGT) into its constituent amino acids (36). GGT is thus critical in maintaining the availability of cysteine (36). In contrast, GGT correlates directly with BMI, central adiposity, and plasma triacylglycerol, LDL, and total cholesterol concentrations (36). Whether these associations are mediated through GGT's role in maintaining plasma tCys is a question for further study.

Several mechanisms link cysteine to increased energy production. In rat liver, cysteine suppresses 3-phosphoglycerate dehydrogenase, which initiates serine synthesis from 3-phosphoglycerate (37). Excess 3-phosphoglycerate can thus be diverted toward oxidative energy production in the Krebs cycle. Moreover, cysteine itself is gluconeogenic, but only under conditions of increased cysteine availability, as shown by the conversion of labeled dietary cysteine to glucose in rats (38). In addition, α-ketobutyrate, a byproduct of cysteine formation via transsulfuration, is ultimately metabolized to CO_2 (39), which raises the question of whether tCys is a marker for an association of α-ketobutyrate with energy production. However, in the present study, the tCys-fat mass association was unrelated to plasma lipids and thus was possibly independent of a general positive energy balance.

Cysteine is also utilized in the synthesis of coenzyme A (CoA) (40). Experiments on perfused rat hearts have shown that exogenous cysteine enhances CoA synthesis (40), and that a high CoA concentration enhances the incorporation of free fatty acids into triacylglycerol (41). It is interesting that CoA deficiency resulting from reduced pantothenic acid intake in chicks decreased lipid synthesis and deposition and markedly decreased total body fat (42). Yet CoA plays a role in fat oxidation as well as in synthesis (43), and the ways in which cysteine affects these processes in humans remain to be determined.

Finally, insulin promotes fat synthesis and storage, and inhibits the hydrolysis of adipose tissue triacylglycerol (44). Several (44–47) but not all (48) studies describe various positive effects of cysteine or its analogue, *N*-acetylcysteine, on insulin function, which suggests that cysteine can modulate body fat by augmenting or mimicking insulin action.

Total homocysteine and indexes of body mass

The inverse association between tHcy and BMI we observed after adjustment for tCys is supported by findings from previous prospective studies (14–16) and one large cross-sectional study (49) but are not in line with the findings of most cross-sectional studies (10–12). However, the cross-sectional studies have not considered the possible confounding effect of tCys, which correlates positively with both tHcy and BMI (8). Associations of tHcy with lean mass and fat mass in the dataset in the present study were modest but generally negative. A negative association of tHcy with both fat mass and lean mass during weight loss has been reported (18). Conversely, in other studies, tHcy correlated positively with fat mass (19) and lean mass (17, 50).

The relation between tHcy and lean mass is complex and likely to involve opposing factors. A high lean mass entails high creatine turnover, with subsequently enhanced homocysteine formation (51). However, dietary creatine intake partly obviates the need for creatine synthesis (51), which may explain the absence of an association in the population in the present study. Furthermore, tHcy unfavorably correlates with lower body muscle strength and gait speed (52, 53) and is linked to less calf muscle density (54). Moreover, muscle weakness and electromyographic evidence of myopathy have been shown in homocystinuria (55). Hence, inconsistent results in different studies may be due to differences in diet, physical activity, or health status as well as to tHcy concentrations.

Strengths and weaknesses

Strengths of the study include the large size of the cohort and its recruitment from the general population. Plasma thiols and DXA measurements were available from >5000 persons. Furthermore, BMI, plasma tHcy, and plasma tCys were assessed 6 y apart, which allowed us to examine the effect of change over time. However, this is a cohort study, without intervention. Hence, despite the strong association between tCys and fat mass, our data cannot determine causality, and it remains possible that fat mass influences tCys or that a third, unknown factor can simultaneously alter both fat mass and tCys. Still, we should remember the image of a typical CBS-deficient homocystinuria patient (Internet: http://info.med.yale.edu/pediat/pedres/syndrome/week30_1.jpg) and ask, why are these patients so thin?

In summary, our data from the general population show that subjects with low tCys concentrations have substantially lower fat mass through an unknown mechanism that is independent of total energy intake, physical activity, and concentrations of serum lipids. The association of tHcy with body composition is much weaker but tends to be in the opposite direction. Our data are in line with the thin phenotype observed in homocystinuria due to CBS deficiency. Important implications of this study are that it is impossible to evaluate the association of tHcy with BMI and fat mass without taking tCys concentrations into account and that tCys may be an important confounder in studies involving body composition. Finally, mechanisms underlying the tCys-fat mass association, with possible implications for modulation of body fat, are interesting areas for further study.

We thank AD Smith for his valuable insights and review of the manuscript. We are also grateful to Kari Juul and Elfrid Blomdal for help with setting up the data files and for literature support.

The authors' responsibilities were as follows—AKE: the concept and design of the study, statistical analysis and interpretation, and preparation of the first draft of the manuscript; EN: statistical modeling of the dose-response figures and revision of the manuscript; CGG: data collection and critical revision of the manuscript; GST: planning, design, conduct, and data collection of the Hordaland Health Study and the Hordaland Homocysteine Study II; interpretation of the data; and critical revision of the manuscript; PMU: data collection and critical revision of the manuscript; ON: data collection and critical revision of the manuscript; AT: data collection and critical revision of the manuscript; SEV: data collection and interpretation and critical revision of the manuscript; HR: concept and design of the study, data collection and analysis, and critical revision of the manuscript; and all authors: approved the final version of the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The molecular and metabolic bases of inherited disease. 7th ed. New York, NY: McGraw-Hill, 1995; 1279–327.
2. Brosnan JT, Brosnan ME. The sulfur-containing amino acids: an overview. J Nutr 2006;136(suppl):1636S–40S.
3. Hargreaves IP, Lee PJ, Briddon A. Homocysteine and cysteine-albumin binding in homocystinuria: assessment of cysteine status and implications for glutathione synthesis? Amino Acids 2002;22:109–18.
4. Brenton DP, Dow CJ, James JI, Hay RL, Wynne-Davies R. Homocystinuria and Marfan's syndrome. A comparison. J Bone Joint Surg Br 1972; 54:277–98.
5. Gibson JB, Carson NA, Neill DW. Pathological findings in homocystinuria. J Clin Pathol 1964;17:427–37.
6. Herrmann M, Widmann T, Herrmann W. Letter re: "Elevated serum homocysteine and McKusick's hypothesis of a disturbed collagen cross-linking: what do we really know?" Bone 2006;39:1385–6 (letter).
7. Kraus JP, Kožich V. Cystathionine β synthase and its deficiency. In: Carmel R, Jacobsen DW, eds. Homocysteine in health and disease. Cambridge, United Kingdom: Cambridge University Press, 2001;223–44.
8. El-Khairy L, Ueland PM, Nygard O, Refsum H, Vollset SE. Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: the Hordaland Homocysteine Study. Am J Clin Nutr 1999; 70:1016–24.
9. El-Khairy L, Vollset SE, Refsum H, Ueland PM. Predictors of change in plasma total cysteine: longitudinal findings from the Hordaland homocysteine study. Clin Chem 2003;49:113–20.
10. Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001;73:613–21.
11. Koehler KM, Romero LJ, Stauber PM, et al. Vitamin supplementation and other variables affecting serum homocysteine and methylmalonic acid concentrations in elderly men and women. J Am Coll Nutr 1996; 15:364–76.
12. Poirier LA, Wise CK, Delongchamp RR, Sinha R. Blood determinations of *S*-adenosylmethionine, *S*-adenosylhomocysteine, and homocysteine: correlations with diet Cancer Epidemiol Biomarkers Prev 2001;10:649–55.
13. Brasileiro RS, Escrivao MA, Taddei JA, D'Almeida V, Ancona-Lopez F, Carvalhaes JT. Plasma total homocysteine in Brazilian overweight and non-overweight adolescents: a case-control study. Nutr Hosp 2005; 20:313–9.
14. Dixon JB, Dixon ME, O'Brien PE. Elevated homocysteine levels with weight loss after Lap-Band surgery: higher folate and vitamin B12 levels required to maintain homocysteine level. Int J Obes Relat Metab Disord 2001;25:219–27.
15. Henning BF, Tepel M, Riezler R, Gillessen A, Doberauer C. Vitamin supplementation during weight reduction—favourable effect on homocysteine metabolism. Res Exp Med (Berl) 1998;198:37–42.
16. Sheu WH, Wu HS, Wang CW, Wan CJ, Lee WJ. Elevated plasma homocysteine concentrations six months after gastroplasty in morbidly obese subjects. Intern Med 2001;40:584–8.
17. Rauh M, Verwied S, Knerr I, Dorr HG, Sonnichsen A, Koletzko B. Homocysteine concentrations in a German cohort of 500 individuals: reference ranges and determinants of plasma levels in healthy children and their parents. Amino Acids 2001;20:409–18.

18. Gallistl S, Sudi KM, Erwa W, Aigner R, Borkenstein M. Determinants of homocysteine during weight reduction in obese children and adolescents. Metabolism 2001;50:1220–3.
19. Gallistl S, Sudi K, Mangge H, Erwa W, Borkenstein M. Insulin is an independent correlate of plasma homocysteine levels in obese children and adolescents. Diabetes Care 2000;23:1348–52.
20. Refsum H, Nurk E, Smith AD, et al. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. J Nutr 2006;136(suppl):1731S–40S.
21. Guttormsen AB, Solheim E, Refsum H. Variation in plasma cystathionine and its relation to changes in plasma concentrations of homocysteine and methionine in healthy subjects during a 24-h observation period. Am J Clin Nutr 2004;79:76–9.
22. Pietrobelli A, Formica C, Wang Z, Heymsfield SB. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. Am J Physiol 1996;271:E941–51.
23. Nes M, Frost Andersen L, Solvoll K, et al. Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. Eur J Clin Nutr 1992;46:809–21.
24. Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA 1995;274:1526–33.
25. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem 1993;39:263–71.
26. Refsum H, Grindflek AW, Ueland PM, et al. Screening for serum total homocysteine in newborn children. Clin Chem 2004;50:1769–84.
27. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.
28. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol 1991;44:592–5.
29. Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. Clin Chem 2003;49:286–94.
30. Leuchtenberger C, Leuchtenberger R. L-cysteine or vitamin C influence cellular growth and prolong survival of normal adult human lung tissue in culture. Cell Biol Int Rep 1977;1:317–24.
31. Pieniazek D, Rakowska M, Szkilladziowa W, Grabarek Z. Estimation of available methionine and cysteine in proteins of food products by in vivo and in vitro methods. Br J Nutr 1975;34:175–90.
32. Reid IR, Ames R, Evans MC, et al. Determinants of total body and regional bone mineral density in normal postmenopausal women–a key role for fat mass. J Clin Endocrinol Metab 1992;75:45–51.
33. Namekata K, Enokido Y, Ishii I, Nagai Y, Harada T, Kimura H. Abnormal lipid metabolism in cystathionine beta-synthase-deficient mice, an animal model for hyperhomocysteinemia. J Biol Chem 2004;279:52961–9.
34. Moat SJ, Bonham JR, Allen JC, Powers HJ, McDowell IF. Decreased circulating plasma lipids in patients with homocystinuria. J Inherit Metab Dis 1999;22:243–6.
35. Tsuboyama-Kasaoka N, Shozawa C, Sano K, et al. Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. Endocrinology 2006;147:3276–84.
36. Whitfield JB. Gamma glutamyl transferase. Crit Rev Clin Lab Sci 2001; 38:263–355.
37. Achouri Y, Robbi M, Van Schaftingen E. Role of cysteine in the dietary control of the expression of 3-phosphoglycerate dehydrogenase in rat liver. Biochem J 1999;344(Pt 1):15–21.
38. Simpson RC, Hill FW, Freedland RA. Gluconeogenesis from L-cysteine in the perfused rat liver. J Nutr 1975;105:379–84.
39. Stipanuk MH. Sulfur amino acid metabolism: pathways for production and removal of homocysteine and cysteine. Annu Rev Nutr 2004;24: 539–77.
40. Chua BH, Giger KE, Kleinhans BJ, Robishaw JD, Morgan HE. Differential effects of cysteine on protein and coenzyme A synthesis in rat heart. Am J Physiol 1984;247:C99–106.
41. Lopaschuk GD, Hansen CA, Neely JR. Fatty acid metabolism in hearts containing elevated levels of CoA. Am J Physiol 1986;250:H351–9.
42. Cupo MA, Donaldson WE. Effect of pantothenic acid deficiency on lipogenesis in the chick. Nutr Rep Int 1986;33:147–55.
43. Guyton AC, Hall JE. Textbook of medical physiology. 11th ed. Philadelphia, PA: Elsevier Saunders, 2006.
44. Olefsky JM. Comparison of the effects of insulin and insulin-like agents on different aspects of adipocyte metabolism. Horm Metab Res 1979; 11:209–13.
45. Fulghesu AM, Ciampelli M, Muzj G, et al. N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome. Fertil Steril 2002;77:1128–35.
46. Blouet C, Mariotti F, Azzout-Marniche D, et al. Dietary cysteine alleviates sucrose-induced oxidative stress and insulin resistance. Free Radic Biol Med 2007;42:1089–97.
47. Ammon HP, Hehl KH, Enz G, Setiadi-Ranti A, Verspohl EJ. Cysteine analogues potentiate glucose-induced insulin release in vitro. Diabetes 1986;35:1390–6.
48. Droge W. Oxidative stress and ageing: is ageing a cysteine deficiency syndrome? Philos Trans R Soc Lond B Biol Sci 2005;360:2355–72.
49. Ganji V, Kafai MR. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr 2003;77:826–33.
50. Battezzati A, Bertoli S, San Romerio A, Testolin G. Body composition: an important determinant of homocysteine and methionine concentrations in healthy individuals. Nutr Metab Cardiovasc Dis 2007;17:525–34.
51. McCarty MF. Supplemental creatine may decrease serum homocysteine and abolish the homocysteine 'gender gap' by suppressing endogenous creatine synthesis. Med Hypotheses 2001;56:5–7.
52. Kuo HK, Liao KC, Leveille SG, et al. Relationship of homocysteine levels to quadriceps strength, gait speed, and late-life disability in older adults. J Gerontol A Biol Sci Med Sci 2007;62:434–9.
53. Kado DM, Bucur A, Selhub J, Rowe JW, Seeman T. Homocysteine levels and decline in physical function: MacArthur Studies of Successful Aging. Am J Med 2002;113:537–42.
54. McDermott MM, Ferrucci L, Guralnik JM, et al. Elevated levels of inflammation, d-dimer, and homocysteine are associated with adverse calf muscle characteristics and reduced calf strength in peripheral arterial disease. J Am Coll Cardiol 2007;50:897–905.
55. Hurwitz LJ, Chopra JS, Carson NA. Electromyographic evidence of a muscle lesion in homocystinuria. Acta Paediatr Scand 1968;57:401–4.

The American Journal of Clinical Nutrition