Effect of bariatric surgery on sulphur amino acids and glutamate

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Abstract

Plasma total cysteine (tCys) concentrations are associated with BMI. To study the relationship between tCys and BMI, we monitored the changes in serum concentrations of tCys and metabolically related compounds in sixty obese patients (BMI 50–60 kg/m^2) from before to 1 year after either gastric bypass surgery (mean 30 % weight loss) or duodenal switch surgery (mean 41 % weight loss). A total of fifty-eight healthy persons (BMI 17–31 kg/m^2) served as controls. Before surgery, obese patients had modestly (approximately 17 %) higher mean serum tCys, and markedly ($>$2-fold) higher glutamate concentrations, than controls ($P \leq 0.001$ for both). Serial examinations after surgery revealed that gastric bypass patients had no change in tCys concentrations ($P = 0.22$), while duodenal switch patients showed a modest (approximately 12 %) but significant decrease in tCys ($P < 0.001$). Total homocysteine concentrations increased in duodenal switch patients but not in gastric bypass patients. Independent of surgery type, serum concentrations of methionine and cystathionine decreased ($P < 0.05$ for both), while serum glutathione and taurine remained stable. Glutamate concentrations declined, as did γ-glutamyltransferase activity ($P < 0.001$ for both). These results show that despite 30 % weight loss, and decreases in methionine, cystathionine and glutamate, there was no significant change in serum tCys in patients after gastric bypass surgery. The decrease in tCys in patients undergoing duodenal switch could be related to malabsorption. The present findings do not suggest that BMI is a causal determinant of plasma tCys.

Key words: Obesity surgery: Homocysteine: Folic acid: Cobalamin: Pyridoxine

The sulphur amino acids (SAA) are fundamental to protein synthesis. Both methionine and cysteine are proteinogenic amino acids; methionine is required for translation of all eukaryotic proteins, and cysteine contributes to protein structure by forming disulphide bonds[1]. Although it has not been established that SAA can contribute to the development of obesity, methionine and cysteine intakes can promote growth and weight gain in mammals[2,3]. In humans, plasma total cysteine (tCys) levels are positively associated with BMI and obesity[4–6], mediated through fat mass[6]. Among men and women in a population-based study, an increase from low to high tCys quintile was associated with an increase in fat mass of 6–9 kg, after adjustment for age, diet, lifestyle factors and serum lipids. Longitudinal studies have revealed that an increase or decrease in tCys over 6 years was linked with corresponding changes in BMI and fat mass[6,7].

Obesity is also associated with changes in compounds metabolically related to cysteine. Plasma glutathione (GSH) correlates negatively with BMI[8], while the amino acid glutamate is elevated in the blood[9] and plasma[10] of obese individuals. Glutamate is linked to cysteine via GSH metabolism: cysteine and glutamate combine to form γ-glutamylcysteine in the rate-limiting step of GSH synthesis[11]. The membrane enzyme γ-glutamyltransferase (GGT) hydrolyses GSH, with the subsequent release of cysteine and glutamate[12]. Serum GGT activity is elevated in obese persons[5,13], and decreases

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with weight loss[14]. Conversely, plasma concentrations of the cysteine precursor, homocysteine, often increase after weight loss[15].

Despite the diverse evidence pointing to a link between cysteine metabolism and body weight, the effect of weight loss on cysteine metabolism is largely unexplored. The causal direction for the relationship between tCys and fat mass remains uncertain: does tCys promote body fat accumulation; or does fat mass influence cysteine production, catabolism, uptake or release? In favour of tCys promoting fat accumulation, intake of cysteine-rich diets has been associated with weight gain both in animal models[16,17] and in patients with cancer[18]. However, recent studies on the role of adipose tissue in regulating cysteine conversion to taurine[19] have suggested that adipose tissue mass could be an important determinant of cysteine and taurine levels. The hypothesis that fat mass determines plasma tCys has not been addressed in humans. Severely obese patients undergoing bariatric surgery may be an ideal population for testing the hypothesis: if fat mass determines tCys, then tCys concentrations would be expected to be markedly elevated before and drop significantly after bariatric surgery due to major weight loss. We, therefore, studied the effects of bariatric surgery in obese patients on serum concentrations of tCys and metabolically related compounds, such as methionine, homocysteine, cystathionine, taurine, GSH and glutamate, and serum GGT activity.

Experimental methods

The patients have been described in detail previously[20]. Briefly, sixty obese patients aged 20–50 years, from two centres (Oslo University Hospital Aker, n 30; Sahlgrenska University Hospital, n 30), were included in a randomised trial of gastric bypass and duodenal switch for weight loss (February 2006–August 2007; ClinicalTrials.gov identifier: NCT00327912). All patients had a BMI between 50 and 60 kg/m^2. In addition to the baseline visit, the patients were examined 6 weeks, 6 months and 1 year after surgery. Patients followed a 4184 kJ (1000 kcal) diet for 3 weeks before surgery. During surgery, the alimentary limb, biliopancreatic limb and common channel were created as follows: gastric bypass – 150, 50 cm and variable; duodenal switch – 200 cm, variable and 100 cm[20]. After surgery, all patients were daily prescribed Fe, Ca, vitamin D and multivitamin/mineral supplements (selected contents: 2 mg vitamin B$_6$, 200 μg folic acid and 1 mg vitamin B$_{12}$ (Nycoplus multi; Nycomed, Asker, Norway)). Gastric bypass patients also received cyanocobalamin supplementation (intramuscular injections, 1 mg every 3 months (Betolvex; Actavis, Oslo, Norway) or oral supplement, 1 mg daily (Behepan; Pfizer, Sollentuna, Sweden)). Ursodeoxycholic acid was given until 6 months after surgery. Duodenal switch patients were more often taking additional fat-soluble vitamin supplements, but otherwise, supplement use was similar in the two groups[20].

The controls were fifty-eight healthy men and women with BMI between 17 and 31 kg/m^2 and aged 19–59 years, recruited January–May 2007 at Oslo University Hospital

Aker[21]. The controls were recruited as a convenience sample, with no attempt made to match controls and patients. Exclusion criteria were chronic disease and regular medication or multivitamin use. Persons using contraceptive medication (n 10) or thyroxine substitution (n 4) were included. As a population-based control group for plasma tCys and BMI comparisons (see Fig. S1 of the supplementary material, available online at http://www.journals.cambridge.org/bjn), we used the Hordaland Homocysteine Study participants (n 3721, men and women aged 47–49 years)[6].

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the appropriate regional ethics committees for medical research. Written informed consent was obtained from all subjects.

Biochemical analysis

Overnight fasting blood samples were clotted for 30 min at room temperature, and serum was separated by centrifugation. Samples collected at Oslo University Hospital Aker were frozen and stored at −80°C until assayed. Samples collected at Sahlgrenska University Hospital were thawed once for the analysis of 25-hydroxyvitamin D[20] and were subsequently stored at −20°C until assayed. Assays were performed within 2 years of blood sampling.

Serum concentrations of SAA and glutamate were analysed by liquid chromatography–tandem MS using modifications of described methods[16,22]. Methionine, total homocysteine (tHcy), cystathionine, tCys and total glutathione (tGSH) were analysed in a single run. A separate assay was used to simultaneously measure taurine and glutamate. Inter-assay CV was <4% for tCys, tHcy and taurine; <8% for methionine, cystathionine and tGSH; and 10·5% for glutamate. Serum cobalamin was determined using *Lactobacillus leichmannii* microbiological assays[23].

Serum creatinine, alanine aminotransferase, GGT and folate were measured during the follow-up with Hitachi (717 or 800) Modular multianalysers (Boehringer Mannheim, Mannheim, Germany)[20]. Serum pyridoxal-5′-phosphate (vitamin B$_6$) was measured by HPLC (Chromsystems, Munich, Germany). Plasma tHcy concentrations measured during the follow-up in some patients (using Hitachi 717 multianalyser) correlated well with the reported serum concentrations obtained by liquid chromatography–tandem MS (Pearson's r 0·95, $P < 0·001$; n 18).

In total, frozen serum samples were available from 223 out of 240 study visits (93%). As outlined above, serum handling and storage varied between the study sites (Oslo and Sahlgrenska). In agreement with our laboratory's experience that sample processing and freeze–thaw cycles may influence assay values, the serum concentrations for methionine, taurine, tGSH and glutamate differed markedly between the centres. We, therefore, report data on those metabolites only for patients with specimens kept at −80°C (n 30; followed in Oslo), and data on tHcy, cystathionine and tCys from all patients (n 60).

Statistical analysis

For cross-sectional analyses, we compared patients and controls separately for men and women, using Fisher's exact test for proportions and unpaired Student's *t* tests for continuous data. These tests, correlation analyses and multiple linear regressions were calculated using SPSS 14.0 (SPSS, Inc., Chicago, IL, USA).

For longitudinal analyses, we used linear mixed-effects models (with a random intercept and assuming compound symmetric correlation structure) for continuous dependent variables to assess a trend (time effect) across the four time points in each patient. Estimates of fixed-effects coefficients and their standard errors were extracted after fitting the model. Standardised values of the outcome variables were computed as predicted values from the model. These predictions were done for each time point in a new dataset, where potential confounders (age, sex and type of surgery) were replaced by their overall average values in the full dataset. Standard errors of the predicted values were estimated by assuming the predictors as non-random and using the estimated variance–covariance matrix for the fixed-effects estimates. Non-normally distributed variables (including tHcy, cystathionine, cobalamin, creatinine, alanine aminotransferase and GGT) were log-transformed before analysis. Data are expressed as standardised means or geometric means with 95 % CI. Linear mixed-effects models (lme function in the R-library nmle) were computed in R 2.8.1 for Windows[24]. The *P* values are two-sided, and the significance level was 0·05.

Results

Participant characteristics

Most participants (97 %) were of North European descent. Mean age was not significantly different between patients and controls, who had a mean BMI of 55 and 24 kg/m². Patients had lower serum pyridoxal-5′-phosphate and folate concentrations than controls (Table 1).

Total cysteine and related compounds in obese patients and healthy controls

Before surgery, the severely obese patients had significantly but modestly (approximately 17 %) higher serum tCys concentrations than controls. Compared with controls, the obese patients also had significantly higher serum concentrations of amino acids both upstream (tHcy) and downstream (taurine) of cysteine, as well as of glutamate (Table 1).

Effect of bariatric surgery on total cysteine and related compounds

In the same obese patients, serum tCys and related compounds were also measured 6 weeks, 6 months and 1 year after a bariatric surgical procedure, which was either gastric bypass or duodenal switch. Unless stated otherwise, patients in the two surgical groups had similar trends in biomarker

Table 1. Baseline characteristics of obese patients and healthy controls
(Mean values and standard deviations)

	Women					Men				
	Controls (*n* 30)		Patients (*n* 42)			Controls (*n* 28)		Patients (*n* 18)		
	Mean	SD	Mean	SD	*P* *	Mean	SD	Mean	SD	*P* *
Age (years)	39	11	35†	7	0·08	39	11	37	5	0·43
BMI (kg/m²)	23	3	55	4	<0·001	25	3	55	3	<0·001
Current smoker					0·002					0·28
n	1		14			4		5		
%	3		33			14		28		
Type 2 diabetes					0·04					0·02
n	0		7			0		4		
%	0		17			0		22		
Creatinine (μmol/l)	65	7	61	9	0·06	76	10	73	11	0·42
ALT† (U/l)	18	8	23	7	0·007	23	9	44	17	<0·001
GGT† (U/l)	15	7	43	33	<0·001	23‡	13	56	29	<0·001
FAD (nmol/l)	279	39	305	47	0·01	319	40	320	53	0·93
FMN (nmol/l)	20	7	22	10	0·30	25	14	29	15	0·39
PLP (nmol/l)	46	24	24	14	<0·001	58	31	32	20	0·001
Folate† (nmol/l)	16	4	13	6	0·01	16	5	12	4	0·04
Vitamin B₁₂ (pmol/l)	305	104	419	694	0·65	364	103	344	139	0·37
tHcy (μmol/l)	9·3	2·1	11·4	4·8	0·02	10·3	2·1	14·4	5·8	0·001
Cystathionine (μmol/l)	0·19	0·09	0·22	0·10	0·13	0·22	0·10	0·30	0·17	0·03
tCys (μmol/l)	257	28	303	40	<0·001	275	34	320	50	0·001
Methionine† (μmol/l)	22·6	2·8	20·7	1·9	0·007	24·4	4·0	24·7	2·4	0·84
tGSH† (μmol/l)	4·7	0·8	5·0	1·2	0·27	5·5	1·3	5·2	1·8	0·57
Taurine† (μmol/l)	59	14	81	17	<0·001	47	13	79	21	<0·001
Glutamate† (μmol/l)	79	14	201	68	<0·001	98‡	25	227	58	<0·001

ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; PLP, pyridoxal-5′-phosphate; tHcy, total homocysteine; tCys, total cysteine; tGSH, total glutathione.
* Comparisons by the *t* test or Fisher's exact test. ALT, GGT, PLP, folic acid, vitamin B₁₂, homocysteine and cystathionine were compared using log-transformed values.
† Data available only from the Norwegian patients in the study (twenty-two women and eight men).
‡ One male control (outlier) was excluded from comparisons of GGT and glutamate.

values during the follow-up (i.e. no significant time × procedure interaction was found).

SAA upstream of cysteine changed considerably after bariatric surgery (Fig. 1). Methionine concentrations declined ($P<0.001$), with the lowest concentrations (79 % of pre-surgery levels) measured 6 months postoperatively. Trends for serum tHcy concentrations differed in the two surgical groups ($P<0.001$ for time × procedure interaction): concentrations increased during weight loss after duodenal switch ($P<0.001$) but did not change significantly after gastric bypass ($P=0.63$). Cystathionine dropped steeply from baseline to 6 weeks after surgery (by approximately 36 %) and then remained stable ($P<0.001$). Trends for serum tCys differed according to the surgical procedure ($P=0.02$ for time × procedure interaction): duodenal switch patients showed a gradual decline in tCys concentrations, reaching 12 % decrease 1 year post-surgery ($P<0.001$), while gastric bypass patients showed no significant change ($P=0.22$). Duodenal switch patients did not have significantly higher tCys than controls 1 year after surgery ($P=0.11$).

Biomarkers downstream of cysteine are shown in Fig. 2. No significant changes were found for concentrations of taurine ($P=0.51$) or tGSH ($P=0.98$) during the follow-up. However, glutamate concentrations dropped briskly after surgery ($P<0.001$). Serum GGT activity also decreased ($P<0.01$ in both surgical groups); this trend was more pronounced after gastric bypass than duodenal switch ($P=0.03$ for time × procedure interaction).

Effect of bariatric surgery on other variables

For the same patients, we have previously reported 1-year outcomes for the following variables: mean weight loss (30 % after gastric bypass and 41 % after duodenal switch); serum folate and pyridoxal-5′-phosphate (stable and increased concentrations after both procedures); Hb, total cholesterol and plasma albumin (greater decreases after duodenal switch)[20].

Further variables relevant to SAA metabolism are shown in Fig. 3. Serum cobalamin concentrations increased in gastric bypass patients, who received additional vitamin B_{12} supplementation. Blood FAD concentrations were mostly stable after surgery ($P=0.054$). Serum creatinine declined ($P<0.001$). Alanine aminotransferase activity increased transiently 6 weeks after surgery, before declining ($P=0.005$).

Associations of serum total cysteine and related compounds with other variables

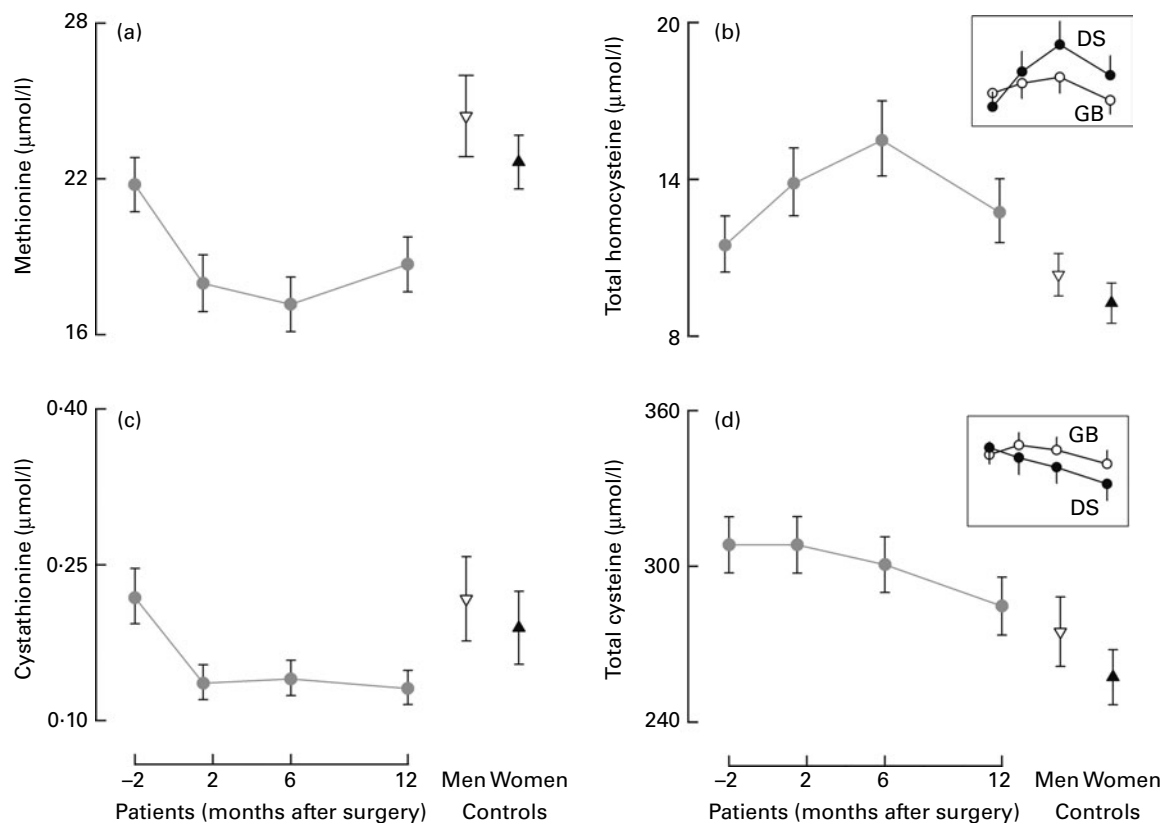The relationships (Spearman's correlations) of tCys and related compounds with other biomarkers are shown in Table S1

Fig. 1. Biomarkers upstream of cysteine in bariatric surgery patients (●, n 60), healthy men (▽, n 28) and healthy women (▲, n 30). Linear mixed-effects model: there was a significant trend for (a) methionine (μmol/l), n 30 (patients, fifteen in each surgical group), (b) total homocysteine (μmol/l), (c) cystathionine (μmol/l) and (d) total cysteine (μmol/l) after surgery ($P<0.05$, time effect); insets: trend for the two surgical procedures was significantly different ($P<0.05$, time × procedure interaction). Values are means, with 95 % CI represented by vertical bars, adjusted for age, sex and type of surgery in patients and unadjusted in controls. DS (●), duodenal switch; GB (○), gastric bypass. The precise illustrated patient values are shown in Table S2 of the supplementary material (available online at http://www.journals.cambridge.org/bjn).
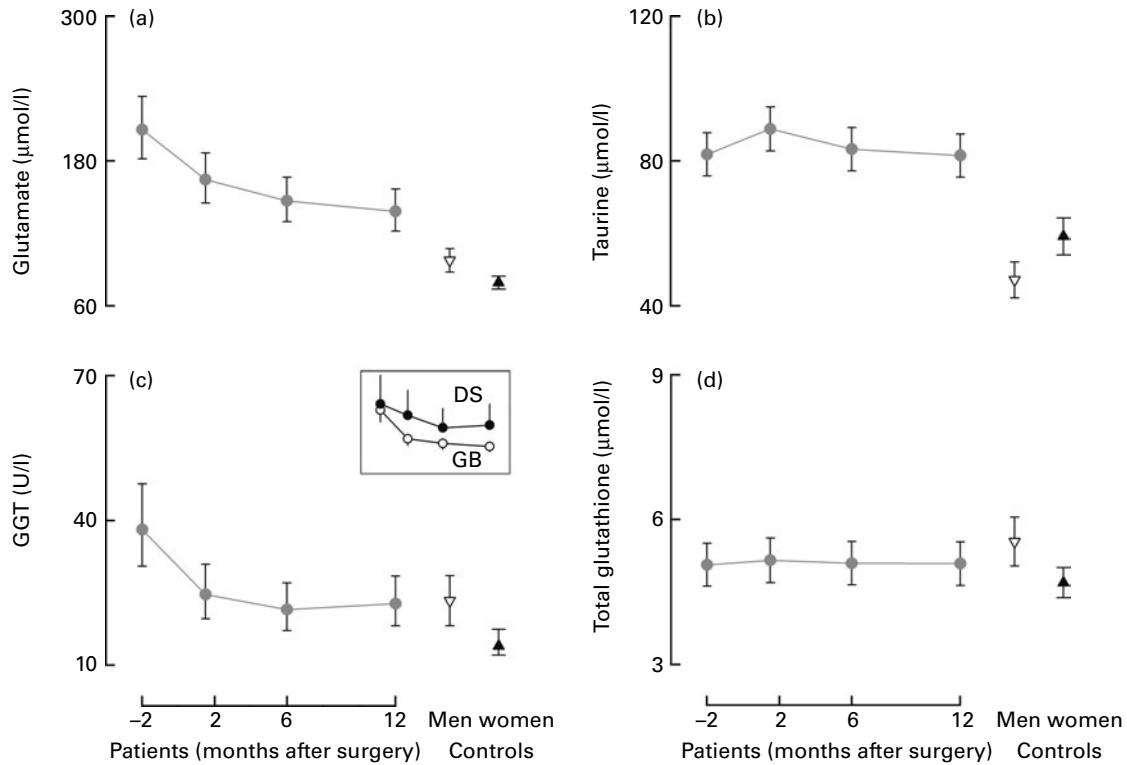
Fig. 2. Biomarkers downstream of cysteine in bariatric surgery patients (●, n 30), healthy men (▽, n 28) and healthy women (▲, n 30). Linear mixed-effects model: there was a significant trend for (a) glutamate (μmol/l) and (c) γ-glutamyltransferase (GGT) (U/l) after surgery ($P < 0.05$, time effect); insets: trend for the two surgical procedures was significantly different ($P < 0.05$, time × procedure interaction). No significant changes observed for (b) taurine (μmol/l) and (d) total glutathione (μmol/l). DS (●), duodenal switch; GB (○), gastric bypass. Values are means, with 95 % CI represented by vertical bars, adjusted for age, sex and type of surgery in patients and unadjusted in controls. The precise illustrated patient values are shown in Table S2 of the supplementary material (available online at http://www.journals.cambridge.org/bjn).

(supplementary material for this article can be found at http://www.journals.cambridge.org/bjn). No consistent associations were found with BMI. However, at all patient visits, serum GGT activity correlated positively with glutamate ($\rho = 0.79$ to 0.84) and inversely with tGSH ($\rho = -0.41$ to -0.76) concentrations (Fig. 4). Significant correlations were also noted for creatinine (with tHcy and cystathionine), alanine aminotransferase (with glutamate), folate (with tHcy) and albumin (with tCys).

Mixed-effects models identified the following associations with tCys and related compounds in the patients: age was associated positively with tCys ($P = 0.001$) and inversely with tGSH ($P = 0.03$), while male sex was associated with higher methionine ($P = 0.003$), tHcy ($P = 0.002$), cystathionine ($P = 0.02$) and tCys ($P = 0.03$) concentrations.

Relationship between total cysteine and weight loss

The relationship between tCys and weight loss was explored in multiple linear regression models, using percentage of weight loss (from baseline to 1 year after surgery) as a dependent variable and surgical procedure (gastric bypass or duodenal switch) as well as baseline BMI, age, sex and tCys as independent variables. In this model (r^2 0.59), the only significant predictors of weight loss were surgical procedure and baseline BMI ($P < 0.001$ for both) (baseline tCys: $P = 0.43$).

Replacing baseline tCys with change in tCys (from baseline to 1 year after surgery) did not change the findings (r^2 0.59; change in tCys: $P = 0.64$).

Total cysteine in bariatric surgery patients and the general population

Finally, we compared the obese patients with a large population-based cohort. Before surgery, the obese patients had a BMI much higher than the 95th percentile (32 kg/m²) of the Hordaland Homocysteine Study population, while serum tCys was below the 95th percentile. After surgery, duodenal switch patients had a modest but significant decline in tCys, whereas the gastric bypass patients had no significant change in tCys despite a BMI loss of 16.3 kg/m² (see Fig. S1 of the supplementary material, available online at http://www.journals.cambridge.org/bjn).

Discussion

In the present study, we showed changes in serum tCys and metabolically related amino acids in severely obese patients undergoing surgery-induced weight loss. The patients had modestly elevated tCys concentrations before surgery. At 1 year after surgery, gastric bypass patients showed no significant change in tCys, despite 30 % weight loss. Given the
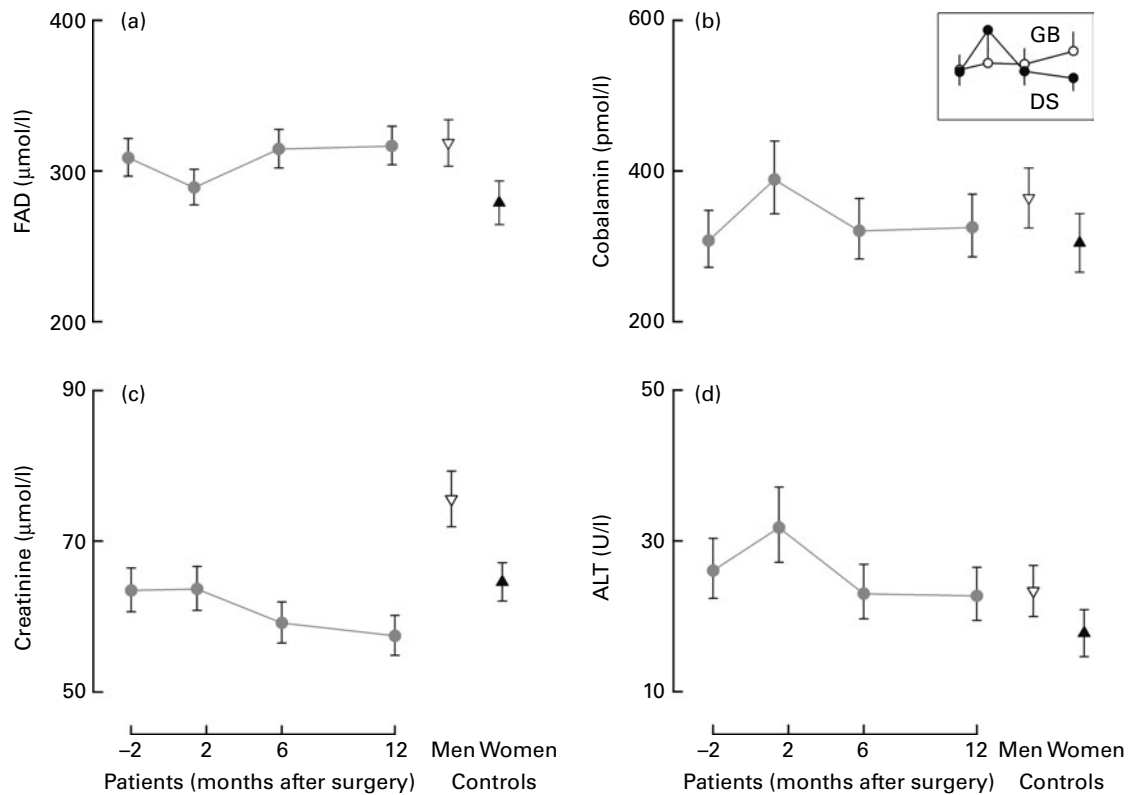
Fig. 3. Biomarkers in bariatric surgery patients (●, n 60), healthy men (▽, n 28) and healthy women (▲, n 30). Linear mixed-effects model: there was a significant trend for (b) cobalamin (pmol/l), (c) creatinine (μmol/l) and (d) alanine aminotransferase (ALT, U/l), n 30 (patients, fifteen in each surgical group) after surgery (P<0·05, time effect); insets: trend for the two surgical procedures was significantly different (P<0·05, time × procedure interaction). No significant changes observed for FAD (nmol/l) (a). Values were means, with 95 % CI represented by vertical bars, adjusted for age, sex and type of surgery in patients (cobalamin was additionally adjusted for site) and unadjusted in controls. DS (●), duodenal switch; GB (○), gastric bypass. The precise illustrated patient values are shown in Table S2 of the supplementary material (available online at http://www.journals.cambridge.org/bjn).

strong association between tCys and fat mass in the general population[6], the present data suggest that fat mass does not cause elevation of tCys, since these very obese patients neither had very high tCys at baseline nor showed a marked drop in tCys after 30 % weight loss. This contrasts with glutamate concentrations, which were markedly elevated before surgery and decreased with both procedures; reflecting the changes in GGT.

It has been known for decades that obese persons may have elevated plasma amino acid levels[25]. Given that type of dietary protein influences the risks of having obesity and insulin resistance[26,27], it is conceivable that specific amino acids could contribute to causing these conditions. Recent interest in this field has been fuelled by the finding that certain plasma amino acid profiles, in particular elevated branched-chain amino acid (BCAA) and glutamate levels, may promote insulin resistance in obesity[10,27]. Then again, obese rodents with elevated BCAA levels had a reduction in BCAA-metabolising enzymes; and in obese humans, after gastric bypass, a decrease in plasma BCAA was coupled with an increase in BCAA-metabolising enzymes[28]. Hence, the elevated plasma BCAA in obesity could be due to disturbances in metabolising enzymes.

The present study targets a different group of amino acids, the SAA. SAA are relevant to clinical obesity not only because of the association of tCys with BMI and fat mass in large cohort studies[4−6], but also because rodent experiments show that restricted intake of methionine, the precursor of all SAA, protects against obesity and insulin resistance[29,30]. Furthermore, adding cysteine to a methionine-restricted diet reverses the effect of the diet on rat adiposity and the serum fatty acid profile[31], raising the possibility that cysteine may influence lipid metabolism and weight in humans.

To our knowledge, these are the first data on tCys levels in patients undergoing current bariatric procedures. Early studies found high tCys and glutamic acid levels in obese patients, with varying changes after jejunoileal bypass surgery[32,33]. Jejunoileal bypass more often led to metabolic complications (e.g. liver cirrhosis)[34], which may hamper comparison with modern operations. More recent studies have found both increases[15,35] and decreases[36,37] in tHcy in obese patients following bariatric surgery; perhaps related to differences in vitamin supplements, or, as in the present study, the procedure used.

Possible mechanisms for the changes in total cysteine and related compounds

Methionine and cysteine are normally efficiently digested and absorbed in the intestines, and are removed from the portal blood by the liver for use in the synthesis of proteins and GSH, or for catabolism to taurine[19] (ses Fig. S2 of the
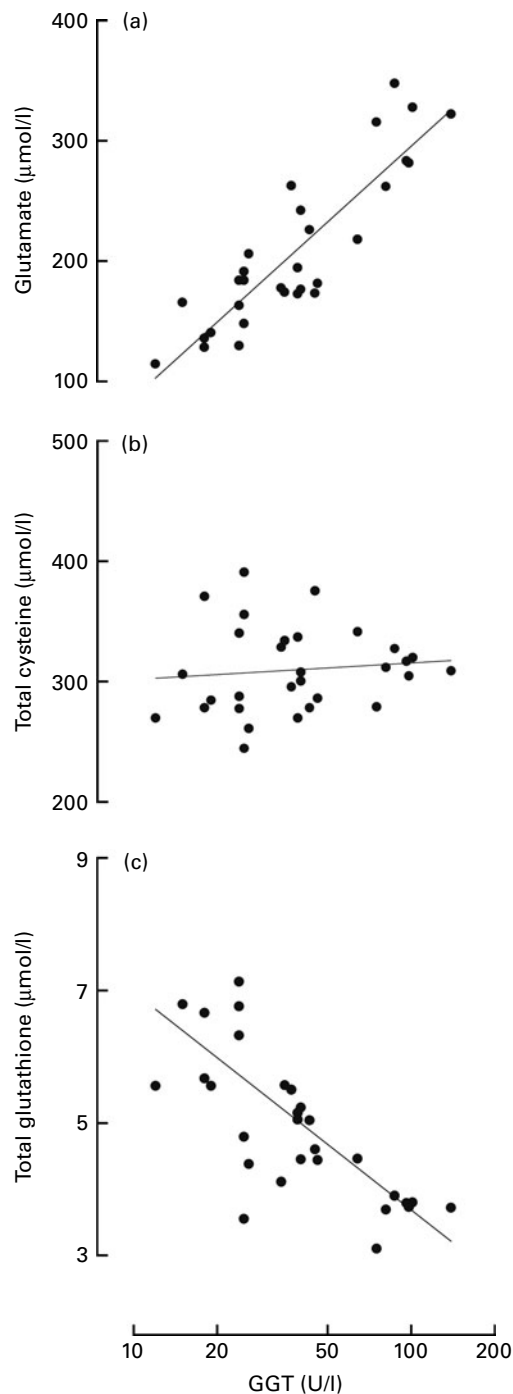
Fig. 4. Relationships between γ-glutamyltransferase (GGT) and serum concentrations (μmol/l) of (a) glutamate (*r* 0·84, *P*<0·001), (b) total cysteine (*r* 0·18, *P*=0·35), and (c) total glutathione (*r* −0·76, *P*<0·001) in obese patients before bariatric surgery (*n* 30). Spearman's correlation coefficients are shown.

activity: this limits trans-sulphuration of homocysteine to cystathionine to preserve methionine[40,41]. Sparse protein availability in duodenal switch patients could thus help explain their higher tHcy concentrations and, secondary to diminished flux through the trans-sulphuration pathway, their lower tCys concentrations. Elevated tHcy could potentially in itself also contribute to decreasing serum tCys by displacing protein-bound cysteine from albumin[42]. Finally, given that the gastrointestinal tract is an active site for SAA metabolism[43], it is possible that differences in tCys after gastric bypass and duodenal switch could in part relate to varying intestinal SAA metabolism following these procedures.

Changes in dietary intakes and food preferences[44] and use of vitamin supplements could have contributed to changes in SAA concentrations after surgery. It has been suggested that higher serum cobalamin and folate levels are needed to maintain tHcy during weight loss[15]. Cobalamin is a cofactor in homocysteine remethylation to methionine[1]. Duodenal switch patients did not receive parenteral cobalamin supplementation, so a relative cobalamin deficiency may have contributed to the elevation of their tHcy concentrations. Both surgical groups showed increases in serum vitamin B_6 postoperatively, which probably explains the abrupt 36% reduction in cystathionine concentrations after surgery[45]: vitamin B_6 supplementation increases the conversion of cystathionine to cysteine by the cystathionine γ-lyase enzyme, which is very sensitive to pyridoxal-5′-phosphate depletion[46].

Taurine can be obtained from the diet, or via conversion of cysteine by cysteine dioxygenase[47]. Since cysteine dioxygenase is up-regulated in the liver and adipose tissue in response to high cysteine availability[19], the higher taurine concentrations in obese patients compared with controls could be secondary to increased conversion of cysteine to taurine. Glutamate was much higher in obese patients than controls, as shown by others[9,10], and decreased after surgery. Elevated GGT levels in obesity, and their decrease during weight loss, also correspond with previous research[14]. Perhaps surprisingly, despite losing more weight, duodenal switch patients had higher GGT levels than gastric bypass patients during the follow-up. This could reflect either higher alcohol intake, or less regression of non-alcoholic fatty liver disease[48] in duodenal switch patients relative to gastric bypass patients post-surgery. Strong correlations were found for GGT with tGSH (inverse) and glutamate (positive) before and after surgery. Despite a decline in GGT activity, however, patients had stable tGSH concentrations. Other researchers reported increased erythrocyte GSH after gastric banding[49]. The varying results could relate to our assessment of tGSH in the serum; and not erythrocytes, where GSH is more abundant. Our finding of a strong correlation (*r* 0·8) between serum GGT activity and glutamate concentrations may reflect the role of GGT in hydrolysing GSH to ultimately yield glutamate (and cysteine), and could suggest that GGT influences plasma glutamate. Increased glutamate levels in obesity may thus be secondary to disturbances in metabolic enzymes, similar to BCAA[28].

supplementary material, available online at http://www.journals.cambridge.org/bjn). While gastric bypass patients presumably lose weight mainly by lowering energy intake, biliopancreatic diversion with duodenal switch causes more protein and fat malabsorption[38,39] and more often leads to nutrient deficiencies[20]. Protein depletion and methionine restriction lead to impaired cystathionine β-synthase enzyme

A longitudinal study of predictors of change in tCys in a population-based cohort showed that a decrease in BMI of 1kg/m^2 or more was associated with a corresponding decrease in tCys[7]. In the present trial, despite 30% weight loss, there was no significant change in tCys after gastric bypass surgery. This suggests that the epidemiological association between changes in tCys and changes in BMI[7] is not explained by an effect of body weight on plasma tCys levels. The 12% decrease in tCys seen in the present study after duodenal switch surgery may relate to additional malabsorption with this procedure[38,39]. Consistent with this, duodenal switch patients had lower serum albumin than gastric bypass patients[20], and serum albumin correlated positively with tCys in the combined patient group.

The strengths of the present study include a broad biochemical characterisation of patients throughout major weight loss. Unlike previous reports on other amino acids[28], we did not investigate changes in metabolic enzymes, apart from plasma GGT. However, the serial examinations enabled us to distinguish between amino acid changes that occurred rapidly (e.g. decrease in cystathionine) or more gradually (e.g. decrease in glutamate) during weight loss. The randomised trial design allowed us to compare the effects of two different surgical techniques. We did not assess dietary SAA content, but monitored the intake of dietary supplements[20], and use of fasting blood samples minimised bias from acute variations in SAA intake[50].

In summary, the stability of tCys during weight loss contrasts with the marked drops in serum methionine, cystathionine and glutamate. This may illustrate that elevated plasma amino acid levels associated with obesity fall into at least two categories: those that are an adjunct to obesity and those that may contribute towards the development of obesity. This can potentially have implications for the modulation of dietary protein content in future strategies to prevent obesity.

Acknowledgements

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