Determinants of Homocysteine During Weight Reduction in Obese Children and Adolescents

Siegfried Gallistl, Karl Michael Sudi, Wolfgang Erwa, Reingard Aigner, and Martin Borkenstein

Plasma homocysteine levels have been shown to be associated with indexes of obesity and insulin resistance in obese children and adolescents. We, therefore, investigated the contribution of changes in body composition, markers of insulin resistance, folate, and vitamin B_{12} to changes in homocysteine during a weight reduction program in obese children and adolescents. Thirty-seven obese white girls (mean SD; age, 12 ± 1.8 years, body mass index [BMI], 26.9 ± 5.25) and 19 obese white boys (age, 11.9 ± 1.7 years; BMI, 26.2 ± 5.2) were investigated for body composition, fasting total plasma homocysteine (tHcy), insulin, C-peptide, folate, and vitamin B_{12} before and after a 3-week weight reduction program including physical activities. During weight reduction BMI, fat mass (FM), percentage fat mass, insulin, and C-peptide decreased significantly, whereas homocysteine and vitamin B_{12} showed a significant increase. Folate and lean body mass (LBM) remained unchanged. tHcy concentration before weight reduction was a function of age, folate, and C-peptide, whereas tHcy concentration after weight reduction was a function of folate and baseline LBM. Changes in tHcy during weight reduction correlated significantly with baseline LBM and were related inversely to changes in LBM during weight reduction. Children who increased LBM showed lower increases in tHcy compared with children who lost LBM. In multiple linear regression analysis, only baseline LBM contributed independently and significantly to changes in tHcy. Our study suggests that LBM has a significant impact on tHcy metabolism during weight reduction.

CHILDHOOD OBESITY IS associated with an increased cardiovascular risk in later life.[1] Besides dyslipidemia[2] and disturbances in the hemostatic system,[3-5] plasma homocysteine concentrations have also been shown to be linked to indexes of childhood obesity and hyperinsulinism.[6] Hyperhomocysteinemia, an independent risk factor for occlusive vascular disease in adults[7] and even in childhood[8] has been demonstrated to correlate with fat intake,[9] suggesting that fat restriction might improve plasma homocysteine concentrations. On the other hand, some investigators found increased homocysteine concentrations during weight reduction,[10,11] which has been ascribed to inadequate folate supplementation, because folate is an essential cofactor in homocysteine metabolism.[12]

Loss of fat mass (FM) during weight reduction programs might be accompanied by substantial loss of lean body mass (LBM),[13] reflecting protein catabolism. Because homocysteine is derived from the metabolic conversion of the essential amino acid, methionine, we investigated the effects of changes in body composition on changes in homocysteine concentrations in obese children and adolescents during a weight reduction program.

SUBJECTS AND METHODS

Subjects

Thirty-seven obese white girls (mean SD; age, 12 ± 1.8 years, body mass index [BMI], 26.9 ± 5.25) and 19 obese white boys (age, 11.9 ± 1.7 years; BMI, 26.2 ± 5.2) were investigated. Obesity was defined as a BMI greater than 85th percentile for age and sex.[14] All children and adolescents had normal liver and renal function as assessed by standard clinical chemistry analyses. None of the participants were taking multivitamin preparations or medications known to affect lipid metabolism. Children were judged healthy by medical examination, and written informed consent was given by the parents. The study protocol was approved by the investigation review board of the University of Graz, Austria.

Weight Reduction Program

Children participated in a weight reduction program including physical activities for 3 weeks during summer holidays, which took place in a school in a rural area. Physical training consisted of different activities and was performed 3 times a day. Children were assigned to a mixed diet of 3,800 to 5,000 kJ/d. Energy intake consisted of approximately 50% carbohydrate, 20% protein, and 30% fat. The approximate daily intake of folate, B_{12}, and B_6 was 230 μg, 4 μg, and 1.5 mg, respectively.

Laboratory Methods

Blood samples were taken after an overnight fast. Total homocysteine (tHcy) was determined by high-performance liquid chromatography with fluorometric detection. Vitamin B_{12}, folate, insulin, and C-peptide were assessed using radioimmunoassays.

Measurement of Body Composition

Measurements for LBM were performed by means of bioelectrical impedance (BIA Akern-RJL 101/S, Firenze, Italy) with an applied current of 0.8 mA at 50 kHz. FM was calculated as the difference between body mass and LBM. Percentage fat mass (%FM) was expressed as the relative amount of FM for a given body weight.[15,16]

Statistics

Insulin was skewed and therefore $\log_{10}$ transformed. Analysis of variance was used to compare parameters between boys and girls where appropriate. A 2 (sex) × 2 (time) design with repeated measurements on time was used to compare parameters before and after weight loss. Correlations between variables of interest were calculated using Pearson's correlation coefficient and Spearman's rank correlation. Partial correlation was performed to adjust for the influence of confounding

From the Ludwig-Boltzmann Research Institute for Pediatric Haemostasis and Thrombosis, Department of Pediatrics, Institute for Sport Sciences, and Department of Radiology and Nuclear Medicine, University of Graz, Graz, Austria.

Submitted December 4, 2000; accepted February 23, 2001.

Address reprint requests to Siegfried Gallistl, MD, Univ. Klinik fuer Kinder- und Jugendheilkunde, Auenbruggerplatz 30, A-8036 Graz, Austria.

0026-0495/01/5010-0029$35.00/0
doi:10.1053/meta.2001.25646

variables. The independence and significance of variables was tested by stepwise, multiple regression analysis based on results of the bivariate correlations. The significance level of P values was set at 5%.

RESULTS

Variables before and after weight reduction are shown in Table 1. No significant gender-differences were found for all available anthropometric and metabolic characteristics before and after the program. BMI, FM, %FM, insulin, and C-peptide decreased significantly during the program. tHcy and vitamin B_{12} increased significantly, whereas folate and LBM showed no differences. tHcy concentrations before and after the program correlated significantly with age ($P < .001$). After adjustment for age, baseline tHcy correlated significantly with baseline BMI, FM, log insulin, and C-peptide and showed an inverse correlation with baseline folate (Table 2). tHcy after weight loss correlated with baseline LBM and LBM determined after the program. Inverse correlations were found between tHcy and folate and vitamin B_{12} assessed after weight loss. Insulin and C-peptide did not correlate with tHcy after the program.

Table 3 shows the results of multiple regression analysis before and after weight reduction. Age, folate, and C-peptide contributed to 36% of the variance in baseline tHcy. Surprisingly, baseline LBM contributed independently and significantly to tHcy after weight reduction.

The correlations between the changes in tHcy and the independent variables are shown in Table 4. LBM before and after weight reduction showed the strongest correlation with changes in tHcy. As shown in Fig 1, all except 1 subject with unchanged or decreased tHcy concentrations increased LBM during weight reduction. Children who increased their LBM (n = 30) showed significant lower increases in homocysteine concentrations (0.99 ± 1.12 μmol/L) compared with children who lost LBM (n = 26), (1.72 ± 1.14 μmol/L; $P = .02$). The baseline tHcy concentrations in children who increased their LBM were not different from the baseline tHcy concentration in children who lost LBM (8.9 ± 1.4 *v* 9.4 ± 2.0 μmol/L, $P = .3$).

Stepwise multiple regression analysis showed that only baseline LBM contributed independently and significantly to the variance in changes in tHcy (adjusted $R^2 = .16$, $P = .003$).

DISCUSSION

To our knowledge, this is the first study investigating the influence of changes in body composition on homocysteine concentrations in obese children and adolescents during weight reduction. Homocysteine concentrations increased significantly, although indexes of obesity and insulin resistance, which have been shown to contribute to the variance of homocysteine in obese children,[6] improved. The most intriguing outcome of the present study was the observation that changes in homocysteine concentrations during weight reduction were determined by changes in LBM. Surprisingly, age and C-peptide, which independently and significantly explained proportions of the variance in homocysteine before weight reduction, did not contribute to the variance of homocysteine after the program. Only folate, which did not change from before to after the program, contributed to the variance of homocysteine before and after weight loss.

Hyperhomocysteinemia has been described recently in severe obese patients 1 year after gastroplasty.[10] In this study, the increase in homocysteine concentrations was correlated to weight loss and to decrease in plasma folate concentrations, whereby the correlation between changes in homocysteine and body weight was stronger ($P < .001$) compared with the correlation between changes in homocysteine and folate ($P < .01$). Because reduction of FM in the absence of physical activity has been shown to be accompanied by loss of LBM,[13] the observed hyperhomocysteinemia might have been also due to a reduction of LBM. In agreement with this assumption, increased plasma concentrations of homocysteine have been shown in anorexia nervosa,[17] although folate and vitamin B_{12} were within the normal range in the investigated patients. On the other hand, beneficial effects of folate supplementation on plasma homocysteine concentrations during weight reduction have been demonstrated,[18] although there was no correlation between folate concentrations and changes in homocysteine.

The observation that baseline LBM contributed to the variation in homocysteine after weight reduction is intriguing. It has been suggested that in adolescents the effect of muscle mass may be related to the amount of homocysteine formed in conjunction with creatine-creatinine synthesis.[19] High LBM represents a high amount of protein, which can be metabolized during weight reduction, especially in the absence of physical training. It has been shown that brief starvation is associated

Table 1. Anthropometric and Metabolic Characteristics From Before to After the Weight Reduction Program

	Before	After	*P*
BMI (kg/m^2)	26.6 ± 5.2	25 ± 4.9	<.001
FM (kg)	31.3 ± 12.5	27.7 ± 11.9	<.0001
%FM	45.8 ± 6.8	42.7 ± 7.3	<.0001
LBM (kg)	35 ± 6.7	34.9 ± 6.4	.3
Homocysteine (μmol/L)	7.8 ± 1.4	9.2 ± 1.7	<.0001
Folate (ng/mL)	10.8 ± 3.4	10.7 ± 2.9	.6
B_{12} (pg/mL)	461 ± 119	560 ± 216	<.0001
Insulin (mU/L)	11.5 ± 6.4	7.4 ± 3	<.0001
C-peptide (mU/L)	2.5 ± 1.2	1.2 ± 0.6	<.0001

NOTE. Data are means (±SD).

Table 2. Age-Adjusted Correlations Between Homocysteine and Independent Variables Before and After the Weight Reduction Program

	Homocysteine*		Homocysteine†	
	r	*P*	*r*	*P*
BMI*	.26	.024		
FM*	.26	.024		
LBM*	.18	.08	.42	.0004
LBM†			.39	.001
Folate*	−.35	.003		
Folate†			−.36	.002
B_{12}*	−.2	.05		
B_{12}†			−.24	.03
Log insulin*	.26	.022		
C-peptide*	.34	.005		

*Before weight reduction program.

†After weight reduction program.

Table 3. Multiple Regression Analysis With Homocysteine Before and After the Program as Dependent Variable

		Multiple Regression Model		Stepwise Regression Model		
Dependent Variable	Independent Variables	β	95% CI	β	95% CI	P
Homocysteine*	Age*	.32; $P = .0004$	±.17	.32	±.17	.0004
	Folate*	−.12; $P = .01$	±.09	−.12	±.09	.01
	C-peptide*	.29; $P = .02$	±.26	.29	±.25	.027
				adj. $R^2 = .36$, $P < .0001$		
Homocysteine†	Age*	−.03; $P = .8$	±.32			
	LBM*	.15; $P = .002$	±.09	.14	±.06	.00005
	Folate†	−.17; $P = .005$	±.11	−.17	±.11	.005
				adj. $R^2 = .39$; $P < .0001$		

NOTE. The regression coefficient (β), the 95% confidence interval (95% CI) for each independent variable, and the adjusted R^2 for each model are shown.

*Before weight reduction program.

†After weight reduction program.

with an increased muscle release of methionine and other glycogenic amino acids.[20] Increased methionine concentration raises the concentration of S-adenosylmethionine,[21] thus leading to the inhibition of methylenetetrahydrofolate reductase activity,[22] a key enzyme in the remethylation pathway of homocysteine.

We are aware of the fact that the reasons behind our observations remain to be demonstrated and that the mechanisms for elevated homocysteine will require further study. In addition, there is no evidence that the elevated homocysteine levels after short-term energy restriction represented an increased cardiovascular risk, as it is very likely that the homocysteine levels ultimately return to baseline levels.

It has been shown that reduction in LBM during weight reduction programs correlates with weight regain[16] and that rapid, massive weight loss might be accompanied by cardiac dysfunction due to loss of body (and myocardial) protein.[23] Therefore, our data confirm the recommendation that weight reduction programs for obese children should focus not only on weight loss, but also on maintenance or increase of LBM.

Table 4. Age-Adjusted Correlations Between Δ Homocysteine and LBM

	Δ Homocysteine
LBM*	$r = .41$; $P < .001$
LBM†	$r = .34$; $P < .005$
Δ LBM	$r = -.3$; $P = .01$

*Before weight reduction program.

†After weight reduction program.

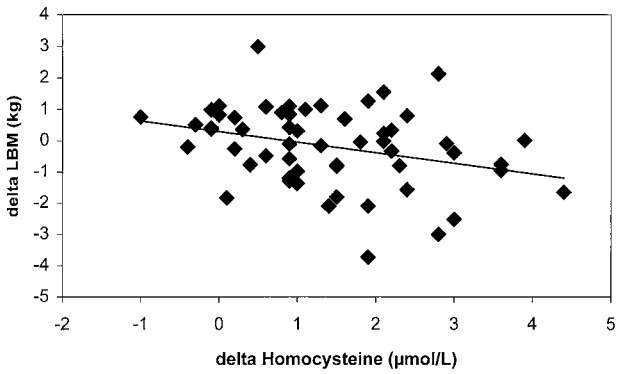

Fig 1. Correlation between Δ LBM and Δ homocysteine ($r = -.3$, $P = .01$).

REFERENCES

1. Must A, Strauss RS: Risks and consequences of childhood and adolescent obesity. Int J Obes Relat Metab Disord 23:S2-S11, 1999 (suppl 2)

2. Berenson GS, Srinivasan SR, Bao W, et al: Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 338:1650-1656, 1998

3. Gallistl S, Sudi KM, Borkenstein M, et al: Determinants of haemostatic risk factors for coronary heart disease in obese children and adolescents. Int J Obes Relat Metab Disord 24:1459-1464, 2000

4. Sudi K, Gallistl S, Muntean W, et al: The relationship between plasminogen activator inhibitor-1 antigen, fat mass, and leptin in obese children and adolescents. Metabolism 49:890-895, 2000

5. Sudi K, Gallistl S, Payerl D, et al: Interrelationship between estimates of adiposity and body fat distribution with metabolic and hemostatic parameters in obese children. Metabolism (in press)

6. Gallistl S, Sudi K, Mangge H, et al: Insulin is an independent correlate of plasma homocysteine levels in obese children and adolescents. Diabetes Care 23:1348-1352, 2000

7. Cattaneo-M: Hyperhomocysteinemia, atherosclerosis and thrombosis. Thromb Haemost 81:165-176, 1999

8. Van Beynum IM, Smeitink JAM, den Heijer M, et al: Hyperhomocysteinemia—A risk factor for ischemic stroke in children. Circulation 99:2070-2072, 1999

9. Oshaug A, Bugge KH, Refsum H: Diet, an independent determinant for plasma total homocysteine. A cross sectional study of Norwegian workers on platforms in the North Sea. Eur J Clin Nutr 52:7-11, 1998

10. Borson-Chazot F, Harthe C, Teboul F, et al: Occurrence of hyperhomocysteinemia 1 year after gastroplasty for severe obesity. J Clin Endocrinol Metab 84:541-545, 1999

11. Henning BF, Tepel M, Riezler R, et al: Unfavourable changes in

homocysteine metabolism during weight reduction. Med Sci Res 25: 555-556, 1997

12. Finkelstein JD: Methionine metabolism in mammals. J Nutr Biochem 1:228-237, 1990

13. Hoie LH, Bruusgaard D, Thom E: Reduction of body mass and change in body composition on a very low calorie diet. Int J Obes Relat Metab Disord 17:17-20, 1993

14. Must A, Dallal GE, Dietz WH: Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht2) and triceps skinfold thickness. Am J Clin Nutr 53:839-846, 1991

15. Lukasi HC, Johmson PE, Bolonchuk WW, et al: Assessment of fat free mass using bio-electrical impedance measurements of the human body. Am J Clin Nutr 41:810-817, 1985

16. Schwingshandl J, Borkenstein M: Changes in lean body mass in obese children during a weight reduction program: Effect on short-term and long-term outcome. Int J Obes Relat Metab Disord 19:752-755, 1995

17. Mayona D, Vilaseca MA, Artuch R, et al: Plasma total-homocysteine in anorexia nervosa. Eur J Clin Nutr 52:172-175, 1998

18. Henning BF, Tepel M, Riezler R, et al: Vitamin supplementation during weight reduction—Favourable effect on homocysteine metabolism. Res Exp Med (Berl) 198:37-42, 1998

19. De Laet C, Wautrecht JC, Brasseur D, et al: Plasma homocysteine concentrations in a Belgian school-age population. Am J Clin Nutr 69:968-972, 1999

20. Pozefsky T, Tancredi RG, Moxley RT, et al: Effects of brief starvation on muscle amino acid metabolism in nonobese man. J Clin Invest 57:444-449, 1976

21. Krebs HA, Hems R, Tyler B: The regulation of folate and methionine metabolism. Biochem J 158:341-353, 1976

22. Kutzbach C, Stokstad EL: Mammalian methylenetetrahydrofolate reductase. Partial purification, properties, and inhibition by S-adenosylmethionine. Biochim Biophys Acta 250:459-477, 1971

23. Van Itallie TB, Yang MU: Cardiac dysfunction in obese dieters: A potentially lethal complication of rapid, massive weight loss. Am J Clin Nutr 39:695-702, 1984