The association of plasma homocysteine levels with serum leptin and apolipoprotein B levels in childhood obesity

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BACKGROUND: An elevated plasma total homocysteine (tHcy) level has recently been established as an independent risk factor for thrombosis and vascular disease. However, the relationship between hyperhomocysteinemia and cardiovascular disease and obesity remains controversial. The aim of the study was to investigate a possible relationship between plasma tHcy levels and measures of childhood obesity.

SUBJECTS AND METHODS: Forty children and adolescents with exogenous obesity and 20 non-obese subjects in an age-and sex-matched control group were investigated. Fasting samples were collected for plasma tHcy, serum insulin, leptin, vitamin B12, folate, creatinine and lipid parameters. Anthropometric characteristics and body compositions were assessed in both groups.

RESULTS: The obese patients had significantly higher tHcy levels than the non-obese controls (14.3±11.8 μmol/L vs 8.7±5.9 μmol/L; *P*=0.017). In both groups, plasma tHcy was positively related to serum leptin, but serum apolipoprotein B (apo B) levels were positively related to plasma tHcy levels only in obese patients.

CONCLUSIONS: Our study demonstrates for the first time that leptin and apo B are main correlates of tHcy in obese children and adolescents and suggests that hyperleptinemia and increased apo B may contribute to impairment of tHcy metabolism in childhood obesity.

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Hyperhomocysteinemia is a well-established independent risk factor for atherosclerotic and thromboembolic vascular disease. Hereditary enzymatic deficiencies and nutritional deficiencies of folate, pyridoxine or cobalamin (B12), as well as chronic renal failure are associated with elevated blood total homocysteine (tHcy) and accelerated atherosclerosis.[1-3] In addition, individuals homozygous for the thermolabile form of methylenetetrahydrofolate reductase (MTHFR) also show higher levels of tHcy, mainly in the presence of low folate.[3] One further dietary determinant of plasma tHcy level may be fat intake because fat intake is associated with higher tHcy levels in healthy men, probably because of a lower intake of essential vitamins.[4] High fat intake has been shown to be linked with childhood obesity,[5] which is associated with an unfavorable profile of risk factors for cardiovascular disease (CVD) such as hyperinsulinemia, hyperlipidemia, and elevated blood pressure.[6]

Leptin, the product of the obesity (ob) gene,[7] is a 16-kDa circulating hormone that is best known as a regulator of food intake and energy expenditure via hypothalamic-mediated effects.[8] It is now appreciated that this hormone has many additional effects, often as a consequence

of direct peripheral actions. These include angiogenesis, hematopoiesis, lipid and carbohydrate metabolism and effects on the reproductive, cardiovascular and immune systems; these effects are reviewed elsewhere.[9-11] In obese humans, an increased fat mass correlates with increased plasma leptin levels[12] and is also associated with the development of insulin resistance.[13] Currently, no data are available that deal with a possible relationship among tHcy levels, insulin levels, leptin levels, body composition, and metabolic risk factors for CVD in childhood obesity. Therefore, the aim of the present study was to investigate a possible relationship between plasma tHcy levels and measures of childhood obesity.

Subjects and Methods

Subjects consisted of 40 obese (BMI>95th percentile for age and sex) children and adolescents, including 23 boys and 17 girls, and 20 age- and sex-matched non-obese children and adolescents, including 11 boys and 9 girls. The age of subjects ranged from 7 to 17 years in both the obese and non-obese control groups. Developmental stage was not formally assessed, although 18 subjects in the obese group and 9 subjects in non-obese group were Tanner stage II and bigger.[14,15] At admission, a medical history and physical examination were performed to ensure that the subjects were healthy. All children and adolescents had normal liver and renal function as assessed by standard clinical chemistry analyses.

Before entering a course of treatment, they were instructed to visit the clinic in the morning, after an overnight fast. Blood was then drawn and, at the same time, anthropometric measurements were made, including body weight, height, blood pressure, waist girths, hip girth, and triceps and subscapular skinfold thicknesses. Waist-hip ratio (WHR) was calculated by division of the two parameters. Skinfold thickness was measured by calipers at the triceps and subscapular region. Fat mass and lean body mass were calculated by DEXA (dual energy x-ray absorptiometry). A fan beam X-ray bone densitometer (Hologic QDR-4500A; Hologic Inc.) with human software version 9.8 was used. Resting blood pressure was measured in the sitting position after a 15-minute rest using a mercury sphygmomanometer and a cuff appropriately sized for the arm size of the subject. Informed consent was obtained from each child and a legal guardian.

Venous blood samples were taken between 7:30 and 9:30 A.M after an overnight fast. The tHcy concentration in plasma was determined by high-performance liquid chromatography with fluorometric detection. The sample, internal standard and phosphate buffered saline (PBS, pH 7.4) were mixed. Tris-2-carboxy-ethylphosphine (TCEP) in water-soluble was added to reduce the thiols and mixed sulfides. After incubation at room temperature for 30 minutes, trichloroacetic acid was added to precipitate proteins. Next, the centrifuged supernatant was mixed with NaOH, EDTA and SBD-F (ammonium-7-fluorobenzo-2-oxa-1.3-diazole-4-sulfonate) in borate buffer. Samples were incubated at 60°C in a water bath for 1 hour, to form fluorescent derivates. After cooling at 4°C, 10 μL of the samples were injected to HPLC. HPLC was carried out on a solvent delivery system and a fluorescence detector; 0.1 mol/L acetic acid-acetate buffer, pH 5.5 containing 30 ml/L methanol was used to elute the constituents as mobile phase. Flow rate was 0.7 mL/minute. L-homocysteine calibrators (5-100 μmol/L) were prepared in PBS, pH 7.4 and in pooled EDTA plasma. The tHcy peaks separated with analytic HPLC column were provided in 3.3 minutes. The tHcy concentrations were calculated according to peak levels. Variation co-efficient was 3.5%.[16]

Serum folate and vitamin B12 were measured by Simultrac-SNB Radioassay kit vitamin B12 [^{57}Co]/Folate [^{125}]. Serum glucose, serum lipids [total cholesterol (TC), triglyceride, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol] and serum lipoprotein [Apolipoprotein A (apo A), Apolipoprotien B (apo B) and Lipoprotein a] concentrations were assayed on a Konelab 60i autoanalyzer (Thermo Electron Corp, *www.thermo.com*). Serum concentrations of insulin were measured by Coat-A-Count Insulin from DPC (Diagnostic Products Corporation, Los Angeles, CA). Obese subjects with fasting insulin levels >20 μU/ml were considered hyperinsulinemic.[17] Serum concentrations of leptin were measured with commercially available IRMA kits (Human Leptin Irma DSL-23100).

Data were expressed as mean ±SD. Differences between data were studied using Student's t-test. Correlations were assessed using Pearson's correlation coefficient. The significance of variables was tested by multiple regression analysis based on the results of the bivariate correlations. In the regression model we verified tHcy as a dependent variable and included age, BMI, total fat mass, leptin, apo B, insulin, folate values as independent variables. Statistical significance was taken as $P<0.05$. Data were analyzed using SPSS for Windows (version 11.0, SPSS).

Results

In the obese group (n=40), all anthropometric parameters were higher than in the non-obese control group (n=20) except for height (Table 1). Systolic and diastolic blood pressure, total fat mass and lean body mass were significantly higher in obese subjects than non-obese subjects. There were no differences between males and females.

The obese subjects had significantly higher tHcy levels than the non-obese controls (Table 2). The mean leptin and insulin concentrations were also significantly higher, and the mean serum TC, tryglyceride and serum apo B values were significantly higher in the obese group compared with those in the control group. All obese subjects were hyperinsulinemic according to fasting insulin levels.

Among several parameters analyzed, only leptin and apo B correlated significantly with tHcy in obese subjects (Table 3). tHcy concentrations correlated positively with leptin and apo B concentrations in the obese group (Figure 1). tHcy concentrations correlated positively only with leptin in non-obese subjects. None of the other anthropometric and laboratory parameters were correlated with tHcy concentration in either group.

In the multiple regression analyses, the contribution of the independent variables leptin, Apo B, total body fat, insulin, age, BMI and folate to the variance in tHcy was investigated. In stepwise multiple regression analysis, only leptin contributed to the variance in tHcy (R=0.55, β=0.42, *P*=0.006).

Discussion

Homocysteine is a nonprotein-forming, thiol-containing amino acid formed by demethylation of methionine. It is metabolized by remethylation to methionine or by transsulfuration to cysteine. An elevated plasma tHcy level has recently been established as an independent risk factor for thrombosis and vascular disease.[18] However, the relationship between hyperhomocysteinemia and CVD remains controversial. Although some prospective studies have confirmed that tHcy is an independent risk factor for CVD[19-21] other studies have not found such a relationship.[22] Data are relatively sparse on variations in tHcy levels among obese subjects at increased risk for type 2 diabetes or CVD. Our study demonstrated an unfavorable relationship among fasting plasma tHcy levels, leptin levels and apo B levels in obese children and adolescents.

No study has demonstrated a relationship between tHcy and leptin levels in obesity. Leptin

Table 1. Anthropometric parameters for both groups.

	Obese n=40	Control n=20	*P*
Age (years)	11.4±2.5	11.6±2.6	0.81
Height (cm)	147.1±15.4	141.5±15.2	0.18
Weight (kg)	60.2±16.2	37.3±11.1	0.001
BMI (kg/m^2)	27.4±4.3	18.2±2.2	0.001
WHR	0.89±0.06	0.83±0.05	0.001
Triceps SFT (cm)	27.4±5.5	10.8±4.1	0.001
Subscapular SFT (cm)	29.7±7.7	6.7±2.2	0.001
Sistolic BP (mmHg)	116.1±16.8	104.5±12.6	0.004
Diastolic BP (mmHg)	72.8±13.9	65.7±12.0	0.047
Total fat mass (kg)	22.7.0±6.5	8.4±2.9	0.001
Lean body mass (kg)	36.9±19.6	28.7±8.9	0.003

BMI: Body mass index, WHR: Waist-hip ratio
Data are means (±SD), *P*<0.05.

Table 2. Laboratory data for both groups.

	Obese n=40	Control n=20	*P*
tHcy ((mol/L)	14.3±11.8	8.7±5.9	0.017
Vitamin B12 (pg/ml)	602.3.±283.8	592.2±314.5	0.90
Folate (ng/ml)	10.7.±5.1	10.8±8.5	0.95
Leptin (ng/ml)	27.5±16.0	6.8±8.3	0.001
Glucose (mg/dl)	99.7±16.4	92.1±13.7	0.06
Insulin (µIU/ml)	45.3±32.1	14.5±5.3	0.001
Creatinine (mg/dl)	0.57±0.13	0.57±0.14	0.90
Tryglyceride (mg/dl)	174.7±91.1	101.0±34.5	0.001
Total cholesterol (mg/dl)	176.2±35.8	153.0±35.0	0.02
LDL-cholesterol (mg/dl)	97.6±32.8	84.3±35.8	0.17
HDL-cholesterol (mg/dl)	43.7±13.8	48.4±13.2	0.21
Apolipoprotein A (mg/dl)	136.3±21.4	136.8±20.6	0.93
Apolipoprotein B (mg/dl)	93.3±23.3	78.7±16.2	0.007
Lipoprotein a (mg/dl)	29.5±33.9	36.6±36.4	0.46

tHcy: plasma total homocysteine, HDL:high-density lipoprotein, LDL:low-density lipoprotein
Data are means (±SD), P<0.05.

Table 3. Correlations among tHcy levels and measures of obesity in obese children and adolescents.

	Homocysteine	
	r	*P*
Age (years)	0.10	0.53
BMI (kg/m²)	0.21	0.17
Sistolic BP (mmHg)	0.007	0.96
Total fat mass (kg)	0.12	0.46
Lean body mass (kg)	0.04	0.80
Leptin (ng/ml)	0.42	0.006
Insulin (µIU/ml)	0.23	0.14
Folate (ng/ml)	0.06	0.67
Creatinine (mg/dl)	0.13	0.41
Tryglyceride (mg/dl)	0.28	0.07
Total cholesterol (mg/dl)	0.08	0.62
Apolipoprotein A (mg/dl)	0.18	0.25
Apolipoprotein B (mg/dl)	0.34	0.027
Lipoprotein a (mg/dl)	0.30	0.05

BMI: Body mass index
P<0.05.

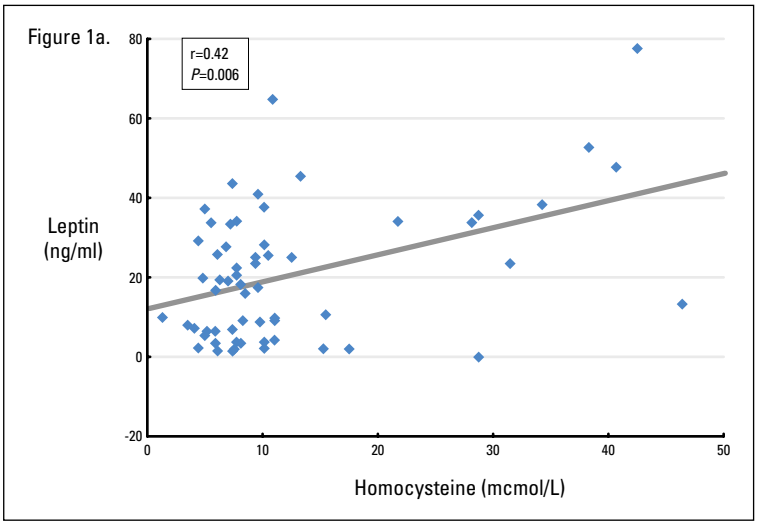
deficiency and resistance to the effects of leptin are each associated with weight gain. Leptin resistance, which is associated with hyperleptinemia, is much more common than leptin deficiency in human obesity.[23] In addition to hypothalamic receptors, there are receptors for leptin on the endothelium[24] and on vascular smooth muscle cells.[25] Accordingly, leptin can exert a receptor-mediated influence on vessel tone and growth. In cell culture, leptin stimulates vascular smooth muscle proliferation and migration.[23] Vascular calcification is also accelerated by leptin in experimental models.[26] Additionally, leptin induces oxidative stress in endothelial cells; this action triggers the transcription of oxidant-sensitive genes that participate in atherogenesis, including monocyte chemotactic protein.[27] Since tHcy seems to induce oxidative stress,[28] the increase in tHcy levels observed in obese subjects with hyperleptinemia may contribute to the pathophysiology of these obese subjects. In the present study, we found that increased total tHcy levels were present in obese subjects with hyperleptinemia and that there was a positive correlation between leptin and tHcy. Hyperleptinemia present in childhood obesity may worsen the angiotoxicity of tHcy, increasing the risk for atherosclerosis. On the other hand, the increase in tHcy levels may also worsen leptin resistance, closing a possible dangerous vicious circle. The mechanism may be oxidative stress. Furthermore, leptin increases sympathetic nervous activity, and chronic administration of leptin increases blood pressure in experimental models.[29] According to our data, it is possible that the high levels of leptin observed in childhood obesity could contribute to its adverse effects on cardiovascular health by increasing tHcy levels.

In the present study, obese subjects had higher TC, triglyceride and apo B levels compared with non-obese subjects of similar age. However, we only found a significant positive correlation between tHcy and apo B levels. Apo B, which is a useful predictor of CVD in adults, was found to be useful in another study in children, particularly for detecting genetic disorders of lipid metabolism.[30] However, no study has demonstrated a correlation between tHcy and apo B in the literature. In 482 patients already at high risk for atherosclerotic vascular disease by virtue of hyperlipidemia, 3.7% had high plasma tHcy31. Plasma tHcy has been shown to be associated with a parental history of CVD in children with familial hypercholesterolemia.[32] Elevated HDL cholesterol is well accepted as a protective factor against atherosclerosis. However, high HDL cholesterol is not necessarily protective in the setting of an elevated plasma tHcy.[33] Considerable in vitro data exist to suggest that tHcy may interact with elevated TC by increasing oxidation of LDL cholesterol. However, this area remains controversial. In one study, LDL cholesterol isolated from two patients with homocystinuria showed a similar degree of copper-catalyzed oxidation as LDL cholesterol from a group of healthy control subjects.[34] According to our data, in the long term, childhood obesity associated with increased apo B may cause CVD. Thus, more studies are needed on the relationship of tHcy to lipids and lipoproteins in patients with childhood obesity.

Our study did not demonstrate an unfavorable relationship among fasting plasma tHcy levels, insulin, vitamin B12, folate, creatinine and body composition in obese children and adolescents. The few studies in which hyperinsulinemia in obese subjects has been assessed directly have shown positive associations with tHcy levels. Sanchez-Margalet et al.[35] reported a significant positive correlation between fasting insulin and tHcy plasma levels. In another study, Gallistl et al.[36] suggested that insulin is an independent correlate of plasma tHcy levels in obese children and adolescents. In that study, it was report-

ed that BMI was significantly correlated with tHcy levels and was an independent predictor of tHcy levels. In the present study, although there were elevated fasting insulin levels in obese subjects, insulin was not correlated with tHcy levels. Moreover, tHcy concentrations were not related to indexes of obesity (BMI and fat mass). In our study, serum creatinine did not alter associations between hyperinsulinemic obese subjects and tHcy levels, confirming findings in some[37] but not all[38,39] epidemiological studies. However, an important limitation of our study is that serum creatinine is probably not an adequately sensitive marker of true glomerular filtration rate among subjects with normal renal function.[40] As in studies performed in adults, folate correlated inversely with tHcy and contributed independently and significantly to the variance in tHcy in stepwise multiple regression analysis, but we did not find a relationship between tHcy and folate or vitamin B12 levels in obese subjects, probably because of adequate intake of folate and vitamin B12.

Our analysis showed that leptin was the main independent correlate explaining the variation in tHcy levels in multiple regression analyses, but the present study does not permit conclusions about whether elevation of plasma tHcy is the cause or result of leptin resistance. Moreover, other possible sources of elevated tHcy, such as genetic variation of MTHFR activity or functional vitamin B6 deficiency[41] have not been assessed in the present study. Nevertheless, in the present study, there seems to be an association with increased tHcy levels in obese subjects with hyperleptinemia and increased apo B levels, which may contribute to the development of the cardiovascular complications that are so common in obesity.



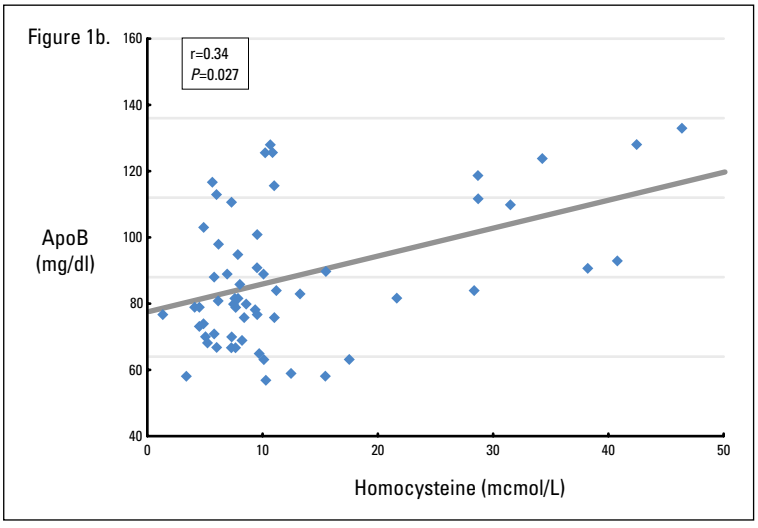


Figure 1. Correlation between tHcy and leptin concentrations (Figure 1a) and between tHcy and apo B concentrations (Figure 1b) in obese children and adolescents.

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References

1. McCully KS. Homocysteine and vascular disease. *Nature Med.* 1996;2:386-389.

2. Kang S, Wong PW. Genetic and nongenetic factors for moderate hyperhomocys(e)inemia. *Atherosclerosis.* 1996;119:135-138.

3. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thromb Haemost.* 1997;78:523-526.

4. 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.* 1998;52:7-11.

5. Nguyen VT, Larson DE, Johnson RK, Goran MI. Fat intake and adiposity in children of lean and obese parents. *Am J Clin Nutr.* 1996;63:507-513.

6. Likitmaskul S, Kiattisathavee P, Chaichanwatanakul K, Punnakanta L, Angsusingha K, Tuchinda C. Increasing prevalence of type 2 diabetes mellitus in Thai children and adolescents associated with increasing prevalence of obesity. *J Pediatr Endocrinol Metab.* 2003;16:71-77.

7. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;372:425-432.

8. Schwartz MW, Baskin DG, Kaiyala KJ, Woods SC. Model for the regulation of energy balance and adiposity by the central nervous system. *Am J Clin Nutr.* 1999;69:584-596.

9. Wauters M, Considine RV, Van Gaal LF. Human leptin; from an adipocyte hormone to an endocrine mediator. *Eur J Endocrinol.* 2000;143:293-311.

10. Freedman DS. Clustering of coronary heart disease risk factors among obese children. *J Pediatr Endocrinol Metab.* 2002;15:1099-1108.

11. Friedland O, Nemet D, Gorodnitsky N, Wolach B, Eliakim A. Obesity and lipid profiles in children and adolescents. *J Pediatr Endocrinol Metab.* 2002;15:1011-1106.

12. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996;334:292-295.

13. Caprio S. Insulin resistance in childhood obesity. J Pediatr Endocrinol Metab. 2002;15:487-492.

14. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child.* 1970;45:13-23.

15. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44:291-303.

16. Pfeiffer CM, Huff DL, Gunter EW. Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in aclinical laboratory setting. *Clin Chem.* 1999;45:290-292.

17. Kauffman RP, Baker VM, DiMarino P, Gimpel T, Castracane VD. Polycystic ovarian syndrome and insulin resistance in white and Mexican American women: a comparison of two distinct populations. *Am J Obstet Gynecol.* 2002;187:1362-1369.

18. Kluijtmans LA, van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oost BA et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet.* 1996;58:35-41.

19. Glowinska B, Urban M, Koput A, Galar M. New atherosclerosis risk factors in obese, hypertensive and diabetic children and adolescents. *Atherosclerosis.* 2003;167:275-286.

20. Wald NJ, Watt HC, Law MR, Weir DG, McPartlin J, Scott JM. Homocysteine and ischemic heart disease: results of a prospective study with implications regarding prevention. *Arch Int Med.* 1998;158:862-867.

21. Mangge H, Schauenstein K, Stroedter L, Griesl A, Maerz W, Borkenstein M. Low grade inflammation in juvenile obesity and type 1 diabetes associated with early signs of atherosclerosis. *Exp Clin Endocrinol Diabetes.* 2004;112:378-382.

22. Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH et al. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation.* 1998;98:204-210.

23. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med.* 1995;1:1155-1161.

24. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC et al. Biological action of leptin as an angiogenic factor. *Science.* 1998;281:1683-1686.

25. Oda A, Taniguchi T, Yokoyama M. Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe J Med Sci.* 2001;47:141-150.

26. Parhami F, Tintut Y, Ballard A, Fogelman AM, Demer LL. Leptin enhances the calcification of vascular cells: artery wall as a target of leptin. *Circ Res.* 2001;88:954-960.

27. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzman M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem.* 2001;276:25096-25100.

28. Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA, Hyperhomocyst(e)inemia is associated with inpaired endothelium-de-pendent vasodilation in humans. *Circulation.* 1997;95:1119-1121.

29. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest.* 1997;100:270-278.

30. Sarria A, Moreno LA, Mur M. Usefulness of serum apolipoprotein B levels for screening children with primary dyslipoproteinaemias. *Am J Dis Child.* 1992;146:1230-1231.

31. Glueck CJ, Shaw P, Lang JE, Tracy T, Sieve-Smith L, Wang Y. Evidence that homocysteine is an independent risk factor for atherosclerosis in hyperlipidemic patients. *Am J Cardiol.* 1995;75:132-136.

32. Tonstad S, Refsum H, Ueland PM. Association between plasma total homocysteine and parental history of cardiovascular disease in children with familial hypercholesterolemia. *Circulation.* 1997;96:1803-1808.

33. Superko HR. Elevated high-density lipoprotein cholesterol, not protective in the presence of homocysteinemia. *Am J Cardiol.* 1997;79:705-706.

34. Halvorsen B, Brude I, Drevon CA, Nysom J, Ose L, Christiansen EN, Nenseter MS. Effect of homocysteine on copper ion-catalyzed, azo compound-initiated, and mononuclear cell-mediated oxidative modification of low density lipoprotein. *J Lipid Res.* 1996;37:1591-1600.

35. Sanchez-Margalet V, Valle M, Ruz FJ, Gascon F, Mateo J, Goberna R Elevated plasma total homocysteine levels in hyperinsulinemic obese subjects. *J Nutr Biochem.* 2002;13:75-79.

36. 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;23:1348-1352.

37. Jager A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD: Microalbuminuria is strongly associated with NIDDM and hypertension, but not with the insulin resistance syndrome: The Hoorn Study. *Diabetologia.* 1998;41:694-700.

38. Forsblom CM, Eriksson JG, Ekstrand AV, Teppo AM, Taskinen MR, Groop LC. Insulin resistance and abnormal albumin excretion in non-diabetic first-degree relatives of patients with NIDDM. *Diabetologia.* 1995;38:363-369.

39. Mykkanen L, Zaccaro DJ, Wagenknecht LE, Robbins DC, Gabriel M, Haffner SM. Microalbuminuria is associated with insulin resistance in nondiabetic subjects. *Diabetes.* 1998;47:793-800.

40. Wollesen F, Brattstrom L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int.* 1999;55:1028-1035.

41. Kang SS, Wong PW. Genetic and nongenetic factors for moderate hyperhomocyst(e)inemia. *Atherosclerosis.* 1996;119:135-138.