Hyperhomocysteinemia as a Component of Syndrome X

Mor Oron-Herman, Talma Rosenthal, and Ben-Ami Sela

Syndrome X, a cluster of several metabolic disorders that includes hyperinsulinemia, hypertriglyceridemia, and hypertension, is associated with severe vascular morbidity. Hyperhomocysteinemia is another risk factor for cardiovascular and cerebrovascular diseases, often exhibited by insulin-resistant patients. In the current study, we investigated the relationship between syndrome X and hyperhomocysteinemia in a rat model. Two groups of rats were fed either fructose-enriched diet or standard rat chow for 5 weeks. Systolic blood pressure (SBP), as well as fasting plasma insulin, triglycerides, total cholesterol, and total homocysteine levels, were determined at the beginning and at the end of the study. A complete metabolic syndrome was induced by the fructose-enriched diet, including hyperinsulinemia, hypertriglyceridemia, and hypertension. Homocysteine concentration was 72% higher after 5 weeks on the fructose diet (8.49 ± 1.6 *v* 4.92 ± 0.9 μmol/l, $P < .01$). Insulin, triglycerides, SBP, and homocysteine levels were insignificantly changed during 5 weeks on standard rat chow. Homocysteine was positively and significantly correlated with any original component of syndrome X ($r = 0.565$, $P = .014$ with insulin, $r = 0.662$, $P = .001$ with triglycerides, and $r = 0.774$, $P < .001$ with SBP). The results of the present study indicate that hyperhomocysteinemia is an integral component of this rat model of syndrome X. It is thus highly likely that hyperhomocysteinemia is an integral component of the human syndrome X as well, and thereby contributes to the overall high vascular risk associated with this condition.

IMPAIRED glucose tolerance (IGT), glucose intolerance, insulin resistance syndrome (IRS), and non–insulin-dependent diabetes mellitus (NIDDM) are all clinical definitions for states in which the normal response of insulin secretion to glucose uptake is not adequate to efficiently transfer the glucose from blood into cells. Age-related decline in insulin sensitivity is common, although even within any given age group, a wide range of glucose clearance rate is frequent.[1] With the decline in insulin action, a considerable amount of insulin is secreted by β cells of the pancreas as a compensatory mechanism aimed at maintaining normal plasma glucose level. Unfortunately, even when glucose level is successfully maintained, the resultant extra insulin secretion is in itself harmful.

Chronic hyperinsulinemia has been known for decades as an important risk factor for atherosclerotic cardiovascular morbidity and mortality, as documented in both prospective[2,3] and retrospective[1,4-6] epidemiological studies. Moreover, the coexistence of insulin resistance and compensatory hyperinsulinemia is often accompanied by additional disorders such as hypertension and dyslipidemia, which are also known risk factors for coronary artery disease (CAD) and stroke. In 1988 Reaven proposed the general name "syndrome X" for the cluster of abnormalities stemming from insulin resistance: hyperinsulinemia, hypertriglyceridemia, and hypertension.[7] Since each component of syndrome X is a vascular risk factor by itself,[2-6,8,9] the combination of all of them together makes it an extremely high-risk syndrome. An expert panel of the National Cholesterol Education Program recently recognized syndrome X as a new target of risk reduction therapy.[10]

Homocysteine emerges as another important independent risk factor for atherosclerotic morbidity and mortality. This thiol-containing amino acid is produced during metabolism of the essential amino acid methionine, which is consumed via dietary proteins. Direct correlation, without threshold effect, between plasma level of homocysteine and vascular morbidity was found in many population studies.[11-20] The involvement of homocysteine in vascular diseases was recently systematically reviewed by Ford et al.[21] The summarized hazard odds ratio for a 5-μmol/L increase in homocysteine concentration, as gathered from publications of case-control studies, was 1.70 (95% confiedence interval [CI], 1.50 to 1.93) for coronary heart disease and 1.58 (95% CI, 1.35 to 1.85) for cerebrovascular morbidity. These values are similar to those previously reported by Boushey et al.[15]

Recently, conflicting data have been accumulating on the relationship between insulin resistance and homocysteine metabolism.[22-30] In the present study we used a fructose-induced hyperinsulinemic rat model[31] to determine whether hyperhomocysteinemia is an integral component of syndrome X.

MATERIALS AND METHODS

Animals, Diets, and Study Design

Twenty male Sprague-Dawley rats weighing 200 ± 20 g were purchased from Harlan, Israel. The animals were kept on a 14 hour/10 hour light/dark cycle and at a constant temperature (22°C). Food and water were supplied ad libitum. All rats were fed a standard rat chow prior to beginning the study, and then divided randomly into 2 equal groups. For 5 weeks the control group continued to consume standard rat chow (Koffolk, Israel) composed of 21% protein, 4% fat, 50% carbohydrate (vegetable starch), and 4.5% cellulose, while the study group was fed fructose-enriched chow (Harlan Teklad, Madison, WI) consisting of 21% protein, 5% fat, 60% carbohydrate (mainly fructose), and 8% cellulose. Both diets contained a standard mineral and vitamin mixture. Fasting plasma insulin, triglycerides, total cholesterol, and homocysteine levels, as well as systolic blood pressure (SBP), were measured at the beginning of the study (t = 0) and once again at the end (t = 5 weeks). The study protocol was approved by the hospital supervisory committee for animal studies and adhered to their guidelines.

From the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; Chorley Institute of Hypertension, Sheba Medical Center, Tel Hashomer; and the Institute of Chemical Pathology, Sheba Medical Center, Tel Hashomer, Israel.

Submitted February 18, 2003; accepted April 12, 2003.

Address reprint requests to Mor Oron-Herman, MSc, Chorley Institute of Hypertension, Sheba Medical Center, Tel Hashomer, 52621, Israel.

0026-0495/03/5211-0051$30.00/0
doi:10.1016/S0026-0495(03)00262-2

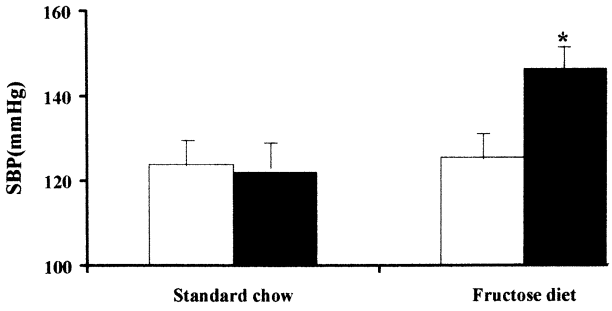

Fig 1. SBP of Sprague-Dawley rats fed either fructose-enriched diet or standard rat chow for 5 weeks. *$P < .001$ compared with baseline. (□) t = 0; (■) t = 5 weeks.

Blood Sampling

Blood samples were taken from the retro-orbital sinus under light ether anesthesia, after 5 hours of fasting. Collecting tubes contained 100 μL of 0.1% EDTA in saline. The samples were centrifuged for separation, and plasma was split into aliquots and frozen in polypropylene tubes at −20°C until assayed.

Plasma Insulin Level Determination

An insulin ^{125}I radioimmunassay (RIA) kit (Incstar, Stillwater, MN) was used for quantitative determination of plasma insulin concentration.

Plasma Triglycerides and Total Cholesterol Levels Determination

Triglycerides and total cholesterol were measured by automatic analyzer (Olympus AU 2700).

Total Plasma Homocysteine Level Determination

Total plasma homocysteine concentration was determined by high-pressure liquid chromatography system, using fluorescent detection.[32]

Systolic Blood Pressure Measurement

SBP was measured in conscious rats by the indirect tail-cuff method, using an electrosphygmomanometer and a pneumatic pulse transducer (Narco Biosystems, Houston, TX). The animals were kept at 37°C for 30 minutes before measurements were performed. The average of 5 consecutive readings was used for blood pressure evaluation.

Statistical Analysis

Results are presented as means ± SD. Paired, 2-tailed Student's *t* test (Excel, Microsoft Office 2000, Microsoft, Redmond, WA) and analysis of variance (ANOVA) with repeated measurments (SPSS Inc, Chicago, IL) were performed to compare measurements pre-and post-diets. Pearson's correlation coefficients were calculated using SPSS version 11. Statistical significance was defined as $P < 0.05$.

RESULTS

The effect of fructose feeding on SBP is illustrated in Fig 1. SBP rose significantly from 125.6 ± 9.5 to 146.3 ± 5.0 mm Hg ($P < .001$) during the fructose feeding period, while there was no significant elevation in the control group (121.9 ± 3.7 *v* 123.9 ± 4.7 mm Hg, $P > .05$).

Fig 2 summarizes the metabolic effects of 5 weeks of fructose feeding. Plasma insulin level doubled from 22.4 ± 6 to 43.2 ± 15.8 μU/mL ($P < .001$); triglycerides increased 3.5-fold from 91.8 ± 23.4 to 322.3 ± 93.5 mg/dL ($P < .001$); and total homocysteine concentration rose by 72% from 4.92 ± 0.9 to 8.49 ± 1.6 μmol/L ($P < .001$). Insulin, triglycerides, and homocysteine were insignificantly changed in the control group, which consumed standard rat chow during the study period (insulin from 21.72 ± 6 to 22.31 ± 4.55 μU/mL, triglycerides from 107.73 ± 35.3 to 104.64 ± 49.63 mg/dL and total homocysteine concentration from 4.82 ± 1.07 to 4.57 ± 0.9 μmol/l, $P > .05$ for all parameters).

Cholesterol level was not significantly changed during consumption of either standard or fructose diet: 98.4 ± 14.5 versus 92 ± 18.1 mg/dL on standard chow, and 91.1 ± 15 versus 100.6 ± 8.76 mg/dL on fructose diet at t = 0 and t = 5, respectively ($P > .05$).

A significant direct relationship between homocysteine and each of the other components of syndrome X was found.

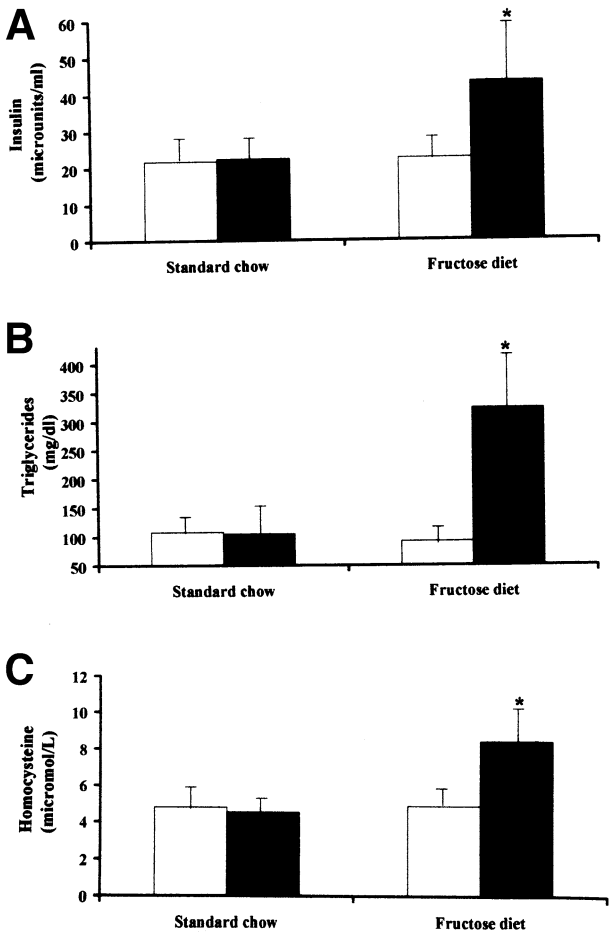

Fig 2. Metabolic parameters of Sprague-Dawley rats fed either standard rat chow or fructose-enriched diet for 5 weeks. (A) Fasting insulin concentration; (B) fasting triglycerides level; (C) fasting total homocysteine concentration. *$P < .001$ compared with baseline. (□) t = 0; (■) t = 5 weeks.

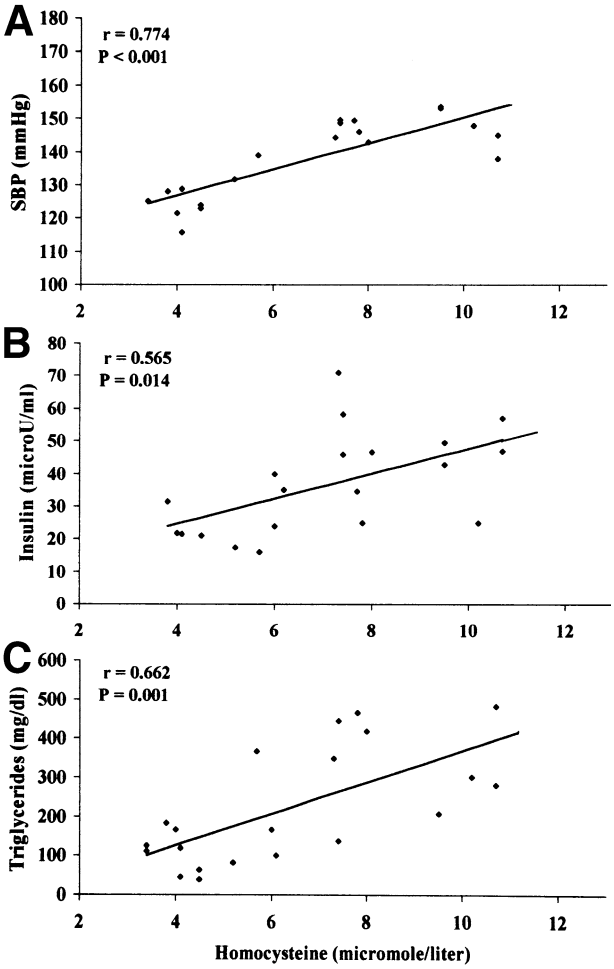

Fig 3. Correlations between fasting plasma total homocysteine and other components of syndrome X. (A)Correlation with SBP; (B) correlation with insulin; (C) correlation with triglycerides. Pearson's correlation coefficient (*r* value), *P* for trend, and linear regression are noted in each part of the figure.

Fasting plasma homocysteine was positively correlated with SBP ($r = 0.774$, $P < .001$, Fig 3A), insulin ($r = 0.565$, $P = .014$, Fig 3B), and triglycerides ($r = 0.662$, $P = .001$, Fig 3C). While calculating the correlation coefficients among the original components of syndrome X in the present study, the following values were found: between triglycerides and insulin, $r = 0.482$, $P < .05$; between triglycerides and SBP, $r = 0.624$, $P < .001$, and between SBP and insulin, $r = 0.633$, $P = .005$.

DISCUSSION

The results of the present study lend further support to a possible regulatory role of insulin in the metabolism of homocysteine. They also indicate that hyperhomocysteinemia is an integral component of syndrome X. The metabolic syndrome obtained using fructose-fed rats included an impressive rise of insulin, SBP, and triglycerides, as was expected. In addition, a significant elevation of plasma homocysteine level was inducd. Correlation coefficients between homocysteine and other components of syndrome X did not differ from those of the components among themselves, indicating that its relation to this syndrome is as strong as the relation of the original components.

The relationship between insulin, or insulin resistance, and homocysteine is controversial. Some human studies[23-25,30,33-35] found a significant positive correlation between the 2 entities, whereas others found no such correlation.[26,28,29] One study[36] even reported a negative correlation between the 2 parameters, although this observation was defined by the authors as "unexpected." In some studies the association between insulin sensitivity and hyperhomocysteinemia was restricted to specific populations and not seen in others.[27,37]

Animal studies also support the hypothesis that insulin may regulate homocysteine metabolism. In one important study, type 1 diabetes mellitus was induced in rats by injection of streptozotocin.[38] This intervention resulted in a 30% reduction in homocysteine level parallel to the reduction in endogenous insulin secretion. Treatment with insulin prevented the decline in homocysteine level. Similar results were recently obtained by another group.[39] These studies coincide with the observation of lower than normal plasma homocysteine levels in human type 1 diabetes mellitus patients who do not present with renal insufficiency.[40,41]

An in vitro study[42] using hepatocytes demonstrated reduction in the specific activity of 2 key enzymes of the homocysteine metabolism, methylene tetrahydrofolate reductase and cystathionine beta-synthase, in response to chronic insulin addition to culture medium. This should cause an accumulation of homocysteine, which is transformed to either methionine or cysteine at a reduced rate.

In the present study the correlation coefficient between insulin and homocysteine was calculated as $r = 0.565$. Similar values were reported in another 2 studies: by Fonseca et al[43] following long-term feeding of rats with a high-fat/sucrose diet ($r = 0.51$), and by Sanchez-Margalet et al[35] in hyperinsulinemic obese human subjects ($r = 0.6$). Taken altogether, similar correlation between those 2 entities found in 3 different models of syndrome X, both animal and human, strongly support the hypothesis that hyperhomocysteinemia does belong to this syndrome.

We conclude that hyperhomocysteinemia is an integral component of syndrome X, and thus may contribute to the characteristic vascular complications of patients with insulin resistance. Based on accumulating data, we suggest that the plasma level of homocysteine be routinely measured in the risk evaluation of hyperinsulinemic patients. Once hyperhomocysteinemia is found, it must be treated by folic acid and vitamins of the B group in an effort to reduce the vascular risk.

Since syndrome X was originally proposed by Reaven, serum uric acid[44,45] and plasminogen activator inhibitor type I (PAI-I)[46,47] were found to be significantly correlated to this metabolic disorder, leading Reaven to state[47] that hyperurice-

mia and PAI-I belong to the cluster of abnormalities comprising syndrome X. The results presented here indicate that hyperhomocysteinemia should also be added to the cluster of abnormalities derived from insulin resistance.

ACKNOWLEDGMENT

This work was performed in partial fulfillment of the requirements for a PhD degree of Mor Oron-Herman, Sackler Faculty of Medicine, Tel Aviv University, Israel.

REFERENCES

1. DeFronzo RA, Ferrannini E: Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

2. Ducimetiere P, Eschwege E, Papoz L, et al: Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. Diabetologia 19:205-210, 1980

3. Pyorala M, Miettinen H, Laakso M, et al: Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: The 22-year follow-up results of the Helsinki Policemen Study. Circulation 98:398-404, 1998

4. Dhawan J, Bray CL, Warburton R, et al: Insulin resistance, high prevalence of diabetes, and cardiovascular risk in immigrant Asians: Genetic or environmental effect? Br Heart J 72:413-421, 1994

5. Sheu WH, Jeng CY, Young MS, et al: Coronary artery disease risk predicted by insulin resistance, plasma lipids, and hypertension in people without diabetes. Am J Med Sci 319:84-88, 2000

6. Despres JP, Lamarche B, Mauriege P, et al: Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 334:952-957, 1996

7. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

8. Gotto AM Jr: Triglycerides as a risk factor for coronary artery disease. Am J Cardiol 82:22Q-25Q, 1998

9. Stamler J, Stamler R, Liu K: High blood pressure role in coronary heart disease and implication for prevention and control, in Connor W, Bristow D (eds): Coronary Heart Diseases. Philadelphia, PA, Lippincott, 1985, pp 85-109

10. Executive Summary of The Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATPIII). JAMA 285:2486-2497, 2001

11. Israelsson B, Brattstrom LE, Hultberg BL: Homocysteine and myocardial infarction. Atherosclerosis 71:227-233, 1988

12. Verhoef P, Kok FJ, Kruyssen DACM, et al: Plasma total homocysteine, B vitamins and risk of coronary atherosclerosis. Arterioscler Thromb Vasc Biol 17:989-995, 1997

13. Clarke R, Daly L, Robinson K, et al: Hyperhomocysteinemia: An independent risk factor for vascular disease. N Engl J Med 324: 1149-1155, 1991

14. Graham I, Daly LE, Refsum HM, et al: Plasma homocysteine as a risk factor for vascular disease. European Concerted Action Project. JAMA 277:1775-1781, 1997

15. Boushey CJ, Beresford SAA, Omenn GS, et al: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. JAMA 274:1049-1067, 1995

16. Wald NJ, Watt HC, Law MR, et al: Homocysteine and ischemic heart disease: results from a prospective study with implications regarding prevention. Arch Intern Med 158:862-867, 1998

17. Perry IJ, Refsum H, Morris RW, et al: Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 346:1395-1398, 1995

18. Stampfer MJ, Malinow MR, Wilett WC, et al: A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 268:877-881, 1992

19. Arneson E, Refsum H, Bonaa KH, et al: Serum total homocysteine and coronary heart disease. Int J Epidemiol 24:704-709, 1995

20. Nygard O, Nordrehaug JE, Refsum H, et al: Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 337:230-236, 1997

21. Ford ES, Smith SJ, Stroup DF, et al: Homocyst(e)ine and cardiovascular disease: A systematic review of the evidence with special emphasis on case-control studies and nested case-control studies. Int J Epidemiol 31:59-70, 2002

22. House JD, Jacobs RL, Stead LM, et al: Regulation of homocysteine metabolism. Adv Enzyme Regul 39:69-91, 1999

23. 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

24. Giltay EJ, Hoogeveen EK, Elbers JM, et al: Insulin resistance is associated with elevated plasma total homocysteine levels in healthy, non-obese subjects. Atherosclerosis 139:197-198, 1998

25. Meigs JB, Jacques PF, Selhub J, et al: Fasting plasma homocysteine levels in the insulin resistance syndrome. The Framingham Offspring Study. Diabetes Care 24:1403-1410, 2001

26. Pixa A, Pietzsch J, Julius U, et al: Impaired glucose tolerance (IGT) is not associated with disturbed homocysteine metabolism. Amino Acids 18:289-298, 2000

27. Fonseca VA, Mudaliar S, Schmidt B, et al: Plasma homocysteine concentrations are regulated by acute hyperinsuliemia in nondiabetic but not type 2 diabetic subjects. Metabolism 47:686-689, 1998

28. Abbasi F, Facchini F, Humphreys MH, et al: Plasma homocysteine concentrations in healthy volunteers are not related to differences in insulin-mediated glucose disposal. Atherosclerosis 146:175-178, 1999

29. Godsland IF, Rosankiewiez JR, Proudler AJ, et al: Plasma total homocysteine concentrations are unrelated to insulin sensitivity and components of the metabolic syndrome in healthy men. J Clin Endocrinol Metab 86:719-723, 2001

30. Emoto M, Kanda H, Shoji T, et al: Impact of insulin resistance and nephropathy on homocysteine in type 2 diabetes. Diabetes Care 24:533-538, 2001

31. Tobey TA, Mondon CE, Zavaroni I, et al: Mechanism of insulin resistance in fructose-fed rats. Metabolism 31:609-612, 1982

32. Jacobsen DW, Gatautis VJ, Green R, et al: Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: Sex differences and correlation with cobalamin and folate concentrations in healthy subjects. Clin Chem 40:873-881, 1994

33. Lee KU: Oxidative stress markers in Korean subjects with insulin resistance syndrome. Diabetes Res Clin Pract 54:S29-33, 2001 (suppl 2)

34. De Pergola G, Pannacciulli N, Zamboni M, et al: Homocysteine plasma levels are independently associated with insulin resistance in normal weight, overweight and obese pre-menopausal woman. Diabetes Nutr Metab 14:253-258, 2001

35. Sanchez-Margalet V, Valle M, Ruz FJ, et al: Elevated plasma total homocysteine levels in hyperinsulinemic obese subjects. J Nutr Biochem 13:75-79, 2002

36. Rosolova H, Simon J, Mayer O Jr, et al: Unexpected inverse relationship between insulin resistance and serum homocysteine in healthy subjects. Physiol Res 51:93-98, 2002

37. Sheu WH, Lee WJ, Chen YT: Plasma homocysteine concentrations and insulin sensitivity in hypertensive subjects. Am J Heart 13:14-20, 2000

38. Jacobs RL, House JD, Brosnan ME, et al: Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. Diabetes 47:1967-1970, 1998

39. Gursh MF, Baydas G, Cikim G, et al: Insulin increases homocysteine levels in a dose-dependant manner in diabetic rats. Arch Med Res 33:305-307, 2002

40. Robillon JF, Canivet B, Candilo M, et al: Type 1 diabetes mellitus and homocyst(e)ine. Diabete Metab 20:494-496, 1994

41. Cronin C, McPartlin JM, Barry DG, et al: Plasma homocysteine concentrations in patients with type 1 diabetes. Diabetes Care 21:1843-1847, 1998

42. Dicker-Brown A, Fonseca VA, Fink LM, et al: The effect of glucose and insulin on the activity of methylene tetrahydrofolate reductase and cystathionine-β-synthase: Studies in hepatocytes. Atherosclerosis 158:297-301, 2001

43. Fonseca V, Dicker-Brown A, Ranganathan S, et al: Effects of a high-fat-sucrose diet on enzymes in homocysteine metabolism in the rat. Metabolism 49:736-741, 2000

44. Facchini E, Chen Y-DL, Hollenbeck CB, et al: Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. JAMA 266:3008-3011, 1991

45. Zavaroni I, Mazza S, Fantuzzi M, et al: Changes in insulin and lipid metabolism in males with asymptomatic hyperuricaemia. J Intern Med 234:24-30, 1993

46. Potter van Loon BJ, Kluft C, Radder JK, et al: The cardiovascular risk factor plasminogen activator inhibitor type I is related to insulin resistance. Metabolism 42:945-949, 1993

47. Reaven GM: Syndrome X: 6 years later. J Intern Med 236: 13-22, 1994 (suppl 736)