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The Effects of Insulin on Serum Levels of Apo A-I Containing Lipoprotein Particles in Syndrome X Patients With Coronary Heart Disease

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Abstract: Syndrome X consists of low serum high density lipoprotein-cholesterol (HDL-C), raised serum triglyceride, glucose intolerance, increased blood pressure, abdominal obesity and insulin resistance which is associated with increased risk of coronary heart disease (CHD). Apolipoprotein (apo) A-I containing lipoprotein particles, lipoprotein A-I (Lp A-I) and lipoprotein A-I:A-II (Lp A-I:A-II.) may have different metabolic functions. The purpose of this study was to evaluate the relationship between insulin and apo A-I containing lipoproteins in syndrome X patients with or without CHD. We selected 38 male patients with syndrome X and divided into two groups: The one with CHD (n=21) and the other without CHD (n=17). Third group including 22 normal male subjects was the control group. We measured fasting blood glucose, cholesterol, triglyceride, low density lipoprotein-cholesterol (LDL-C), HDL-C, apo A-I, apo B, Lp A-I, Lp A-I:A-II and insulin, and 2-h glucose and insulin levels after a 75 g oral glucose tolerance test in all subjects. In both syndrome X groups, serum levels of triglyceride, apo B, fasting glucose and insulin, and 2-h glucose and insulin were significantly increased ($p<0.01$ for fasting insulin, $p<0.001$ for others) in comparison with the control group, whereas HDL-C, apo A-I and Lp A-I concentrations were significantly lower ($p<0.001$ for all). However Lp A-I:A-II levels were not different between three groups. Syndrome X group with CHD had significantly higher 2-h and fasting insulin levels than syndrome X group without CHD ($p<0.02$). Lp A-I and Lp A-I:A-II levels were correlated inversely with triglyceride, fasting and 2-h insulin levels (varying degrees between $p<0.05$ and $p<0.001$) only in syndrome X group with CHD. These results suggest that (1), low HDL-C levels observed in syndrome X patients could be attributed to only decreased Lp A-I concentration; (2), the serum levels of apo A-I containing lipoproteins can be effected by insulin; (3), the greater insulin levels observed in syndrome X patients with CHD may be responsible in part for increased risk of CHD.

Key Words: High density lipoprotein-cholesterol, Apolipoprotein A-I, Lipoprotein A-I, Lipoprotein A-I:A-II, Glucose intolerance, Atherosclerosis, Insulin resistance, Hyperinsulinaemia.

Introduction

Resistance to insulin stimulated glucose uptake is a common phenomenon which named as syndrome X, and is associated with a number of conditions, like hyperinsulinaemia, abnormal glucose tolerance, non-insulin dependent diabetes mellitus (NIDDM), increased plasma triglyceride, low plasma high density lipoprotein-cholesterol (HDL-C), abdominal obesity and hypertension, which they are also related to coronary heart disease (CHD) (1,2).

Hyperinsulinaemia, which can be considered as a characteristic feature of obesity (3), NIDDM (4), essential hypertension (5) and primary hypertriglyceridemia (6), usually reflects underlying insulin resistance. It generally has been assumed that patients with CHD are insulin resistant (2). There is also considerable evidence from longitudinal survey that high plasma level of insulin, which has some important effects in regulation of lipoprotein metabolism, may be an independent predictor of CHD (1,7,8). Another risk factor for CHD is low plasma HDL-C, which may directly promote atherosclerosis by decreasing reverse cholesterol transport (9). On the other hand, many patients with low HDL-C also have high levels of triglyceride-rich lipoproteins (10). It has been shown by the Framingham

Table 1. Group characteristics in the patients with syndrome X and the controls

	Control (n=22)	Syndrome X without CHD (n=17)	Syndrome X with CHD (n=21)
Age	53.4 ± 7.3	53.1 ± 9.3	52.8 ± 7.8
BMI (kg/m2)	26.5 ± 0.91	27.6 ± 1.5*	27.8 ± 1.4*
Waist/Hip ratio	0.87 ± 0.04	0.97 ± 0.07**	0.98 ± 0.07**
Systolic BP (mm Hg)	122.7 ± 7.9	160.2 ± 14.9**	161.1 ± 13.9**
Diastolic BP (mm Hg)	77.2 ± 6.1	100.8 ± 7.1**	101.9 ± 7.1**

*, p <0.01 when compared with control

**, p <0.001 when compared with control

BP, Blood pressure.

Study that patients with a combination of low HDL-C and high triglyceride have particularly increased risk for CHD (11).

High density lipoproteins (HDL) include two main apolipoproteins (apo), apo A-I and apo A-II (66% and 21% of total protein mass, respectively). Structurally, HDL represent a heterogeneous group of particles, and the current tendency is to differentiate them according to their apo composition, those containing apo A-I but no apo A-II, referred to as Lp A-I particles, and those containing both apo A-I and apo A-II, named Lp A-I:A-II. (12,13). This kind of classification has the potential importance, because of the observation that Lp A-I, but no Lp A-I:A-II, may be a predictive marker of CHD (14). This finding has stimulated extensive investigations about possible physiological differences between these two classes of HDL. It has been reported by Barkia et al (15) that Lp A-I promotes the efflux of cholesterol from adipocytes. In the other study, Ohta et al (16) measured the effect of Lp A-I and Lp A-I:A-II on the efflux of cholesterol from cholesterol loaded macrophages, and observed that a significant amount of unesterified cholesterol can be depleted from the cells only by Lp A-I, although both particles may deplete cholesterol from the cells.

To our knowledge, there is no study which examine the status of apo A-I containing lipoproteins in syndorme X patients and the relationship between these particles and insulin levels. The aim of this study was to examine the relationships between apo A-I containing lipoproteins, insulin and some characteristic features of syndrome X by comparing serum levels of these HDL subparticles in syndrome X patients with angiographically confirmed CHD positive and negative, and age -matched control subjects.

Material and Methods

Subjects: The patients with syndrome X were 31 to 60 years aged old male patients undergoing coronary angiography in our cardiology department. The inclusion criteria for this study were 1, a status with impaired glucose tolerance based on an oral glucose tolerance test (OGTT) according to the World Health Organization (WHO) criteria (17) (fasting blood glucose between 100-140 mg/dL or 2-h glucose 140-200 mg/dL); 2, an elevated systolic and/or diastolic blood pressure (>160/95 mmHg); 3, a waist to hip circumference ratio > 0.9; 4, fasting serum triglyceride level > 150 mg/dL and HDL-C level <40 mg/dL measured at least at two separate days; 5, non-smoker at least for 10 years. The study population selected according to the above criteria divided into two groups: First, syndrome X patients with CHD confirmed by greater than 50% occlusion of one or more main coronary arteries as determined by angiography. Second, syndrome X patients without CHD having a normal coronary angiogram and without a history of myocardial infarction or coronary artery bypass surgery. Patients were excluded if they had a history of congestive heart failure, endocrine disorders (except insulin resistance), gastrointestinal or renal dysfunction, and took a lipid lowering medication. Control subjects who living in Ankara were selected from among healthy male population having a normal lipid profile (cholesterol

Table 2. Serum triglycerides, total cholesterol, HDL- C, LDL-C, fasting glucose and insulin, 2-h glucose and insulin, apo B, apo A-I, Lp A-I, Lp A-I:A-II in the patients with syndrome X and the controls.

	Control (n=22)	Syndrome X without CHD (n=17)	Syndrome X with CHD (n=21)
Triglyceride (mg/dL)	98.9 ± 24.3	187.0 ± 27.1**	195.4 ± 27.8**
Total cholesterol (mg/dL)	195.8 ± 23.6	206.3 ± 22.0	212.6 ± 24.0§
HDL-C (mg/dL)	47.7 ± 5.9	34.9 ± 3.3**	34.3 ± 3.2**
LDL- C (mg/dL)	128.2 ± 22.4	133.9 ± 19.8	139.0 ± 26.0
Fasting Glucose (mg/dL)	92.4 ± 9.3	103.2 ± 9.4**	105.4 ± 9.1**
Fasting Insulin (mg/dL)	8.0± 3.0	10.5 ± 3.0 *	12.8 ± 3.2 *⊥
2-h Glucose (mg/dL)	110.4 ± 18.0	160.7 ± 16.0**	163.0 ± 16.2**
2-h Insulin (mg/dL)	67.9 ± 17.7	132.6 ± 31.6**	163.3 ± 44.9**⊥
Apo B (mg/dL)	96.6 ± 14.1	120.4 ± 12.7**	129.0 ±14.2**
Apo A-I (mg/dL)	126.2 ± 13.2	110.8 ± 12.6**	108.2 ± 14.8**
Lp A-I (mg/dL)	49.7 ± 7.7	38.2 ± 9.0**	35.6 ± 5.5**
Lp A-I:A-II (mg/dL)	75.4 ± 7.8	72.5 ± 6.0	72.6 ± 9.5

§, p <0.02 when compared with control

*, p <0.01 when compared with control

**, p <0.001 when compared with control

⊥, p <0.02 when compared with Syndrome X without CHD

level <240 mg/dL, triglyceride level <150 mg/dL and HDL-C level >40 mg/dL), a normal glucose tolerance, without a history of CHD or the other significant diseases including hypertension. The clinical characteristics of the patients and the control subjects are presented in Table 1.

Methods: Height, weight and waist to hip circumference ratio of the study population were measured in light clothing without shoes. Waist circumference was measured at the mid-point between the inferior border of the costal margin and the anterior superior iliac crest and hip circumference at the level of the greater trochanters. The body mass index was calculated as weight (kg) divided by height squared (in meters). Systolic and diastolic blood pressure were determined, using a mercury sphyngomanometer, two times in the sitting position after a 5 minutes rest. The mean of two measurements was used.

Coronary angiography was done by the percutaneous transfemoral technique according to a standard protocol and was recorded on cine film. All of the cine angiograms were assessed by two cardiologists and two cardiovascular surgeons who were unaware of the biochemical profiles of the subjects.

Table 3. Pearson correlation analyses among Lp A-I, Lp A-I:A-II, and the other parameters which are the characteristics of syndrome X in the patients with syndrome X and the controls.

	Control (n=22)		Syndrome X without CHD (n=17)		Syndrome X with CHD(n=21)	
	LpA-I	LpA-I:A-II	LpA-I	LpA-I:A-II	LpA-I	LpA-I:A-II
HDL-C	r=88**	r.66⊥	r=.76**	r=.63⊥	r=.73*	r.62⊥
Triglycerides	r=-.21	r=-.19	r=-.39	r=-.24	r=-.49*	r=-.59⊥
Fasting Glucose	r=-.14	r=-.34	r=.14	r=.13	r=.24	r=.23
Fasting Insulin	r=.39	r=.18	r=-.37	r=-.12	r=-.57⊥	r=-.69**
2-h Glucose	r=-.11	r=-.36	r=.15	r=.08	r=.39	r=.27
2-h Insulin	r=-.25	r=.26	r=-.25	r=-.18	r=-.61⊥	r=-.70**
BMI	r=.36	r=-.26	r=-.33	r=.26	r=-.22	r=-.32
Waist/Hip ratio	r=-.20	r=-17	r=-.35	r=-.16	r=-19	r=-23
Systolic BP	r=-.08	r=-.04	r=-07	r=-.05	r=-.14	r=-.20
Diastolic BP	r=.14	r=.12	r=-.22	r=-.32	r=-.07	r=.11

*, p<0.05

⊥, p<0.01

**, p<0.001

BP, Blood pressure.

All subjects with a minimum fasting period of 12 h underwent an OGTT with 75 g of glucose according to the WHO standard (17). Serum samples for determination of blood lipids, glucose and insulin levels were obtained before the OGTT. A second serum sample was taken after 2 h. The serum samples were frozen at -20 ˚C within 3 h and later analyzed.

Serum glucose, triglyceride and cholesterol were measured by enzymatic methods using commercial kits (Boehringer Mannheim GmBH, Mannheim Germany). Analysis of HDL-C was performed after precipitating of other lipoproteins with polyethylene glycol 6000 as previously described (18). Low density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald formula (19). Serum concentrations of apo A-I and apo B were determined by immunoturbidimetry (Biomerieux, Marcy-L`Etoile, France). The coefficients of intra-assay variation of serum cholesterol, triglyceride, HDL-C, apo A-I and apo B were 1.8%, 2.6%, 4.1%, 3.4% and 3.1%, respectively and inter-assay coefficients of variation were 2.2%, 3.1%, 5.1%, 3.8% and 3.3%, respectively. The serum insulin level was determined by a commercial solid-phase radioimmunassay kit (Diagnostic Products Corporation, Los Angeles, USA). We estimated the concentration of Lp A-I in samples of serum as described by Parra et al (20) using a Hydragel Lp A-I Particles Kit (Sebia, Issy-les-Moulineaux, France) for electroimmunoassay. The concentration of Lp A-I:A-II particles was calculated as a difference between the total concentrations of apo A-I and Lp A-I.

Statistical Analysis: Data were compared by using Student`s t-test. Pearson correlation analysis was performed to test for relationships between Lp A-I, Lp A-I:A-II and other parameters. A p value of less than 0.05 was considered to be significant.

Results

As shown in Table 1, the patient groups with or without CHD have significantly higher BMI, waist/hip ratio, and systolic and diastolic blood pressure than the control group. But there were no significant differences between both patient groups.

Serum triglyceride, total cholesterol, LDL-C, HDL-C, glucose, insulin, apo B, apo A-I, Lp A-I and Lp A-I:A-II levels in the study subjects are shown in Table 2. In the syndrome X patients with or without CHD, serum levels

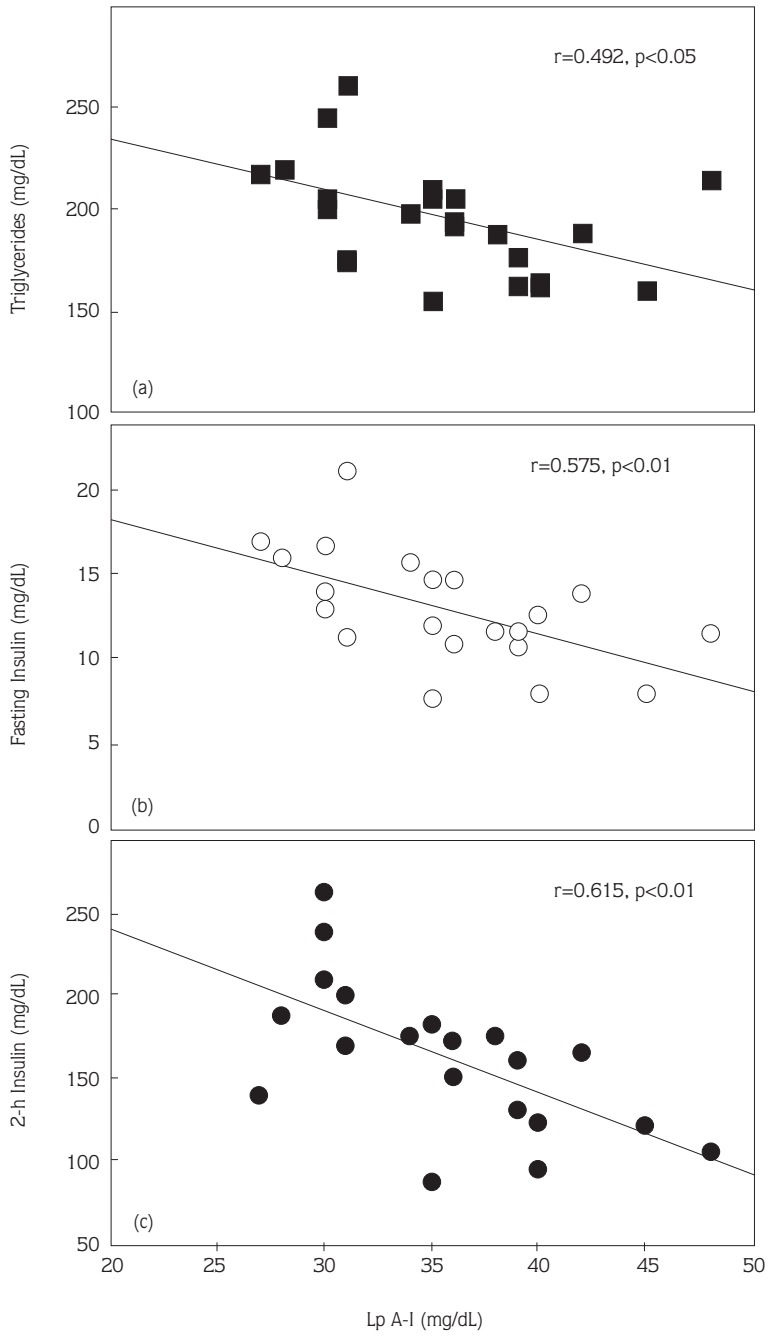

Figure 1. Graphs show the correlations between Lp A-I and triglycerides (a), fasting insulin (b), and 2-h insulin (c) in syndrome X patients with CHD.

of triglyceride, fasting glucose and insulin, 2-h glucose and insulin and apo B were all significantly increased in comparison with the control group, whereas HDL-C, apo A-I and Lp A-I concentrations were significantly lower. Total cholesterol was significantly high only in syndrome X patients with CHD when compared with the control group. Serum fasting insulin and 2-h insulin in syndrome X patients with CHD were significantly higher than syndrome X patients without CHD. But, there is no difference in LDL-C and Lp A-I:A-II levels among the three groups.

The correlations between Lp A-I, Lp A-I:A-II and some parameters including triglyceride, HDL-C, fasting glucose, fasting insulin, 2-h glucose, 2-h insulin, BMI, waist/hip ratio, systolic and diastolic blood pressure in three groups are shown in Table III. Lp A-I and Lp A-I:A-II levels were

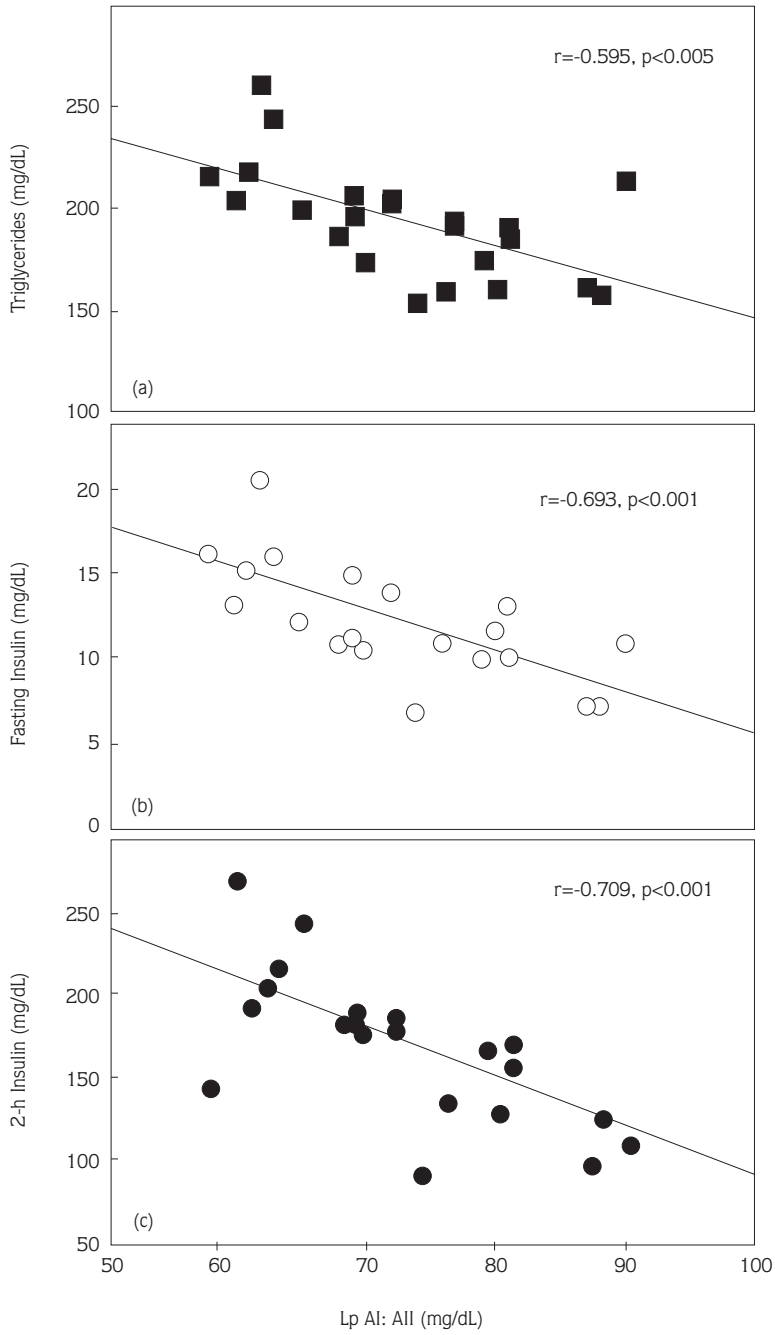

Figure 2. Graphs show the correlations between Lp A-I:A-II and triglycerides (a), fasting insulin (b), and 2-h insulin (c) in syndrome X patients with CHD.

significantly correlated to HDL-C in three groups. But, they had inversely significant correlation with triglyceride, fasting and 2-h insulin levels only in the syndrome X patients with CHD (Figures 1 and 2).

Discussion

Syndrome X and its characteristic metabolic abnormalities, especially insulin resistance, have been receiving much attention due to their potential relationship to CHD risk (2). Also, apo A-I containing lipoprotein particles may be the other factors which their serum levels are considered to have the significant relation to CHD. Especially, Lp A-I was reported by Puchosis et al. (14) as the antiatherogenic fraction of HDL. Even though, Rader et al. (21) suggested that Lp A-I was the best single discriminating factor in a case control study of lipids and lipoproteins in adolescent

children of parents with premature CHD compared with matched control subjects. For this reason, in the present work, we attempted to evaluate the relationship between CHD, insulin levels, the characteristics of syndrome X and apo A-I containing lipoprotein particles.

In this study, the subjects who were non-smoker at least for 10 years were especially selected for avoiding or minimizing the effect of nicotine on insulin action, because it was suggested that smoking can acutely impair insulin action, and chronic smoking can be of importance for the development of the insulin resistance syndrome, and a risk factor for CHD (22). As shown in Table I, BMI, waist/hip ratio, systolic and diastolic pressure of the patient groups are significantly different from the control group, but not different from each other. This is a suitable condition for comparing of both patient groups in regard to syndrome X characteristics we measured.

Apo A-I and Lp A-I levels in both patient groups were significantly less than those in the control group, while Lp A-I:A-II levels did not have any significant change (Table II). For this reason, low HDL-C we observed in the patients with syndrome X could be attributable to only the decrease in Lp A-I levels. The mechanism of the decrease of Lp A-I particles in insulin resistance is not obvious. However insulin resistance and/or hyperinsulinemia lead to enhanced hepatic secretion and decreased catabolism of triglyceride rich lipoproteins (23). Montali et al. (24) demonstrated a preferential reduction in Lp A-I levels compared with Lp A-I:A-II in hypertriglyceridemic low-HDL-C patient. Therefore the reason of the decrease in Lp A-I levels without significant alterations in Lp A-I:A-II levels observed in syndrome X patients seems to be primarily due to the effects of hyperinsulinaemia on triglyceride metabolism.

The serum levels of apo B in the patient groups were significantly higher than in the control group, without significant changes between LDL-C levels of all groups. Observed high apo B should be dependent high VLDL levels in the patient groups. The mean LDL-C level, 128.2 mg/dL, in our control group is paid attention to which is near to upper limit of normal LDL range described by NCEP (25). This should be acceptable as normal for Turkish population. Because Turkish Heart Study showed that Turkish population have high LDL-C levels, especially for who living in big cities in Türkiye (26). We did not measure LDL particle size. Since small, dense LDL have been shown to be associated with insulin resistance and with CHD (27).

We did not observed significant differences in parameters studied except 2-h insulin and fasting insulin levels between syndrome X patients with and without CHD (Table II). Both insulin levels were significantly higher in patients with CHD than those of the patients without CHD. This is an important finding, which is emphasizing that hyperinsulinaemia or insulin resistance could be much efficient factor than the serum levels of apo A-I, Lp A-I, apo B, HDL-C, triglyceride and glucose in regard to development of CHD in syndrome X. As a matter of fact that there is a widespread belief from longitudinal surveys that high insulin levels may be an independent predictors of CHD (7,8,28). There is a widespread believe that endothelial dysfunction is an early and prominent event in atherogenesis (29). It is well establish that hyperinsulinemia and insulin resistance is related to endothelial dysfunction (30). It is also well known that high insulin concentration is associated with lipid and lipoprotein changes favoring atherosclerosis (31). Furthermore, hyperinsulinaemia promotes LDL receptor activation (32), increases cellular 3-hydroxy-3-methylglutaryl coenzyme A reductase synthesis (33), decreases cholesterol efflux from cell membrane (34), leading to the accumulation of cholesterol in vascular endothelium, and it also stimulates the proliferation of these cells (35,36). Meanwhile a positive association between plasma insulin levels and plasminogen activator inhibitor type 1, a potent inhibitor of fibrinolysis, has been demonstrated (37). More recently, it has been indicated that endogenous hyperinsulinaemia contributes to the development of atherosclerosis by accelerating cholesterol ester accumulation in the arterial wall (38). All of these effects are potentially atherogenic and may explain possible mechanisms leading to CHD in our syndrome X patients with CHD. Not being significant differences of apo A-I containing lipoproteins between CHD positive and negative patient groups indicates that the decreased serum levels of these lipoproteins are not the primary factors on developing of CHD in syndrome X patients. Their decreased levels we observed should be the result of high insulin level. The other hand, presence of significant correlation between apo A-I containing lipoproteins and triglyceride, fasting and 2-h insulin levels in CHD positive group suggest that more insulin has much determinative effect on serum level of apo A-I containing lipoprotein and triglyceride metabolism (Table III, Fig. 1 and 2). Also, it is suggested that efforts must be concentrated on decreasing insulin resistance and hyperinsulinaemia to prevent macrovascular disease in subjects with glucose intolerance (39).

Several reports, which examining the relationship between apo A-I containing lipoproteins and CHD in patient with angiographically confirmed CHD positive,

suggested that Lp A-I is cardioprotective, whereas Lp A-I:A-II is not (14,15,27). But, some others (40-42) have found lower levels of both Lp A-I and Lp A-I:A-II in patients with CHD than control subjects. In a recent study (43) examining atherosclerotic plaques at carotid, aortic and femoral sites, any significant difference in the Lp A-I and Lp A-I:A-II particles has not been observed between the patients with and without atherosclerotic plaque, except at the femoral site where Lp A-I:A-II was significantly lower in those with than without plaque. Whereas, we did not observed any significant differences in Lp A-I and Lp A-I:A-II particles between the syndrome X patients with and without CHD, although a significant decrease in Lp A-I levels without changes in Lp A-I:A-II levels in all syndrome X patients as compared with the control subject. These results of in vivo studies on the antiatherogenic role of these particles have been inconsistent. These conflicting reports may be originated from different features of the subjects studied (e.g., obese, normolipemic, hypertriglyceridemic, hypoalpha-lipoproteinemic or hyperinsulinaemic) and different laboratory methods used to measure Lp A-I and Lp A-I:A-II particles.

In summary, we found decreased Lp A-I levels without any changes in Lp A-I:A-II levels in patients with syndrome X. The factors which are responsible for low HDL-C may apparently affect Lp A-I particles more than Lp A-I:A-II particles. However apo A-I containing lipoproteins should not be useful for assessment coronary risk in syndrome X patients. Furthermore the greater insulin levels observed in syndrome X patients with CHD may be responsible in part for increased risk of CHD as compared with syndrome X patients without CHD.

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