Hypertriglyceridemia Is Associated With Increased Insulin Resistance in Subjects With Normal Glucose Tolerance: Evaluation in a Large Cohort of Subjects Assessed With the 1999 World Health Organization Criteria for the Classification of Diabetes

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The current study retrospectively examined the association between insulin resistance and plasma triglycerides (TG) in a group of subjects with normal glucose tolerance. Among 1,434 subjects consecutively undergoing a standard oral glucose tolerance test (OGTT) between 1993 and 1998, 567 (age, 15 to 78 years) were classified as having a normal glucose tolerance according to the 1999 World Health Organization (WHO) criteria and were selected for the study. Serum insulin was measured by radioimmunoassay (INSI-CTK, Dia Sorin, Saluggia, Italy). Intra-assay and interassay coefficients of variation for the method were less than 4% and less than 8.5%, respectively. Insulin resistance was calculated by a homeostasis model assessment ($HOMA_{IR}$ = fasting serum insulin [mU/mL] × fasting blood glucose [mmol/L]/22.5). A very significant correlation was found between $HOMA_{IR}$ and plasma TG ($r = 0.27$, $P < 1.02E^{-10}$). Multiple regression analyses confirmed plasma TG as independent variables explicative of $HOMA_{IR}$. When subjects were evaluated according to tertiles of TG, those in the upper two tertiles were older ($P < .001$) and presented higher body mass index (BMI) values ($P < .0001$) in comparison to subjects in the lower tertile. A positive trend (analysis of variance [ANOVA]) was found in regard to systolic ($P < .05$) and diastolic blood pressure ($P < .0001$), fasting blood glucose ($P < .01$), fasting serum insulin ($P < .0001$), and total cholesterol ($P < .0001$), while a negative trend was found in regard to high-density lipoprotein cholesterol (HDL-C) ($P < .0001$). Insulin resistance, calculated as $HOMA_{IR}$, was higher in the upper two tertiles of TG in comparison to the lower tertile ($P < .001$ and $P < .0001$, respectively), with a statistically significant trend for the entire group (first tertile, 1.85 ± 0.94; second tertile, 2.28 ± 1.10; third tertile, 2.65 ± 1.71; ANOVA: $P < .0001$). In conclusion, this study shows an association between high levels of circulating TG and insulin resistance in patients with normal glucose tolerance seen in an atherosclerosis prevention clinic. This association is also present at levels of plasma TG considered to be normal and is associated with a cluster of cardiovascular risk factors.

THE ROLE OF TRIGLYCERIDES (TG) as a risk factor for coronary artery disease (CHD) is controversial.[1-3] While epidemiological studies have demonstrated a positive relationship between the increase of plasma TG and the risk of CHD,[4-6] multivariate analysis, including high-density lipoprotein cholesterol (HDL-C), has usually eliminated or substantially reduced their predictive role.[1] Neverthless, the Copenhagen Male Study demonstated that a high level of fasting TG is a strong predictor of CHD mortality, independently of the other major risk factors.[7] The pathogenetic link between hypertriglyceridemia and CHD is, however, not completely understood. In addition to a direct atherogenic effect, it has been suggested that hypertriglyceridemia might increase CHD risk by determining hyperinsulinemia and insulin resistance.[8] The association between hyperinsulinemia and CHD has been largely reported,[9-13] even if this association is generally weakened or abolished when closely associated risk factors such as obesity, glucose tolerance, and dyslipidemia are taken into account.[9-13] The aim of the present study was to investigate the relationship between insulin resistance and triglyceridemia in a large group of subjects with normal glucose tolerance, according to 1999 World Health Organization (WHO) criteria,[14] while taking into account other CHD risk factors. We selected subjects with normal glucose levels in response to a glucose load to make sure that islet function was adequate and therefore circulating insulin would reflect the degree of insulin resistance.[15-18] Insulin resistance was measured by a homeostasis model assessment ($HOMA_{IR}$).[16] This method has been validated[17] and represents a useful tool for assessing insulin resistance in population studies across a range of glucose tolerance from normal to diabetes.[17,18]

MATERIALS AND METHODS

We retrospectively evaluated a group of 1,434 subjects who were not known to be diabetics (none had a fasting serum glucose level > 7.8 mmol/L or were taking oral hypoglycemic agents or insulin) and who consecutively underwent a standard oral glucose tolerance test (OGTT) between 1993 and 1998 to establish their glucose tolerance. The subjects all belonged to a larger group of patients screened for coronary risk profile in a clinic for the "prevention of atherosclerosis." Patients arrived to this clinic, operating in Venice since 1970 and well-known to the population, by the referral of their family doctor. The most common reasons for referral were patients asking their family doctors for a cardiovascular screening, or the doctors asking for consultation due to the presence of dyslipidemia with or without other cardiovascular risk factors. The reasons for suggesting an OGTT were borderline fasting blood glucose levels with or without other cardiovascular risk factors. Patients who were overweight, had hyperlipemia, and/or had a positive family history of diabetes were more likely to undergo an OGTT for lower glucose levels. Subjects with heart or renal failure, chronic liver disease, or endocrine disease were excluded from the study. Subjects who were taking medications known to alter glucose or insulin metabolism and/or treated with lipid-lowering drugs were excluded from the study. None of the females selected for the study were on hormone-replacement therapy. A total of 567 subjects (60% male;

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Table 1. Linear Correlation of $HOMA_{IR}$ With the Main Clinical Characteristics and Laboratory Parameters

Variable	r	P
Age (yr)	0.0208	.6208
Sistolic blood pressure (mm Hg)	0.1554	.0004
Diastolic blood pressure (mm Hg)	0.2539	4.0022E-09
HbA_{1c} (%)	0.0555	.2078
BMI (kg/m^2)	0.4323	1.7520E-37
TC (mmol/L)	0.0345	.4117
TG (mmol/L)	0.2672	1.0258E-10
Ln TG (ln mmol/L)	0.2730	3.8679E-11
HDL-C (mmol/L)	−0.2650	1.4682E-10

age, 15 to 78 years), who were classified as having a normal glucose tolerance based on fasting and 2-hour glucose values using the 1999 WHO criteria[14] for diagnosis of diabetes (fasting serum glucose level < 6.1 mmol/L and second hour of a standard glucose tolerance test < 7.8 mmol/L) were considered for the study.

Body mass index (BMI) was calculated as weight (kg)/(height [m^2]). Subjects with BMI less than 18 kg/m^2 were considered underweight and excluded from the study.[19] Sitting blood pressure was measured with a standard mercury sphygmomanometer after at least 10 minutes of rest. Mean values were determined from 2 independent measurements.

In the morning, after an overnight fast, venous blood was sampled for the measurement of plasma concentrations of glucose, total cholesterol (TC), HDL-C, TG, and serum insulin. A 75-g oral glucose load was administered to all subjects and blood was drawn for 2 hours thereafter at 30-minute intervals. Plasma glucose was measured by the glucose-oxidase method on a Beckman glucose analyzer (Beckman Instruments, Fuellerton, CA). TC and TG concentrations were determined enzimatically with specific test kits from Menarini (Milano, Italy). HDL-C was measured according to Kostner et al.[20] Serum insulin was measured by radioimmunoassay (INSI-CTK, Dia Sorin, Saluggia, Italy). The intra-assay coefficient of variation of the method was less than 4% and the interassay coefficient of variation was less than 8.5%. The estimate of insulin resistance by HOMA ($HOMA_{IR}$) was calculated by the equation fasting serum insulin (mU/mL) × fasting blood glucose (mmol/L)/22.5, as described by Matthews et al.[16]

Calculations and Statistical Analyses

Results are expressed as means ± SD. Analysis of variance (ANOVA) was performed to compare clinical and laboratory parameters of the subjects subdivided in tertiles of triglycerides. Post hoc multiple comparisons were performed using the Bonferroni test. Pearson correlation coefficients were computed to quantify the relationships among $HOMA_{IR}$ and age, BMI, TC, HDL-C, plasma TG, and diastolic and systolic blood pressure. A multiple linear regression analysis was performed, considering $HOMA_{IR}$ as a dependent variable and all others as explanatory variables. Since the levels of $HOMA_{IR}$ strictly depended on fasting insulin and glucose concentration, we did not consider these parameters in multiple regression analysis. A P value less than.05 was considered significant.

RESULTS

Linear correlations between $HOMA_{IR}$ and the other variables evaluated in the study are shown in Table 1. $HOMA_{IR}$ was positively correlated with BMI, plasma TG, and diastolic and systolic blood pressure and negatively correlated with plasma HDL-C levels. In contrast, age and TC had no significant relationship with $HOMA_{IR}$. Multiple regression analyses, with $HOMA_{IR}$ as a dependent variable, confirmed BMI, plasma TG, plasma HDL-C, and diastolic blood pressure as independent variables explicative of $HOMA_{IR}$ (Table 2). As plasma TG was selected as an independent variable explicative of insulin resistance, we evaluated (Table 3) the clinical characteristics and the laboratory parameters of the subjects divided in tertiles of TG. Subjects in the upper two tertiles of TG were significantly older ($P < .001$ for both tertiles) and presented higher BMI values ($P < .0001$ for both tertiles) compared to subjects in the first tertile, without statistically significant differences between the upper two tertiles (Bonferroni post hoc test). A statistically significant positive trend (ANOVA) was found with regard to systolic ($P < .05$) and diastolic blood pressure ($P < .0001$), fasting blood glucose ($P < .01$), fasting insulinemia ($P < .0001$), and TC ($P < .0001$), while a negative trend was found for HDL-C ($P < .0001$). Insulin resistance, calculated as $HOMA_{IR}$, was higher in the upper two tertiles of TG in comparison to the lower tertile ($P < .001$ and $P < .0001$, respectively) with a statistically significant trend (first tertile, 1.85 ± 0.94; second tertile, 2.28 ± 1.10; third tertile, 2.65 ± 1.71; ANOVA: $P < .0001$) for the entire group (Fig 1).

DISCUSSION

Data of the present study confirm the strong association between insulin resistance and obesity, hypertension, hypertriglyceridemia, and low HDL-C[21-24] even in patients with a normal glucose tolerance evaluated using the 1999 WHO criteria for the classification of diabetes.[14] The association between insulin resistance and the risk of CHD is controversial.[25,26] Some case-control and cross-sectional studies have demonstrated an important association between raised insulin levels and CHD,[11,12,26] but others failed to do so.[13,27] In particular, it has been demonstrated that the predictive value of insulin resistance for CHD is strong when found in a peculiar cluster of risk factors such as obesity, hypertension, hypertriglyceridemia, and hypo-HDL cholesterolemia, independently from low-density lipoprotein cholesterol and smoking.[27,28] The insulin resistance hypothesis does not necessarily assume that insulin directly promotes atherogenesis.[8] It is possible that the association between insulin resistance and CHD is mediated through a disturbance of lipoprotein metabolism.[23] In agreement with this hypothesis, we found that the correlation be-

Table 2. Multiple Regression Analyses Showing Independent Associations Among Triglycerides, HDL-C Levels, and $HOMA_{IR}$

Predictor	Slope Coefficient	SE of Slope Coefficient	F to R*	P value	t Value
Diastolic blood pressure (mm Hg)	0.01	0.0053	4.84	0.0283	2.20
TG (mmol/L)	0.02	0.06	11.57	0.0007	3.40
HDL-C (mmol/L)	−0.62	0.17	12.81	0.0004	−3.58
BMI (kg/m^2)	0.12	0.01	73.44	1.432E-16	8.57

*F to R evaluates how significant is the contribution of the variable to total R^2 through a distribution F.

Table 3. Main Clinical Features of the Study Population, Subdivided in Tertiles of Triglycerides

Variable	Tertiles of TG Levels		
	0.33-1.07	1.08-1.85	1.86-6.02
Age (yr)‡	46.06 ± 15.43	50.21 ± 12.29	50.64 ± 11.43
BMI (kg/m^2)§	25.22 ± 4.06	27.13 ± 4.49	27.26 ± 3.52
Systolic blood pressure (mm Hg)*	131.38 ± 20.16	134.71 ± 19.46	136.55 ± 19.33
Diastolic blood pressure (mm Hg)§	80.69 ± 10.42	84.43 ± 10.72	85.67 ± 10.71
Fasting plasma glucose (mmol/L)†	5.12 ± 0.60	5.29 ± 0.58	5.32 ± 0.58
Fasting serum insulin (mU/L)§	8.15 ± 3.98	9.66 ± 4.85	11.19 ± 7.40
TC (mmol/L)§	5.17 ± 0.99	6.05 ± 1.09	6.53 ± 1.19
TG (mmol/L)§	0.77 ± 0.19	1.42 ± 0.22	2.82 ± 0.92
HDL-C (mmol/L)§	1.37 ± 0.33	1.21 ± 0.31	1.06 ± 0.28

NOTE. Data are expressed as mean ± SD; TG are expressed as natural logarithm transformed.
ANOVA: *$P < .05$, †$P < .01$; ‡$P < .001$; §$P < .0001$.

tween insulin resistance and both hypertriglyceridemia and hypo-HDL cholesterolemia was independent of TC, obesity, and hypertension. In particular, the association between the increase of insulin resistance and the rise of plasma TG was present even in subjects with a range of plasma TG that is usually considered normal (1.08 and 1.85 mmol/L). We consider these results particularly interesting in view of the results of the Copenhagen Male Study,[7] showing that not only hypertriglyceridemia is an independent predictor of cardiovascular mortality, but also that cardiovascular mortality increases with the rise of TG, even in patients with "normal" TG levels (1.10 and 1.59 mmol/L). Our data show that insulin resistance increases in a similar range of TG. This finding supports the hypothesis that insulin resistance could be an important risk factor of CHD also in patients with normal glucose tolerance and with "normal" plasma TG levels. The pathogenetic link between hypertriglyceridemia and insulin resistance remains a matter of speculation. It is controversial whether genetic conditions might be primarily responsible for the increase of serum TG that secondarily causes peripheral insulin resistance[29] or other genetic conditions might be primarily responsible for induced insulin resistance that secondarily increases serum TG via enhanced peripheral lipolysis.[30] Probably, increased TG and free fatty acid (FFA) levels could be secondary to insulin resistance, as has been shown in animal models.[31] Moreover, several studies have shown that insulin suppresses very–low-density lipoprotein production in insulin-sensitive subjects, partly by reducing plasma FFA levels and partly by non–FFA-mediated mechanisms,[32,33] but not in chronically insulin-resistant hyperinsulinemic individuals.[34,35]

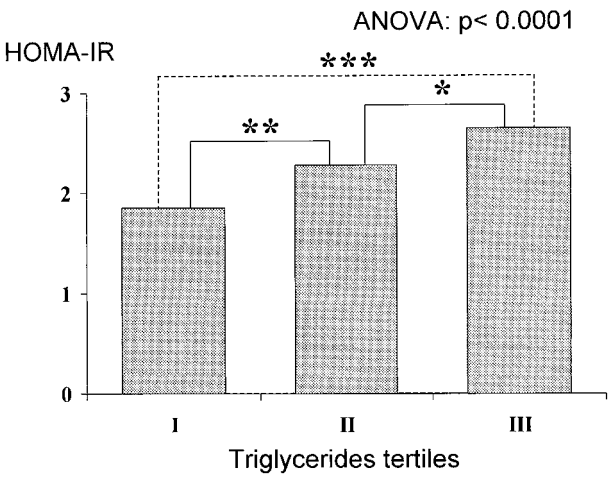

Fig 1. $HOMA_{IR}$ in 567 subjects with normal glucose tolerance according to 1999 WHO criteria for diagnosis of diabetes, subdivided into TG tertiles. Results are expressed as mean ± SD. Bonferroni post hoc test: *$P < .01$, **$P < .001$, ***$P < .0001$.

Several limitations are present in our study: the first is that $HOMA_{IR}$ is not the gold standard in the evaluation of insulin resistance. Nevertheless, this surrogate index correlates moderately well with insulin resistance as measured by hyperinsulinemic euglycemic clamp, which is considered to be the gold standard in the evaluation of insulin resistance.[36] Moreover, several other methods, which have been proposed to evaluate insulin resistance in a metabolic ward setting, including the insulin suppression test[37] and the intravenous glucose tolerance test with computer modeling,[38] have too limited patients' acceptance to be used in large-scale population-based studies. On the other hand, the measure of fasting insulin is a surrogate index that is unable to explain more than 30% to 40% of the variance in insulin resistance as measured by hyperinsulinemic euglycemic clamp.[39] Finally, several large population studies have shown that the application of $HOMA_{IR}$ is a good method for assessing insulin resistance across a range of glucose tolerance from normal to diabetes.[40,41] A second limitation is that we measured serum insulin by a radioimmunoassay method using a nonspecific antibody that also picked up proinsulin. Regarding this aspect, a recent study has reported no evident advantages of specific radioimunoassay methods for insulin measurements over less specific ones in the assessment of insulin resistance.[17] On the other hand, an increased ratio of fasting proinsulin to insulin is considered to be a marker of insulin resistance in nondiabetic subjects.[18]

In conclusion, our study confirmed the association between hypertriglyceridemia and insulin resistance, even in patients with a normal glucose tolerance, evaluated by the 1999 WHO criteria for diagnosis of diabetes, in an atherosclerosis prevention clinic. This association was independent of obesity and was present even at levels of plasma TG that are commonly considered normal in clinical evaluation. Although the pathogenetic link between hypertriglyceridemia and insulin resistance remains a matter of speculation, probably increased TG and FFA levels could be secondary to insulin resistance, as has been shown in animal models.

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