Asymptomatic Atherosclerosis and Insulin Resistance

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High plasma insulin has been shown to be associated with the risk of coronary heart disease in nondiabetic subjects in prospective population studies. Furthermore, insulin resistance measured by the euglycemic glucose clamp technique has been shown to be related to lipid and lipoprotein changes favoring atherosclerosis and to high blood pressure. No study, however, has demonstrated that insulin resistance per se is directly associated with atherosclerosis. With this aim, we studied 30 middle-aged nonobese subjects with asymptomatic atherosclerosis in the femoral or carotid arteries and 13 corresponding control subjects. Fasting blood glucose, insulin, and C-peptide levels were only slightly and nonsignificantly higher in subjects with atherosclerosis than in controls, and during the oral glucose tolerance test 1- and 2-hour glucose, insulin, and C-peptide levels were similar in both groups. During the euglycemic hyperinsulinemic (1,200 pmol/l) clamp studies, subjects with atherosclerosis had a 20% reduced whole-body glucose uptake (58 ±2 versus 71 ±4 μmol/kg/min, p=0.004). Glucose oxidation, lipid oxidation, suppression of free fatty acid levels, and potassium disposal were similar in both groups. In contrast, nonoxidative glucose disposal was significantly reduced in patients compared with that in controls (37 ±2 versus 50 ±4 μmol/kg/min, p=0.004). When glucose uptakes were matched during the hyperglycemic clamp studies, the rate of nonoxidative glucose uptake was normalized in the patients. These results provide the first direct evidence that asymptomatic atherosclerosis is associated with insulin resistance. This insulin resistance is characterized by reduced whole-body and nonoxidative glucose uptake. In contrast, glucose and lipid oxidation, potassium disposal, and suppression of free fatty acid levels during hyperinsulinemia did not differ between the subjects with and without atherosclerosis.

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is directly associated with atherosclerosis. Therefore, we performed a study to investigate whether middle-aged men with asymptomatic atherosclerosis in the femoral or carotid arteries would be more insulin resistant than men without these findings.

Methods

Subjects

The subjects for this study were selected from a large ongoing population study (the Kuopio Ischemic Heart Disease Risk Factor Study) in which risk factors for coronary heart disease and extracoronary atherosclerosis are studied in middle-aged men. Exclusion criteria applied in the selection of the subjects were 1) any chronic disease; 2) any symptom suggesting coronary heart disease (angina pectoris according to the Rose cardiovascular questionnaire, cerebrovascular disease, or peripheral vascular disease); 3) any abnormality in the oral glucose tolerance test (impaired glucose tolerance or diabetes according to the criteria of the World Health Organization); 4) any abnormality in the oral glucose tolerance test (impaired glucose tolerance or diabetes according to the criteria of the World Health Organization); 5) hypertension (use of antihypertensive drugs or systolic/diastolic blood pressure greater than 160/95 mm Hg); 6) obesity (body mass index, weight in kilograms divided by height in meters squared, >27.0); 7) abnormal liver, kidney, or thyroid function tests; or 8) a positive exercise test suggestive of coronary heart disease.

All subjects eligible for the study (N=43) underwent an ultrasound investigation in which the femoral and carotid arteries were evaluated for atherosclerotic plaques, as described in detail below. Altogether, 30 male subjects were identified who had asymptomatic atherosclerosis in the femoral (n=20) or carotid (n=2) arteries or in both arteries (n=8), and they formed the patient group. Thirteen male subjects carefully matched for age and body mass index of the patients and who had no signs of atherosclerotic lesions in the femoral or carotid arteries, as assessed by ultrasonography, served as controls.

Study Protocol

Study 1. The subjects selected for the study were admitted to the metabolic ward for 2 days. On day 1 an oral glucose tolerance test (75 g glucose in 10% solution) was performed, and samples for blood glucose, serum insulin, and plasma C-peptide were drawn at 0, 1, and 2 hours to exclude those with impaired glucose tolerance or diabetes. On day 2 the euglycemic clamp test was performed.

Study 2. At least 3 weeks after study 1, the 10 most insulin-resistant subjects having asymptomatic atherosclerosis were admitted for 2 days for performance of the hyperglycemic clamp.

Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio.

Ultrasound Investigation

As a noninvasive imaging technique without any known risks, ultrasonography is a useful screening method for the assessment of asymptomatic atherosclerosis. According to the available data, high-resolution B-mode ultrasonography has proven to be a reliable method for diagnostic screening of atherosclerosis. In our study the extracranial carotid arteries and the common and superficial femoral arteries of all subjects were evaluated with high-resolution B-mode ultrasonography by a single experienced radiologist (M.S.). All ultrasound studies were performed with an Aloka SSD-650 B-mode scanner. A 7.5-MHz mechanical sector probe and a 7.5-MHz linear small-part probe were both employed to achieve the best possible visualization of the arteries. The extracranial carotid arteries were scanned, beginning with the lowest portion visible in the supraclavicular fossa and continuing to the carotid bifurcation. The femoral arteries were examined from the level of the inguinal ligament down to the adductor canal. Both longitudinal and transverse views were obtained, and several scanning directions were used in both planes to optimize visualization of the arterial walls. Subjects were classified as controls if they had no signs of atherosclerotic lesions in either the carotid or femoral arteries. The subjects were classified as positive for the diagnosis of atherosclerosis (the patient group) if they had either at least one minimal plaque (localized smooth and homogenous plaque >1 mm thick without calcification, ulceration, or significant stenosis) or at least one major plaque (localized dense or nonhomogeneous plaque, with an irregular surface, possible calcification, and mild to significant stenosis of the artery). Atherosclerotic changes of the carotid arteries were classified as present if they were localized in the common carotid artery.

Euglycemic Clamp

The degree of insulin resistance was evaluated by the euglycemic clamp technique. At 8 AM after a 12-hour overnight fast, an intravenous catheter was placed in an antecubital vein for infusion of insulin and 20% glucose. Another cannula for blood sampling was inserted into a wrist vein surrounded by a heated box (70°C). After baseline blood collection and measurement of gas exchange (see “Indirect Calorimetry” section), a priming dose of insulin (Velosulin Human, Nordisk Insulin, Gentofte, Denmark) was administered during the initial 10 minutes in a logarithmically decreasing manner to acutely raise serum insulin to the desired level, where it was maintained by a continuous insulin infusion at a rate of 574 pmol/m^2 body surface area/min. Blood glucose was clamped at 5.0 mmol/l for the next 180 minutes by infusing 20% glucose at varying rates according to blood glucose measurements performed.
at 5-minute intervals. The data were calculated for each 20-minute interval.

**Hyperglycemic Clamp**

In the 10 most insulin-resistant subjects with asymptomatic atherosclerosis in study 1, the hyperglycemic clamp was performed to evaluate whether the decrease in insulin-mediated glucose uptake in these subjects was due to a defect in glucose oxidation or nonoxidation. In each of these subjects the glucose infusion rate was raised to the mean level of that in control subjects in study 1 to match the glucose fluxes. The glucose level during the clamp was allowed to rise while the insulin infusion rate was kept constant and similar to that in study 1 (574 pmol/m² body surface area/min). None of the study subjects had glucosuria during the hyperglycemic clamp, and therefore the correction of glucose uptake for urinary glucose loss was not necessary.

**Indirect Calorimetry**

Indirect calorimetry was performed with a computerized flow-through canopy gas analyzer system (DELTATRAC, TM Datex, Helsinki, Finland) as previously described. This device has a precision of 2.5% for O₂ consumption and 1.0% for CO₂ production. On the day of the experiment, gas exchange (O₂ consumption and CO₂ production) was measured for 30 minutes after a 12-hour fast before the clamp and during the last 30 minutes of the euglycemic and hyperglycemic clamps. The values obtained during the first 10 minutes of each data set were discarded, and the mean value of the remaining 20-minute data was used for calculation. Protein, glucose, and lipid oxidation were calculated according to Ferrannini. Protein oxidation was calculated on the basis of the urinary nonprotein N₂ excretion rate before and during the clamp studies by multiplying this value by 6.25. The fraction of carbohydrate nonoxidation during glucose clamp studies was estimated by subtracting the carbohydrate oxidation rate (determined by indirect calorimetry) from the glucose infusion rate (determined by the euglycemic or hyperglycemic clamp).

**Analytical Methods**

Blood glucose in the fasting state and during glucose clamp studies was measured by the glucose oxidase method (Glucose Auto & Stat HGA-1120 analyzer, Daiichi Co., Kyoto, Japan). For the determination of serum insulin and plasma C-peptide, blood was collected in untreated chilled tubes and allowed to clot. After centrifugation, the serum was stored at -20°C until the analysis. Plasma insulin and C-peptide were determined by radioimmunoassay (Phadeseq Insulin RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden, and C-peptide of insulin by 125J RIA kit, Incstar Co., Stillwater, Minn.). Serum lipid and lipoprotein levels were determined from fresh serum samples drawn after a 12-hour overnight fast. Lipoprotein fractionation was performed by ultracentrifugation and selective precipitation as previously described.

Cholesterol and triglyceride levels from the whole serum and from lipoprotein fractions and plasma lactate were assayed by automated enzymatic methods (Boehringer Mannheim, Mannheim, FRG). Serum free fatty acids were determined by an enzymatic method from Wako Chemicals GmbH, News, FRG. Plasma potassium was measured by flame photometry. Nonprotein urinary N₂ was measured by an automated Kjeldahl method.

**Data Analysis**

All calculations were performed with the SPSS/PC+ programs (SPSS Inc., Chicago, Ill.). Data are presented as mean±SEM. The two-tailed Student's t test for unpaired and paired samples was used to compare the two groups when appropriate. Correlations were calculated by Pearson's correlation coefficients.

**Results**

Table 1 shows the characteristics of patients (subjects with asymptomatic atherosclerosis) and controls (subjects without atherosclerosis). The study groups were comparable with respect to age, weight, body mass index, blood pressure readings, total cholesterol, HDL cholesterol, and very low density lipoprotein (VLDL) triglycerides. Figure 1 depicts glucose and insulin responses to an oral glucose load. Fasting glucose (5.6±0.1 versus 5.4±0.1 mmol/l, p=NS), insulin (60±5 versus 47±4 pmol/l, p=NS) and C-peptide levels (0.57±0.05 versus 0.46±0.04 nmol/l, p=NS) were slightly although not significantly higher in patients than in controls. Also, at 1 and 2 hours insulin and C-peptide responses to the glucose load did not differ between the patients and controls (1-hour insulin: 367±43 versus 331±47 pmol/l; C-peptide: 2.39±0.18 versus 2.12±0.21 pmol/l; 2-hour insulin: 199±37 versus 158±33 pmol/l; C-peptide: 1.73±0.18 versus 1.58±0.23 nmol/l).

During the euglycemic clamp studies, the blood glucose level was 5.0±0.05 mmol/l in patients and controls, with a coefficient of variation of less than 4% during the last 2 hours of the clamp. The steady-state insulin levels during the clamp were 1,191±57 pmol/l in controls and 1,227±39 pmol/l in patients (p=NS between the groups). As shown in
Figure 2, the glucose uptake rate was significantly lower ($p<0.01$) in patients than in controls during the last 2 hours of the clamp. During the last hour of the euglycemic clamp, the mean glucose infusion rate was $57.7 \pm 2.4$ μmol/kg/min in patients and $71.4 \pm 3.7$ μmol/kg/min in controls ($p=0.005$). When the subjects with somewhat elevated blood pressure readings (blood pressure $>140/90$ but $<160/90$ mm Hg) were excluded, the patients ($n=20$) still had lower whole-body glucose uptakes than did the controls ($n=7$) ($58.1 \pm 3.3$ versus $74.5 \pm 5.3$ μmol/kg/min, $p=0.016$).

In the patient group, the glucose infusion rate was similar in subjects having atherosclerosis either in the femoral or carotid arteries ($n=22$) compared with that in subjects having atherosclerosis in both femoral and carotid arteries ($n=8$) ($59 \pm 3$ versus $55 \pm 4$ μmol/kg/min, $p=NS$). Glucose oxidation in the fasting state was similar in patients and controls ($7.4 \pm 0.4$ and $8.5 \pm 0.8$ μmol/kg/min, respectively). Similarly, the fasting lipid oxidation did not differ between patients and controls ($3.8 \pm 0.3$ and $3.5 \pm 0.4$ μmol/kg/min, respectively). As shown in Figure 3, glucose oxidation during the last 30 minutes of the euglycemic clamp was similar in patients and controls ($20.8 \pm 0.6$ and $21.4 \pm 1.2$ μmol/kg/min), but glucose nonoxidation was significantly lower in patients than in controls ($37.0 \pm 2.1$ versus $50.4 \pm 3.6$ μmol/kg/min, respectively, $p=0.004$). Lipid oxidation during the clamp was almost completely suppressed and did not differ between patients and controls ($0.1 \pm 0.4$ versus $0.2 \pm 0.4$ μmol/kg/min). However, when the glucose infusion rate of patients was raised to the level of that
of control subjects during the hyperglycemic clamp (the mean glucose concentration during the last 30 minutes of the clamp was 6.7±0.4 mmol/l), glucose nonoxidation increased to 49.6±0.9 μmol/kg/min, which was not significantly different from that in control subjects (Figure 3, clamp II).

Plasma lactate concentrations were similar in patients and controls in the fasting state (0.63±0.04 and 0.68±0.04 mmol/l, respectively) and increased significantly (p<0.001) during euglycemic clamp studies (Figure 4). Although the lactate level in patients was somewhat lower than that in controls (1.33±0.08 versus 1.13±0.05 mmol/l during the third hour of the clamp), the difference was not statistically significant. During the hyperglycemic clamp, lactate levels increased in the patients to 1.40±0.09 mmol/l. In the pooled data, the increment in blood lactate (the mean blood lactate level during the third hour of the euglycemic clamp minus the fasting blood lactate level) was significantly correlated with total glucose uptake (r=0.59, p<0.01) and with glucose nonoxidation (r=0.64, p<0.01) but not with glucose oxidation (r=0.01, p=NS).

**FIGURE 3.** Bar graph of mean glucose oxidation and nonoxidation rates (μmol/kg/min) during the euglycemic (I) and hyperglycemic (II) clamp studies in the patients and controls.

**FIGURE 4.** Bar graph of plasma lactate concentrations (mean±SEM) in the fasting state and during the euglycemic (I) and hyperglycemic clamp (II) studies in the patients and controls.
Serum free fatty acid levels did not differ between the patients and controls in the fasting state (0.53±0.04 versus 0.41±0.06 mmol/l) and were suppressed in a similar manner during the euglycemic clamp (0.06±0.01 versus 0.06±0.01 mmol/l during the third hour of the clamp). Plasma potassium levels were similar in patients and controls in the fasting state (4.1±0.1 versus 4.1±0.1 mmol/l) and fell by similar amounts, so that at the end of the clamp, potassium levels were 3.6±0.1 mmol/l in both patients and controls.

Discussion

Previous studies have established an association between insulin resistance and abnormal lipid and lipoprotein levels\(^\text{12-14}\) and hypertension.\(^\text{9,10}\) However, no previous study has directly demonstrated that insulin resistance is related to atherosclerosis. Thus, our study investigating insulin resistance by the euglycemic glucose clamp technique in lean subjects with and without asymptomatic atherosclerosis who did not differ with respect to obesity as well as blood pressure levels is the first to provide direct evidence about the association of insulin resistance with atherosclerosis.

The finding that subjects with atherosclerosis have abnormalities in insulin and glucose metabolism was already noted in the 1960s. Several clinical studies demonstrated that plasma insulin levels during oral glucose tolerance tests were often elevated in patients with clinically established coronary heart disease,\(^\text{24-27}\) cerebrovascular disease,\(^\text{28}\) or peripheral vascular disease.\(^\text{29-31}\) The drawback of all these studies was that only subjects having symptoms due to atherosclerotic complications were studied. Furthermore, subjects with established atherosclerosis are often treated with cardiovascular drugs that influence carbohydrate metabolism. Thus, hyperinsulinemia or insulin resistance in subjects with atherosclerotic complications could be a secondary phenomenon. To avoid all these problems, we studied asymptomatic individuals who were not taking any medication, who did not have any chronic diseases, and who had normal glucose tolerance. Only men were studied because men develop atherosclerosis earlier than do women. Atherosclerosis was defined on the basis of atherosclerotic changes assessed by ultrasonography in the femoral or common carotid arteries. The atherosclerotic processes occurring in the coronary arteries, extracranial carotid arteries, and peripheral arteries in the leg appear pathologically identical.\(^\text{32}\) A correlation between coronary and carotid atherosclerosis has been noted in previous autopsy studies,\(^\text{33,34}\) as well as an association between peripheral and coronary atherosclerosis.\(^\text{35}\) Although we were unable to assess noninvasively the status of the coronary arteries, it is reasonable to assume that our patients with a negative exercise test also represent those having asymptomatic coronary artery lesions.

Subjects with asymptomatic atherosclerosis exhibited an approximate 20% decrease in insulin-mediated glucose uptake. Patients with atherosclerosis in only one arterial territory, either in the femoral or carotid arteries, were found to be equally insulin resistant as patients having atherosclerosis in both these arterial territories. The lack of a relation between insulin resistance and the extent of atherosclerosis in a cross-sectional study is not a completely unexpected finding if one assumes that insulin resistance is a metabolic antecedent for the development of atherosclerosis.

We studied our subjects when they had an insulin concentration of 1,200 pmol/l, which is a high physiological insulin concentration and which stimulates glucose uptake to greater than 80% of the maximum response.\(^\text{36}\) With the insulin concentration obtained in our study, liver glucose production is completely suppressed in normoglycemic individuals.\(^\text{37,38}\) Even if the hepatic glucose production from the basal value (about 10 \(\mu\)mol/kg/min) had been suppressed by only 50% in the patient group compared with 100% in the control group, we still would have seen a statistically significant difference (\(p<0.05\)) in whole-body glucose uptake between these groups. Therefore, our results indicate that the defect in insulin action in atherosclerosis lies in the peripheral tissues, most likely in muscle.

Measurements based on indirect calorimetry to assess the intracellular metabolism of glucose showed that glucose and lipid oxidation did not differ between patients and controls either in the fasting state or during the euglycemic clamp. In contrast, nonoxidative glucose disposal (glycogen synthesis, lipid synthesis, and anaerobic glycolysis) was significantly reduced, accounting for virtually all the decrease in total glucose uptake in patients. A decrease in glucose nonoxidation in our study is not a surprise because the enzymatic steps involved in glucose oxidation become saturated at considerably lower insulin concentrations than are those involved in glucose nonoxidation. At an insulin concentration of 1,200 pmol/l, glucose oxidation has been shown to be greater than 90% of its maximum value.\(^\text{39}\) Therefore, a possible glucose oxidation defect in patients with atherosclerosis could have been masked by the insulin concentration we used.

A recent study\(^\text{40}\) has shown that muscle glycogen synthesis accounts for almost all of the nonoxidative glucose disposal in normal subjects. Therefore, impaired glycogen synthesis must be the main factor responsible for the decrease in nonoxidative and whole-body glucose disposal in subjects with atherosclerosis. A part of the decrease in glucose nonoxidation is also likely to be due to impaired anaerobic glycolysis because when glucose fluxes were matched, the plasma lactate levels of patients increased to the levels of those in controls. Although the total nonoxidative glucose uptake was clearly reduced, this could simply be a result of less glucose entering the cell rather than an independent defect. Our results demonstrated, however, that when glucose fluxes were matched, glucose oxidation and nonoxidation did not differ between the groups (study 2). Thus, the intra-
cellular defect in glucose metabolism could be overcome in subjects with atherosclerosis by increasing glucose concentrations. This does not, however, exclude the existence of an intracellular defect in insulin-mediated glucose metabolism. 41

In this connection it is interesting to compare our results of insulin resistance in subjects with atherosclerosis with those related to other insulin-resistant states such as obesity, NIDDM, and essential hypertension. As summarized by Ferrannini et al, 9 whole-body glucose as well as nonoxidative glucose disposal is reduced in all these insulin-resistant states as in the relatives of patients with NIDDM. 42 Furthermore, glucose oxidation is reduced in obesity and NIDDM. Lipid oxidation, suppression of lipolysis, and promotion of potassium uptake may be disturbed in obesity and NIDDM, but in hypertension no defects in these parameters were found by Ferrannini et al. 9 Our results are surprisingly similar to those found in hypertension. We also noted a decrease in whole-body glucose uptake and glucose nonoxidation in patients with atherosclerosis, but glucose oxidation, lipid oxidation, suppression of free fatty acid levels, or promotion of potassium uptake during hyperinsulinemia did not differ between patients and controls.

The presence of hypertension in our patients cannot explain these findings because our study subjects were clearly normotensive (Table 1). Therefore, our results indicate that the etiology of insulin resistance associated with atherosclerosis must be quite similar to that in hypertension.

Previous studies have shown that hyperinsulinemia is associated with high blood pressure levels, 5, 8, 43 low HDL cholesterol, 5, 8 and high VLDL triglyceride concentrations, 5, 8 a cluster of risk factors known to promote atherosclerosis. 45, 46 Furthermore, subjects with a parental history of diabetes are often characterized not only by hyperinsulinemia 47 but also by an atherogenic pattern of cardiovascular risk factors. 48 Therefore, it has been proposed that hyperinsulinemia could be a risk indicator for atherosclerosis, and prospective epidemiological studies 1-3 support this notion. A high insulin level could promote the progression of the atheroma through its biologic effects because in physiological concentrations, insulin stimulates the proliferation 49-51 and migration 52 of arterial smooth muscle cells. Even in states of insulin resistance, high insulin concentrations may have growth-promoting effects on the arterial wall directly or indirectly through a second receptor, for example, that for insulin-like growth factor I. 53-55 Our results indicate that insulin resistance is associated with asymptomatic atherosclerosis even without significant hyperinsulinemia. Therefore, the primary events responsible for atherosclerosis could be related to insulin resistance per se rather than to elevated insulin levels, as suggested in previous studies. 24-31 Thus, in atherosclerosis impaired glucose uptake in peripheral tissues is likely to be a primary phenomenon and the elevation of insulin levels only a secondary mechanism.

With respect to the pathogenesis of insulin resistance in atherosclerosis, two main possibilities should be considered. Theoretically, reduced insulin-mediated glucose uptake in patients could be due to one or both of the components that, according to the Fick principle, 56 determine glucose uptake in insulin-sensitive tissues, namely arteriovenous glucose difference (glucose extraction) and/or blood flow into insulin-sensitive tissues. The current prevailing view is that insulin's action to stimulate glucose uptake in peripheral tissues is mediated via its effect to stimulate cellular glucose uptake, 45 which would be predicted to increase glucose extraction in skeletal muscle. Although we measured only whole-body glucose uptake, we showed that the patients with atherosclerosis were more insulin resistant than were corresponding controls, without any intracellular defect in glucose metabolism that could not be overcome by increasing the glucose concentration. These results are in accordance with the notion that atherosclerosis is associated with the glucose extraction defect, and this postbinding defect of insulin's action could lie at the glucose transport or at some other step proximal to the intracellular metabolism of glucose, as has been suggested in NIDDM. 57

On the other hand, glucose uptake is a function not only of insulin action but also of the delivery of glucose to insulin-sensitive tissues. Thus, insulin's impaired ability to generate increments in blood flow to insulin-sensitive tissues in the patients could also lead to reduced rates of glucose uptake. Although we did not measure blood flow, recent studies have indicated that the insulin-mediated increment in skeletal muscle blood flow is a significant determinant for the decrease of in vivo rates of insulin-mediated glucose uptake in other insulin-resistant states, obesity, 58 and NIDDM. 59 Therefore, it is possible that insulin resistance in atherosclerosis is also associated with a defect in insulin's ability to stimulate microvascular blood flow into skeletal muscle.

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