Evidence That Acute Insulin Administration Enhances LDL Cholesterol Susceptibility to Oxidation in Healthy Humans

Alfredo Quiñones-Galvan, Anna Maria Sironi, Simona Baldi, Fabio Galetta, Ulisse Garbin, Anna Fratta-Pasini, Luciano Cominacini, Ele Ferrannini

Abstract—Increased free radical production and hyperinsulinemia are thought to play a role in experimental and human atherosclerosis, but the relation between the 2 abnormalities has not been studied. In 23 healthy volunteers, we measured the susceptibility of circulating low-density lipoprotein (LDL) cholesterol particles to in vitro copper sulfate oxidation (measured as the lag phase) and cell-mediated oxidative modification (measured as malondialdehyde generation in LDL during incubation with human umbilical vein endothelial cells), as well as the vitamin E content of LDL cholesterol at baseline and after 2 hours of physiological hyperinsulinemia (euglycemic insulin clamp). The lag time of LDL oxidation decreased from control values of 108±3 and 107±3 minutes (at baseline and after 2 hours of saline infusion) to 101±3 minutes after 2 hours of clamping (P<0.0001). At corresponding times, cell-mediated malondialdehyde generation in LDL rose from 4.96±0.11 and 4.98±0.10 to 5.28±0.10 mmol/L (P=0.0006), whereas the LDL vitamin E content decreased from 6.78±0.06 and 6.77±0.06 to 6.64±0.06 μg/mg (P<0.04). The insulin-induced shortening of the lag phase was directly related to the decrement of vitamin E in LDL; furthermore, in subjects with higher baseline serum triglyceride levels, insulin induced a greater shortening of the lag phase than in subjects with low baseline triglycerides. We conclude that in healthy humans acute physiological hyperinsulinemia enhances the oxidative susceptibility of LDL cholesterol particles. This effect may have pathogenic significance for atherogenesis in insulin resistant states. (Arterioscler Thromb Vasc Biol 1999;19:2928-2932.)

Key Words: insulin LDL cholesterol oxidation free radicals

Increased free radical production and lipid peroxidation play an important role in experimental and human atherosclerosis.1,2 A variety of clinical conditions, such as smoking,3 diabetes mellitus,4 essential hypertension,5 and low vitamin E consumption,6 have been associated with increased oxidative stress and higher prevalence of atherosclerosis. Conversely, epidemiological studies have suggested that diets enriched in antioxidants are associated with lower rates of coronary artery disease7,8 and that antioxidant supplementation may reduce the incidence of coronary artery disease–related events.9 Insulin resistance/hyperinsulinemia is also present in several conditions of enhanced atherogenic risk, such as essential hypertension, diabetes mellitus, dyslipidemia, smoking, obesity, and sedentary lifestyle (reviewed in Reference 10). Thus, both hyperinsulinemia and increased peroxidation have been implicated in atherogenesis, but the relation between the 2 phenomena has not been investigated.

In vitro studies have shown that insulin induces H2O2 formation in human peritoneal adipocytes, macrophages, and brain stem cells.11–13 In suspended human fat cells, physiological amounts of insulin stimulate H2O2 release via activation of a membrane-bound dehydrogenase, an effect that is still seen in isolated adipocyte plasma membranes and is not prevented by blockade of the insulin receptor tyrosine kinase.11 The latter findings have led to the idea that hydrogen peroxide generation may be an alternative insulin-signaling pathway in adipocytes. In diabetic rats, the production of free radicals is stimulated by the intraperitoneal administration of insulin.14 Recent clinical studies have demonstrated that the urinary excretion of 8-iso-prostaglandin F2α, a bioactive product of arachidonic acid peroxidation, is increased in patients with diabetes and that this abnormality is improved by vitamin E supplementation.15 In studies carried out in our laboratory in healthy volunteers, acute insulin administration induced a decrement in total plasma vitamin E concentrations.16 Because vitamin E is the main tissue scavenger of free radicals,17 one interpretation of that finding was that acute insulin administration would enhance oxidative stress. However, because other parameters of free-radical activation were not concomitantly measured, an alternative explanation was that the reduction of circulating vitamin E levels may be due to accelerated transport from the intravascular to the extravascular space, which is analogous to the insulin effect on LDL cholesterol transport.18

The present study was therefore undertaken to establish whether acute euglycemic hyperinsulinemia within the phys-
TABLE 1. Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47±2</td>
<td>30–60</td>
</tr>
<tr>
<td>BMI, kg · m⁻²</td>
<td>26.6±0.5</td>
<td>23.2–30.0</td>
</tr>
<tr>
<td>WHR, cm/cm</td>
<td>0.87±0.02</td>
<td>0.72–0.98</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>106±3</td>
<td>90–128</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.1±0.1</td>
<td>4.4–6.0</td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol/L</td>
<td>70±10</td>
<td>18–196</td>
</tr>
<tr>
<td>Steady-state plasma insulin, pmol/L</td>
<td>671±40</td>
<td>455–1117</td>
</tr>
<tr>
<td>Insulin sensitivity, μmol · min⁻¹ · kg⁻¹</td>
<td>40±3</td>
<td>23–70</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; WHR, waist-to-hip ratio.

iologial range has a pro-oxidant activity in vivo and whether such an effect may be related to insulin sensitivity.

Methods

Subjects

We studied 23 healthy volunteers, 20 men and 3 women (Table 1). All subjects were free of intercurrent acute or chronic illness, were nonsmokers, were not engaged in competitive sports, and were not consuming vitamin supplements.

The study protocol was approved by the Institutional Ethics Committee, and the purpose, nature, and risks involved in the study were explained to all subjects before obtaining their written consent to participate.

Protocol

All subjects were studied at 9 AM after an overnight (10- to 12-hour) fast. A 20-gauge polyethylene catheter was inserted into an antecubital vein for infusion of test substances. A wrist vein was cannulated to participate.

The plasma glucose and insulin concentrations at baseline and during the clamp are given in Table 1. The effect of insulin on serum lipid concentrations is summarized in Table 2. Saline infusion had no detectable effect on serum lipids. After 2 hours of euglycemic hyperinsulinemia, serum total cholesterol decreased by 2%; this change was due to a 22% decline in VLDL cholesterol levels and to a small (2%) decline in LDL cholesterol (P<0.01 for the difference between time 0 and 120 minutes). Serum total triglycerides decreased by 4%; this was the result of decrements in the triglyceride content of both the LDL (−7%) and VLDL fraction (−4%). Both cholesterol and triglycerides were essentially unchanged in the HDL fraction. Plasma free fatty acid concentrations decreased by 65±2% (average value during 2 hours of hyperinsulinemia).

Results

The plasma glucose and insulin concentrations at baseline and during the clamp are given in Table 1. The effect of insulin on serum lipid concentrations is summarized in Table 2. Saline infusion had no detectable effect on serum lipids. After 2 hours of euglycemic hyperinsulinemia, serum total cholesterol decreased by 2%; this change was due to a 22% decline in VLDL cholesterol levels and to a small (2%) decline in LDL cholesterol (P<0.01 for the difference between time 0 and 120 minutes). Serum total triglycerides decreased by 4%; this was the result of decrements in the triglyceride content of both the LDL (−7%) and VLDL fraction (−4%). Both cholesterol and triglycerides were essentially unchanged in the HDL fraction. Plasma free fatty acid concentrations decreased by 65±2% (average value during 2 hours of hyperinsulinemia).

Lag phase, MDA generation, and LDL vitamin E content were all interrelated at baseline (lag phase versus vitamin E, r=0.73, P<0.0001; lag phase versus MDA, r=0.85, P<0.0001; and vitamin E versus MDA, r=0.69, P=0.0004). Furthermore, the lag phase was significantly shorter in subjects with higher mean blood pressure levels (r=0.42, P<0.05).

The lag phase of in vitro LDL oxidation was unchanged after 2 hours of saline infusion, whereas it decreased significantly after insulin infusion (Table 3). This effect of insulin...
was seen in 20 of 23 subjects (Figure). Likewise, MDA generation and LDL vitamin E content were not altered by saline infusion, whereas MDA generation was significantly increased and LDL vitamin E content was significantly decreased at the end of the clamp (Table 3). The observed changes in oxidative parameters were consensual. Thus, the reduction in lag phase after insulin was directly related to the decrement of LDL vitamin E content ($r = 0.55$, $P = 0.01$) and tended to be related to the rise in MDA generation ($r = 0.36$, $P = 0.09$). A greater insulin-induced reduction in lag phase was associated with higher fasting triglyceride levels both in whole plasma and in the VLDL fraction ($r = 0.46$, $P < 0.03$ for both). When the study group was subdivided according to the median serum triglyceride concentration, the insulin-induced decrease in lag phase was directly related ($P = 0.01$) to baseline serum triglycerides in the 11 subjects with higher triglyceride levels (2.04 ± 0.28 mmol/L) but not in those with lower triglyceride levels (0.84 ± 0.08 mmol/L, $P = NS$). In contrast, none of the insulin-induced changes in oxidative parameters was related to insulin sensitivity or any other anthropometric or metabolic variable.

### Discussion

In the present study, acute in vivo insulin administration increased the susceptibility of LDL cholesterol to both copper-induced and cell-mediated oxidation. This effect of insulin was consistent and specific (no effect of saline infusion). The increased susceptibility of the LDL cholesterol particles to oxidation was closely related to a decrease in their vitamin E content but not to the insulin sensitivity of glucose metabolism. Using a different probe (in vitro incubation of LDL with alanine) to estimate the amount of partially oxidized LDL present in vivo, Carantoni et al reported that in nondiabetic subjects the presence of insulin resistance of glucose metabolism was associated with increased serum levels of partially oxidized LDL. Although we cannot exclude that this was also the case in our subjects, we found that in vivo insulin administration enhanced LDL oxidizability similarly in insulin-sensitive and -resistant individuals. This suggests that dyslipidemia, especially hypertriglyceridemia, is associated both with insulin resistance of glucose metabolism and with increased partially oxidized LDL particles. However, the pathway through which insulin generates...
oxygen-reactive species may be independent of the glucose pathway or may already be saturated at the plasma insulin concentrations (≈700 pmol/L) that we used in these clamp studies.

It must be noted that insulin promotes LDL cholesterol uptake by upregulating LDL receptors. If large, more buoyant LDL particles were preferred to small, dense LDL molecules in such a process, insulinization would be associated with an enrichment of plasma with small, dense LDL particles, intrinsically more susceptible to oxidation. In the present study, insulin did cause a small decrease in serum LDL concentrations; in addition, the possibility that insulin may cause an in vivo selection of lipoprotein has been recently documented in studies measuring LDL size after an oral glucose load.

Higher serum triglyceride levels are generally associated with a predominance of small, dense LDL. In the present study, the lack of correlation between fasting triglyceride levels and LDL lag phase could be due to the relatively small sample size or could reflect the fact that very few subjects were hypertriglyceridemic (only 3 subjects >2.3 mmol/L) or could be a combination of these 2 factors. On the other hand, the presence of higher triglycerides, in whole serum or the VLDL fraction, was associated with an enhanced effect of insulin, suggesting that higher triglycerides do signal an increased susceptibility of LDL to oxidative stimuli. Insulin also induced a small decrement (4% on average) in circulating triglyceride levels. Thus, one could surmise either that the insulin-induced shortening of the lag phase would have been even larger had the triglycerides remained unchanged or that the kinetics of the insulin effect was faster than any secondary effect mediated by changes in triglycerides. In general, the nature of the relation between triglycerides and LDL oxidizability is imperfectly understood. On the one hand, when plasma triglycerides are reduced with intensive exercise and dieting or by fibrate therapy, in vitro LDL cholesterol oxidizability is reduced. On the other hand, recent studies have shown that if triglyceride levels are acutely increased (by an infusion of Intralipid [triglyceride emulsion]), the susceptibility of LDL cholesterol to oxidation is decreased rather than increased. From these results, it has been suggested that enriching the LDL molecule with triglycerides reduces its oxidizability, whereas the subsequent hydrolysis of triglycerides within LDL can lead to atherogenic changes. Collectively, these data suggest that one should distinguish between the acute and chronic effects of lipid exchange between lipoprotein particles on their susceptibility to oxidation. Clearly, additional work is needed to gain insight into these mechanisms.

The insulin effect observed in the present studies was small in size (averaging 7%). However, the experiment was designed to test whether insulin alone (ie, without hyperglycemia) at physiological concentrations had any acute effects on LDL oxidizability. On the other hand, chronic hyperinsulinemia, such as prevails in insulin-resistant individuals, may translate into a larger insulin pro-oxidant effect. In this respect, it is relevant that treatment of type 2 diabetic patients with troglitazone, a thiazolidinedione compound with an α-tocopherol moiety, leads to an improvement in insulin sensitivity (and plasma insulin levels) and a reduction in LDL susceptibility to oxidation. The extent to which the ex vivo measurements of LDL cholesterol oxidation reflect the in vivo oxidative damage of lipoproteins has been generally uncertain. Although this uncertainty has not prevented a wide application of the methodology to a number of clinical conditions, the presence of oxidized LDL in vivo has recently been demonstrated in human serum by NMR spectroscopy. Importantly, the results of this assay agreed well with the simultaneously measured in vitro LDL oxidizability, indicating that the latter does reflect a biological phenomenon.

A series of studies using human aortic intimal cells was reviewed by Sobenin et al, who demonstrated that sera from type 2 diabetic patients show increased atherogenic properties, as measured by the ability of LDL cholesterol to accumulate in these cells. The modifications to the LDL molecule included nonenzymatic glycosylation and loss of sialic acid. Some of these atherogenic modifications of the LDL cholesterol particle can be induced by an excessive activity of free radicals. In vivo, hyperglycemia has been invariably regarded as the principal culprit of the increased free radical production observed in type 2 diabetic patients. In line with this notion, in type 2 diabetic patients the total plasma antioxidant capacity, erythrocyte lipid peroxidation activity, the susceptibility of LDL cholesterol to oxidation, and the excretion of oxidized products of arachidonic acid are all increased, in proportion to the severity of hyperglycemia. Even in nondiabetic subjects, the relative hyperglycemia induced by an oral glucose tolerance test stimulates the production of free radicals. Furthermore, increased reactive oxygen species activity has been documented in hyperinsulinemic nonhyperglycemic conditions, such as obesity, essential hypertension, smoking, and dyslipidemia. Also, a long latency between the histological evidence of atherosclerosis and the onset of hyperglycemia in type 2 diabetes is the rule. Atherogenesis is likely to set in when the only abnormality (in future type 2 diabetic or hypertensive patients) is insulin resistance/hyperinsulinemia and its metabolic correlates. Activation of peroxidation (lipid-conjugated dienes and MDA) is already present in the initial lesion of atherosclerosis, the fatty streak.

In conclusion, insulin at physiological doses is associated with increased LDL peroxidation independent of the presence of hyperglycemia. This result supports the possibility that insulin resistance may be implicated in the genesis of the early atherosclerotic lesions (and/or the progression of atherosclerosis) through the attendant hyperinsulinemia.

References

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