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Oxidative Stress Is Associated with Adiposity and Insulin Resistance in Men

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To investigate the direct relationship of oxidative stress with obesity and insulin resistance in men, we measured the plasma levels of 8-epi-prostaglandin F2α (PGF2α) in 14 obese and 17 nonobese men and evaluated their relationship with body mass index; body fat weight; visceral, sc, and total fat areas, measured by computed tomography; and glucose infusion rate during a euglycemic hyperinsulinemic clamp study.

Obese men had significantly higher plasma concentrations of 8-epi-PGF2α than nonobese men ($P < 0.05$). The plasma levels of 8-epi-PGF2α were significantly correlated with body mass index ($r = 0.408$; $P < 0.05$), body fat weight ($r = 0.467$; $P < 0.05$), visceral ($r = 0.387$; $P < 0.05$) and total fat area ($r = 0.359$; $P < 0.05$) in all (obese and nonobese) men. There was also a significant correlation between the plasma levels of 8-epi-PGF2α and glucose infusion rate in obese men ($r = -0.552$; $P < 0.05$) and all men ($r = -0.668$; $P < 0.01$). In all subjects, the plasma levels of 8-epi-PGF2α were significantly correlated with fasting serum levels of insulin ($r = 0.487$; $P < 0.01$).

In brief, these findings showed that the circulating levels of 8-epi-PGF2α are related to adiposity and insulin resistance in men. Although correlation does not prove causation, the results of this study suggest that obesity is an important factor for enhanced oxidative stress and that this oxidative stress triggers the development of insulin resistance in men. (*J Clin Endocrinol Metab* 88: 4673–4676, 2003)

CHANGES IN LIFESTYLE and diet have resulted in an increased number of obese subjects, and obesity is currently an important causative factor of health-related problems in Japan (1, 2).

It has been recently reported that F2-isoprostanes are the most reliable index of lipid peroxidation in humans (3–6); Davi *et al.* (7) reported increased urinary levels of 8-iso prostaglandin F2α (8-iso PGF2α) in android obese women. However, there is no report showing a direct relationship of F2-isoprostane levels with adiposity in men (8).

Oxidative stress has been linked to insulin resistance, and several clinical trials have demonstrated improvement of insulin sensitivity in insulin-resistant and diabetic patients treated with antioxidants (9–12). Gopaul *et al.* (13) have previously reported correlation of plasma levels of 8-epi-PGF2α with insulin resistance evaluated by homeostasis model assessment (14) in normal, impaired glucose-tolerant, and type 2 diabetic subjects. However, the relationship of oxidative stress with insulin resistance assessed during a euglycemic hyperinsulinemic clamp study (the clamp study) has not as yet been reported in men.

In the present study, we measured the plasma levels of 8-epi-PGF2α in obese and nonobese men to investigate the relationship of oxidative stress with adiposity and insulin resistance.

Abbreviations: BMI, Body mass index; FFA, free fatty acid(s); GIR, glucose infusion rate; OGTT, oral glucose tolerance test; PGF2α, prostaglandin F2α; ROS, reactive oxygen species.

Subjects and Methods

Subjects

This study comprised 14 men with obesity [body mass index (BMI) $\geq$ 25.0] and 17 age-matched nonobese (BMI $<$ 25.0) men (Table 1) (Ref. 1).

BMI was calculated as the body weight (in kilograms) divided by the square of the height (in meters).

None of the subjects had diabetes mellitus according to the diagnostic criteria of the American Diabetes Association on the 75-g oral glucose tolerance test (OGTT) (Trelan G 75, Shimizu, Shimizu, Japan) (15).

There were no subjects with hyperlipidemia (total cholesterol $\geq$ 5.69, triglyceride $\geq$ 1.69), hypertension (blood pressure $\geq$ 140/90 mm Hg), hyperuricemia, or smoking history.

None of the subjects were receiving any medication that could affect insulin levels, insulin sensitivity, or oxidative stress, and they were not under any regular exercise or dietary therapy before the beginning of this study.

Informed consent was obtained from all subjects before the beginning of the study.

Study protocol and methods

Several variables in blood samples, insulin resistance, body fat weight, body fat distribution, and blood pressure were evaluated in all subjects. Venous blood was collected at 0800 h after overnight bed rest. After centrifugation, the plasma and serum samples were separated in small aliquots and then frozen at −80 C until use.

The plasma levels of free 8-epi-PGF2α were measured using a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Briefly, 50 μl of standard or plasma samples were placed in a 96-well plate that was precoated with mouse monoclonal antibody after purification using a C-18 solid phase extraction cartridge. Thereafter, 50 μl of 8-isoprostane tracer and 8-isoprostane antiserum were added to each well and incubated for 18 h at room temperature. After washing with wash buffer, 200 μl of Ellman's reagent containing the substrate of acetylcholinesterase was added. The plates were read at 412 nm, and the values of plasma 8-epi-PGF2α levels were calculated from a curve drawn using standard concentrations of 8-epi-PGF2α. This assay

TABLE 1. Clinical characteristics of the subjects

	Nonobese men (n = 17)	Obese men (n = 14)
Age (yr)	31.1 ± 1.5	35.5 ± 1.7
BMI (kg/m^2)	21.4 ± 0.4	26.9 ± 0.6^a
Body fat weight (kg)	12.4 ± 0.8	22.2 ± 1.6^a
Visceral fat area (cm^2)	72.3 ± 9.8	156.9 ± 10.4^a
Subcutaneous fat area (cm^2)	104.7 ± 9.0	187.6 ± 13.8^a
Fasting plasma glucose (mmol/liter)	5.1 ± 0.1	5.1 ± 0.2
Fasting serum insulin (pmol/liter)	25.1 ± 2.1	35.3 ± 3.8^a
GIR (μmol/kg·min)	58.9 ± 4.3	45.9 ± 2.9^a
Total cholesterol (mmol/liter)	4.72 ± 0.19	4.92 ± 0.14
Triglyceride (mmol/liter)	0.84 ± 0.09	1.11 ± 0.08
HDL cholesterol (mmol/liter)	1.31 ± 0.08	1.19 ± 0.07
Serum FFA (mmol/liter)	0.46 ± 0.06	0.47 ± 0.05
Serum Vitamin E		
α-tocopherol (μmol/liter)	25.7 ± 1.5	26.2 ± 1.2
γ-tocopherol (μmol/liter)	3.2 ± 0.2	3.7 ± 0.5
Systolic blood pressure (mm Hg)	121.1 ± 2.1	130.9 ± 2.5^a
Diastolic blood pressure (mm Hg)	74.0 ± 1.6	81.4 ± 1.3^a

Data are mean ± SE. HDL, High-density lipoprotein.
^{a} $P < 0.05$, nonobese *vs.* obese men.

showed no significant cross-reactivity with or interference by other factors (8-iso-PGE2, 2,3-dinor-8-iso-PGF2α, 8-iso-PGE1, PGF1α, PGF3α, PGE1, PGD2, and PGF2α). The intra- and interassay coefficients of variation were 7.5 and 9.2%, respectively. The lower detection limit of the assay was 1.5 pg/ml.

The plasma levels of glucose, and serum levels of total cholesterol, triglyceride, and high-density lipoprotein cholesterol, and free fatty acid (FFA) levels were measured by an automated enzymatic method. The serum levels of vitamin E (α- and γ-tocopherol) were measured by HPLC. Serum insulin was measured using an immunoradiometric assay kit (Insulin Riabead II kit, Dainabot, Tokyo, Japan). The intra- and interassay coefficients of variation of the assay were 1.9 and 2.0%, respectively. No significant cross-reactivity or interference was observed between insulin and proinsulin, C-peptide, glucagon, secretin, and gastrin-I.

Insulin resistance was evaluated by the euglycemic hyperinsulinemic clamp technique using an artificial pancreas (Nikkiso STG-22, Tokyo, Japan) (16–19). At 0800 h, a priming dose of insulin (Humulin R, Shionogi, Osaka, Japan) was administered during the initial 10 min in a logarithmically decreasing manner to rapidly raise serum insulin to the desired level (1200 pmol/liter); this level was then maintained by continuous infusion of insulin at a rate of 13.44 pmol/kg·min for 120 min. The mean insulin level from 90–120 min after starting the clamp study was stable (obese men, 1144.8 ± 36.0 pmol/liter; nonobese men, 1089.0 ± 53.4 pmol/liter). Blood glucose was monitored continuously and maintained at the target clamp level (5.24 mmol/liter) by infusing 10% glucose. The mean amount of glucose given during the last 30 min was defined as the glucose infusion rate (GIR) and was used as a measure of peripheral insulin sensitivity.

Body fat weight was measured by bioelectric impedance using a TBF-101 (Tanita, Tokyo, Japan).

Body fat area was evaluated by a previously described method (20). At 0800 h, after an overnight fast of 12 h, all patients underwent single abdominal computed tomography scanning at the umbilical level. Any intraperitoneal region having the same density as the sc fat layer was defined as a visceral fat area; this area was measured by tracing object contours on films using a computerized planimetric method.

In addition, we measured blood pressure in the supine position after a rest of 5 min.

Statistical methods

Data were expressed as the mean ± SE. Comparisons between obese and nonobese subjects were done using the Mann-Whitney *U* test. Correlations were evaluated by Spearman's rank correlation. All statistical analyses were performed with the StatView 4.0 software program (Abacus Concepts, Berkeley, CA) for the Macintosh. $P < 0.05$ was taken as statistically significant.

Results

The plasma concentrations of 8-epi-PGF2α were significantly increased in obese men compared with nonobese men ($P < 0.05$) (Fig. 1).

Obese men showed significant increased values of the ratio between plasma 8-epi-PGF2α and serum total cholesterol levels (9.00 ± 2.80; $P < 0.05$) compared with nonobese men (1.90 ± 0.35). No significant differences in the serum levels of vitamin E were observed between obese and nonobese men (Table 1).

The plasma levels of 8-epi-PGF2α were proportionally correlated with BMI in all (obese and nonobese) subjects (r = 0.408; $P < 0.05$) (Fig. 2). The plasma levels of 8-epi-PGF2α were significantly correlated with body fat weight (r = 0.467; $P < 0.05$), visceral fat area (r = 0.387; $P < 0.05$), and total fat area (r = 0.359; $P < 0.05$) in all men. There was a significant correlation between the plasma levels of 8-epi-PGF2α and GIR in obese subjects (r = −0.552; $P < 0.05$) and all subjects (r = −0.668; $P < 0.01$) (Fig. 3). Significant positive correlations were observed between the plasma levels of 8-epi-PGF2α (r = 0.487; $P < 0.01$) or visceral fat area (r = 0.534; $P < 0.01$) and the serum levels of insulin in all men. No significant correlations were observed between the plasma levels of 8-epi-PGF2α and serum levels of FFA (r = 0.221; $P = 0.23$) or vitamin E (α-tocopherol, r = 0.190, $P = 0.30$; γ-tocopherol, r = −0.03, $P = 0.85$) in all men. There was a significant correlation between the visceral fat area and serum levels of FFA in all men (r = 0.386; $P < 0.05$).

Discussion

The present study showed that the plasma levels of 8-epi-PGF2α are significantly related to adiposity and insulin resistance in men.

Recently, Dandona *et al.* (21) reported that the ratio of oxidative damage to lipids, proteins, and amino acids is increased in obese subjects. Significant decrease in oxidative stress after dietary restriction and weight loss has also been reported in obese subjects (7, 21). However, whether the

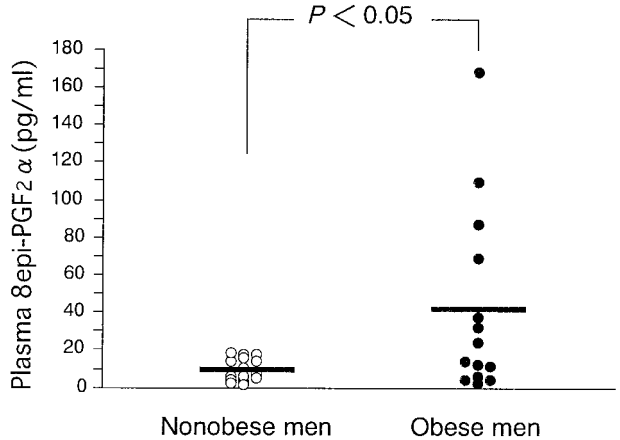

FIG. 1. Plasma levels of 8-epi-PGF2α in obese (●) and nonobese (○) men. Plasma levels of 8-epi-PGF2α in obese men were significantly increased compared with those in nonobese men ($P < 0.05$).

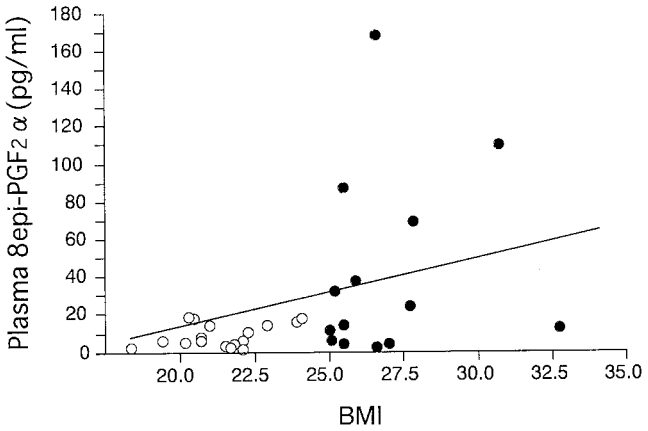

FIG. 2. Correlation between the plasma levels of 8-epi-PGF2α and BMI in all (obese and nonobese) men. A significant positive correlation was observed between the plasma levels of 8-epi-PGF2α and BMI ($r = 0.408$; $P < 0.05$). Nonobese men, ○; obese men, ●.

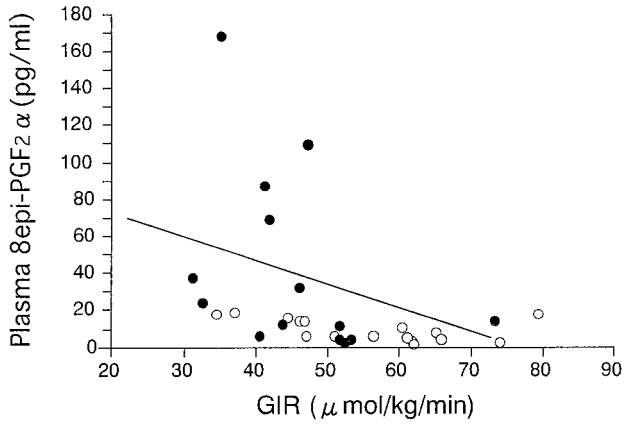

FIG. 3. Correlation between the plasma levels of 8-epi-PGF2α and GIR in all (obese and nonobese) men. A significant negative correlation was observed between the plasma levels of 8-epi-PGF2α and GIR ($r = -0.668$; $P < 0.01$). Nonobese men, ○; obese men, ●.

main cause of oxidative stress is overnutrition or adiposity remains unknown. Nutritional intake may be a major causative factor of oxidative stress because reactive oxygen species (ROS) generation from leukocytes is increased during OGTT in normal and obese subjects, and it is decreased after 48-h fasting in normal subjects (21–23). Plasma levels of 8-epi-PGF2α are also elevated during OGTT in type 2 diabetic patients (5). Measurement of plasma 8-epi-PGF2α levels in obese subjects before and after dietary restriction or several days of fasting and during OGTT may provide information to answer the above question.

Oxidative stress has been considered the major mechanism responsible for endothelial dysfunction in human obesity, endothelial dysfunction being the early event in the pathogenesis of atherosclerosis (24, 25). Endothelial dysfunction may occur by reduced bioavailability of nitric oxide, and this mainly depends on the balance between nitric oxide production and its reaction with ROS. Cytokines released from adipose tissue and low-density lipoprotein, as well as abnormalities in the renin-angiotensin system, may be the potential causative factors of ROS-mediated endothelial dysfunction in obese subjects. However, the direct relationship between adiposity and oxidative stress has not been evaluated as yet in the previous studies (24, 25). Regarding this point, the results of our present study are clinically relevant because a clear and direct correlation between oxidative stress and obesity was observed.

Several studies have shown correlation between hyperinsulinemia and free radical production in human fat cells and rats (26–28). In the present study, there was a significant correlation between fasting insulin concentration and 8-epi-PGF2α. This suggests that hyperinsulinemia and insulin resistance may play a role in the pathogenesis of oxidative stress. On the other hand, it was previously reported that insulin exerts a potent antiinflammatory effect and that it reduces ROS generation by mononuclear cells in obese subjects (29). Shamir *et al.* (30) reported insulin-mediated reduction of oxidative stress in apolipoprotein E-deficient mice. These observations suggest that insulin may have a protective role against increased oxidative stress.

Previous studies have demonstrated that FFA induces increased oxidative stress (2, 31–33). In muscles, it is believed that increased FFA enhances diacylglycerol synthesis and activates protein kinase C by enhancing generation of long-chain fatty acyl CoA; activation of these signal pathways leads to ROS generation and activation of nuclear factor-κB (31). In the present study, the serum levels of FFA were not correlated with the plasma levels of 8-epi-PGF2α. We found that both obese and nonobese have similar circulating levels of FFA, which is not usual (34). Measurement of circulating levels of FFA may be a very crude marker of FFA generation, and this may explain these discordant results.

Recently, a great body of studies has reported that oxidative stress is linked to insulin resistance (3, 9, 12, 35–37). The results of our present study support these previous findings. The results of our present study are of clinical significance because a reliable marker of oxidative stress was measured and insulin resistance was evaluated by the clamp study, which is the gold standard method. However, correlation does not prove causation. Administration of antioxidants to obese subjects may be useful for clarifying the cause and effect relationship.

In brief, the present study showed for the first time that the circulating level of 8-epi-PGF2α is associated with adiposity and insulin resistance in men. These findings suggest that obesity is an important factor for enhanced oxidative stress and that this oxidative stress triggers the development of insulin resistance in men.

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