Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 48, No. 12, 2006
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2006.08.040

Insulin Resistance as a Determinant of Platelet Activation in Obese Women

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OBJECTIVES We tested the hypothesis that insulin resistance, per se, contributes to increased platelet activation in obesity, independently of underlying inflammation.

BACKGROUND Obesity, insulin resistance, and atherosclerosis are closely linked phenomena associated with low-grade inflammation. Obesity is associated with persistent platelet activation in otherwise healthy women.

METHODS We performed a cross-sectional study in 40 obese and 20 non-obese healthy women using urinary thromboxane metabolite excretion as a non-invasive index of platelet activation. An index of insulin sensitivity, S_I, and plasma adiponectin, C-reactive protein (CRP), and CD40 ligand (CD40L) levels were measured.

RESULTS Obese women had significantly ($p < 0.0001$) higher 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2) excretion (median 718 vs. 211 pg/mg creatinine), CRP (1.13 vs. 0.48 mg/l), and CD40L levels (4.45 vs. 0.90 ng/ml) than controls. Obese women had lower S_I (median 2.51 vs. 5.0 10^4 $min^{-1}/[\mu U/ml]$, $p < 0.002$) and adiponectin (6.3 vs. 10 $\mu g/ml$, $p < 0.01$) than control subjects. On multiple regression analysis, waist-to-hip ratio ($\beta = 0.27$, $p < 0.05$) and S_I ($\beta = -0.72$, $p < 0.04$) predicted 11-dehydro-TXB_2 excretion rate, independently of adiponectin, CRP, CD40L, and lipid patterns. In order to investigate the cause-effect relationship of these associations, we examined the effects of a 12-week weight loss program or a 3-week pioglitazone treatment on urinary 11-dehydro-TXB_2 in 10 women with impaired S_I and visceral obesity. Successful weight loss (0.6 kg loss/week) achieved in 5 subjects was associated with increased S_I (+92%) and decreased CD40L (−27%), CRP (−37%), and 11-dehydro-TXB_2 (−53%) ($p < 0.05$). Consistently, improvement of insulin sensitivity achieved with pioglitazone significantly decreased urinary 11-dehydro-TXB_2 excretion (−43%, $p < 0.05$) without changes in body weight.

CONCLUSIONS Insulin resistance is a major determinant of platelet activation in female obesity. (J Am Coll Cardiol 2006;48:2531–8) © 2006 by the American College of Cardiology Foundation

Obesity, insulin resistance, and atherosclerosis are closely linked phenomena, often associated with low-grade inflammation (1). It has been suggested that insulin resistance or its associated hyperinsulinemia are independent risk factors for coronary artery disease, with a level of risk similar to that of hyperlipidemia (2,3). Coronary artery disease has been related to chronic, subclinical inflammation, as indicated by elevated circulating levels of inflammatory proteins (4). Proinflammatory proteins have been related to insulin resistance cross sectionally (5–7).

Obesity is associated with insulin resistance (8). Although visceral obesity is much more strongly linked to insulin resistance, this relation is not present in all obese individuals (8).

We previously reported that visceral obesity is associated with enhanced lipid peroxidation and persistent platelet activation in otherwise healthy women (9). This abnormality appeared to be driven by inflammatory triggers that were, at least in part, down-regulated after a successful weight-loss program (9). The finding that human platelets have insulin receptors that participate in the regulation of platelet function (10) led to the hypothesis that platelets are potential sites of insulin resistance, and that the latter is associated with impairment in the physiological antiaggregating action exerted by insulin (11). In the present report, we tested the hypothesis that insulin resistance, per se, contributes to increased platelet activation in obesity, independently of underlying inflammation.

METHODS

Forty non-diabetic obese women (age 24 to 63 years) were studied on an outpatient basis as a follow-up investigation of cardiovascular risk evaluation. Subjects had to be in good general health and physical condition and had to have a body mass index (BMI) >30 kg/m^2 at the time of screening. None had a family history of premature cardiovascular

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Manuscript received May 15, 2006; revised manuscript received August 2, 2006, accepted August 7, 2006.

Abbreviations and Acronyms

11-dehydro-TXB_2	= 11-dehydro-thromboxane B_2
AUC	= area under the response curve
BMI	= body mass index
CD40L	= CD40 ligand
CRP	= C-reactive protein
DI	= disposition index
FSIGT	= insulin-modified frequently sampled intravenous glucose tolerance test
OGIS	= oral glucose insulin sensitivity
OGTT	= oral glucose tolerance test
S_I	= insulin sensitivity index
WHR	= waist-to-hip ratio
ΔAIR_G	= incremental acute insulin response

disease or a personal history of thyroid or pituitary disease, anorexia, or bulimia. To avoid confounding by other determinants of platelet activation, women were excluded if they had a history or evidence of atherothrombotic diseases, diabetes mellitus, cigarette smoking, dyslipidemia, or arterial hypertension. Women were also excluded if they were pregnant; had given birth in the previous 6 months; or were taking hormonal contraception or replacement therapy, low-dose aspirin, non-steroidal anti-inflammatory drugs, or vitamin supplements. Three women were post-menopausal. A standard 75-g oral glucose tolerance test (OGTT) was performed, and glucose tolerance status was based on the World Health Organization criteria (12). Twenty healthy women (BMI $\leq$25 kg/m^2), age 24 to 49 years, were also recruited as a control group. All women were recruited at the Eating Disorders Clinic of the University of Chieti, after they were interviewed and agreed to participate in an outpatient study. Their characteristics are detailed in Table 1. All subjects gave written informed consent, and the study protocol was approved by the institutional review board.

Anthropometric measurements. Anthropometric measurements were taken according to standardized procedures. Height, weight, and waist and hip circumferences were measured while the subjects wore indoor clothes without shoes. Body mass index and waist-to-hip ratio (WHR) were computed. The WHR was defined as the minimal abdominal circumference between the xiphoid process and the iliac crests (waist) divided by the circumference determined over the femoral heads (hip). The cut-off point used to distinguish between android and gynoid fat distribution was 0.86 (android type, $\geq$0.86; gynoid type, $<$0.86). Fat mass (kg) was determined using bioelectrical impedance analysis (B.I.A.101-F-Akern System SRL, Florence, Italy).

Design of the studies. In the first study, we performed a cross-sectional comparison of urinary 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2), a major enzymatic metabolite of thromboxane A_2 (13), insulin sensitivity (S_I), plasma adiponectin, C-reactive protein (CRP), and CD40 ligand (CD40L) levels, in the 2 groups of women.

In order to investigate the cause-effect relationship of associations characterized in the cross-sectional study, we examined the effects of a short-term weight loss program on urinary 11-dehydro-TXB_2 in 10 of the 20 obese women with impaired S_I ($<2.5\ 10^4\ min^{-1}/[\mu U/ml]$), who agreed to participate in this additional study. This involved a caloric restriction to about 1,200 kcal/day in order to achieve approximately 0.6 kg loss/week, during a 12-week period. Successful weight loss was defined as a reduction of at least 5 kg of the initial body weight. Before and after the weight loss program, participants were instructed to perform an overnight urine collection and had a fasting blood sample drawn the following morning. Plasma, serum, and urine were stored in aliquots at −20°C until used for the various analyses.

Finally, to further investigate the relationship between insulin resistance and platelet activation and to avoid confounding by weight loss, we performed a single-blind, placebo-controlled 3-week study with pioglitazone, 30 mg daily (Actos, Takeda Ireland Limited, Kilruddery, Ireland), a peroxisome proliferator-activated receptor-γ ligand that acts as an insulin-

Table 1. Clinical and Laboratory Parameters in Nonobese and Obese Women

Parameters	Nonobese Women (n = 20)	Obese Women (n = 40)	p Value
Age (yrs)	37 ± 7	41 ± 10	0.1048
Body mass index (kg/m^2)	23 ± 2	38 ± 6	<0.0001
Waist-to-hip ratio	0.80 ± 0.06	0.91 ± 0.10	<0.0001
Total cholesterol (mmol/l)	4.37 ± 0.51	4.8 ± 0.72	0.0179
Triglycerides (mmol/l)	0.83 ± 0.41	1.19 ± 0.62	0.0239
HDL-C (mmol/l)	1.37 ± 0.23	1.37 ± 0.36	0.8577
S_I ($10^4\ min^{-1}/[\mu U/ml]$)*	5.00 (3.82–8.03)	2.51 (1.73–4.99)	0.0023
ΔAIR_G (μU/ml)*	23.7 (10.0–34.3)	41.8 (21.7–72.0)	0.0079
Disp index ($10^{-2}\ min^{-1}$)*	1.39 (0.72–1.89)	0.97 (0.53–1.90)	0.6991
Adiponectin (μg/ml)	10.0 (6.8–12.5)	6.3 (4.6–10.5)	0.0121
C-reactive protein (mg/l)	0.48 (0.29–0.57)	1.13 (0.67–2.13)	<0.0001
CD40L (ng/ml)	0.90 (0.60–1.35)	4.45 (2.1–6.5)	<0.0001
U-11-dehydro-TXB_2 (pg/mg creatinine)	211 (135–300)	718 (522–1,280)	<0.0001

Data are expressed as mean ± SD or median (interquartile range). *Metabolic parameters from insulin-modified frequently sampled intravenous glucose tolerance test; insulin-sensitivity index (S_I), incremental acute insulin response (ΔAIR_G), Disp index ($S_I \times \Delta AIR_G$)

11-dehydro-TXB_2 = 11-dehydro-thromboxane B_2; CD40L = CD40 ligand; HDL-C = high-density lipoprotein cholesterol; U = urinary.