

## Insulin Resistance, Platelets, and Obesity

# Insulin Resistance as a Determinant of Platelet Activation in Obese Women

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| <b>OBJECTIVES</b>  | We tested the hypothesis that insulin resistance, per se, contributes to increased platelet activation in obesity, independently of underlying inflammation.  |
| <b>BACKGROUND</b>  | 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.   |
| <b>METHODS</b>     | 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.   |
| <b>RESULTS</b>     | Obese women had significantly ( $p < 0.0001$ ) higher 11-dehydro-thromboxane $B_2$ (11-dehydro-TXB <sub>2</sub> ) 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 $10^4 \text{ min}^{-1}/[\mu\text{U/ml}]$ , $p < 0.002$ ) and adiponectin (6.3 vs. 10 $\mu\text{g/ml}$ , $p < 0.01$ ) 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 <sub>2</sub> 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 <sub>2</sub> 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 <sub>2</sub> (-53%) ( $p < 0.05$ ). Consistently, improvement of insulin sensitivity achieved with pioglitazone significantly decreased urinary 11-dehydro-TXB <sub>2</sub> excretion (-43%, $p < 0.05$ ) without changes in body weight. |
| <b>CONCLUSIONS</b> | 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  |

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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)  $> 30 \text{ kg/m}^2$  at the time of screening. None had a family history of premature cardiovascular

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**Abbreviations and Acronyms**

|                             |  |
|-----------------------------|--|
| 11-dehydro-TXB <sub>2</sub> | = 11-dehydro-thromboxane B <sub>2</sub>  |
| AUC                         | = area under the response curve  |
| BMI                         | = body mass index  |
| CD40L                       | = CD40 ligand  |
| CRP                         | = C-reactive protein   |
| DI                          | = disposition index  |
| FSIGT                       | = insulin-modified frequently<br>sampled intravenous glucose<br>tolerance test |
| OGIS                        | = oral glucose insulin sensitivity   |
| OGTT                        | = oral glucose tolerance test  |
| S <sub>I</sub>              | = insulin sensitivity index  |
| WHR                         | = waist-to-hip ratio   |
| ΔAIR <sub>G</sub>           | = 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 ≤25 kg/m<sup>2</sup>), 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, ≥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<sub>2</sub> (11-dehydro-TXB<sub>2</sub>), a major enzymatic metabolite of thromboxane A<sub>2</sub> (13), insulin sensitivity (S<sub>I</sub>), 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<sub>2</sub> in 10 of the 20 obese women with impaired S<sub>I</sub> (<2.5 10<sup>4</sup> min<sup>-1</sup>/[μU/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<br>(n = 20) | Obese Women<br>(n = 40) | p Value |
|--|----------------------------|-------------------------|---------|
| Age (yrs)  | 37 ± 7                     | 41 ± 10                 | 0.1048  |
| Body mass index (kg/m <sup>2</sup> )                         | 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 <sub>I</sub> (10 <sup>4</sup> min <sup>-1</sup> /[μU/ml])* | 5.00 (3.82-8.03)           | 2.51 (1.73-4.99)        | 0.0023  |
| ΔAIR <sub>G</sub> (μU/ml)*                                   | 23.7 (10.0-34.3)           | 41.8 (21.7-72.0)        | 0.0079  |
| Disp index (10 <sup>-2</sup> min <sup>-1</sup> )*            | 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 <sub>2</sub> (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<sub>I</sub>), incremental acute insulin response (ΔAIR<sub>G</sub>), Disp index (S<sub>I</sub> × ΔAIR<sub>G</sub>)

11-dehydro-TXB<sub>2</sub> = 11-dehydro-thromboxane B<sub>2</sub>; CD40L = CD40 ligand; HDL-C = high-density lipoprotein cholesterol; U = urinary.

sensitizing agent (14). During this period, the 5 obese women who failed to achieve successful weight loss after 12 weeks of caloric restriction were allocated to pioglitazone (30 mg/day), whereas the 5 obese women with impaired  $S_I$ , who did not participate in the weight loss program, received placebo. Before and after the intervention, participants were instructed to perform an overnight urine collection and had a fasting blood sample drawn the following morning. Moreover, a standard 75-g OGTT was performed after a 10-h overnight fast at baseline and at the end of the 3-week treatment period.

**Assessment of insulin sensitivity.** Insulin sensitivity was assessed using insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) for the cross-sectional and weight loss studies. All studies were performed in the recumbent position beginning at 8 AM, after a 10- to 12-h overnight fast. A Teflon catheter was inserted into a peripheral vein in each forearm for blood sampling and for glucose and insulin administration, respectively. Basal blood samples were collected at time  $-10$  and  $-1$  min, after which glucose (300 mg/kg body weight) was infused intravenously within 30 s, starting at time 0. At time 20 min, rapid insulin (0.03 IU/kg, Humulin R, Eli Lilly, Indianapolis, Indiana) was infused for 5 min. The sampling schedule was 2, 4, 8, 19, 27, 30, 40, 50, 70, 100, and 180 min according to Steil et al. (15) with slight modifications.

For the pioglitazone intervention study, insulin sensitivity was evaluated using 75-g 2-h OGTT values. Plasma samples were obtained at 0, 30, 60, 90, and 120 min after glucose loading.

**Analytical measurements.** Blood glucose was measured by the glucose-oxidase method, plasma insulin was measured by radioimmunoassay (Coat-A-Count Insulin Kit, Diagnostic Products Corporation, Los Angeles, California). Total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol concentrations were determined as previously described (9).

Plasma CD40L was determined by enzyme-linked immunoadsorbent assay (R&D Systems, Minneapolis, Minnesota). Plasma CRP levels were measured with a highly sensitive immunoassay (16). Plasma adiponectin levels were measured by enzyme-linked immunoadsorbent assay (R&D Systems). Inter-assay and intra-assay variations of all measurements were  $<10\%$ . Urinary 11-dehydro-TXB<sub>2</sub> was measured by a previously described and validated radioimmunoassay method (17).

**Data analysis and statistics.** Glucose and insulin concentrations obtained by FSIGT were analyzed using the minimal model method (18) that provides an index of insulin sensitivity ( $S_I$ ) (i.e., the effect of insulin on glucose uptake [19] by taking into account glucose disposal in the tissues and net hepatic balance).

Insulin secretion was evaluated as the incremental acute insulin response,  $\Delta AIR_G$ , calculated by averaging insulin concentrations above basal from 3 to 10 min after glucose injection. Plasma insulin clearance was calculated as the ratio of insulin dose to dynamic area under the insulin

concentration curve from 20 to 180 min (20).  $S_I \times \Delta AIR_G/100$  gives the disposition index (DI), a measure of the combined effect of insulin secretion and sensitivity on glucose disposal (21,22).

The plasma glucose response and total insulin secretion obtained by OGTT were evaluated from the area under the response curve (AUC) for plasma glucose and insulin (2-h glucose AUC and 2-h insulin AUC), calculated from the fasting, 30-, 60-, 90-, and 120-min plasma concentrations using the trapezoid rule. The oral glucose insulin sensitivity (OGIS) index, proposed by Mari et al. (23), was calculated as previously described. The parameters obtained by the different methods used in the 2 intervention studies ( $S_I$  and OGIS) have been previously shown to be significantly correlated (24).

The data were analyzed by non-parametric methods to avoid assumptions about the distribution of the measured variables. Comparisons between groups were made with the Kruskal-Wallis method and Mann-Whitney  $U$  test. The association between different measurements was assessed by the Spearman rank correlation test. Multiple linear regression analysis was conducted to assess independent predictors of 11-dehydro-TXB<sub>2</sub> levels. The differences between baseline and post-treatment values were analyzed with the Wilcoxon signed rank test. All values are reported as mean  $\pm$  SD or median (interquartile range). A  $p$  value lower than 0.05 was regarded as statistically significant. All tests were 2-tailed, and analyses were performed using a computer software package (Statistica version 6, StatSoft Inc., Tulsa, Oklahoma or Statistical Package for the Social Sciences, version 13.0, SPSS Inc., Chicago, Illinois).

## RESULTS

Clinical and laboratory parameters of the non-obese and obese women are detailed in Table 1. Obese women had higher BMI, WHR, total cholesterol, and triglyceride serum levels than control subjects. By contrast,  $S_I$  was lower and  $\Delta AIR_G$  was higher indicating insulin resistance and hyperinsulinemia in obese women. Plasma adiponectin was lower in obese women and significantly correlated with  $S_I$  ( $R_s = 0.83$ ,  $p < 0.0001$ ) and DI ( $R_s = 0.48$ ,  $p = 0.0017$ ).

Consistent with our earlier findings (9), obese women had markedly higher urinary 11-dehydro-TXB<sub>2</sub> excretion rate, an index of in vivo platelet activation, when compared with non-obese controls (Table 1). Plasma CD40L and CRP levels were also significantly higher in obese than in non-obese women (Table 1). As shown in Table 2, a statistically significant direct correlation was found between urinary 11-dehydro-TXB<sub>2</sub> excretion rate and WHR, CD40L or CRP, and a significant inverse correlation between 11-dehydro-TXB<sub>2</sub> and  $S_I$ , plasma adiponectin or DI. No significant correlation was observed between urinary 11-dehydro-TXB<sub>2</sub> and  $\Delta AIR_G$ .

When the association between urinary 11-dehydro-TXB<sub>2</sub> excretion and  $S_I$  was investigated in non-obese women, a

**Table 2.** Spearman's Correlation Coefficients Among the Various Parameters Analyzed in 40 Obese Women

|                                      | CD40L              | CRP                | BMI               | WHR                | Adiponectin         | $\Delta$ AI <sub>R<sub>G</sub></sub> | DI                 | S <sub>I</sub>      |
|--------------------------------------|--------------------|--------------------|-------------------|--------------------|---------------------|--------------------------------------|--------------------|---------------------|
| U-11-dehydro-TXB <sub>2</sub>        | 0.66<br>p < 0.0001 | 0.67<br>p < 0.0001 | 0.20<br>p = 0.206 | 0.32<br>p = 0.043  | -0.56<br>p < 0.0002 | 0.16<br>p = 0.315                    | -0.37<br>p = 0.017 | -0.72<br>p < 0.0001 |
| CD40L                                | —                  | 0.55<br>p < 0.0003 | 0.26<br>p = 0.109 | 0.21<br>p = 0.188  | -0.47<br>p < 0.003  | 0.41<br>p = 0.0085                   | -0.18<br>p = 0.257 | -0.73<br>p < 0.0001 |
| CRP                                  | —                  | —                  | 0.11<br>p = 0.489 | 0.16<br>p = 0.326  | -0.64<br>p < 0.0001 | 0.09<br>p = 0.555                    | -0.39<br>p = 0.012 | -0.65<br>p < 0.0001 |
| BMI                                  | —                  | —                  | —                 | 0.55<br>p < 0.0003 | -0.02<br>p = 0.874  | 0.19<br>p = 0.240                    | 0.11<br>p = 0.487  | -0.02<br>p = 0.904  |
| WHR                                  | —                  | —                  | —                 | —                  | -0.03<br>p = 0.837  | 0.01<br>p = 0.964                    | 0.01<br>p = 0.937  | -0.04<br>p = 0.799  |
| Adiponectin                          | —                  | —                  | —                 | —                  | —                   | -0.016<br>p = 0.316                  | 0.48<br>p < 0.002  | 0.83<br>p < 0.0001  |
| $\Delta$ AI <sub>R<sub>G</sub></sub> | —                  | —                  | —                 | —                  | —                   | —                                    | 0.64<br>p < 0.0001 | -0.28<br>p = 0.0833 |
| DI index                             | —                  | —                  | —                 | —                  | —                   | —                                    | —                  | 0.50<br>p < 0.001   |

$\Delta$ AI<sub>R<sub>G</sub></sub> = incremental acute insulin response; BMI = body mass index; CD40L = CD40 ligand; CRP = C-reactive protein; DI = disposition index; S<sub>I</sub> = insulin-sensitivity index; U-11-dehydro-TXB<sub>2</sub> = urinary 11-dehydro-thromboxane B<sub>2</sub>; WHR = waist-to-hip ratio.

significant inverse correlation was also observed (R<sub>s</sub> = -0.62, p < 0.006).

To further define the relationship between 11-dehydro-TXB<sub>2</sub>, anthropometric measurements (WHR, BMI), metabolic variables (S<sub>I</sub>, DI, serum cholesterol, triglycerides), plasma adiponectin, CD40L and CRP, a multiple regression analysis was performed with 11-dehydro-TXB<sub>2</sub> as the dependent variable. This analysis yielded a model in which S<sub>I</sub> ( $\beta$  = -0.72, SEM = 0.32, p < 0.04) and WHR ( $\beta$  = 0.27, SEM = 0.13, p < 0.05) independently predicted urinary 11-dehydro-TXB<sub>2</sub> excretion rate.

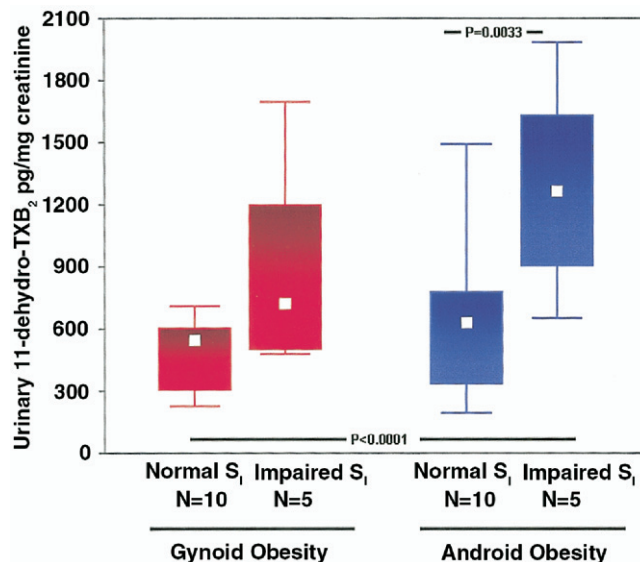
Therefore, we stratified obese women on the basis of these 2 parameters. Using a S<sub>I</sub> value of 2.5 10<sup>4</sup> min<sup>-1</sup>/[ $\mu$ U/ml], corresponding to the median value of obese women, we separated subjects with normal (4.99 [3.82 to 7.0] 10<sup>4</sup> min<sup>-1</sup>/[ $\mu$ U/ml], median [interquartile range], n = 20) or impaired (1.73 [1.21 to 2.05] 10<sup>4</sup> min<sup>-1</sup>/[ $\mu$ U/ml], n = 20) S<sub>I</sub>. Using a WHR value of 0.86, we characterized women with gynoid or android obesity.

Figure 1 shows the excretion rate of 11-dehydro-TXB<sub>2</sub> in gynoid obesity (n = 15) and in android obesity (n = 25) according to S<sub>I</sub> categorization. In android obesity, impaired S<sub>I</sub> discriminated women with significantly higher urinary excretion of 11-dehydro-TXB<sub>2</sub> (p = 0.0033). In addition, women with android obesity and impaired S<sub>I</sub> showed higher plasma levels of CRP (1.98 [1.20 to 2.56] vs. 0.81 [0.60 to 1.10] mg/l, p < 0.0008) and CD40L (8.2 [5.1 to 8.9] vs. 3.2 [2.3 to 5.2] ng/ml, p < 0.006) compared with women with android obesity but normal insulin sensitivity.

To characterize the cause-and-effect relationship of these associations, we examined the effects of a 3-month weight loss program, by assessing changes associated with caloric restriction in 10 of the 15 android obese women with impaired S<sub>I</sub> (<2.5 10<sup>4</sup> min<sup>-1</sup>/[ $\mu$ U/ml]). Successful weight loss (108.7  $\pm$  13.2 to 93.2  $\pm$  6.8 kg; median reduction: -9.5%) was achieved in 5 subjects and was associated with

a statistically significant increase in S<sub>I</sub> (+92%, p < 0.05) and decreases in plasma CD40L (-27%, p < 0.05) and CRP (-37%, p < 0.05) as well as in urinary 11-dehydro-TXB<sub>2</sub> excretion (-53%, p < 0.05) (Fig. 2). In the other 5 obese women, the weight loss program failed (101.8  $\pm$  15.74 to 101.4  $\pm$  14.4 kg; median: -1%), and the urinary 11-dehydro-TXB<sub>2</sub> excretion decreased non-significantly by 15% (697 [638 to 797] vs. 595 [525 to 705] pg/mg creatinine, p > 0.05) (Fig. 2), in association with substantially unaltered S<sub>I</sub> levels (from 2.0 [1.7 to 2.06] to 2.0 [1.5 to 2.0] 10<sup>4</sup> min<sup>-1</sup>/[ $\mu$ U/ml], p > 0.05).

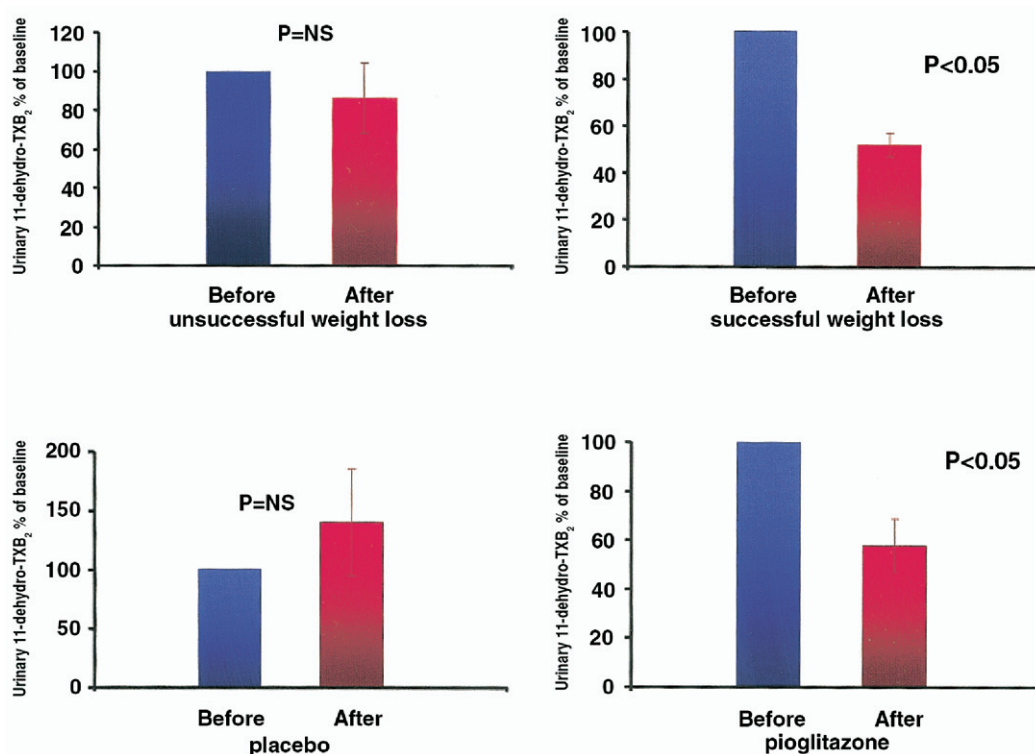
In the 10 patients undergoing the weight loss program, a statistically significant direct correlation was observed when



**Figure 1.** Urinary 11-dehydro-thromboxane B<sub>2</sub> (11-dehydro-TXB<sub>2</sub>) excretion in obese women. Urinary 11-dehydro-TXB<sub>2</sub> excretion in android obesity (waist-to-hip ratio  $\geq$ 0.86, n = 25) and in gynoid obesity (waist-to-hip ratio <0.86, n = 15) according to normal or impaired insulin sensitivity (insulin sensitivity index [S<sub>I</sub>] above or below the median value of 2.5 10<sup>4</sup> min<sup>-1</sup>/[ $\mu$ U/ml]).



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**Figure 2.** Effects of weight loss or pioglitazone on urinary 11-dehydro-thromboxane B<sub>2</sub> (11-dehydro-TXB<sub>2</sub>). Effects of weight loss or pioglitazone treatment on urinary 11-dehydro-TXB<sub>2</sub> excretion in android obese women with impaired insulin sensitivity. Mean ± SD percentage of baseline metabolite excretion rates are represented for 15 women participating in the 2 studies. The p values were assessed by Wilcoxon signed rank test.

relating changes in body weight to changes in urinary 11-dehydro-TXB<sub>2</sub> excretion ( $R_s = 0.71$ ,  $p < 0.03$ ), plasma CD40L ( $R_s = 0.66$ ,  $p < 0.04$ ) and CRP ( $R_s = 0.75$ ,  $p < 0.02$ ). An inverse correlation was found when relating changes in body weight to changes in S<sub>1</sub> ( $R_s = -0.77$ ,  $p < 0.01$ ).

Because the concurrent weight loss may confound the interpretation of the reduction in platelet activation, a short-term treatment with pioglitazone (30 mg daily for 3 weeks) was carried out in the 5 obese women in whom the weight loss program failed, whereas the 5 obese women with impaired S<sub>1</sub> who did not participate in the weight loss program were allocated to a short-term placebo treatment as a control group. This single-blind placebo-controlled study demonstrated that pioglitazone treatment was associated with an improvement in insulin sensitivity (OGIS 446 [444 to 471] vs. 490 [481 to 505] mg/min/m<sup>2</sup>,  $p = 0.04$ ) and with significantly decreased urinary 11-dehydro-TXB<sub>2</sub> excretion (891 [629 to 974] vs. 469 [438 to 550] pg/mg creatinine,  $p < 0.05$ ) (Fig. 2) and plasma CD40L (1.79 [1.60 to 1.89] vs. 1.20 [1.13 to 1.36] ng/ml,  $p < 0.011$ ). Placebo was not associated with any statistically significant changes (OGIS 547 [492 to 561] vs. 546 [491 to 562] mg/min/m<sup>2</sup>,  $p = NS$ ; 11-dehydro-TXB<sub>2</sub> 658 [439 to 699] vs. 851 [556 to 936] pg/mg creatinine,  $p > 0.05$ ; and plasma CD40L 1.80 [1.59 to 1.81] vs. 1.66 [1.62 to 1.76] ng/ml,  $p > 0.05$ ). No statistically significant changes in body weight ( $98.8 \pm 8.9$  kg to  $97.6 \pm 8.5$  kg in the pioglitazone

group vs.  $95.1 \pm 11.2$  kg to  $93.6 \pm 11.1$  kg in the placebo group), CRP levels, or serum lipids (data not shown) were observed in either group.

**DISCUSSION**

Severe overweight and particularly visceral obesity is associated with increased morbidity and mortality (25) through a variety of molecular mechanisms linking the metabolic syndrome to hemostatic and vascular abnormalities (26). Obesity is associated with impaired insulin signalling, and intra-abdominal fat deposition is highly related to insulin resistance (8). However, the association of obesity and insulin resistance is not necessarily present in all obese subjects, and nonobese, nondiabetic individuals may be insulin resistant (27).

We have previously characterized a novel mechanism through which visceral obesity may affect cardiovascular morbidity and mortality in women (i.e., thromboxane-dependent platelet activation) (9). Biochemical evidence of increased platelet activation in vivo was obtained through non-invasive measurements of thromboxane metabolite excretion (13) that avoid artifactual platelet activation during and after blood sampling (28).

The abnormal metabolic state that accompanies insulin resistance renders arteries susceptible to atherosclerosis, by altering the functional properties of multiple cell types, including platelets (29). In vitro and in vivo studies show

that insulin inhibits platelet aggregation in healthy non-obese subjects, an effect that is blunted in obese individuals (30), raising the possibility that platelets, which exhibit insulin receptors, may be sites of insulin resistance (11). Thus, an impaired platelet response to insulin could be another feature of the insulin resistance syndrome.

In the present study, we tested the hypothesis that thromboxane-dependent platelet activation in obesity is related to insulin resistance, per se, independently of vascular inflammation or adipocyte-derived adipokines associated with insulin resistance. Because comorbidity associated with the obese state might confound the association with platelet activation, we selected a group of obese women free of other cardiovascular risk factors previously associated with persistent platelet activation (31-33). Our results in obese women without abnormal fasting glucose or impaired glucose tolerance demonstrate that thromboxane-dependent platelet activation is related to insulin sensitivity and not to impaired insulin secretion, because  $S_I$ , but not  $\Delta AIR_G$ , independently predicted thromboxane metabolite excretion.

There is a large body of evidence that insulin secretion and insulin sensitivity are strictly related (22). It has been known since the 1960s that obesity is associated with increased insulin secretion (34), which is not caused by obesity per se—or at least not exclusively—but by the accompanying reduced insulin sensitivity.

In the setting of insulin resistance, the secretion of several adipokines from adipocytes is altered (35). Adiponectin plasma levels are usually found to be decreased in obese individuals, and the reduction in adiponectin levels may be related to insulin resistance (36). Adiponectin, one of the most abundant proteins in human fat cells, has insulin-sensitizing properties (37). Moreover, it has anti-inflammatory effects on the cellular components of the vessel wall (38), and adiponectin administration lowers CRP and TNF- $\alpha$  levels in adiponectin knockout mice (39). A reciprocal association of CRP with adiponectin has been reported in both human plasma and adipose tissue (40). We asked whether adiponectin levels may predict thromboxane-dependent platelet activation. Although obese women had lower  $S_I$  and adiponectin levels than controls, a multiple regression analysis with 11-dehydro-TXB<sub>2</sub> as the dependent variable indicated that only  $S_I$  and WHR independently predicted 11-dehydro-TXB<sub>2</sub> levels. Moreover, impaired  $S_I$  significantly discriminated subjects with higher urinary excretion of 11-dehydro-TXB<sub>2</sub> among those with android obesity but not among those with gynoid obesity (Fig. 1).

There is increasing evidence that features of the insulin resistance syndrome, including visceral obesity, are associated with increased CRP levels (41), suggesting that an expanded abdominal fat depot may be responsible for a low-grade inflammatory state, by providing a source of increased production of interleukin-6, a stimulus to CRP synthesis by the liver. Previously, CRP and CD40L were found elevated in healthy women with visceral obesity

(9,42). In the present study both inflammatory proteins were elevated in obese individuals with insulin resistance; however, their relation to increased platelet activation appeared to be largely explained by differences in WHR and insulin sensitivity.

Further evidence for a cause-and-effect relationship between insulin resistance and persistent platelet activation was obtained through a short-term, diet-induced weight loss program. This demonstrated that a 10% reduction in body weight obtained through a successful program over a 12-week period was associated with a doubling in  $S_I$  levels and normalization of a noninvasive index of platelet activation. The obese women who failed to achieve a significant weight loss during this period provided an interesting control group, which demonstrated the reproducibility of the relationship between  $S_I$  levels and 11-dehydro-TXB<sub>2</sub> excretion over time in the presence of relatively constant body weight.

Given the concurrent weight loss, one might argue that weight loss, per se, may modulate platelet function, independently of the improvement in insulin sensitivity. To avoid this potential confounding, we performed a single-blind, placebo-controlled study with pioglitazone, an agent known to ameliorate insulin resistance (14,43) without a detectable effect on body weight (or in some cases with a modest weight gain). A 3-week treatment with pioglitazone led to a significant reduction in thromboxane-dependent platelet activation, with no significant change in body weight. This result is consistent with recent findings in a mouse model of obesity and insulin resistance, where pioglitazone afforded protection against occlusive arterial thrombosis by lowering platelet P-selectin expression (44). Moreover, pioglitazone has no direct inhibitory effects on agonist-induced platelet aggregation (45), suggesting that the reduction in in vivo platelet activation after pioglitazone described in the present study is mediated through improved insulin sensitivity. As persistent platelet activation in obese women is driven by inflammatory triggers (9), we cannot exclude the possibility that concurrent anti-inflammatory effects exerted by pioglitazone may have contributed to down-regulating platelet activation.

The pathophysiological mechanisms coupling platelet activation with insulin resistance remain elusive. Recently, a large body of evidence from cellular, physiological, clinical, and epidemiologic studies strongly supports a reciprocal relationship between insulin resistance and endothelial function, raising the hypothesis that endothelial dysfunction may provide the missing link between insulin resistance and platelet activation, thereby connecting disorders of metabolic and cardiovascular homeostasis (46).

Several limitations of the intervention studies should be emphasized. These include self-selection of the participating women, lack of randomization to weight loss versus weight maintenance or to pioglitazone versus placebo, lack of generalizability of the findings to gynoid obese women as

well as to women from ethnic minorities, and small sample size of these pilot studies.

Despite these limitations, these preliminary findings may have clinical implications, by supporting the hypothesis that insulin resistance, per se, is a major determinant of enhanced platelet activation in the setting of visceral obesity. Thus, our results suggest that a substantial reduction in thromboxane-dependent platelet activation in this setting may be achieved by increasing insulin sensitivity and provide a rationale for performing adequately sized randomized studies comparing the effects of caloric restriction and insulin sensitizing agents.

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