Platelet Activation in Obese Women
Role of Inflammation and Oxidant Stress

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Context Obesity, in particular abdominal adiposity, is associated with increased cardiovascular morbidity and mortality through mechanisms possibly linking the metabolic disorder to platelet and vascular abnormalities.

Objective To investigate the clinical and biochemical determinants of lipid peroxidation and platelet activation in obese women.

Design, Setting, and Participants Cross-sectional comparison, conducted between September 1999 and September 2001, of urinary 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$) and 11-dehydrothromboxane B_2 (11-dehyhdro-TxB_2) excretion levels in 93 women: 44 with a body mass index (BMI) higher than 28 and a waist-to-hip ratio (WHR) of 0.86 or higher, *android obesity*; 25 with a BMI higher than 28 and a WHR lower than 0.86, *gynoid obesity*; and 24 nonobese women with a BMI lower than 25. An additional study was conducted to determine the short-term effects of weight loss in 20 of the 44 women with android obesity.

Intervention During a 12-week period, 20 women with android obesity followed a weight loss program to reduce caloric intake to about 1200 kcal/d.

Main Outcome Measures Plasma C-reactive protein, insulin and leptin levels, and urinary 8-iso $PGF_{2\alpha}$ (marker of in vivo lipid peroxidation) and 11-dehyhdro-TxB_2 (marker of in vivo platelet activation) excretion. Weight loss was defined as successful when the initial body weight decreased by at least 5 kg after a 12-week period of caloric restriction.

Results Women with android obesity had higher levels of 8-iso $PGF_{2\alpha}$ (median [interquartile range {IQR}] 523 [393-685] vs 187 [140-225] pg/mg creatinine) and 11-dehyhdro-TxB_2 (median [IQR], 948 [729-1296] vs 215 [184-253] pg/mg creatinine) than nonobese women ($P<.001$). Both 8-iso $PGF_{2\alpha}$ and 11-dehyhdro-TxB_2 were higher in women with android obesity than women with gynoid obesity ($P<.001$). Based on multiple regression analysis, C-reactive protein levels and WHRs of 0.86 or higher predicted the rate of 8-iso $PGF_{2\alpha}$ excretion independently of insulin and leptin levels. Of 20 women with android obesity, 11 achieved successful weight loss, which was associated with statistically significant reductions in C-reactive protein (median change, 23%; $P<.05$), 8-iso $PGF_{2\alpha}$ (median change, 32%; $P=.04$) and 11-dehydro-TxB_2 (median change, 54%; $P=.005$).

Conclusions Android obesity is associated with enhanced lipid peroxidation and persistent platelet activation. These abnormalities are driven by inflammatory triggers related to the degree of abdominal adiposity and are, at least in part, reversible with a successful weight-loss program.

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THROMBOXANE-DEPENDENT platelet activation is associated with cardiovascular risk factors, such as cigarette smoking,[1] hypercholesterolemia,[2] and diabetes mellitus[3] and may contribute to increased risk of myocardial infarction and stroke as suggested by aspirin trials.[4] These risk factors are also associated with low-grade inflammation[5] and enhanced oxidant stress.[6] We have reported that bioactive products of lipid peroxidation, such as the F_2-isoprostane 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$)[7] are enhanced and may contribute to persistent platelet activation in the setting of hypercholesterolemia,[8] diabetes mellitus[9] and severe hyperhomocysteinemia.[10] Similar studies investigating abnormally increased body weight have not been conducted although obesity, in particular android or visceral obesity, is independently associated with increased cardiovascular morbidity and mortality.[11-13]

In our study, we have tested the hypothesis that lipid peroxidation and platelet activation are increased in obese women, in the absence of other known cardiovascular risk factors, and are modifiable after body weight reduction. Because visceral fat is an important source of inflammatory cytokines,[14] we measured C-reactive protein, along with insulin and leptin levels, as potential determinants of increased lipid peroxidation and platelet activation in this setting.

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METHODS

Subjects

We studied 44 obese women, who were aged 24 to 63 years; who had a body mass index (BMI), calculated as weight in kilograms divided by the square of height in meters, higher than 28; and who had a waist-to-hip ratio (WHR) of 0.86 or higher, *android obesity*. We compared them with 25 obese women, aged 36 to 60 years; with a BMI higher than 28; and with a WHR lower than 0.86, *gynoid obesity*, and with 24 apparently healthy nonobese women, aged 20 to 49 years with a BMI lower than 25. Both groups were recruited as 2 distinct control groups. All women were recruited at the eating disorders clinic of the University of Chieti between September 1999 and September 2001, after they were interviewed and had agreed to participate in an outpatient study. All subjects gave written informed consent, and the study protocol was approved by the Chieti University institutional review board.

Women had to be in good general health, with a normal medical history and physical examination. None had a family history of premature cardiovascular disease or a personal history of thyroid or pituitary disease, anorexia, or bulimia. To avoid confounding by other determinants of oxidant stress and platelet activation, women were excluded if they had a history or evidence of atherothrombotic diseases, diabetes mellitus, cigarette smoking, dyslipidemia, or arterial hypertension. Women also were excluded if they were pregnant; had given birth in the previous 6 months; or were taking hormonal contraception or replacement therapy, low-dose aspirin, nonsteroidal anti-inflammatory drugs, or vitamin supplements. All participants underwent a nondiabetic glucose tolerance test by National Diabetes Data Group criteria.[15] Four women were postmenopausal. Their characteristics are detailed in TABLE 1.

Anthropometric Measurements

Anthropometric measurements were taken according to standardized procedures.[16] Height, weight, and waist and hip circumferences were measured while the subjects wore indoor clothes without shoes. Body mass index and 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 cutoff point between android and gynoid fat distribution was 0.86 (android type, ≥0.86; gynoid type, <0.86).[17]

Design of the Studies

In the first study, a cross-sectional comparison of urinary 8-iso $PGF_{2\alpha}$ and 11-

Table 1. Baseline Characteristics of Nonobese and Obese Gynoid and Android Women*

Variable	Nonobese (n = 24)	Obese		*F* or *H* Statistic	*P* Value†		
		Gynoid (n = 25)	Android (n = 24)		Gynoid Obese vs Nonobese	Gynoid vs Android Obese	Android Obese vs Nonobese
Age, y	38 (8)	40 (8)	45 (10)	$F = 5.4$; $P<.01$	.39	.09	.04
Body weight, kg	64 (8)	86 (10)	97 (17)	$F = 46.5$; $P<.001$	<.001	<.01	<.001
Body mass index, kg/m^2	22.5 (2.3)	33 (4)	39 (6)	$F = 82.4$; $P<.001$	<.001	<.001	<.001
Waist-to-hip ratio	0.79 (0.01)	0.80 (0.05)	0.96 (0.05)	$F = 92.5$; $P<.001$	.34	<.001	<.001
Blood pressure, mm Hg							
Systolic	120 (15)	123 (16)	130 (17)	$F = 2.9$; $P = .06$	.50	.10	.09
Diastolic	78 (9)	79 (10)	83 (9)	$F = 2.8$; $P = .07$	.71	.10	.09
Serum lipid levels, mg/dL							
Total cholesterol‡	172 (18)	174 (28)	178 (28)	$F = 0.5$; $P = .63$	.77	.51	.34
Triglycerides§	120 (16)	125 (51)	144 (52)	$F = 2.8$; $P = .07$	.65	.13	.09
HDL-C‡	56 (10)	50 (12)	48 (12)	$F = 3.8$; $P = .03$	.06	.56	.02
LDL-C‡	104 (18)	101 (30)	102 (29)	$F = 0.1$; $P = .94$	.68	.93	.76
Plasma glucose, mg/dL‖							
Fasting	88 (9)	93 (10)	96 (8)	$F = 6.4$; $P<.01$	.07	.18	<.01
2 hours after oral glucose challenge	111 (16)	111 (17)	115 (19)	$F = 0.6$; $P = .56$	.10	.39	.38
Fasting insulin, μU/mL	10 (8-12)	10 (8-17)	27 (24-42)	$H = 56.4$; <.001	.36	<.001	<.001
Plasma leptin, median (IQR), ng/mL	9.9 (6.2-13.4)	14.6 (7.6-22.6)	22.9 (11.6-39.5)	$H = 19.3$; $P<.001$	.03	.04	<.001
C-reactive protein, median (IQR), mg/L	0.40 (0.25-0.53)	0.65 (0.45-0.92)	1.67 (0.99-2.53)	$H = 45.2$, $P<.001$	<.001	<.001	<.001
U 8-iso $PGF_{2\alpha}$	187 (140-225)	275 (220-349)	523 (393-685)	$H = 53.9$; $P<.001$	<.001	<.001	<.001
U 11-dehydro-TxB_2	215 (184-253)	610 (421-759)	948 (729-1296)	$H = 53.8$; $P<.001$	<.001	<.001	<.001

*The definition of gynoid is a waist-to-hip ratio (WHR) less than 0.86; android; 0.86 or higher. Data are presented as mean (SD) unless otherwise indicated. *F* indicates analysis of variance (the *df* between groups is 2 and within group is 90); *H*, Kruskal-Wallis test; IQR, interquartile range; U 8-iso $PGF_{2\alpha}$, urinary 8-iso prostaglandin $F_{2\alpha}$; and U 11-dehydro-TxB_2, urinary dehydrothromboxane B_2.
†*P* values are compared by a modified least significant difference (Bonferroni) test.
‡To convert total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) from mg/dL to mmol/L, multiply by 0.0259.
§To convert triglycerides from mg/dL to mmol/L, multiply by 0.0113.
‖To convert glucose from mg/dL to mmol/L, multiply by 0.0555.

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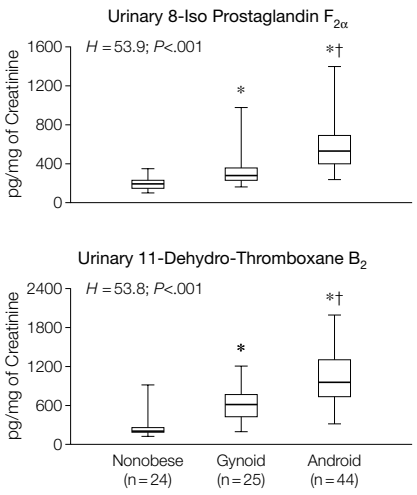
Figure 1. Urinary 8-Iso Prostaglandin $F_{2\alpha}$ and 11-Dehydro-Thromboxane B_2 Excretion in Nonobese and Gynoid and Android Obese Women



The *H* statistic was calculated by the Kruskal-Wallis test. The error bars represent minimum and maximum values, the heavy horizontal rules indicate median values, and the ends of the boxes indicate interquartile range.
*$P<.001$, gynoid and android vs nonobese.
†$P<.001$, android vs gynoid.

dehydro-thromboxane B_2 (11-dehydro-TxB_2), a major enzymatic metabolite of thromboxane A_2,[18] was performed among the 3 groups. All the participants were studied as outpatients after a 12-hour fast. Blood samples were obtained in the early morning. Each participant performed an overnight urine collection before blood sampling. Urine samples were added with the antioxidant 4-hydroxy-Tempo (Sigma Chemical Co, St Louis, Mo) (1 mmol/L) and stored at –20°C until extraction.

To assess the causal relationship between abnormal body weight and indexes of lipid peroxidation and platelet activation in android obesity, an open pilot intervention study was performed. Thus, we investigated the short-term effects of a diet-induced weight loss program on urinary 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 excretion in 20 out of the 44 android obese women, who agreed to participate in this additional study. During a 4-week baseline period, all participants were following a weight maintenance diet (55%-60% carbohydrate, 15%-20% protein, and 20%-25% fat). A weight loss program was designed to achieve approximately a loss of 0.6 kg/wk, during a 12-week period, through a reduction in caloric intake to about 1200 kcal/d. These women were also encouraged to increase their physical activity during this 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, the participants were instructed to perform an overnight urine collection and underwent 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.

Assays

Urinary 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 excretion rates were measured by previously described radioimmunoassay methods.[19,20] These methods have been validated using different antisera and by comparison with gas chromatography/mass spectrometry, as detailed elsewhere.[19,20]

Blood glucose was measured by the glucose-oxidase method, hemoglobin A_{1c} (HbA_{1C}) level was measured by automated high-performance liquid chromatography (Menarini, Italy), and plasma insulin was measured by radioimmunoassay (Coat-A-Count Insulin kit, Diagnostic Products Corp, Los Angeles, Calif). Total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were measured as previously described.[2,7] Plasma leptin was measured by enzyme-linked immunosorbent assay (DRG Diagnostics GmbH, Marburg, Germany). Plasma C-reactive protein levels were measured with a highly sensitive immunoassay.[21]

Statistical Analysis

Statistical analysis was performed by χ^2 statistics, Pearson correlation coefficient, and 1-way analysis of variance with the Bonferroni adjustment to assess differences among the groups (android obesity, gynoid obesity, and nonobese women). When necessary, log transformation was used to normalize the data, or appropriate nonparametric tests were used (Spearman correlation coefficient, Kruskall-Wallis method, and Mann-Whitney *U* test). Because the distributions of urinary prostanoid metabolites, C-reactive protein, insulin, and leptin were skewed, the concentration of these variables was also analyzed statistically by quartiles. Thus, a multiple-linear regression analysis was performed to further quantify the relationship between 8-iso $PGF_{2\alpha}$ excretion and the above mentioned variables in the 69 obese women. Specifically, the dependent variable, 8-iso $PGF_{2\alpha}$ was regressed for leptin, insulin, and C-reactive protein plasma levels, all of which were divided into quartiles. Age, BMI, and WHR (<0.86 and ≥ 0.86) were also used as explanatory factors. Comparability of baseline urinary prostanoid metabolites among C-reactive protein quartiles groups (quartile 1, ≤0.63; quartile 2, 0.64-1.14; quartile 3, 1.15-2.35; quartile 4, >2.35 mg/L) was assessed by the Kruskall-Wallis test.

A sample size of 20 patients with android obesity participated in the intervention study with 80% power to detect a 50% reduction in urinary 11-dehydro-TxB_2 following the weight-loss program, assuming at least a 50% success rate. The differences between baseline and posttreatment values were analyzed with the Wilcoxon signed-rank test.

Data are presented as mean (SD) or as median (interquartile range [IQR]). Only *P* values lower than .05 were regarded as statistically significant. All tests were 2-tailed and analyses were performed using a computer software package (Statistical 4.5, StatSoft Inc, Tulsa, Okla, or Statistical Package for the Social Sciences, version 6.0, SPSS Inc, Chicago, Ill).

RESULTS

The 2 groups of obese women were similar in age, serum lipid levels, systolic and diastolic blood pressure, and plasma glucose concentration. However, body weight; BMI; and fasting plasma insulin, leptin and C-reactive protein levels were significantly different between the 2 groups (Table 1).

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Both groups of obese women had markedly higher levels of lipid peroxidation and platelet activation, as reflected by urinary 8-iso $PGF_{2\alpha}$ ($P<.001$) and 11-dehydro-TxB_2 ($P<.001$) excretion, respectively, when compared with nonobese women (Table 1 and FIGURE 1). Moreover, urinary 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 excretion rates were significantly higher in android than in gynoid obese women ($P<.001$, Figure 1).

The Spearman correlation coefficients among all the measured variables and their statistical significance are detailed in TABLE 2. In particular, a statistically significant correlation was found between urinary excretion rates of 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 in both groups of obese women. A multiple regression analysis indicated that C-reactive protein levels (regression coefficient, 0.49; SE, 0.104; standardized coefficient, 0.50; $P<.001$) and WHR $\geq$0.86 (regression coefficient, 0.27; SE, 0.104; standardized coefficient, 0.62; $P<.02$) predicted the rate of 8-iso $PGF_{2\alpha}$ excretion independently of insulin and leptin levels (R^2 for the entire model, 0.44). Thus, when concentrations of C-reactive protein of the entire sample were divided into quartiles the excretion rates of 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 significantly increased from the first to the fourth quartile (by Kruskall-Wallis test; H, 29.9; $P<.001$ and H, 13.1; $P=.004$, respectively). As shown in FIGURE 2, the highest rates of both lipid peroxidation and platelet activation measured in women with android obesity were associated with the third and fourth quartiles of C-reactive protein levels while normal excretion rates of both metabolites were associated with the 2 lowest quartiles of C-reactive protein levels.

To characterize the cause-and-effect relationship of these associations, we examined the effects of a short-term weight loss program, by assessing in 20 android obese women changes in urinary 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 associated with caloric restriction. In 11 of the 20 subjects, mean (SD) weight loss averaged 15.3 (10.5) kg (from 111 [16] to 96 [9] kg), with a parallel mean (SD) decrease in BMI (from 45 [6] to 38 [3]). Successful weight loss was associated with statistically significant reductions in fasting plasma, insulin, leptin, and C-reactive protein levels (TABLE 3). Small changes in HDL-C and LDL-C levels were noted while HbA_{1c} levels remained unchanged. Moreover, the rates of 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 excretion were also significantly reduced following successful weight loss, by 32% and 54%, respectively (FIGURE 3). Changes in urinary 11-dehydro-TxB_2 excretion correlated with the amount of weight that was lost (R_s, 0.67; $P=.02$). In fact, all the values of thromboxane metabolite excretion, a noninvasive index of platelet activation, fell within the normal range at the end of the study (Figure 3).

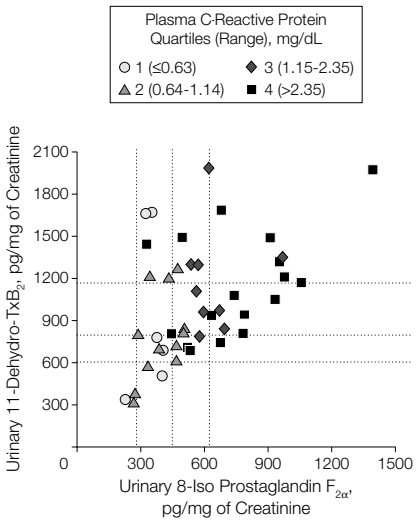
In the other 9 obese women, the weight loss program failed (Table 3). Their mean (SD) weight increased slightly from 93 (12) to 95 (11) kg. In these subjects, the urinary excretion of 8-iso $PGF_{2\alpha}$ and 11-dehydro-TxB_2 remained substantially unchanged, in association with unaltered levels of C-reactive protein (Table 3).

Table 2. Spearman Correlation Coefficients Among the Various Parameters Analyzed in 69 Obese Women With Android and Gynoid Obesity*

	U 8-iso $PGF_{2\alpha}$	CRP	Leptin	Insulin	WHR	BMI
U 11-dehydro-TxB_2	0.61‡	0.44‡	0.35†	0.46‡	0.31†	0.28†
U 8-iso $PGF_{2\alpha}$		0.67‡	0.22	0.47‡	0.40‡	0.23
CRP			0.23	0.44‡	0.38†	0.09
Leptin				0.35†	0.13	0.17
Insulin					0.55‡	0.26
WHR						0.49‡

*For the definition of gynoid and android obesity see Table 1. U 8-iso $PGF_{2\alpha}$ indicates urinary 8-iso prostaglandin $F_{2\alpha}$; CRP, C-reactive protein; WHR, waist-to-hip ratio; BMI, body mass index; and U 11-dehydro-TxB2, urinary-11-dehydrothromboxane B_2.
†$P<.05$.
‡$P<.001$.

Figure 2. Relation Between 8-Iso Prostaglandin $F_{2\alpha}$ and 11-Dehydro-Thromboxane B_2 (11-Dehydro-TxB_2) Excretion Rates in Women



Vertical and horizontal dotted lines mark the boundaries of quartiles of both urinary metabolites. Different symbols represent individual measurements, according to plasma C-reactive protein level.

COMMENT

There is a well-established increase in the risk of cardiovascular death associated with severe overweight as well as a gradient of increasing risk associated with moderate overweight.[22,23] This is true for both men and women, and the relative risk associated with greater BMI is higher among younger individuals.[22] Android or visceral obesity is associated with increased cardiovascular morbidity and mortality[13] through a variety of molecular mechanisms possibly linking the metabolic syndrome to hemostatic and vascular abnormalities.[24,25] Thus, abdominal adiposity, as determined by an increased WHR, was a strong independent predictor of vascular endothelial dysfunction in healthy overweight adults.[26]

In our study, we have identified a novel mechanism through which android obesity may affect cardiovascular morbidity and mortality, ie, thromboxane-dependent platelet activation. Because comorbidity associated with the obese state might confound the as-

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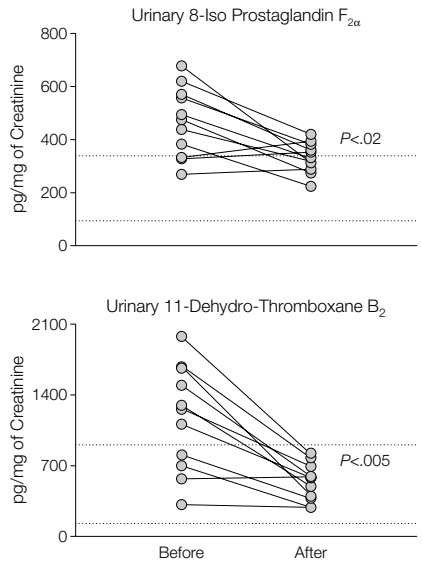
Table 3. Clinical and Biochemical Variables Measured Before and After Successful vs Unsuccessful Weight Loss

Variable	Successful Weight Loss* (n = 11)		*P* Value†	Unsuccessful Weight Loss (n = 9)	
	Median (IQR) Baseline Values	Median (IQR) Change		Median (IQR) Baseline Values	Median (IQR) Change
Body weight, kg	109 (101 to 128)	−10 (−22.5 to −8.0)	<.001	91 (83.2 to 97.3)	1.8 (1.0 to 2.3)
BMI, kg/m^2	44.2 (41.1 to 47.7)	−5.4 (−9.2 to −3.4)	<.001	36.5 (34.6 to 37.2)	0.5 (−0.1 to 1.1)
Waist-to-hip ratio	0.98 (0.95 to 1.0)	0.02 (0.01 to 0.02)	.57	0.92 (0.88 to 0.97)	0.02 (−0.04 to 0.04)
Lipid levels, mg/dL					
Total cholesterol	176 (161 to 229)	−11 (−17 to 5)	.19	198 (173 to 212)	−3 (−12 to 9)
HDL-C	46 (35 to 67)	5 (2 to 6)	<.02	51 (39 to 58)	−2 (−7 to 2)
Triglycerides	82 (69 to 192)	−4 (−11 to 12)	.81	113 (82 to 136)	−2 (−15 to 16)
LDL-C	119 (93 to 146)	−15 (−20 to −1)	.05	124 (94 to 127)	1 (−14 to 7)
HbA1c levels, %	5.5 (5.4 to 5.7)	0 (−0.4 to 0.3)	.99	5.5 (5.2 to 5.6)	−0.1 (−0.2 to 0.1)
Fasting insulin, μU/mL	30 (21 to 45)	−17 (−30 to −7)	<.01	25 (20 to 34)	1.0 (−3 to 3)
Plasma leptin, ng/mL	30.0 (20.6 to 45.3)	−20 (−37.1 to −10)	.04	23.2 (15.9 to 25.9)	−5 (−21 to 3.2)
U-8-iso-PGF$_{2\alpha}$, pg/mg creatinine	476 (334 to 571)	−166 (−201 to 16)	.04	327 (272 to 338)	−17 (−94 to −5)
U-11-dehydro-TxB$_2$, pg/mg creatinine	1265 (691 to 1659)	−582 (−907 to −407)	<.01	684 (378 to 747)	−112 (−228 to 11)
CRP, mg/L	0.90 (0.70 to 1.25)	−0.20 (−0.8 to −0.14)	.05	0.95 (0.85 to 1.30)	0.14 (−0.46 to 0.25)

*Weight loss is defined as loss of at least 5 kg of the initial body weight. To convert total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) from mg/dL to mmol/L, multiply by 0.0259; triglycerides from mg/dL to mmol/L, multiply by 0.0113. IQR indicates interquartile range; BMI, body mass index; HbA$_{1c}$, hemoglobin A$_{1c}$; U 8-iso PGF$_{2\alpha}$, urinary 8-iso prostaglandin F$_{2\alpha}$; U 11-dehydro-TxB$_2$, urinary dehydrothromboxane B$_2$; and CRP, C-reactive protein.
†*P* values for intergroup comparisons of median changes (Mann-Whitney *U* test).

Figure 3. Effects of Weight Loss on 8-Iso Prostaglandin F$_{2\alpha}$ and 11-Dehydro-Thromboxane B$_2$ Excretion in 11 Android Obese Women



Circles and lines connecting circles represent metabolite measurements performed in each participant; dotted lines indicate the range of metabolite excretion in nonobese women.

Differences between before and after weight loss program were analyzed by Wilcoxon signed-rank test.

sociation with platelet activation, we selected a group of obese women free of other cardiovascular risk factors, previously associated with persistent platelet activation, such as diabetes mellitus,[3] cigarette smoking,[1] and hypercholesterolemia.[2] Biochemical evidence of increased platelet activation in vivo was obtained through noninvasive measurements of thromboxane metabolite excretion[18,27] that avoid artifactual platelet activation during and after blood sampling.[28] Although we did not measure platelet aggregation in our study, it should be emphasized that the ex-vivo measurement of platelet responses to various agonists represents an index of functional capacity that by no means reflects the extent of platelet activation in vivo.[28] Android obesity was associated with 4-fold higher rate of thromboxane metabolite excretion than measured in nonobese women. These changes are comparable to those previously described in association with type 2 diabetes mellitus,[3] cigarette smoking,[1] and hypercholesterolemia.[2]

Furthermore, we characterized a putative biochemical link between obesity and platelet activation, by investigating the in vivo formation of F$_2$-isoprostanes,[7] as reflected by the urinary excretion of a PGF$_{2\alpha}$ isomer, 8-iso PGF$_{2\alpha}$.[20] This family of bioactive iso-eicosanoids is produced from arachidonic acid through a process of nonenzymatic free radical-catalyzed lipid peroxidation[7] and their biological activities may transduce the effects of oxidant stress associated with complex metabolic disorders into specialized forms of cellular activation.[29] The linear relationship between the excretion rates of 8-iso PGF$_{2\alpha}$ and 11-dehydro-TxB$_2$ demonstrated in obese women in our study confirms and extends previous findings in patients with hypercholesterolemia,[8] non–insulin-dependent diabetes mellitus,[9] and homozygous homocystinuria.[10] Experimental evidence indicates that obesity is associated with increased oxidative stress.[30,31] Thus, markedly elevated levels of 8-iso PGF$_{2\alpha}$ have been reported in the obese Zucker rat.[32] This F$_2$-isoprostane induces vasoconstriction and can amplify the response of human platelets to other agonists.[29] Although both hyperinsulinemia and increased leptin production may trigger increased generation of oxygen radicals,[33,34] possibly contributing to enhanced lipid peroxidation, we found that C-reactive protein levels and a WHR ≥0.86 predicted the rate of 8-iso PGF$_{2\alpha}$ excretion independently of insulin and leptin levels.

There is increasing evidence that features of the insulin resistance syndrome, including abdominal obesity, are associated with increased

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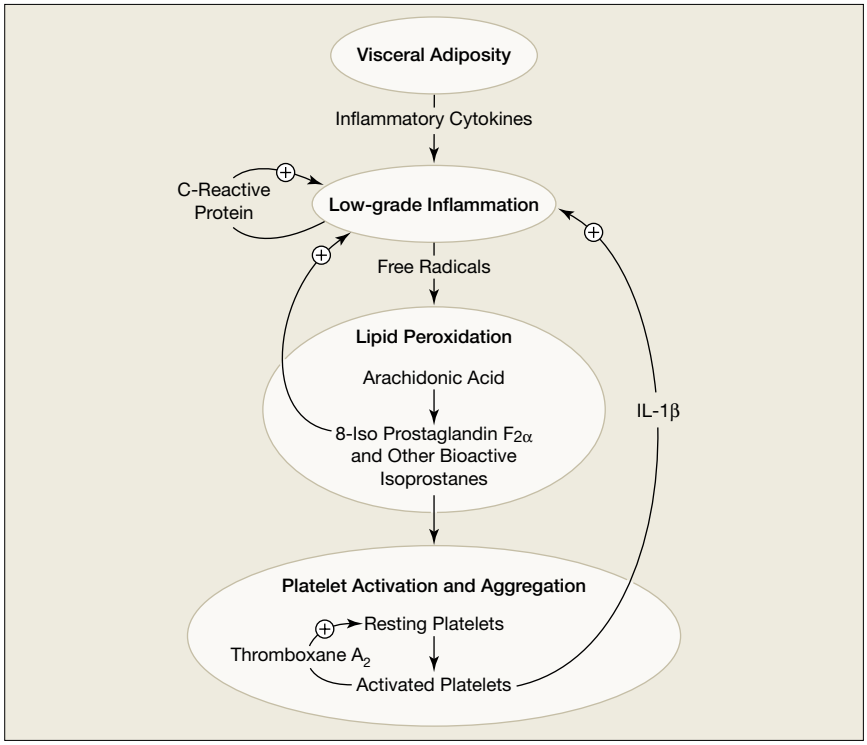
C-reactive protein levels.[26,35-37] In the most recent study by Lemieux et al[37] plasma C-reactive protein levels showed positive and significant correlations with waist girth and visceral adipose tissue accumulation as measured by computed tomography at L4 to L5. These results have been interpreted[24] to suggest that the expanded abdominal fat depot may be responsible for a low-grade inflammatory state, by providing a source of increased production of IL-6, a potent stimulus of C-reactive protein synthesis by the liver.[38] Our cross-sectional findings confirm and extend these earlier observations and suggest that a low-grade inflammatory state associated with abdominal adiposity may be the primary trigger of thromboxane-dependent platelet activation mediated, at least in part, through enhanced lipid peroxidation (FIGURE 4). Several feed-forward mechanisms are likely to amplify and sustain the relationship between systemic inflammation and platelet activation, such as a direct proinflammatory effect of C-reactive protein,[39] the effects of F_2-isoprostanes on inflammatory gene expression,[40] and the synthesis and release of inflammatory cytokines from activated platelets[41] (Figure 4).

Further evidence for a cause-and-effect relationship between the obese state 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 more than a 50% reduction in thromboxane biosynthesis in 11 android obese women. This led to a normalization of this noninvasive index of platelet activation. Body weight reduction was also associated with statistically significant changes in C-reactive protein levels and 8-iso $PGF_{2\alpha}$ excretion, consistently with the hypothesis outlined in Figure 4. A similar reduction in C-reactive protein levels following energy restriction and weight loss in obese, healthy women was recently reported.[42] The group of 9 obese women who failed to achieve a significant weight loss during this period provided an interesting control group, that demonstrated the reproducibility of these biochemical indexes over time in the presence of constant body weight.

Several limitations of the intervention study should be emphasized. These include self-selection of the participating women, lack of randomization to weight loss vs weight maintenance, lack of generalizability of the findings to gynoid obese women as well as to women from ethnic minorities, and small sample size of the study.

Despite these limitations, these findings may have clinical implications for the primary prevention of myocardial infarction in obese women. Although primary prevention studies have demonstrated that low-dose aspirin can reduce the risk of myocardial infarction,[43] the balance of benefits and risk of major bleeding complications is substantially uncertain in this setting.[43] Whether supplementing vitamin E to modulate F_2-isoprostane formation and platelet activation[44] may have a similar impact on the risk of a first myocardial infarction in obese, otherwise healthy, women remains formally untested although it represents an option that is often considered.[45] Within the limits of a relatively small mechanistic study with biochemical end points, our results suggest that a substantial reduction in thromboxane-dependent platelet activation can be achieved by a successful weight loss program. For android obese women who fail to achieve substantial weight reduction, low-dose aspirin may be considered as an option after evaluating the potential benefits and hemorrhagic risk of the individual patient,[46] taking into account the limited amount of randomized trial evidence for the efficacy and safety of antiplatelet prophylaxis in high-risk women without prior vascular complications.[47]

Figure 4. Biochemical Mechanisms Linking Android Obesity to Persistent Platelet Activation



The illustration depicts the role of inflammation and nonenzymatic peroxidation of arachidonic acid in triggering persistent platelet activation, as suggested by the experimental findings of the present study. It also illustrates potential amplification loops sustaining this mechanistic chain of events. IL indicates interleukin.

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