



# LEPTIN INCREASES THROMBOXANE A<sub>2</sub> FORMATION IN THE RAT

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## Abstract

Chronic hyperleptinemia may contribute to various complications of obesity including atherosclerosis, however, the underlying mechanisms are incompletely clear. We examined the effect of leptin on platelet activity by measuring stable metabolites of thromboxane A<sub>2</sub> (TXA<sub>2</sub>), TXB<sub>2</sub>, 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-TXB<sub>2</sub>, in plasma and urine. *In vitro*, leptin stimulated TXB<sub>2</sub> formation by platelet-rich plasma (PRP). *In vivo*, leptin (1 mg/kg ip.) increased urinary excretion of 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-TXB<sub>2</sub>. Urinary excretion of these metabolites was also elevated in rats made hyperleptinemic by administration of recombinant leptin (0.5 mg/kg/day) for 8 days. The stimulatory effect of leptin on TXB<sub>2</sub> formation in PRP isolated from hyperleptinemic animals was impaired in comparison to the control group. In rats made obese, hyperleptinemic and hyperinsulinemic/insulin resistant by cafeteria diet administered for 3 months, acute stimulatory effect of leptin on TXB<sub>2</sub> formation by PRP was not impaired. In rats made insulin resistant by fructose feeding for 8 weeks, stimulatory effect of leptin on TXB<sub>2</sub> formation in PRP was augmented in comparison to the control group. Insulin sensitizer, rosiglitazone, decreased insulin level and attenuated the stimulatory effect of leptin on TXB<sub>2</sub> formation in obese and fructose-fed animals. In contrast, rosiglitazone had no effect on insulin level or leptin-induced TXB<sub>2</sub> formation in control rats and rats receiving recombinant leptin for 8 days. These results indicate that: (i) leptin stimulates platelet TXA<sub>2</sub> formation both *in vitro* and *in vivo*, (ii) chronic hyperleptinemia impairs acute stimulatory effect of leptin on platelet activity if insulin sensitivity is normal, (iii) insulin resistance/hyperinsulinemia augments the stimulatory effect of leptin on TXA<sub>2</sub> formation, which results in normal platelet sensitivity to leptin in obesity associated with both hyperleptinemia and hyperinsulinemia, and (iv) PPAR-γ agonists such as rosiglitazone decrease platelet sensitivity to leptin by reducing insulin resistance.

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**Key words:** platelets, obesity, metabolic syndrome, atherosclerosis, rosiglitazone

## Introduction

Recent studies indicate that an adipose tissue hormone, leptin, is involved in atherogenesis. Plasma leptin concentration is markedly increased in obese humans and in animals with obesity induced by high-calorie diet. In experimental studies leptin has been demonstrated to have many potentially proatherogenic effects. This adipokine induces endothelial dysfunction, oxidative stress, vascular smooth muscle cells hypertrophy and proliferation, stimulates macrophage cholesterol synthesis, and reduces HDL cholesterol level (1). Deficiency of leptin or its receptor reduce atherosclerosis in classic animal models such as apolipoprotein E and LDL receptor knockout mice, whereas administration of exogenous leptin in supraphysiological doses or transgenic hormone overexpression have the opposite effect (2). In addition, many clinical studies indicate the link between high leptin level and atherosclerosis, acute cardiovascular events and ischemic stroke in humans (3).

Platelets play a crucial role in atherosclerosis. Formation of platelet-rich thrombus on the ruptured plaque, with resulting complete or almost complete vessel occlusion, is the ma-

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major mechanism of acute complications of atherosclerosis such as myocardial infarction and ischemic cerebral stroke. In addition, chronic low-grade platelet activation facilitates growth of atherosclerotic plaque. Platelets secrete several mediators which potentially stimulate hypertrophy and proliferation of vascular smooth muscle cells including platelet-derived growth factor and thromboxane A<sub>2</sub> (TXA<sub>2</sub>). Moreover, activated platelets facilitate leukocyte recruitment to the endothelium by secreting chemotactic and proinflammatory mediators such as a chemokine platelet factor-4, a CD40 ligand, P-selectin, thrombospondin-1 and RANTES (4).

The effect of leptin on platelet function is controversial. Some studies have demonstrated that leptin augments ADP-induced platelet aggregation (5), but other authors observed no effect (6). These studies were performed *in vitro*, and it is well-known that platelet aggregation *in vitro* is at best only a crude estimate of their function in the intact organism (7). Although the positive correlation between serum leptin and urinary excretion of TXA<sub>2</sub> metabolite, 11-dehydro-TXB<sub>2</sub>, has been observed in obese women (8), this correlation disappeared after adjustment for anthropometric variables suggesting that it represents the effect of obesity and not necessarily of leptin itself. In addition, resistance to many effects of leptin has been described in obesity. It is unclear if platelets' sensitivity to leptin is preserved or impaired in obesity. If platelets remain sensitive to leptin, chronic hyperleptinemia could contribute to platelets' hyperactivity observed in the metabolic syndrome. However, if platelets become resistant to leptin, the contribution of this adipokine to atherothrombotic complications would be less likely.

To address these issues, in the present study we examined the effect of leptin on platelet function, measured as TXA<sub>2</sub> production, in the rat both *in vitro* and *in vivo*. In addition, we compared the effect of leptin in lean animals and in selected models of the metabolic syndrome. We also tested the effect of rosiglitazone, a PPAR-γ agonist, on leptin-induced TXA<sub>2</sub> formation.

## Materials and methods

### Animals

All studies were performed on adult male Wistar rats weighing 314 ± 8 g. The animals were housed under controlled conditions of temperature (20-22°C), humidity (60-70%), lighting (12 h light/dark cycle) and provided with food and water *ad libitum*. The study protocol was reviewed and approved by the local institutional ethical committee.

### Effect of leptin on TXA<sub>2</sub> production *in vitro*

Rats were anesthetized with pentobarbital (50 mg/kg ip.) and

blood was withdrawn from the abdominal aorta using sodium citrate as an anticoagulant. Blood was centrifuged at 150×g for 15 min at a room temperature to obtain platelet-rich plasma (PRP). PRP was diluted with Tyrode's buffer (5 mM HEPES, 2 mM MgCl<sub>2</sub>, 0.1% BSA and 0.1% D-glucose) to a standard concentration of 3 × 10<sup>8</sup> platelets/ml. To obtain platelet-poor plasma (PPP), PRP was recentrifuged at 1000 × g for 15 min and the supernatant was collected. PRP was incubated with leptin for 10 min and TXB<sub>2</sub> concentration was measured (see below).

### Effect of leptin on TXA<sub>2</sub> production *in vivo*

Rats were anesthetized with ethylurethane (1.25 g/kg ip.). Then, a polyethylene catheter was inserted into the urinary bladder for urine collection. Urine was collected for 1 hour and then either leptin (1 mg/kg in 0.5 ml) or an equivolume amount of phosphate-buffered saline (PBS) was injected intraperitoneally. Urine collection was continued for additional 2 hours. After the end of urine collection, animals were euthanized by the lethal dose of pentobarbital.

### Induction of hyperleptinemia, dietary-induced obesity and fructose-induced metabolic syndrome

Experimental hyperleptinemia was induced by administration of exogenous recombinant leptin (0.25 mg/kg twice daily sc.) for 8 days as described by us previously (9). Leptin was injected between 7.00 and 8.00 AM and between 7.00 and 8.00 PM. The last dose was given in the morning and plasma for *in vitro* experiments was obtained 6 hours after this injection. Animals in this group received standard rat chow *ad libitum*. Obesity was induced by offering the animals a highly palatable "cafeteria diet" for either 1 or 3 months. This diet consisted of standard chow combined 1:1 (wt/wt) with a liquid diet containing equal amounts of sucrose, glucose, whole milk powder and soybean powder suspended in tap water (10). The composition of this diet was similar to standard chow (66% carbohydrates, 20% protein, and 14% fat). In the separate group of animals, fructose was administered in the drinking water at a concentration of 20% for 8 weeks to induce hyperlipidemia and insulin resistance not associated with obesity. In subgroups of control, hyperleptinemic, 3-month obese and fructose-fed animals, insulin sensitizer, rosiglitazone, was administered at 10 mg/kg/day by oral gavage for 8 days before blood collection for acute *in vitro* experiments.

### Measurement of TXA<sub>2</sub> metabolites

TXA<sub>2</sub> is rapidly converted non-enzymatically to its immediate

metabolite, TXB<sub>2</sub>, which is further enzymatically metabolized to several derivatives such as 2,3-dinor-TXB<sub>2</sub>, 2,3-dinor-TXB<sub>2</sub> and 11-dehydro-TXB<sub>2</sub> (11). Measurement of these terminal metabolites in urine provides a reliable estimate of whole-body platelet-derived TXA<sub>2</sub>. In contrast, because TXB<sub>2</sub> has a short half-life (5-7 min), most of this compound found in urine originates from local intrarenal production rather than from systemic sources. On the other hand, concentrations of 2,3-dinor-TXB<sub>2</sub> and 11-dehydro-TXB<sub>2</sub> in plasma are below detection limit of most assays until the sample is concentrated. However, most of TXB<sub>2</sub> found in plasma is synthesized by platelets *ex vivo* after blood withdrawal. Therefore, we used plasma TXB<sub>2</sub> and urinary 11-dehydro-TXB<sub>2</sub>/2,3-dinor-TXB<sub>2</sub> as markers of TXA<sub>2</sub> formation for *in vitro* and *in vivo* studies, respectively. TXB<sub>2</sub>, 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-TXB<sub>2</sub> were measured by competitive enzyme immunoassay (EIA) methods using Cayman Chemical kits (Cat. #519031, 519501 and 519051, respectively), according to the manufacturer's instruction. The detection limits are 11 pg/ml for TXB<sub>2</sub>, 16 pg/ml for 11-dehydro-TXB<sub>2</sub>, and 7 pg/ml for 2,3-dinor-TXB<sub>2</sub>.

#### Other assays

Plasma insulin and leptin concentrations were measured by EIA methods using Rat Insulin EIA Kit (SPIbio, Massy, France) and Leptin Enzyme Immunoassay Kit (Cayman Chemical), respectively (10). Lipid profile and plasma glucose were assayed by routine laboratory methods.

#### Reagents

Recombinant rat leptin was obtained from R&D Systems. Rosiglitazone was purchased from Cayman Chemical. Other reagents were from Sigma-Aldrich.

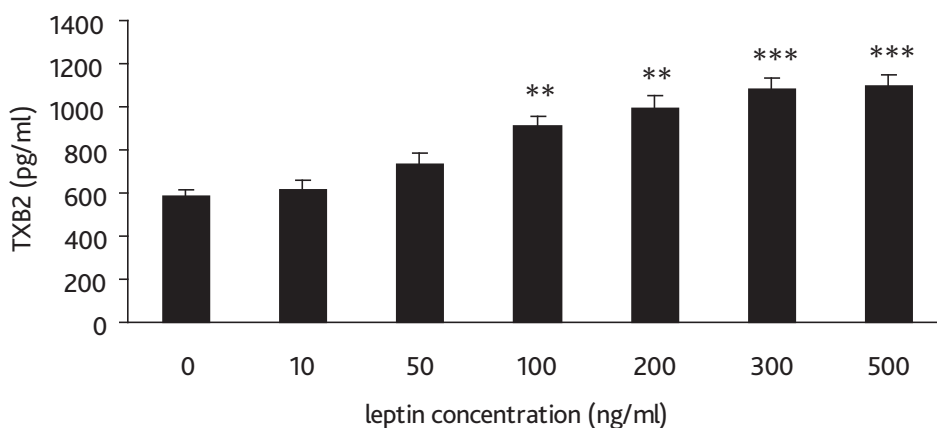
#### Statistical analysis

Baseline (without leptin) and leptin-stimulated values of TXA<sub>2</sub> metabolites in plasma and urine of the same animal were compared by repeated-measures ANOVA. Between-group comparisons of TXA<sub>2</sub> metabolites, plasma lipids, glucose, leptin and insulin were done by single-measures ANOVA.  $P < 0.05$  was considered significant.

#### Results

##### Leptin stimulates TXA<sub>2</sub> formation *in vitro*

Leptin increased TXB<sub>2</sub> concentration in PRP isolated from control lean rats in a concentration-dependent manner (Fig. 1). Leptin had no significant effect at physiological concentration (10 ng/ml) as well as at a moderately elevated concentration (50 ng/ml), but significantly increased TXB<sub>2</sub> at 100 ng/ml. Maximal stimulatory effect of leptin was observed at 300 ng/ml (Fig. 1). Addition of COX inhibitor, indomethacin (10  $\mu$ M) to the blood before isolation of PRP decreased baseline TXB<sub>2</sub> concentration to  $243 \pm 29$  pg/ml ( $p < 0.001$  vs. sample without indomethacin) and abolished the stimulatory effect of leptin (100 ng/ml leptin + indomethacin:  $279 \pm 31$  pg/ml). In addition, leptin had no significant effect on TXB<sub>2</sub> concentration in platelet-poor plasma (without leptin:  $479 \pm 57$  pg/ml; with 100 ng/ml leptin:  $512 \pm 53$  pg/ml,  $p = \text{NS}$ ).



**Figure 1.** Effect of leptin on thromboxane B<sub>2</sub> concentration in platelet-rich plasma (PRP) of control rats *in vitro*. PRP was incubated in the presence of various leptin concentrations for 10 min and then TXB<sub>2</sub> was measured. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. TXB<sub>2</sub> concentration in PRP not treated with leptin.

### Leptin stimulates TXA<sub>2</sub> formation *in vivo*

Urinary excretion of 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-TXB<sub>2</sub> increased within 2 hours after a single intraperitoneal leptin injection (Fig. 2). In contrast, leptin had no acute effect on urinary TXB<sub>2</sub>. Injection of vehicle (PBS) instead of leptin did not change any TXA<sub>2</sub> metabolite in urine (not shown).

### TXA<sub>2</sub> formation is increased in rats receiving leptin for 8 days

Plasma TXB<sub>2</sub> concentration in rats receiving exogenous leptin for 8 days was 612 ± 57 pg/ml and did not differ from control group. However, urinary excretion of 11-dehydro-TXB<sub>2</sub>, 2,3-dinor-TXB<sub>2</sub>, and TXB<sub>2</sub> was higher in hyperleptinemic than in control animals (Fig. 3). These results indicate that chronic hyperleptinemia increases systemic and intrarenal TXA<sub>2</sub> formation.

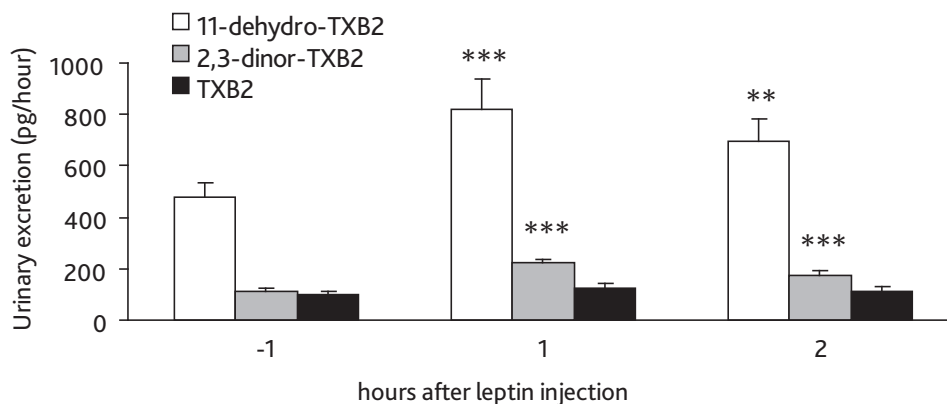
### Acute effect of leptin on TXA<sub>2</sub> formation is impaired in hyperleptinemic rats

To examine if chronic hyperleptinemia induces resistance of

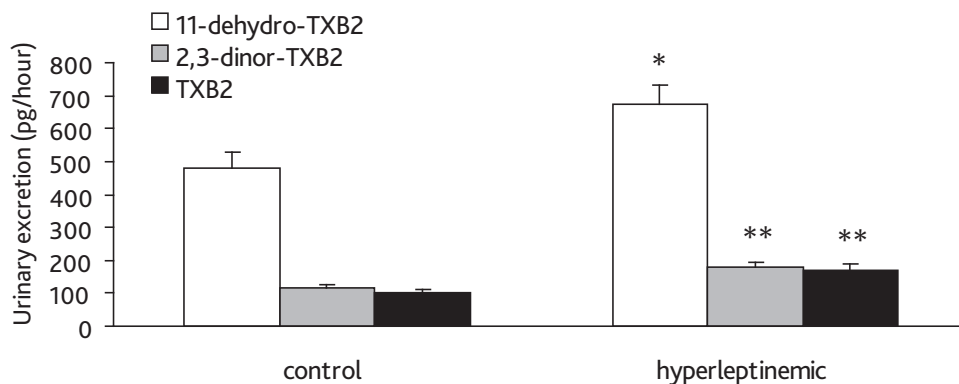
platelets to leptin, we obtained PRP from rats made hyperleptinemic by previous 8-day leptin administration and then studied acute effect of leptin on TXB<sub>2</sub> formation in these samples *in vitro* (Fig. 4). As can be seen, in hyperleptinemic rats leptin significantly stimulated TXB<sub>2</sub> formation at a concentration no less than 300 ng/ml and did it to a lesser extent than in control animals at both 300 ng/ml and 500 ng/ml. Thus, acute stimulatory effect of leptin on platelet TXA<sub>2</sub> formation is impaired in hyperleptinemic rats.

### Effect of leptin on TXA<sub>2</sub> formation in obese and fructose-fed rats

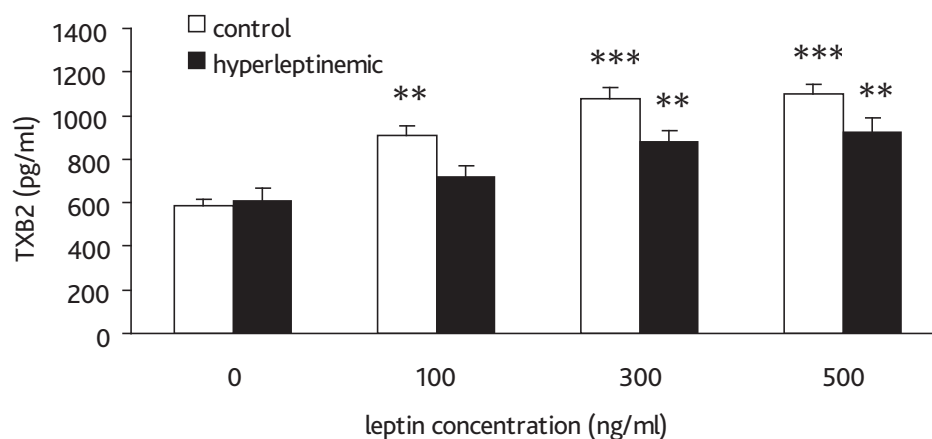
To examine if chronic “endogenous” hyperleptinemia associated with obesity impairs acute effect of leptin on TXA<sub>2</sub> formation, we investigated the effect of leptin on TXB<sub>2</sub> concentration in PRP of rats made obese by high-calorie diet administered for either 1 or 3 months (Fig. 5). Baseline (without exogenous leptin) TXB<sub>2</sub> concentration was slightly but significantly higher in the 3-month but not in the 1-month obese group. After 10-min



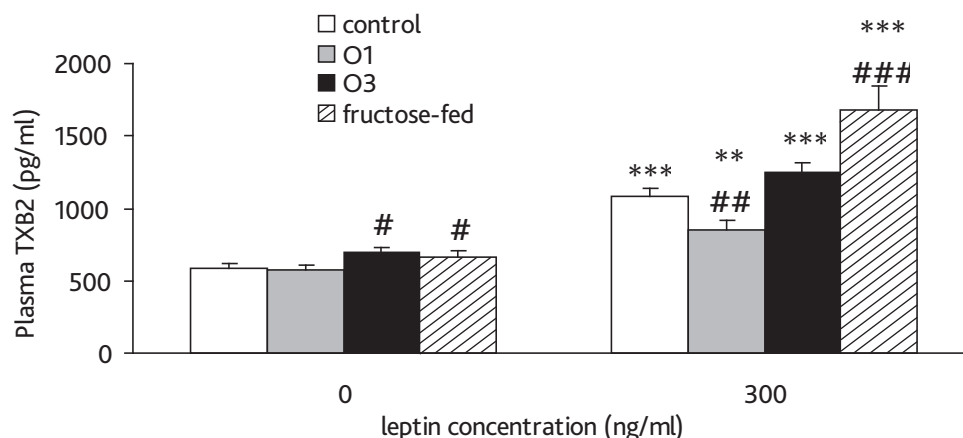
**Figure 2.** Urinary excretion of TXA<sub>2</sub> metabolites in lean control rats before (-1) and during the first (1) and the second (2) hour after intraperitoneal leptin injection at a dose of 1 mg/kg. \*\*p<0.01, \*\*\*p<0.001 vs. pre-injection values.



**Figure 3.** Urinary excretion of TXA<sub>2</sub> metabolites in control rats and in animals receiving exogenous leptin (0.25 mg/kg sc.) for 8 days. Urine was collected for 1 hour in anesthetized animals. Urine collection was started 6 hours after the last leptin injection. \*p<0.05, \*\*p<0.01 vs. control group.



**Figure 4.** Concentration-dependent effect of leptin on TXB<sub>2</sub> in PRP obtained from control rats and from rats made hyperleptinemic by previous 8-days leptin administration. \*\*p<0.01, \*\*\*p<0.001 vs. TXB<sub>2</sub> concentration in PRP not treated with leptin.



**Figure 5.** Effect of leptin on TXB<sub>2</sub> formation in PRP of control, 1-month obese (O1), 3-month obese (O3) and fructose-fed rats. \*\*p<0.01, \*\*\*p<0.001 vs. TXB<sub>2</sub> concentration in the respective sample not treated with leptin, #p<0.05, ##p<0.01, ###p<0.001 vs. respective (treated or not treated with leptin) control group.

incubation, leptin (300 ng/ml) increased TXB<sub>2</sub> concentration in PRP of control animals by 84.1% but in 1-month obese group by only 49.6%. Surprisingly, in contrast to short-time obesity, the effect of leptin in 3-month obese group was not impaired in comparison to the control group (stimulation by 80.9%).

Metabolic characteristics of both obese groups is presented in Table 1. As can be seen, plasma leptin is similarly elevated in both obese groups but in contrast to 1-month group, the 3-month group exhibits hyperinsulinemia (a marker of insulin resistance) and dyslipidemia (hypertriglyceridemia and low HDL-cholesterol). Insulin inhibits platelet function and its effect is impaired in insulin resistance states (12). Therefore, we hypothesized that insulin resistance in the 3-month obese group

might paradoxically increase the sensitivity of platelets to leptin in comparison to 1-month obese group. To verify this hypothesis, we examined the effect of leptin in fructose-fed rats, which are markedly hyperinsulinemic and hypertriglyceridemic but only slightly hyperleptinemic (Table 1). Leptin (300 ng/ml) increased TXB<sub>2</sub> concentration in PRP of fructose-fed animals to a much greater degree (+153.0%) than in control rats. Thus, fructose-induced metabolic syndrome is associated with enhanced platelet response to leptin. Taken together, these results suggest that in the 3-month obese group resistance of platelets to leptin due to chronic hyperleptinemia is counterbalanced by concomitant hyperinsulinemia/insulin resistance which increases platelets' sensitivity to this adipokine.



**Table 1.** Characteristics of experimental groups

	Control	Hyperleptinemic	1-month obesity	3-month obesity	Fructose-fed
Body weight (g)	357 ± 5	342 ± 7	429 ± 7***	497 ± 6***	385 ± 9*
Triglycerides (mmol/l)	0.83 ± 0.06	0.74 ± 0.06	0.85 ± 0.05	1.20 ± 0.08***	2.71 ± 0.19***
Total cholesterol (mmol/l)	2.05 ± 0.21	1.97 ± 0.17	1.83 ± 0.11	1.47 ± 0.12*	2.06 ± 0.15
HDL-cholesterol (mmol/l)	1.24 ± 0.07	1.25 ± 0.10	1.19 ± 0.08	0.76 ± 0.08***	1.30 ± 0.12
Plasma glucose (mmol/l)	6.01 ± 0.29	6.09 ± 0.35	6.34 ± 0.43	6.30 ± 0.41	6.178 ± 0.39
Plasma insulin (ng/ml)	2.38 ± 0.24	2.15 ± 0.20	2.58 ± 0.32	3.89 ± 0.46*	4.99 ± 0.61***
Plasma leptin (ng/ml)	4.22 ± 0.41	13.6 ± 1.23***	9.39 ± 0.81***	12.60 ± 1.27***	5.97 ± 0.52*

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , compared to control group.

### Rosiglitazone attenuates the effect of leptin on TXA<sub>2</sub> formation in the 3-month obese and fructose-fed rats

To further evaluate the above mentioned hypothesis, we examined the effect of peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) agonist, rosiglitazone (RGZ), which increases insulin sensitivity, on platelet response to leptin in various experimental groups. Rosiglitazone had no effect on plasma leptin, insulin and lipid profile in either control or hyperleptinemic rats. In addition, rosiglitazone did not alter leptin-induced increase in TXB<sub>2</sub> in PRP of these animals (not shown). In the 3-month obese group, rosiglitazone had no significant effect on plasma leptin (without RGZ: 12.60 ± 1.27 ng/ml; with RGZ: 10.21 ± 0.98 ng/ml), but decreased plasma insulin (from 3.89 ± 0.46 to 2.57 ± 0.29 ng/ml,  $p < 0.01$ ) and triglycerides (from 1.20 ± 0.08 to 0.74 ± 0.05 mM,  $p < 0.01$ ). Baseline (without exogenous leptin) TXB<sub>2</sub> concentration was lower in RGZ-treated than in non-treated 3-month obese rats (571 ± 27 vs. 693 ± 39 pg/ml,  $p < 0.05$ ). In addition, leptin (300 mg/ml) increased TXB<sub>2</sub> concentration in RGZ-treated obese rats to 878 ± 87 pg/ml (+53.8%), which is significantly less than in either control or RGZ-untreated obese animals. In the fructose-fed group, RGZ decreased plasma insulin (-54.6%) and triglycerides (-41.7%) and reduced the stimulatory effect of 300 ng/ml leptin on TXB<sub>2</sub> from +153.0% ( $p < 0.01$  vs. control group) to +79.4% ( $p = \text{NS}$  vs. control group).

### Discussion

Metabolic syndrome is associated with increased risk of arterial and venous thrombosis (13-15). The pathogenesis of prothrombotic state in the metabolic syndrome is complex and incompletely understood. Increased concentration of some coagulation

factors such as fibrinogen, factor VII and tissue factor, deficiency of a major activator of fibrinolysis, tissue plasminogen activator, and excess of fibrinolysis inhibitor, plasminogen activator inhibitor-1 (PAI-1) have been reported in patients with overweight/obesity. In addition, platelet aggregation is augmented in obese animals and humans (16), as evidenced by increased ADP-induced platelet aggregation (6), higher urinary 11-dehydro-TXB<sub>2</sub> excretion (8), plasma P-selectin (17) and soluble CD40 ligand levels (18). Platelets of obese subjects are resistant to inhibitory effects of prostacyclin and adenosine (which elevate intracellular cAMP) and nitric oxide (which elevates intracellular cGMP), as well as to these cyclic nucleotides themselves (19, 20). Moreover, metabolic syndrome is associated with reduced sensitivity of platelets to at least two groups of antiplatelet drugs, acetylsalicylic acid and P<sub>Y</sub><sub>12</sub> receptor inhibitors, thienopyridines (21). Increased endothelial cell-platelet interaction has been observed in visceral adipose tissue in murine models of obesity (22).

Several studies have demonstrated that leptin augments ADP-, thrombin- or collagen-induced platelet adhesion and/or aggregation in vitro (5, 23, 24). In addition, exogenous leptin administered to wild-type mice augmented vascular thrombosis induced by vessel wall injury (25, 26). In the present study we examined the effect of leptin on TXA<sub>2</sub> formation both in vitro and in vivo. TXA<sub>2</sub> is one of the most common platelet agonists, and the most widely prescribed antiplatelet drug, acetylsalicylic acid, reduces platelet activity by inhibiting its synthesis. Apart from stimulating platelets, TXA<sub>2</sub> is involved in atherogenesis by inducing vascular smooth muscle cell migration and proliferation (27) and expression of adhesion molecules in endothelial cells (28). To the best of our knowledge, only one previous study

addressed the effect of leptin on TXA<sub>2</sub> formation. In that study, Dellas *et al* (29) have demonstrated that leptin increases TXB<sub>2</sub> generation by isolated human platelets and augments the stimulatory effect of ADP. Our results are consistent with these data. Although the minimal effective leptin concentration (100 ng/ml) is above physiological level (except in cases of extreme obesity), leptin was applied only for 10 min. It is possible that more prolonged incubation with leptin would have resulted in platelet stimulation even at lower concentrations of this adipokine. Increased urinary TXA<sub>2</sub> metabolites in animals receiving leptin for 8 days at doses which raised its level to values observed in obesity (Table 1) is consistent with this hypothesis. In addition, leptin concentration in the blood perfusing adipose tissue may be locally much higher than in systemic circulation. Thus, it is likely that stimulatory effect of leptin on platelets is relevant in pathological conditions.

Obesity is associated with resistance not only to central anorectic but also to some peripheral effects of leptin (30), which partially results from downregulation of leptin receptors and/or postreceptor signaling mechanisms due to chronic hyperleptinemia (31). It is controversial if platelets of obese individuals remain sensitive to leptin or become leptin-resistant. Corsonello *et al* (32) have demonstrated that stimulatory effect of leptin on platelet aggregation is impaired in overweight and obese individuals. In contrast, Corica *et al* (33) found that leptin-induced platelet aggregation was impaired only in morbidly obese but not in overweight subjects, and Dellas *et al* (34) observed normal platelet sensitivity to leptin in morbidly obese patients in comparison to normal-weight controls. Herein we demonstrate that both hyperleptinemia induced in lean rats by administration of exogenous leptin and “endogenous” hyperleptinemia in 1-month obese group are associated with resistance to acute TXA<sub>2</sub>-stimulating effect of leptin *in vitro*. Despite this resistance, urinary TXA<sub>2</sub> metabolites are elevated in hyperleptinemic rats. These results indicate that leptin may still contribute to platelet hyperactivity when hormone level is markedly elevated, although the degree of platelet stimulation is undoubtedly lower than would have been if platelets remained leptin-sensitive.

Surprisingly, resistance to leptin was not observed in the 3-month obese group, although endogenous leptin level tended to be higher in these animals than in the 1-month obese group. We hypothesize that despite possible downregulation of leptin signaling, leptin-induced TXA<sub>2</sub> production is augmented by concomitant insulin resistance in these animals. This hypothesis is supported by the following observations: (i) only 3-month obese rats were hyperinsulinemic (and thus presumably insulin resistant), (ii) platelet sensitivity to leptin was augmented in fructose-fed rats which were markedly insulin resistant but

relatively normoleptinemic, (iii) rosiglitazone decreased insulin level and platelets' response to leptin in the 3-month obese and fructose-fed but not in either control or hyperleptinemic groups, and (iv) insulin inhibits platelet function and its effect is impaired in the metabolic syndrome (12,19,35). Nevertheless, other explanations cannot be definitely excluded. For example, hyperlipidemia promotes platelet aggregation (36), and both 3-month obese and fructose-fed animals were hyperlipidemic. In addition, rosiglitazone not only reduced insulin level but also improved lipid profile. Whatever the mechanism, the results suggest that PPAR- $\gamma$  agonists such as rosiglitazone may beneficially modulate platelet function in obesity/metabolic syndrome by reducing their sensitivity to leptin. In addition, variable degree of insulin resistance may determine platelets' sensitivity to leptin in obese subjects and may explain, at least partially, controversial results of previous studies in this field (32-34).

Interestingly, urinary TXB<sub>2</sub> excretion was increased in the hyperleptinemic group. Increased intrarenal TXA<sub>2</sub> production may contribute to the development of arterial hypertension and nephropathy due to its vasoconstricting, antinatriuretic and profibrogenic effects. Increased urinary TXB<sub>2</sub> excretion was observed in many animal models of hypertension such as spontaneously hypertensive rat, Dahl salt-sensitive rat, fructose- or NO synthase inhibitor-induced hypertension as well as in obese Zucker rats which develop severe nephropathy (37-39). Chronic leptin administration induces arterial hypertension (9) and may induce nephropathy (40-41). It remains to be established if excessive intrarenal TXA<sub>2</sub> formation contributes to these leptin-induced complications.

There are several limitations of the present study. First, we measured only TXA<sub>2</sub> formation and did not assess platelet function directly. TXA<sub>2</sub> formation is an established marker of platelet function and allowed us to examine it both *in vitro* and *in vivo* in the same experimental model. Second, we examined the effect of leptin alone and did not address its interaction with other platelet agonists. Third, acutely administered leptin increases natriuresis (42,43). It could be suggested that leptin increased urinary 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-TXB<sub>2</sub> secondarily to increasing sodium excretion. This possibility seems, however, unlikely for the following reasons: (i) in contrast to 11-dehydro-TXB<sub>2</sub> and 2,3-dinor-TXB<sub>2</sub>, acutely administered leptin had no effect on TXB<sub>2</sub> excretion, (ii) 8-day hyperleptinemia is associated with increased excretion of TXA<sub>2</sub> metabolites whereas natriuresis is reduced in this model (9), and (iii) furosemide increased natriuresis while having no effect on urinary TXA<sub>2</sub> metabolites, whereas COX inhibitor, indomethacin, prevented leptin-induced increase in TXA<sub>2</sub> metabolites but had no effect on leptin-induced increase in natriuresis (unpublished observation).

In conclusion, we have demonstrated that both acutely and chronically administered leptin increases TXA<sub>2</sub> formation in the rat. Chronic hyperleptinemia impairs platelets' response to acutely administered leptin if insulin sensitivity is normal, however, the stimulatory effect of leptin is intact if insulin resistance is concomitantly observed. PPAR- $\gamma$  agonists may be useful as adjunctive antiplatelet therapy in obesity/metabolic syndrome because they reduce platelets' sensitivity to leptin by ameliorating insulin resistance.

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