Parametrial Adipose Tissue and Metabolic Dysfunctions Induced by Fructose-rich Diet in Normal and Neonatal-androgenized Adult Female Rats

Ana Alzamendi[1], Daniel Castrogiovanni[1], Hugo H. Ortega[2], Rolf C. Gaillard[3], Andres Giovambattista[1] and Eduardo Spinedi[1]

Hyperandrogenemia predisposes an organism toward developing impaired insulin sensitivity. The aim of our study was to evaluate endocrine and metabolic effects during early allostasis induced by a fructose-rich diet (FRD) in normal (control; CT) and neonatal-androgenized (testosterone propionate; TP) female adult rats. CT and TP rats were fed either a normal diet (ND) or an FRD for 3 weeks immediately before the day of study, which was at age 100 days. Energy intake, body weight (BW), parametrial (PM) fat characteristics, and endocrine/metabolic biomarkers were then evaluated. Daily energy intake was similar in CT and TP rats regardless of the differences in diet. When compared with CT-ND rats, the TP-ND rats were heavier, had larger PM fat, and were characterized by basal hypoadiponectinemia and enhanced plasma levels of non-esterified fatty acid (NEFA), plasminogen activator inhibitor-1 (PAI-1), and leptin. FRD-fed CT rats, when compared with CT-ND rats, had high plasma levels of NEFA, triglyceride (TG), PAI-1, leptin, and adiponectin. The TP-FRD rats, when compared with TP-ND rats, displayed enhanced leptinemia and triglyceridemia, and were hyperinsulinemic, with glucose intolerance. The PM fat taken from TP rats displayed increase in the size of adipocytes, decrease in adiponectin (protein/gene), and a greater abundance of the leptin gene. PM adipocyte response to insulin was impaired in CT-FRD, TP-ND, and TP-FRD rats. A very short duration of isocaloric FRD intake in TP rats induced severe metabolic dysfunction at the reproductive age. Our study supports the hypothesis that the early-androgenized female rat phenotype is highly susceptible to developing endocrine/metabolic dysfunction. In turn, these abnormalities enhance the risk of metabolic syndrome, obesity, type 2 diabetes, and cardiovascular disease.

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INTRODUCTION

Menstrual disturbances, chronic anovulation, and hyperandrogenism in premenopausal women are frequently attributable to polycystic ovary syndrome (1). Hyperinsulinemia is also frequently associated with polycystic ovary syndrome (2–4). Clinical studies suggest that an interaction between insulin and sex hormones takes place in healthy subjects (5). Barraclough (6) reported that a single neonatal subcutaneous treatment with testosterone propionate (TP) in the female rat produces several clinical features of the human polycystic ovary syndrome. We too have previously reported that transient androgenization in normal female rats at neonatal (7) or early postpubertal (8) age impairs insulin sensitivity at the reproductive age.

Nowadays, fructose syrup has become a popular sweetener used in products of mass consumption. In the United States, the annual per capita fructose consumption rose from 0.2 kg in 1970 to 28 kg in 1997 (9). Evidence shows that increase in fructose intake has contributed to the current epidemics of obesity, type 2 diabetes, and metabolic syndrome (10). In humans, metabolic syndrome is defined as a cluster of clinical and metabolic abnormalities including overweight, changes in insulin sensitivity, dyslipidemia, and hypertension (11). Subjects who meet the minimal criteria of the Adult Treatment Panel–III for metabolic syndrome diagnosis (12) have an increased risk of developing cardio- and cerebrovascular diseases. Therefore a better understanding of the factors that

[1]Neuroendocrine Unit, IMBICE (CONICET-CICPBA), La Plata, Argentina; [2]Center for Experimental Biology and Laboratory Animal Sciences, National University of Litoral, Esperanza, Argentina; [3]Division of Endocrinology, Diabetology and Metabolism, University Hospital (Centre Hospitalier Universitaire Vaudois), Lausanne, Switzerland. Correspondence: Eduardo Spinedi (spinedi@imbice.org.ar)

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predispose toward the development of metabolic syndrome has acquired great relevance in the design and implementation of effective prevention strategies.

The administration of a fructose-rich diet (FRD) to a normal male rat induces several features of the metabolic syndrome (13,14), although the physiopathological mechanisms involved are still not fully understood. This allostatic load induced by excessive incorporation of a substrate (fructose) provides the same caloric intake as a normal balanced diet and lacks any direct insulin-enhancing effect (15).

Till date, no studies have been reported dealing with the metabolic consequences of FRD administration to early-androgenized female rats. The aim of this study, therefore, was to evaluate whether neonatal androgenization in the female rat is a predisposing factor for the development of metabolic disturbances shortly after allostasis induced by the intake of FRD.

METHODS AND PROCEDURES

Animals

The method employed for neonatal androgenization of female rats was validated in our laboratory (7). Briefly, on day 5 after birth, female rats (Sprague-Dawley) were subcutaneously injected with either 50 µl sterile corn oil alone (control (CT); n = 35/40) or with a similar volume containing 1.25 mg TP (n = 35/40). After weaning, the rats were housed individually, and their body weights (BW) were recorded daily (at 0730 to 0830 hours) up to the day of the experiment (age 100 days) (7). The animals were maintained under conditions of controlled light (light from 0700 to 1900 hours) and temperature (22 °C).

Design of the experiment

CT and TP rats were divided into two groups each: one group of rats from each type was fed ad libitum with standard lab chow and tap water (normal diet (ND) groups) for 3 weeks preceding the day of the experiment, while the other was fed the same diet with the addition of 10% fructose (wt/vol) to the drinking water (FRD groups). The FRD diet provided daily total calorie intake comparable to that provided by the normal diet (15,16). Fresh fructose solution was provided daily to the animals in the relevant groups. The animals were killed on day 100 of age in a nonfasting condition or after an intraperitoneal glucose tolerance test (IPGTT). Parametrial (PM) fat pads were dissected, weighed, and either kept frozen (at −80 °C) or processed immediately. The experiments were conducted in accordance with international regulations relating to the ethical use of animals, and were approved by our Animal Care Committee.

Peripheral metabolite measurements

Plasma glucose (Wiener Argentina Lab, Rosario, Argentina), total proteins (Wiener Argentina Lab), triglyceride (TG; Wiener Argentina Lab), and non-esterified fatty acid (NEFA; Randox Laboratories, Antrim, UK) levels were measured using commercial kits. Plasma and medium leptin (17) and concentrations of circulating insulin (8), testosterone (7), and corticosterone (18) were determined using specific radioimmunoassays developed in our laboratories. Plasma levels of other adipokines were assayed using commercial kits (Linco Research, St Charles, MO, cat. no. EZRADP-62K for adiponectin; American Diagnostica, Stamford, CT, IMUCLONE, cat. no. 601 for plasminogen activator inhibitor factor-1 (PAI-1); Life Diagnostics, West Chester, PA, cat. no. 2210-2 for C-reactive protein (CRP); and Amersham, GE Healthcare, Buckinghamshire, UK, cat. no. RPN2744 for tumor necrosis factor-α (TNF-α)).

IPGTT

Metabolic responses to high IP glucose load (2 g/kg BW) were measured in rats implanted with indwelling intravenous cannulas (right jugular vein, implanted 48 h earlier). Rats were bled before administration of glucose (time 0 min), and again at 30 and 120 min after glucose administration (19). Plasma samples were kept frozen (−20 °C) until analysis for determining the values of various parameters.

PM adipose tissue histology and adipocyte incubation

For histological study of adipocytes, freshly dissected PM fat pads were fixed in 4% paraformaldehyde (in 0.2 mol/l phosphate buffer) at 4 °C (maximum 3 days), washed (in 0.01 mol/l phosphate-buffered saline), and immersed in 70% ethanol (24 h) before being embedded in paraffin. Four-micrometer sections were obtained at different levels of the blocks and stained with hematoxylin–eosin, and examined using a Jenamed 2 Carl Zeiss light microscope. For quantitative morphometric analysis, a RGB CCD Sony camera was used, together with OPTIMAS software (Bioscan, Edmonds, WA) (×40 objective). For each PM fat sample, 1 section and 3 levels were selected (n = 4/5 animals per group). Systematic random sampling was used to select 10 fields for each section and 852–1,358 cells per group were examined. Adipocyte diameters were measured, and the volumes were calculated ($4/3\pi \cdot r^3$) (20).

Adipocytes isolated from PM fat pads were obtained as previously and extensively described (17,21). These adipocytes were diluted to ~200,000 cells per 900 µl medium and distributed into 15-ml plastic tubes. Cells were incubated with 100 µl of medium either alone (concentration 0) or containing insulin (0.1–10 nmol/l; Novo Nordisk Pharma, Copenhagen, Denmark) (21). The adipocytes were then incubated for 45 min at 37 °C in an atmosphere containing 95% air and 5% CO_2. Finally, the media were carefully aspirated and kept frozen (−20 °C) awaiting measurement of leptin concentrations.

RNA isolation from PM fat and real-time quantitative PCR

Total RNA was isolated from PM fat pads of different groups of animals by the single-step, acid guanidinium isothiocyanate–phenol–chloroform, extraction method (Trizol; Invitrogen, Life Technologies; cat. no. 15596-026) (17). One microgram of total RNA was reverse-transcripted using random primers (250 ng) and Superscript III RNase H-Reverse Transcriptase (200 U/µl; Invitrogen Life Technologies; cat. no. 18989-093). The primers applied were: β-actin (ACTB) (R): 5′-ACCCTCA TAGATGGGCACAG-3′, (F): 5′-AGCCATGTACGTAGCCATCC-3′ (115 pb) (GenBank accession no. (GBAN): NM_031144); ADIPOQ (R): 5′-TCTCCAGGAGTGCCATCTCT-3′, (F): 5′-AATCCTGCC CAGTCATGAAG-3′ (159 pb) (GBAN: NM_144744); LEP (R): 5′-CTCAGCATTCAGGGCTAAGG-3′, (F): 5′-GAGACCTCCTCCA TCTGCTG-3′ (192 pb) (GBAN: NM_013076); IRS1 (R): 5′-ACGG TTTCAGAGCAGAGGAA-3′, (F): 5′-TGTGCCAAGCAACAAGAA AG-3′ (176 bp) (GBAN: NM_012969); and IRS2 (R): 5′-CCAGGGA TGAAGCAGGACTA-3′, (F): 5′-CTACCCACTGAGCCCAAGAG-3′ (151 pb) (GBAN: AF08764). A volume of two microliters of the reverse transcription mix was amplified with QuantiTect SYBER Green PCR kit (Qiagen, cat. no. 204143) containing 0.5 µmol/l of each specific primer, using LightCycler Detection System (MJ MiniOpticon; Bio-Rad). The PCR efficiency was ~1. Threshold cycles (C_t) were measured in separate tubes in duplicate. The identity and purity of the amplified product were checked by electrophoresis on agarose mini-gels, and the melting curve was analyzed at the end of amplification. Values of the differences between the C_t were calculated in every sample for each gene of interest as follows: C_t gene of interest − C_t reporter gene. β-Actin, whose mRNA levels did not differ between CT and test groups, was the reporter gene. Relative changes in expression levels of any specific gene ($\Delta\Delta C_t$) were calculated as ΔC_t of the test group minus ΔC_t of the CT group, and presented as $2^{-\Delta\Delta C_t}$.

Determination of PM fat adiponectin by western blot

PM fat pads were homogenized in RIPA lyses buffer (Santa Cruz Biotechnology). Lysates were centrifuged at 10,000g for 10 min at 4 °C, and the protein concentration in the supernatants was determined. Concentrations of 50 µg protein per lane were resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The proteins were

then transferred onto polyvinylidene fluoride membranes and incubated overnight at 4 °C with specific rabbit antiadiponectin (Chemicon) followed by a 1-h incubation at room temperature with secondary antibody (goat anti-rabbit IgG horseradish peroxidase conjugates; Upstate, Millipore). Immune complexes were visualized using a CN/DAB substrate kit (Pierce). Data were expressed in relation to values obtained in the CT-ND group.

Statistical analysis

Data (mean values ± s.e.m.) were analyzed by ANOVA followed by post hoc comparisons using Fisher's test or the nonparametric Mann–Whitney test. Morphometric data were analyzed using the least significant difference test for multiple comparisons. Where appropriate, data were analyzed by ANOVA-2 factor (neonatal treatment × diet) (20).

RESULTS

Effects of FRD administration in normal and neonatally androgenized female rats on energy intake, BW, and PM fat characteristics

Neonatal androgenization alone did not modify daily total energy intake during the 3-week period before the day of the experiment (average: 18.85 ± 2.07 and 18.48 ± 1.41 cal/day/100 g BW in CT-ND and TP-ND groups, respectively). Similarly, 3-week administration of FRD to CT and TP rats resulted in similar daily total energy intakes in the two groups (average: 21.07 ± 2.01 and 21.09 ± 1.99 cal/day/100 g BW in CT-FRD and TP-FRD groups, respectively). Also, similar daily total energy intakes were recorded, regardless of diet, in the CT rats (CT-ND vs. CT-FRD groups) as well as in the TP rats (TP-ND vs. TP-FRD). It is also important to note that there was no significant difference in daily calorie intake between the FRD-fed groups (5.93 ± 1.15 and 6.89 ± 0.88 cal/day/100 g BW in CT-FRD and TP-FRD groups, respectively). Finally, the average of either daily food intake (in g/day/100 g BW) or daily food-derived calorie intake (cal/day/100 g BW) was significantly ($P < 0.05$) lower in FRD than in ND rats regardless of neonatal treatment (data not shown). Importantly, FRD intake did not modify the levels of circulating total proteins in the two groups examined (data not shown).

We found that BW and PM fat pad mass and adipocyte size/volume were significantly ($P < 0.05$) higher in TP-ND rats than in CT-ND ones (Table 1). Whereas there was no alteration in BW values between CT-FRD and CT-ND, nor between TP-FRD and TP-ND, there were differences in BWs between CT-FRD and TP-FRD groups (Table 1). FRD intake by CT rats induced a significant ($P < 0.05$ vs. CT-ND values) increase in both PM fat mass and PM adipocyte size/volume (Table 1). By contrast, FRD intake by TP rats did not further modify their PM fat characteristics relative to TP-ND rats; however, the PM fat pad mass was significantly ($P < 0.05$) higher in TP-FRD than in CT-FRD (Table 1).

Impact of FRD on the peripheral levels of several metabolites in CT and TP rats

CT and TP rats fed on ND displayed similar circulating levels of glucose (Table 2), insulin (Table 2), total testosterone (109 ± 27 and 82 ± 31 pg/ml, respectively), and corticosterone (5.17 ± 1.91 and 8.13 ± 2.05 µg/dl, respectively). Although neonatal androgenization (TP) did not change (in comparison with CT rats) the levels of total cholesterol (data not shown) and TG in the peripheral circulation in rats fed on ND, circulating NEFA concentrations were higher in TP-ND rats than in CT-ND ones (Table 2). FRD did not alter total cholesterol levels in either group (data not shown); however, CT-FRD rats displayed a significant ($P < 0.05$ vs. CT-ND values) increase in peripheral circulation levels of TG and NEFA (Table 2). A significant rise in triglyceridemia was found in TP-FRD rats ($P < 0.05$ vs. TP-ND values) (Table 2). Nonfasting glycemia was not modified by treatment or by diet (Table 2). Whereas insulinemia was similar in CT-ND and TP-ND rats, FRD significantly

Table 1 Body weight and parametrial fat mass characteristics in CT and TP rats fed on an ND or an FRD

	ND	FRD
CT group		
BW (g)	267.45 ± 12.77	281.39 ± 7.96
PM fat (g/100 g BW)	0.91 ± 0.12	1.26 ± 0.13*
PM cell diameter (µm)	57.9 ± 0.7	60.9 ± 0.6*
PM cell volume (µm^3 × 10^3)	120.9 ± 4.1	139.3 ± 4.5*
TP group		
BW (g)	312.61 ± 13.81*	320.18 ± 6.85**
PM fat (g/100 g BW)	1.59 ± 0.13*	1.78 ± 0.21**
PM cell diameter (µm)	61.1 ± 0.8*	62.6 ± 0.7
PM cell volume (µm^3 × 10^3)	151.1 ± 5.7*	152.7 ± 4.4

Data are mean values ± s.e.m., n = 8–10 rats per group/condition.
BW, body weight; CT, control; FRD, fructose-rich diet; ND, normal diet; PM, parametrial; TP, testosterone propionate.
*$P < 0.05$ vs. respective CT-ND values. **$P < 0.05$ vs. respective CT-FRD values.

Table 2 Peripheral concentrations of lipids, glucose, and insulin in CT and TP rats, fed on an ND or an FRD

	ND	FRD
Triglyceride (g/l)		
CT	0.76 ± 0.07	1.19 ± 0.11*
TP	1.17 ± 0.16	2.03 ± 0.19**,***
NEFA (mmol/l)		
CT	0.49 ± 0.04	0.61 ± 0.03*
TP	0.62 ± 0.04*	0.64 ± 0.07
Glucose (g/l)		
CT	1.06 ± 0.05	1.01 ± 0.04
TP	1.12 ± 0.08	1.03 ± 0.03
Insulin (ng/ml)		
CT	1.37 ± 0.32	1.39 ± 0.28
TP	1.93 ± 0.19	3.07 ± 0.45**,***

Data are mean values ± s.e.m., n = 6–7 rats per group/condition.
CT, control; FRD, fructose-rich diet; ND, normal diet; NEFA, non-esterified fatty acid; PM, parametrial; TP, testosterone propionate.
*$P < 0.05$ vs. respective CT-ND values. **$P < 0.05$ vs. respective CT-FRD values. ***$P < 0.05$ vs. respective TP-ND values.

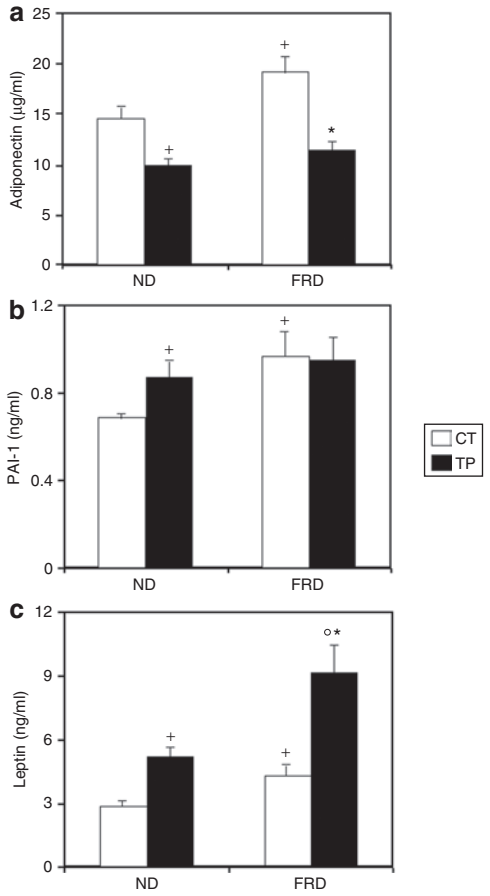

Figure 1 Peripheral concentrations of (**a**) adiponectin, (**b**) plasminogen activator inhibitor-1 (PAI-1), and (**c**) leptin in 100-day-old control (CT) and testosterone propionate (TP) rats fed on a normal diet (ND) or a fructose-rich diet (FRD). Data are expressed as mean values ± s.e.m., $n = 6–7$ animals per group. $^{+}P < 0.05$ vs. CT-ND values. $^{*}P < 0.05$ vs. CT-FRD values. $^{\circ}P < 0.05$ vs. TP-ND values.

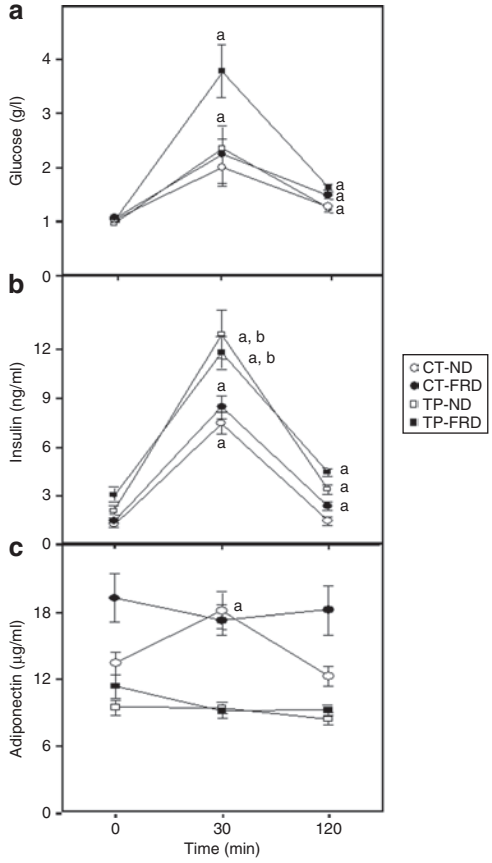

Figure 2 Circulating levels of (**a**) glucose, (**b**) insulin, and (**c**) adiponectin during the intraperitoneal glucose tolerance test in control (CT) and testosterone propionate (TP) rats fed on a normal diet (ND) or a fructose-rich diet (FRD). Data are expressed as mean values ± s.e.m. ($n = 6$ rats per group). $^{a}P < 0.05$ vs. time-zero values in the same group. $^{b}P < 0.05$ vs. CT-ND and CT-FRD values on time 30 min.

($P < 0.05$ vs. TP-ND rats) enhanced insulinemia in TP rats, even in comparison with CT-FRD rats (Table 2).

Effects of FRD administration on the peripheral adipokine levels in normal and neonatally androgenized rats

It has earlier been shown that, when TP rats are fed on ND, there is distortion in the pattern as regards several of the adipokines in the circulation. This was characterized by a significant ($P < 0.05$ vs. CT-ND values) decrease in adiponectinemia and increase in plasma levels of PAI-1 and leptin (Figure 1). The levels of other circulating adipokines remained unchanged: (CRP levels of 0.65 ± 0.18 and 0.62 ± 0.05 mg/ml for CT-ND and TP-ND, respectively; TNF-α levels of 25.45 ± 3.16 and 21.94 ± 1.46 pg/ml for CT-ND and TP-ND, respectively). FRD administration to CT animals significantly ($P < 0.05$ vs. CT-ND values) enhanced the peripheral circulation levels of adiponectin, PAI-1, and leptin (Figure 1a–c). FRD given to TP rats induced a further significant increase ($P < 0.05$ vs. TP-ND values) in circulating leptin levels (Figure 1c). The circulating levels of other adipokines were not altered as a result of FRD in either CT or TP animals (CT-FRD rats, CRP: 0.39 ± 0.11 mg/ml and TNF-α: 20.36 ± 1.66 pg/ml; TP-FRD rats, CRP: 0.46 ± 0.15 mg/ml and TNF-α: 24.31 ± 4.09 pg/ml). This was so even in comparison with corresponding levels in CT-ND and TP-ND rats.

Concentration profiles of glucose, insulin, and adiponectin in peripheral circulation during IPGTT in experimental animals

At 30 min, IPGTT showed similar levels of glycemia in CT-ND, CT-FRD, and TP-ND rats, whereas TP-FRD rats had an ~1.7-fold higher ($P < 0.05$) level of glycemia. At 120 min, the values returned to baseline only in the CT-ND rats (Figure 2a). An analysis of the dynamic changes in insulinemia indicated that peak values (30 min) were higher ($P < 0.05$) in TP animals than in CT animals, regardless of diet (Figure 2b). At 120 min, although CT-ND rats had returned to basal insulinemia levels, the remaining groups had not ($P < 0.05$ vs. respective baseline; graded order: TP-FRD > TP-ND > CT-FRD) (Figure 2b). Interestingly, during the test, only CT-ND rats showed a significant increase in peripheral adiponectin levels (Figure 2c). Data from areas under the curve during IPGTT indicated (Table 3) that only TP-FRD rats displayed glucose intolerance,

although both TP groups showed higher ($P < 0.05$) areas under the curve of insulinemia. Interestingly, TP-FRD rats displayed a lower ($P < 0.05$) area under the curve of peripheral adiponectin peripheral levels.

Effects of neonatal treatment and diet on PM fat genes and adiponectin abundance

In accordance with data relating to peripheral adipokine levels, whereas PM fat ADIPOQ mRNA expression was augmented ($P < 0.05$ vs. CT-ND values) in CT-FRD rats, it was reduced ($P < 0.05$ vs. CT-ND values) in TP rats regardless of diet (Figure 3, upper left). PM fat LEP mRNA expression was higher ($P < 0.05$) in TP rats than in CT rats regardless of diet (Figure 3, lower left). No group-related differences were found as regards the abundance of PM fat IRS1 and IRS2 mRNAs (data not shown). The enhanced PM fat ADIPOQ gene expression found in CT-FRD rats was in agreement with the protein values obtained by western blot (Figure 3, right) (in arbitrary units, 1.49 ± 0.14 vs. 1.01 ± 0.05, CT-FRD vs. CT-ND; $P < 0.05$, $n = 3$ experiments). Also, TP-ND rats displayed a decreased abundance of protein adiponectin in PM fat (0.73 ± 0.08 arbitrary units, $P < 0.05$ vs. CT-ND values; $n = 3$ experiments), and these values remained unaltered in the TP-FRD rats as well (0.64 ± 0.11 arbitrary units).

Table 3 Areas under the curves of circulating glucose, insulin, and adiponectin levels during the IPGTT in CT and TP rats, fed on an ND or an FRD

	ND	FRD
Glucose (g/l/2 h)		
CT	1.45 ± 0.46	1.59 ± 0.57
TP	1.89 ± 0.65	3.42 ± 0.66**,***
Insulin (ng/ml/2 h)		
CT	6.55 ± 1.17	7.91 ± 2.18
TP	10.21 ± 1.99*	11.33 ± 1.26**
Adiponectin (μg/ml/2 h)		
CT	3.09 ± 1.29	–2.88 ± 3.66
TP	0.77 ± 1.69	–6.77 ± 2.98**,***

Data are mean values ± s.e.m., $n = 6–7$ rats per group/condition.
CT, control; FRD, fructose-rich diet; IPGTT, intraperitoneal glucose tolerance test; ND, normal diet; PM, parametrial; TP, testosterone propionate.
*$P < 0.05$ vs. respective CT-ND values. **$P < 0.05$ vs. respective CT-FRD values. ***$P < 0.05$ vs. respective TP-ND values.

In vitro functionality of isolated PM adipocytes

An analysis of the functionality of adipocytes isolated from PM fat indicated that spontaneous (stimulus concentration zero) leptin release followed the order: TP-FRD > TP-ND = CT-FRD > CT-ND (Figure 4). After the cells were incubated with insulin (0.1–10 nmol/l), the release of leptin into the medium occurred in a concentration-related fashion, regardless of cell group (Figure 4). Moreover, we found that CT-ND cells released a higher ($P < 0.05$) amount of leptin than the respective baseline value, regardless of insulin concentration. By contrast, in CT-FRD and TP-ND adipocytes, only insulin ≥1 nmol/l concentration induced an increase ($P < 0.05$) in leptin release over the baseline (Figure 4). The lowering of the insulin threshold was even more pronounced in TP-FRD adipocytes, with an insulin level of 10 nmol/l being capable of increasing ($P < 0.05$ vs. baseline) leptin output (Figure 4).

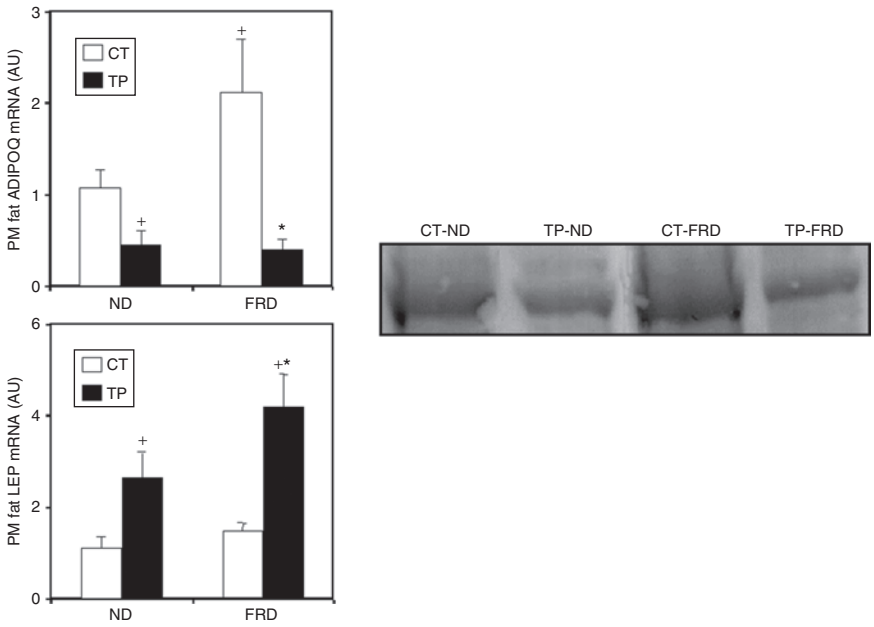

Figure 3 Adiponectin (upper left) and leptin (lower left) mRNA concentrations measured (by quantitative PCR) in parametrial (PM) fat pads from control (CT) and testosterone propionate (TP) rats fed on a normal diet (ND) or a fructose-rich diet (FRD). PM fat pad adiponectin, as detected by western blot, in different groups is also shown (right panel). Data are representative of at least three separate experiments using samples from different groups. Data are expressed as mean values ± s.e.m. ($n = 3–4$ pads per group). +$P < 0.05$ vs. CT-ND values. *$P < 0.05$ vs. CT-FRD values. AU, arbitrary units.

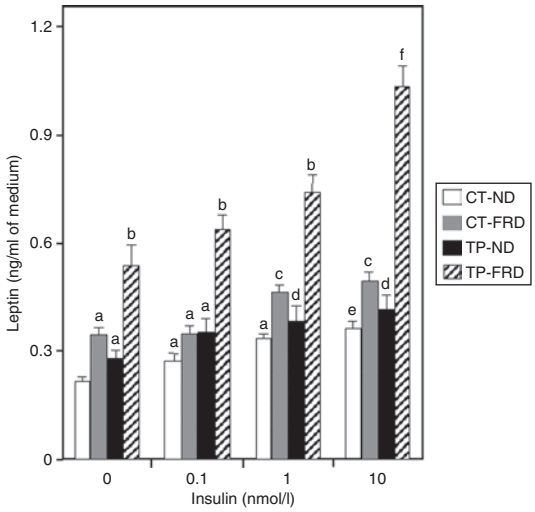

Figure 4 Effect of insulin (0.1–10 nmol/l) on leptin release by adipocytes isolated from parametrial (PM) fat pads of control (CT) and testosterone propionate (TP) rats fed on a normal diet (ND) or a fructose-rich diet (FRD). Data are expressed as mean values ± s.e.m.; each condition was run in five replicates per experiment (n = 3–5 experiments). [a]$P < 0.05$ vs. 0 nmol/l insulin values in the CT-ND group. [b]$P < 0.05$ vs. values in other groups for the corresponding condition. [c]$P < 0.05$ vs. 0 nmol/l insulin values in the CT-FRD group. [d]$P < 0.05$ vs. 0 nmol/l insulin values in the TP-ND group. [e]$P < 0.05$ vs. 0 and 0.1 nmol/l insulin values in the CT-ND group. [f]$P < 0.05$ vs. remaining values in the TP-FRD group.

DISCUSSION

Our study shows, for the first time, the deleterious metabolic effects of FRD administration in neonatally androgenized female rats studied at adult age.

Several metabolic disturbances in the early-androgenized female rat model have been described by us (7) and by several authors (22,23). In the present work we add new data from this rat model, showing comparisons within the group as well as comparisons with normal littermates, as regards: (i) hypoadiponectinemia and high peripheral levels of NEFA and PAI-1 and (ii) PM fat characterized by underproduction of adiponectin and overexpression of the leptin gene, and an increase in the size/volume of adipocytes. Although there are some discrepancies in relation to data from earlier reports, probably on account of differences in the animal model used, early androgenization was shown to induce dyslipidemia. In fact, early androgenization has been reported to raise the levels of serum cholesterol and TG (22,23). Although we did not find similar data, either in our earlier study (7) nor in this one, the rats were shown to display increased serum levels of NEFA. Moreover, while enhanced nonfasting insulinemia has been reported in prenatally androgenized rats (23), we did not observe this in our studies (7 and present data). However, alteration in insulin sensitivity seem to be a common feature regardless of the androgenization model employed (7,22,23 and present data). An examination of regionalized fat depots indicates that hyperadiposity (7,22,23 and present data) and enlarged adipocytes (22) appear to develop as a result of transient hyperandrogenemia. For instance, increases in visceral and mesenteric fat (7,22) as well as in PM fat pads (23 and present data) were reported in neonatally androgenized rats. Additionally, these rats also show enlarged mesenteric (22) and PM (present data) adipocytes. Interestingly, the administration of FRD for 3 weeks to normal rats resulted in enlarged PM fat mass and adipocytes, mimicking the effects of neonatal androgenization. The analysis of changes in PM fat indicates that there was a synergy between neonatal treatment and diet ($P < 0.05$, ANOVA 2 factor). A similar significant interaction ($P < 0.05$) was found with regard to changes in leptinemia.

We also demonstrated, for the first time, that the administration of FRD for 3 weeks to normal female rats induced early changes in the peripheral levels of NEFA, leptin, and PAI-1, and that these events were accompanied by enhanced adiponectin concentration in both body compartments: blood (protein) and PM fat (protein/mRNA). In both CT and TP rats, FRD administration induced a rise in plasma levels of TG. Although the increase was more pronounced in TP rats, it was shown that even in the absence of treatment there was a diet-related effect on the levels of these parameters. FRD intake enhanced the peripheral levels of NEFA in CT rats, reaching values similar to those of TP rats on ND. FRD (vs. ND) administration elevated basal insulinemia in TP rats only; interestingly, these rats were also intolerant to glucose overload. FRD intake also augmented plasma PAI-1 levels in normal rats (vs. CT-ND values), reaching peripheral concentrations similar to those in TP-ND rats.

Of relevance, we show for the very first time the physiological dynamic changes in circulating adiponectin levels during glucose overload as a necessary mechanism for adequate management of peripheral carbohydrate metabolism in normal rats. By contrast, basal metabolism as well as carbohydrate metabolism stimulated by high glucose load CT-FRD rats, probably because of a favorable background involving enhanced endogenous adiponectin production (24). These characteristics were found alongside a slight and probably adaptive reduction in the insulin sensitivity of leptin-releasing adipocytes isolated from CT-FRD PM. Moreover, there was a decrease in PM adipocyte sensitivity to insulin-induced leptin release in cells from TP rats, regardless of diet. Some, though not all, of these features are also observed in the human phenotype of the metabolic syndrome, clearly indicating that an allostatic process is in progress even at an early phase of disease development (25).

Importantly, this is the first study to report that a normal rat fed on FRD displays an enhancement in adiponectin production by its PM adipose tissue. This finding tallies with data from studies in humans indicating that increased adiponectinemia could explain defects in insulin receptor function (26), and those from studies in mice with inactivated adipocyte insulin receptors (27). In contrast to normal rats, TP animals showed that adiponectin could be a main signal that is distorted for the success of insulin action (28). This observation is in accordance with data indicating that androgen treatment in normal men decreases adiponectinemia (29), probably because of an androgen receptor–mediated effect (30) at the adipocyte level (31). Therefore alterations in adipocyte size and/or function could be partly responsible

for the disturbances observed in carbohydrate metabolism in TP rats, the situation being further exacerbated after FRD intake.

Although previous studies have reported that early androgenization in female mammals produces metabolic abnormalities in adulthood (7,22,23), this is the first study on the metabolic consequences of excessive fructose intake, a substrate that does not directly enhance β-cell activity (15), in the androgenized rat. The contribution of the effect of body characteristics of the androgenized rat to different allostatic loads has not yet been fully investigated. A recent work (23) studied the effects of high-fat diet (HFD) administration to the prenatal-androgenized female rat. HFD is hypercaloric and greatly modifies both adiposity mass and lipid function. An important and remarkable difference in metabolism between HFD-induced and FRD-induced allostasis in androgenized rats is that HFD-fed rats remained tolerant to glucose overload (23) whereas the FRD-fed rats in our study did not. In the normal rat, HFD-enhanced insulinemia was attributed to β-cell hyperplasia (32); on the contrary, in rats fed a carbohydrate-rich diet, this characteristic seems to be caused by enhanced β-cell activity (33) and also by changes in their cell glucose transporter system (34). In this study, we demonstrated that leptin overproduction (plasma and PM adipocyte release) in TP rats is another metabolic dysfunction that is exacerbated by FRD intake, leptin being a pivotal signal modifier of insulin activity (7,35). Although neither HFD (31) nor FRD was shown to modify the abundance of IRS1/IRS2, the reduced production of adiponectin, which is an endogenous enhancer of insulin activity (28), could be a crucial factor in this abnormality in TP rats. Nevertheless, androgen/diet-dependent changes in the activity levels of other mediators of insulin action (36) cannot be ruled out. In fact, we found earlier that postpubertal androgenization in the female rat (8) enhances β-cell response and impairs PM fat adipocyte response to insulin. In that study, we also demonstrated that a supraphysiological concentration of testosterone reduces the *in vitro* leptin-releasing action of insulin on normal PM adipocytes (8). These observations further suggest that transient hyperandrogenemia can trigger metabolic adjustments such as a compensatory β-cell hyperfunction (37). As regards underlying physiopathological mechanisms induced by short-term FRD intake, a rise in (general but mainly abdominal) fat tissue oxidative stress has been reported in adult male rats (16). This fat tissue derangement has been clearly demonstrated as an early instigator of metabolic syndrome (38,39). As we have now shown, a condition involving high oxidative stress could favor the enhancement of peripheral levels of lipids and PAI-1.

In summary, we have demonstrated several deleterious metabolic effects of excessive daily fructose consumption, even over a very short period of time. It could silently, but significantly, increase an individual's risk of developing metabolic syndrome, obesity, type 2 diabetes, and cardiovascular disease. This risk would be further exacerbated if the individual is a high androgen–primed phenotype, despite the consumption of an isocaloric low-fat diet.

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DISCLOSURE

The authors declared no conflict of interest.

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