Alterations in Neutrophil Production and Function at an Early Stage in the High-Fructose Rat Model of Metabolic Syndrome

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BACKGROUND
Although neutrophils are crucially involved in inflammation, they have received only little attention in metabolic syndrome (MetS). We hypothesized that neutrophil infiltration into adipose tissue (AT) may occur at an early stage of MetS, in association with modulation of major functions of neutrophils and of their bone marrow production.

METHODS
Fifty-six male Sprague-Dawley rats were fed regular (control rats (CRs)) or high-fructose (60%; fructose-fed rats (FFRs)) diets. After 6 weeks, metabolic parameters were measured. Distribution of neutrophils into AT was investigated by immunohistochemistry. Function of circulating neutrophils (activation, reactive oxygen species production, phagocytosis, and apoptosis) was determined by flow cytometry. Granulopoiesis was evaluated by measuring the number and survival characteristics of neutrophil precursors using bone marrow culture assays and flow cytometry.

RESULTS
Compared with the CR group, the FFR group developed MetS (i.e., arterial hypertension, hypertriglyceridemia, fasting hyperglycemia, and greater intra-abdominal AT volume) and presented higher neutrophil infiltration into AT. At resting state, no significant difference for circulating neutrophil functions was observed between the 2 groups. In contrast, circulating neutrophils from the FFR group exhibited higher responses to phorbol-12-myristate-13-acetate for all studied functions, compared with the CR group, suggesting that early MetS induces neutrophil priming. In parallel, a diminished clonal capacity and an increased apoptosis in bone marrow–derived granulocyte progenitors and neutrophil precursors were observed in the FFR group compared with the CR group.

CONCLUSIONS
These results provide evidence of an increased infiltration into intra-abdominal AT and modified production, function, and phenotype of neutrophils at an early stage of high-fructose diet–induced MetS.

Keywords: blood pressure; fructose; hypertension; metabolic syndrome; neutrophils; rat.

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In recent years, it has become clear that the inflammatory response is intrinsically linked to metabolic pathways in vertebrates. Thus, a chronic and systemic low-grade inflammatory state is observed in metabolic syndrome (MetS), defined by a cluster of metabolic anomalies, including intra-abdominal adiposity, dyslipidemia, high blood pressure, and altered glucose tolerance. The prevalence of MetS in developed countries has dramatically increased worldwide because of modern lifestyle and an increased consumption of high-sugar diets, especially diets high in fructose. However, the pathophysiology of MetS is complex and remains incompletely understood. Our group has reported MetS to be associated with alterations in hematopoietic progenitors. Neutrophils derived from bone marrow colony-forming unit granulocyte/macrophage (CFU-GM) progenitors dominate the early stages of inflammation and set the stage for tissue infiltration by macrophages. By contrast with macrophages, neutrophils have classically received only little attention in MetS. Generally, these cells were rarely detected in adipose tissue (AT).

In this study, we hypothesized that neutrophil infiltration into AT may occur at an early stage of MetS in association with modulation of major functions of peripheral neutrophils and that MetS could alter production of neutrophils by bone marrow. To test these hypotheses, we analyzed circulating neutrophil functions, their AT infiltration levels, and bone marrow efficiency in producing neutrophils at an early stage in a high-fructose-fed Sprague-Dawley rat model. This model is currently used to study MetS-related changes.

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METHODS

Animals, diets, and study design

All animal procedures conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret-87/848) for the care and use of laboratory animals.

A total of 56 male Sprague-Dawley rats (aged 4 weeks; Janvier, Le Genest, France) weighing 125–150 g were housed in cages (n = 4 animals per cage) with a light–dark cycle of 12 hours under controlled environmental conditions. Rats were randomly divided into 2 groups, a control rat (CR) group receiving regular Chow consisting of 60% starch, 16% animal proteins, 4% fat, 4% cellulose, and standard vitamins and mineral mix and a fructose-fed rat (FFR) group receiving Chow consisting of 21% animal proteins, 5% fat, 5% cellulose, and standard vitamins and mineral mix and supplemented with 60% fructose. Food (Safe, Augy, France) and water were available ad libitum during the 6 weeks of the experiment.

At the end of the experiment, systolic blood pressure was measured (mean of 5 consecutive readings) by the tail-cuff method using an automated blood pressure monitoring system in conscious rats (Bioseb, Chaville, France). Total intra-abdominal AT volume measured by in vivo magnetic resonance imaging was performed on a 7T Bruker Biospec imaging system (Ettlingen, Germany) and calculated using OsiriX medical imaging software V3.9.4 (Pixmeo, Geneva, Switzerland).

Before rats were killed, all rats were fasted for 1 night, weighed, and anesthetized with isoflurane (5% for induction and then 2%). Blood collection into heparinized and plain tubes was performed by cardiac puncture. The hearts, livers, and kidneys were isolated and weighed.

Serum glucose, triglycerides, and total cholesterol were measured using the Vet Test 8008 (IDEXX Laboratories, Westbrook, ME). Rat enzyme-linked immunosorbent assay kits were used to measure serum insulin (Mercodia, Uppsala, Sweden), serum adipokines (adiponectin, Euromedex, Mundolsheim, France; resistin and leptin, Gentaur, Paris, France), and plasma myeloperoxidase (MPO, Euromedex). Insulin resistance was assessed using the homeostasis model assessment (HOMA-IR) according to the formula: HOMA-IR (mmol/L × μU/mL) = fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5.8

Neutrophil marker analysis in intra-abdominal AT

Intra-abdominal AT was excised immediately after cardiac puncture, frozen in liquid nitrogen, and stored at −80°C until analysis. Serial 7-μm cryosections were stained with May-Grünwald-Giemsa (May-Grünwald-Giemsa, Glostrup, Denmark). The nuclei were counterstained with nuclear fast red (Sigma Aldrich, St Louis, MO). Neutrophil infiltration was quantified by counting the mean number of Ly6G-positive cells per 10 random high-power fields (from 3 cryosections per rat for 4 individual rats per group) using a ×400 magnification (Leitz, Wetzlar, Germany). The second part of the AT sample was prepared by homogenization with Ultra-Turrax T25 for 30 seconds at 24,000 rpm (Ika, Staufen, Germany) in ice-cold extraction buffer (10 mM Tris hydrochloride, 140 mM sodium chloride, 5 mM ethylenediaminetetraacetic acid, 1% Triton, 1% deoxycholate). After determination of protein concentration using Bradford assay, MPO and proteinase 3 (PR3) were assayed using rat enzyme-linked immunosorbent assay kits (Euromedex).

Whole blood flow cytometric assay

Heparinized blood sample was used to determine neutrophil functions. For data acquisition, a FACS Canto II flow cytometer equipped with the FACS Diva software (BD Biosciences, Franklin Lakes, NJ) was used. One hundred thousand events were collected in the neutrophils’ gate based on their light scatter properties (forward-angle light scatter channel (FSC) intermediate/side-angle light scatter channel (SSC) high) and their high expression of CD11b and CD18.

Surface receptors expression on resting or phorbol-12-myristate-13-acetate–incubated (PMA, 1 μM, 15 minutes at 37°C; Sigma Aldrich) neutrophils was measured using mouse antirat CD18-fluorescein-5-isothiocyanate (FITC) conjugated (clone WT;3; AbD Serotec, Düsseldorf, Germany) and antirat CD11b-phycocerythrin (PE) conjugated (clone MRC0X-42; AbD Serotec). Reactive oxygen species (ROS) production was quantified by measuring the increase in fluorescence generated by hydrogen peroxide–mediated oxidation of dihydroorhodamine-123 (DHR-123, 2.5 μg/ml; Molecular Probes, Eugene, OR). Neutrophil phagocytic activity was determined using the Phagotest Kit (Orpegen-Pharma, Heidelberg, Germany) and reported as the mean fluorescence intensity of cells incubated at 37°C for 10 minutes with FITC-labeled opsonized Escherichia coli, minus the mean fluorescence intensity of cells incubated at 4°C. Apoptosis of neutrophils was determined using FITC-conjugated annexin V (apoptosis marker) and propidium iodide (necrosis marker) following manufacturer’s protocol (Miltenyi-Biotec, Bergisch Gladbach, Germany).

Bone marrow CFU-GM and granulocytic cells apoptosis assays

In isoflurane-anesthetized rats (n = 20 rats per group), bone marrow was collected from femur and tibia in citrate dextrose solution Macoflex (MacoPharma, Mouvaux, France). Ficoll-purified bone marrow cells (FP-BMCs) were obtained by density gradient centrifugation with Ficoll-Paque (density = 1.077; Amersham Pharmacia, Uppsala, Sweden). Immediately after isolation, FP-BMCs were plated on methylcellulose-coated culture dishes for 10 days (Methocult GFR3774; StemCell Technologies, Vancouver, Canada) to obtain CFU-GM colonies. The experiment was done in triPLICATE. Apoptosis of granulocyte and monocytes cell population in FP-BMCs was quantified using the annexin V/propidium iodide method after gating on their respective light scatter properties (granulocyte: FSC intermediate/SSC high; monocyte: FSC high/SSC low). Moreover, total RNA from FP-BMCs was isolated to assess the expression of apoptosis-stimulating processes.

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fragment (FAS) using TRIzol reagent (Life Technologies, Eggenstein, Germany). Two micrograms of total RNA were reverse transcribed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to manufacturer’s procedures. Quantitative real-time reverse transcription polymerase chain reaction was performed with LightCycler (Roche Diagnostics, Indianapolis, IN) involving the SYBR-based method following the manufacturer’s instructions. The polymerase chain reaction primers for FAS were 5′-ACCTGGTGCGGTCAACGTTA-3′ (forward) and 5′-GCCAGACCATGCGTTGAATT-3′ (reverse) and for 18S they were 5′-TCCCAGTAAGTGCGGGTCACA-3′ (forward) and 5′-GATCGAGGGCCTACTAAC-3′ (reverse). Reactions were performed in triplicate and normalized by dividing the relative FAS transcripts level by relative 18S RNA transcripts level. Quantitative values were obtained from the threshold cycle value (Ct), the point at which a significant increase of fluorescence is first detected. The relative changes in gene expression were analyzed with the 2^(-Delta Delta Ct) method.9

Statistical analysis

Values are presented as mean ± SEM, and statistical analyses were performed using Mann–Whitney U test to compare CR and FFR groups, except for flow cytometry results, for which paired data were analyzed by the Wilcoxon signed-rank test. Nonparametric correlation was performed using the Spearman rank correlation coefficient. P < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism version 5.02 (GraphPad Software, San Diego, CA).

Table 1. Metabolic parameters in rats fed with the control or the high-fructose diet (n = 56)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CR</th>
<th>FFR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>7.43 ± 0.004</td>
<td>10.54 ± 0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin, µU/ml</td>
<td>400 ± 50</td>
<td>381.5 ± 47</td>
<td>0.80</td>
</tr>
<tr>
<td>HOMA-IR, mmol/L × µU/mL</td>
<td>3.54 ± 0.6</td>
<td>5.96 ± 0.94</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum triglycerides, mM</td>
<td>0.38 ± 0.02</td>
<td>0.52 ± 0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum total cholesterol, mM</td>
<td>4.9 ± 0.18</td>
<td>6.14 ± 0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>113 ± 1.8</td>
<td>127 ± 2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Relative heart weight, mg/100 g body weight</td>
<td>338 ± 6</td>
<td>360 ± 9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Relative liver weight, mg/100 g body weight</td>
<td>283 ± 9</td>
<td>323 ± 17</td>
<td>0.08</td>
</tr>
<tr>
<td>Relative kidney weight, mg/100 g body weight</td>
<td>331 ± 8</td>
<td>473 ± 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total body weight gain, g</td>
<td>245 ± 32</td>
<td>220 ± 28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intra-abdominal AT volume, cm³</td>
<td>6.48 ± 0.37</td>
<td>8 ± 0.57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum leptin, pg/ml</td>
<td>520 ± 83</td>
<td>710 ± 71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum adiponectin, ng/ml</td>
<td>5,683 ± 418</td>
<td>5,588 ± 406</td>
<td>0.67</td>
</tr>
<tr>
<td>Serum resistin, ng/ml</td>
<td>14.39 ± 1.45</td>
<td>12.29 ± 1.11</td>
<td>0.32</td>
</tr>
<tr>
<td>Adiponectin/leptin ratio</td>
<td>13.6 ± 1.4</td>
<td>8.3 ± 0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma MPO activity, U/µl</td>
<td>0.45 ± 0.05</td>
<td>0.35 ± 0.04</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Statistical analyses were performed using Mann–Whitney U test to compare CRs and FFRs. Abbreviations: AT, adipose tissue; CR, control rat; FFR, fructose-fed rat; HOMA-IR, homeostasis model assessment insulin resistance; MPO, myeloperoxidase.

RESULTS

Effect of high-fructose diet on physiological parameters and adipokines levels

Table 1 summarizes the metabolic parameters of our Sprague-Dawley rats, the CR vs. the FFR group, after 6 weeks of specific diet. All rats survived to the end of the experiment. A higher fasting glycemia and a higher HOMA-IR were observed in the FFR group compared with the CR group. At the same time, no difference in serum insulin levels was observed. Analysis of serum lipid parameters revealed a significantly increased circulating level of triglycerides and total cholesterol in the FFT group compared with the CR group. Moreover, systolic blood pressure was significantly elevated in the FFR group. A significant increase of heart and kidney relative weights (organ-to-body ratio) was noted in the FFR group compared with the CR group. Concerning the relative liver weight, we observed a tendency of increase in the FFR group. Both diets caused a significant positive body weight gain from the baseline weight for all animals. Although the FFR group did not gain much total body weight compared with the CR group, the intra-abdominal AT volume by magnetic resonance imaging was significantly increased. These results are clearly in favor of metabolic anomalies induced by a 6-week high-fructose diet, compatible with the MetS definition.

Circulating leptin levels, but not adiponectin and resistin, were significantly higher in the FFR group compared with the CR group. Inversely, the adiponectin-to-leptin ratio was significantly lower in the FFR group compared with the CR group. No difference in plasma MPO levels was observed between rats.
Effect of high fructose diet on neutrophil infiltration

Significantly higher concentration of MPO and PR3 in intra-abdominal AT homogenates were observed in the FFR group vs. the CR group (5.74 ± 0.79 vs. 2.06 ± 0.3 ng/mg protein, P < 0.005; 10.98 ± 2.07 vs. 5.06 ± 0.7 ng/mg protein, P < 0.01, respectively) (Figure 1a). Neutrophils in intra-abdominal AT in the CR and FFR groups were observed by microscopic analysis of MGG- and Ly6G-stained AT (Figure 1b). Analysis of Ly6G-positive cells of intra-abdominal AT revealed a significantly higher neutrophil count in the FFR group vs. the CR group (35 ± 2 vs. 18.7 ± 2.5; P < 0.05) (Figure 1c).

Figure 1. Neutrophil infiltration into intra-abdominal adipose tissue (AT) from the control rat (CR) and the fructose-fed rat (FFR) group. (a) Myeloperoxidase (MPO) and proteinase 3 (PR3) quantification in AT homogenates (n = 6 per group). (b) Representative histological sections of intra-abdominal AT staining with May-Grünwald-Giemsa (MGG; arrow showing neutrophil) and anti-Ly6G (in blue) counterstained with nuclear red. (c) Mean number of Ly6G-positive cells per 10 fields in AT sections (n = 4 per group). *P < 0.05, **P < 0.01, ***P < 0.005. Values are given as mean ± SEM.
Effect of high-fructose diet on circulating neutrophil functions

Regarding early consequences of a high-fructose diet on resting circulating neutrophils, there was no significant upregulation of CD18 and CD11b surface expression in the FFR group relative to the CR group (1.12 ± 0.04% fold increase, \( P = 0.40 \); 1.13 ± 0.06% fold increase, \( P = 0.60 \), respectively) (Figure 2a,b). Regarding PMA-activated neutrophils, there was a significant upregulation of CD18 and CD11b surface expression in the FFR group relative to the CR group (2.73 ± 0.01% vs. 2.05 ± 0.01% fold increase, \( P < 0.05 \); 3.69 ± 0.03% vs. 3.10 ± 0.02% fold increase, \( P < 0.05 \), respectively) (Figure 2a,b).

Concerning ROS production in resting neutrophils, there was no significant difference in the FFR group relative to the CR group (1.10 ± 0.08% fold increase; \( P = 0.30 \)) (Figure 2c). For PMA-activated neutrophils, ROS production was significantly higher in the FFR group compared with the CR group (2.4 ± 0.02 vs. 1.7 ± 0.01 fold increase; \( P < 0.005 \)) (Figure 2c).

After activation with opsonized E. coli, significantly increased neutrophil phagocytic activity was observed in the FFR group compared with the CR group (1.3 ± 0.02 fold increase; \( P < 0.005 \)) (Figure 2d). Moreover, the percentage of apoptotic neutrophils in the total leukocyte population tended to be higher in the FFR group than in the CR group (20.6 ± 2.4% vs. 15.8 ± 1.6%; \( P = 0.10 \)) (Figure 2e).

Figure 2. Neutrophil surface receptors relative expressions and functions in resting (−) and phorbol 12-myristate 13-acetate (PMA) (+, 1 µM) or Escherichia coli–activated neutrophils. (a) CD18 expression, (b) CD11b expression, (c) reactive oxygen species production, (d) phagocytic activity, and (e) apoptosis by annexin V+/propidium iodide (PI)−. Neutrophils were identified by their light scatter properties (forward-angle light scatter channel (FSC) intermediate/side-angle light scatter channel (SSC) high) and their high expression of CD11b and CD18. Results were expressed as a percentage of resting in a, b, and c or E. coli–activated neutrophils in d or total leukocytes in e. *\( P < 0.05 \), **\( P < 0.005 \). Values are given as mean ± SEM (n = 15 control rats (CRs); n = 15 fructose-fed rats (FFRs)).
Finally, concerning relations between neutrophil functions and circulating adipokines concentrations, only a significant and strong positive correlation between serum leptin levels and relative ROS production by PMA-activated neutrophils from the FFR group was observed ($r = 0.72; P < 0.05$) (Figure 3).

Effect of high-fructose diet on neutrophil production

The CFU-GMs from FP-BMCs were significantly less numerous in the FFR group compared with the CR group ($154.6 \pm 12.77$ vs. $206.5 \pm 14.83; P < 0.05$) (Figure 4a). The result of FAS/18S relative RNA expression in total FP-BMCs was significantly higher in the FFR group compared with the CR group ($2.5 \pm 0.5$ vs. $1.1 \pm 0.3; P < 0.05$) (Figure 4b). Flow cytometry assessment of apoptotic bone marrow cells by annexin V+/propidium iodide− labeling revealed significantly increased apoptotic cells only in the granulocyte population from the FFR group compared with the CR group ($5.6 \pm 0.8\%$ vs. $2.6 \pm 0.5\%; P < 0.05$) (Figure 4c). In the meantime, no differences were found as regards apoptosis in the monocyte population between both groups ($7.4 \pm 0.7\%$ vs. $6.4 \pm 0.8\%; P = 0.90$) (Figure 4d).

DISCUSSION

By contrast with macrophages, to date no study has demonstrated neutrophil presence in AT from patients with MetS.10 Because circulating neutrophils can be rapidly recruited into peripheral tissues,11 we hypothesized that neutrophils infiltrate AT at an early stage of MetS.

It has been reported that the current high dietary intake of carbohydrate-fructose contributed to the epidemic of MetS as a risk factor for heart disease, diabetes, and stroke.12,13 Thus, the FFR model is a model that mimics the human MetS in many aspects, including hypertension, hypertriglyceridemia, insulin resistance, and partial compensatory hyperinsulinemia.

Most of the studies on MetS investigated this rat model after 6–12 weeks of a high-fructose diet.14–17 At 6 weeks, we observed that rats from the FFR group presented hypertriglyceridemia and arterial hypertension associated with cardiac and kidney hypertrophies, as demonstrated by the significant increase in heart and kidney size relative to body weight. Delbosc et al. have observed a similar early heart hypertrophy, as demonstrated by an increase in heart weight index at 6 weeks in the FFR group compared with the CR group.15 In accordance with previous studies, no difference was observed in total body weight gain between the FFR and CR groups,17–19 whereas the FFR group exhibited a significantly higher intra-abdominal AT accumulation, a representative measure of whole abdominal AT in humans.20,21 Moreover, in our study, the FFR group showed fasting hyperglycemia with an increased HOMA-IR index. Furthermore, serum leptin levels were significantly higher in the FFR group, whereas the adiponectin-to-leptin ratio was significantly decreased. These data are in accordance with that observed in animals or in patients with MetS.22–24 Based on these observations, after 6 weeks of treatment, the induction of early stage MetS associated with early organ dysfunction was confirmed.

In this model, we observed neutrophil infiltration into intra-abdominal AT. Until now, neutrophil infiltration into AT has received only recent attention in 1 mouse model of obesity (60% high-fat diet). In this model, Talukdar et al. detected a rapid increase in AT neutrophil content after 3 days of high-fat diet–induced obesity.25 These data are consistent with previous report on a high-fat-fed mouse model of obesity that showed specific interactions between CD11b on neutrophils and ICAM on adipocytes26 and with the concept that extravasation of neutrophils in AT precedes a second wave of emigrating monocytes while releasing proinflammatory cytokines.

The activation of circulating neutrophils changes the expression of their surface receptors, thus influencing neutrophil’s capacity to infiltrate tissues.27 Thus, we investigated circulating neutrophil functions in our early-stage MetS model. In the resting state, no difference was observed for neutrophils from the FFR and the CR groups concerning adhesion molecules expression (CD18 and CD11b) and ROS production.

On the other hand, responses of neutrophils to PMA, a direct activator of protein kinase C and one the most potent activators of neutrophil functions, were significantly increased in the FFR group. These results suggest that MetS induces a priming of neutrophil functions as defined by El-Benna et al.28 Several mechanisms of neutrophil priming have been proposed (NADPH-oxidase phosphorylation and translocation, G-protein reorganization, or phospholipase A2 activation) and this process has been shown to be critical for neutrophil-mediated tissue injury both in...
In humans, consequences of some MetS abnormalities on neutrophil functions have been described. CD11b adhesion molecule of circulating neutrophils is more expressed in severely obese patients and hyperlipidemic patients. Stimulation with PMA caused more rapid neutrophil CD11b adhesion molecule expression in hypertensive patients. Neutrophils of diabetic patients showed enhanced production of ROS. On the other hand, several studies reported a direct impact of neutrophils on MetS components such as blood pressure, abnormal inflammatory response via ROS generation, and insulin resistance. Hadad et al. have shown that neutrophils may well constitute the major driver of adipose tissue inflammation and hepatic insulin resistance, which develop as early as 3 days after initiating a high-fructose diet in mice.

MetS is characterized by elevated oxidative damage. Studies have shown that leptin was oversecreted in intra-abdominal AT accumulation and was a sensitive marker for predicting cardiovascular risk and MetS. We report here, for the first time, a significant positive correlation between ROS production by PMA-activated neutrophils and circulating leptin levels in FFR rats. It is well documented that neutrophils express the short form of the leptin receptor, but leptin function on neutrophils is less convincingly established. In accordance with our results, ex vivo studies have shown that stimulation of human neutrophils by leptin leads to enhanced ROS production. Moreover, it was demonstrated that dexamethasone-stimulated adipocytes, isolated from intra-abdominal AT of rat with MetS, secrete more leptin through an oxidative stress–dependent mechanism. Consequently, exacerbated oxidative damage observed in MetS patients could be explained by a cross-talk between leptin and neutrophil-derived ROS.

Furthermore, we found a tendency to augmentation of apoptotic circulating neutrophils in the FFR group vs. the CR group. Under homeostatic conditions, the half-life of circulating neutrophils is ≤8 hours. MetS via the activation of neutrophils could reduce half-life of neutrophils. On the other hand, a significant increase of the extrinsic apoptosis pathway (FAS ligand receptor mediated) of FP-BMCs and late apoptotic events, including the exposure of phosphatidylserine (measured by annexin V binding), associated with a decrease of granulopoiesis capacity assessed by CFU-GM count, were observed in the FFR group. We previously demonstrated that the number of circulating colony-forming unit endothelial cells was inversely related to the number of MetS components in patients with coronary artery occlusion. A recent study demonstrated that, in the presence of MetS, bone marrow–derived endothelial progenitor cells developed marked functional impairment, resulting

Figure 4. Colony-forming efficiency and apoptosis of the bone marrow-derived granulocyte and monocyte cells from the control rat (CR) and the fructose-fed rat (FFR) group. (a) Number of colony-forming unit granulocyte macrophage (CFU-GM) progenitors for 1.10^5 ficoll-purified bone marrow cells (FP-BMCs) (n = 20 CRs; n = 20 FFRs). (b) Fragment apoptosis stimulating (FAS)/18S relative RNA expression in total FP-BMCs. (c) Percentage of Annexin V+/propidium iodide (PI)+ in bone marrow granulocyte population in 9 CRs and 9 FFRs. (d) Percentage of Annexin V+/PI− in bone marrow monocyte population in 9 CRs and 9 FFRs. Granulocyte and monocyte subpopulations were gated on their distinct forward-angle light scatter channel (FSC) and side-angle light scatter channel (SSC) characteristics for c and d. *P < 0.05. Values are given as mean ± SEM.
in severely reduced angiogenic capacity in vivo. Similarly, granulopoiesis dysfunction could play a role in the pathogenesis of cardiovascular complications associated with MetS.

In conclusion, the results of this study provide evidence that at an early stage of high fructose-induced MetS there is a modification in the production, phenotype, and function of circulating neutrophils and a promotion of their intra-abdominal AT infiltration. These novel findings motivate further investigations on neutrophil functions in MetS in other models of the disease to evaluate the specificity of the observed modifications. The molecular mechanisms underlying such events and their short- and long-term impact on MetS pathophysiology remain to be elucidated. Understanding the contribution of neutrophils at an early stage of MetS would help in the development of novel therapeutic strategies aimed at its detrimental effects.

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DISCLOSURE

The authors declared no conflict of interest.

REFERENCES


