Adaptative Nitric Oxide Overproduction in Perivascular Adipose Tissue during Early Diet-Induced Obesity

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Perivascular adipose tissue (PVAT) plays a paracrine role in regulating vascular tone. We hypothesize that PVAT undergoes adaptative mechanisms during initial steps of diet-induced obesity (DIO) which contribute to preserve vascular function. Four-week-old male C57BL/6J mice were assigned either to a control [low-fat (LF); 10% kcal from fat] or to a high-fat diet (HF; 45% kcal from fat). After 8 wk of dietary treatment vascular function was analyzed in the whole perfused mesenteric bed (MB) and in isolated mesenteric arteries cleaned of PVAT. Relaxant responses to acetylcholine (10^{-9}–10^{-4} M) and sodium nitroprusside (10^{-12}–10^{-5} M) were significantly ameliorated in the whole MB from HF animals. However, there was no difference between HF and LF groups in isolated mesenteric arteries devoid of PVAT. The enhancement of relaxant responses detected in HF mice was not attributable to an increased release of nitric oxide (NO) from the endothelium nor to an increased sensitivity and/or activity of muscular guanilylcyclase. Mesenteric PVAT of HF animals showed an increased bioavailability of NO, detected by 4,5-diaminofluorescein diacetate (DAF2-DA) staining, which positively correlated with plasma leptin levels. DAF-2DA staining was absent in PVAT from ob/ob mice but was detected in these animals after 4-wk leptin replacement. The main finding in this study is that adaptative NO overproduction occurs in PVAT during early DIO which might be aimed at preserving vascular function.

Obesity is an independent risk factor for the development of endothelial dysfunction and vascular disease. Indeed, some studies ensure that the probability of suffering from vascular diseases is four times higher in obese people (body mass index >30 kg/m²) than in normal-weight people (body mass index ≤25 kg/m²) (1).

Adipose tissue is now considered a highly active endocrine organ (2) that releases a variety of inflammatory cytokines, adipokines, and other factors which might influence vascular tone (3–6). In the last years, several studies have demonstrated that the adipose tissue surrounding blood vessels, termed as perivascular adipose tissue (PVAT), has a paracrine role in the regulation of vascular function (7–9). Under physiological conditions, PVAT releases a number of adipokines, such as adipocyte-derived relaxing factor (10–14), adiponectin (15), Ang 1–7 (16), H₂O₂ (14), or leptin (17), that elicit a beneficial anticontractile effect on vascular function and are essential for the maintenance of vascular resistance (9). Interestingly, the anticontractile effect of PVAT is directly dependent on the amount of periadventitial fat (11, 13). Reduction of PVAT mass in lean animals correlates with increased contractile responses in mesenteric artery rings and with a lower release of vasodilatory adipokines (13). The anticontractile effect of PVAT has been also observed in human arteries, such as the thoracic artery (18, 19), leading to the question whether re-
removal of PVAT for coronary artery bypass might be deleterious for the graft patency (19–22). Consequently, in situations of normal weight, PVAT has a protective and beneficial role that is directly related to its amount.

PVAT size appears to be increased in obese humans (23) as well as in animal models of obesity (15, 24, 25). Thus, PVAT would contribute to preserve vascular anticontractive responses in these individuals. However, there is evidence that adipokine production in obesity is unbalanced in favor of vasoconstrictor and proinflammatory substances (9). Gao et al. (26) demonstrated that the anticontractive effect of PVAT appears to be lost in an animal model of obesity despite higher amounts of PVAT. Similarly, New Zealand obese mice, which develop severe metabolic syndrome associated with hypertension (27), show a reduced anticontractile response in obese mice, which develop severe metabolic syndrome as associated with hypertension (27), show a reduced anticontractile response. Moreover, PVAT enlargement is integral to this general adaptation. Therefore, the aim of the current study was to assess vascular function and confocal imaging studies. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication no. 85-23, revised 1996).

Plasma measurements

Plasma leptin and adiponectin concentrations were analyzed by specific RIA for murine leptin (Linco Research, Billerica, MA; 4.9% intraassay variation, 3.3% interassay variation) and adiponectin (Linco Research; 4.43% intraassay variation, 7.13% interassay variation). Insulin was determined by means of a specific EIA kit for mouse insulin (Mercodia, Denmark; 2.2% intraassay variation, 4.9% interassay variation). Glucose was measured by a spectrophotometric method (Glucose Trinder Method; Roche, Madrid, Spain). Triglycerides and nonesterified free fatty acids were determined using the GPO (Biolabo, Maizy, France) methods, respectively.

Vascular reactivity in the perfused mesenteric vascular bed

The superior mesenteric artery was cannulated at its junction with the abdominal aorta. The MB was separated from the intestine, placed in an organ chamber, and perfused using a peristaltic pump (Ismatec, Barcelona, Spain) at constant flow (1.5 ml/min) with oxygenated (95% O2/5% CO2) Krebs-Henseleit solution (KH: 115 mM NaCl, 4.6 mM KCl, 2.5 mM CaCl2·2H2O, 5 mM NaHCO3, 1.2 mM KH2PO4, 1.2 mM MgSO4, 0.01 mM EDTA, 11.1 mM glucose). Perfusion pressure was monitored by a pressure transducer (Grass) and recorded by BIOPACK software. Bubbles in the perfusion buffer were removed by a bubble trap system, and flow was kept at constant rate. Changes in perfusion pressure were used as an index of changes in artery resistance, and therefore increase or decrease of perfusion pressure was indicative of vasoconstriction or vasodilatation, respectively. After equilibration (40 min), vascular contractility was assessed with KCl (75 mM). Relaxant response to acetylcholine (Ach) and sodium nitroprusside (SNP) were analyzed in the MB, precontracted with 10−5 M noradrenaline (NA). Nε-nitro-L-arginine
methylester (t-NAME) and 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one (ODQ) were preincubated for 20 min.

Vascular reactivity in isolated mesenteric arteries cleaned of PVAT

First-order branches of mesenteric arteries were dissected, cleaned of PVAT, and cut in 2-mm segments. Arteries were mounted on 25-μm wires in a wire myograph (Danish Myotech, Aarhus, Denmark). After 30 min of previous incubation, vessel wall tension and diameter were normalized following a standardized method described by Mulvany and Halpern (32). Afterwards, segments were washed with KH solution and stabilized for 30 min. After equilibration, vascular contractility was assessed with KCl (75 mM). Relaxant response to Ach and SNP were determined by the standardized method described by Mulvany and Halpern (32). After 30 min of previous incubation, vessel segments were washed with KH solution and stabilized for 30 min. After equilibration, vascular contractility was assessed with KCl (75 mM). Relaxant response to Ach and SNP were determined by the Bradford method (33). Measurements were made by using a cGMP RIA kit (Amersham, Buckinghamshire, UK).

Determination of cyclic GMP levels

Mesenteric arteries, cleaned of PVAT, were preincubated at 37 °C for 30 min in oxygenated (95% O₂–5% CO₂) KH solution containing zaprinast (10⁻⁵ M), an inhibitor of phosphodiesterase, to prevent cyclic GMP (cGMP) degradation. Afterwards, arteries were incubated with 10⁻⁶ M SNP for 2 min, then homogenized with ethanol/water (2/1) and centrifuged at 4 °C (20 min, 100,000 × g). Positive controls were incubated with 8-bromo-cGMP (10⁻⁶ M) and handled as described. Dry pellets were reconstituted in Tris-EDTA buffer and used for cGMP assay. Measurements were made by using a cGMP RIA kit (Amersham, Spain) as described in the manufacturer’s instructions. Data were expressed as picomoles cGMP/milligram of protein. Proteins were determined by the Bradford method (33).

Determination of basal nitric oxide availability by confocal microscopy

Nitric oxide (NO) availability was determined by using the fluorescent NO indicator 4,5-diaminofluorescein diacetate (DAF-2DA), as previously described (34). Briefly, mesenteric arteries were immediately dissected, cleaned of adipose tissue, and stabilized in KH (30 min, 37 °C), then stained with DAF-2DA (10⁻⁶ M) and washed with KH in the darkness in a shaking bath (30 min, 37 °C for 30 min in oxygenated (95% O₂–5% CO₂) KH solution containing zaprinast (10⁻⁵ M)). After 30 min, vessels were incubated with either 10⁻⁶ M NA, 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one (ODQ) were preincubated for 20 min. Negative and positive controls were incubated with either 10⁻⁶ M NA. L-NAME was preincubated for 20 min.

Statistics

All values are given as mean ± SEM. Statistical significance was analyzed by one-way ANOVA followed by Newman-Keuls post hoc test. Statistical significance was set at P < 0.05. n represents the number of data. Contractions are expressed in mm Hg in the perfused MB, and in mN in isolated mesenteric arteries. relaxations are expressed as the percentage of a previous NA contraction. The maximum response (Eₐ₅₀ values) and the negative logarithm of concentration producing 50% of maximum response (pD₂ values) were calculated by a nonlinear regression analyze of each individual concentration-response curve.

Results

Body weight, adiposity, and plasma parameters

As summarized in Table 1, both HF and ob/ob animals weighed significantly more than LF. In addition, weight of lumbar adipose tissues, expressed in mg per mm tibial length, was 5.2-fold higher in HF and 8.8-fold higher in ob/ob than in the LF group. Mesenteric adipose tissue increase was 1.3-fold in HF and 3-fold in ob/ob compared with LF.

Plasma biochemical parameters (Table 2) were not modified by dietary treatment, with the exception of leptin which exhibited a 296% increase in HF animals. Moreover, plasma leptin concentration positively correlated with the amount of adipose tissue (F (1,9) = 28.9, P < 0.001, compared with HF animals. Newman-Keuls test.

**TABLE 1.** Effect of dietary treatment on body weight and adiposity

<table>
<thead>
<tr>
<th></th>
<th>LF</th>
<th>HF</th>
<th>ob/ob mice</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>25.5 ± 0.6</td>
<td>30.1 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.1 ± 1.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lumbar adipose tissue (mg/mm)</td>
<td>3.9 ± 0.5</td>
<td>20.5 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.4 ± 2.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mesenteric adipose tissue (mg/mm)</td>
<td>23.9 ± 1.8</td>
<td>38.9 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.9 ± 4.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tibial length (mm)</td>
<td>18.9 ± 0.2</td>
<td>19.1 ± 0.1</td>
<td>20.0 ± 0.2</td>
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</table>

Adipose tissue weights were normalized using the tibial length, which was not modified because of the high-fat diet. Data of body and adipose tissues are expressed as mean ± SEM of 14 determinations per group.

<sup>a</sup> P < 0.01 and <sup>b</sup> P < 0.001, compared with their corresponding matched control groups (LF).

<sup>c</sup> P < 0.001, compared with HF animals. Newman-Keuls test.

**TABLE 2.** Effect of dietary treatment on biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>LF ± 0.1</th>
<th>HF ± 0.1</th>
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<tbody>
<tr>
<td>Insulin (µg/liter)</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>5.4 ± 0.3</td>
<td>16.0 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>17.0 ± 1.9</td>
<td>17.0 ± 1.5</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>157.4 ± 3.9</td>
<td>157.7 ± 3.9</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>64.1 ± 6.0</td>
<td>69.5 ± 3.6</td>
</tr>
<tr>
<td>NEFA (mM)</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.02</td>
</tr>
</tbody>
</table>

Data of biochemical parameters are expressed as mean ± SEM of eight determinations per group. NEFA, Nonesterified free-fatty acids.

<sup>a</sup> P < 0.01, compared with their corresponding matched control groups. Newman-Keuls test.

Materials

Ach was dissolved in saline. NA and SNP were dissolved in 0.01% ascorbic acid/saline. t-NAME, SOD, and 8-bromo-cGMP were dissolved in distilled water. Zaprinast was dissolved in PBS. ODQ was dissolved in 50% dimethylsulfoxide (final concentration of dimethyl sulfoxide was less than 0.1% in PBS). All chemicals were provided by Sigma (St. Louis, MO) with the exception of ODQ (Tocris Cookson, Bristol, UK).
Endothelial NO release is not modified by HF diet

Endothelium-dependent relaxation was analyzed in first-order branches of mesenteric arteries cleaned of PVAT. As shown in Fig. 1A, relaxation to Ach $(10^{-9} - 10^{-4} \text{M})$ was unmodified by HF. No differences between groups were observed in either $E_{\text{max}} (84.9 \pm 2.9\% \text{ in LF vs. } 78.7 \pm 4.3\% \text{ in HF})$ or $pD_2 (6.3 \pm 0.3 \text{ in LF vs. } HF = 6.3 \pm 0.1 \text{ in HF}).$ Moreover, inhibition of endothelial-dependent relaxation elicited by $10^{-4}\text{ M L-NAME}$ was also similar in HF and LF animals (Fig. 1A; $E_{\text{max}} = 39.1 \pm 4.9\% \text{ and } pD_2 = 5.5 \pm 0.2 \text{ vs. } E_{\text{max}} = 39.2 \pm 4.7\% \text{ and } pD_2 = 5.9 \pm 0.2 \text{ in LF and HF, respectively}).$ Basal contraction to L-NAME was not different between groups (LF = 0.24 ± 0.2\% vs. HF = 0.21 ± 0.1\%).

Endothelial NO availability, determined by confocal microscopy in mesenteric arteries cleaned of PVAT, was similar between LF and HF animals (Fig. 1B and C). No differences were observed when arteries were preincubated with 15 U/ml SOD, to avoid superoxide-induced inactivation of NO (results not shown). Preincubation with $10^{-4}\text{ M L-NAME}$ reduced fluorescence intensity in a similar extent both in LF and HF animals (results not shown). All the precedent data indicate that 8-wk HF does not induce changes in endothelial NO release.

Smooth muscle sensitivity to NO is not modified by HF diet

To analyze the sensitivity of mesenteric smooth muscle to NO, concentration-response curves to SNP $(10^{-12} - 10^{-5}\text{ M}),$ a NO donor, were performed in first-order branches of mesenteric arteries cleaned of PVAT. As shown in Fig. 1D, relaxation to SNP was similar in LF and HF animals. No differences were observed in either $E_{\text{max}} (84.9 \pm 2.9\% \text{ in LF vs. } 78.7 \pm 4.3\% \text{ in HF})$ or $pD_2 (6.3 \pm 0.3 \text{ in LF vs. } HF = 6.3 \pm 0.1 \text{ in HF}).$ Moreover, cGMP levels after stimulation with SNP $(10^{-6}\text{ M})$ were also similar between groups (Fig. 1E). These data indicate that the sensitivity of guanylyl cyclase to NO is not modified because of HF.

Effect of HF on MB function

We used the whole MB to evaluate the influence of PVAT on vascular function after 8 wk of HF feeding. A perfusion flow rate of 1.5 ml/min was chosen according to previous experiments showing that maximal contraction to 75 mm KCl are obtained at this flow (results not shown).

Basal perfusion pressure was similar between groups (LF = 15.3 ± 1.4 mm Hg; HF = 13.3 ± 1.9 mmHg; $ob/ob = 13.5 \pm 4.3 \text{ mm Hg}$). Contractile responses to 75 mmol/liter KCl were also similar between groups both in the whole perfused MB (LF = 30.7 ± 0.4 mm Hg; HF = 37.2 ± 3.6 mm Hg; $ob/ob = 29.5 \pm 8.2 \text{ mm Hg}$), as well as in isolated mesenteric arteries cleaned of PVAT (LF = 4.95 ± 0.8 mN; HF = 4.45 ± 0.3 mN).

Cumulative doses of Ach $(10^{-9} - 10^{-4}\text{ M})$ elicited a concentration-dependent relaxation in both groups. As shown in Fig. 2A, relaxation was shifted to the left the HF group [one-way ANOVA, $F_{(1,16)} = 8.095, P < 0.01].$ $E_{\text{max}}$ and $pD_2$ values are shown in Table 3. The effect of $10^{-4}\text{ M L-NAME}$ on Ach-induced relaxation was more pronounced in LF (Fig. 2A and Table 3). Indeed, the inhibition by L-NAME of Ach-response was 61.4 ± 6.3\% in LF vs. 23.7 ± 5.1\% in HF.
mesenteric adipocytes of ob/ob mice (Fig. 3E). Leptin administration by means of sc osmotic Alzet minipumps (4 wk) significantly enhanced DAF2-DA fluorescence in mesenteric PVAT (Fig. 3F). Altogether, these results show that the increased production of NO in mesenteric PVAT of HF animals might be related to diet-induced hyperleptinemia.

**Discussion**

In this study, we show a moderate enlargement of PVAT during early DIO which correlates with NO overproduction in this tissue and also with a rise in plasma leptin. We suggest that these changes might be integral to the general adaptation observed at initial steps of HF feeding probably aimed at preserving vascular function.

Feeding mice with chow enriched with saturated fat gradually leads to overweight, hyperleptinemia, and obesity. In the current study we use the term early DIO to refer to the metabolic status induced by 8-wk dietary treatment with HF diet, which is characterized by increased adiposity (overweight rather than obesity) and hyperleptinemia, but preserves peripheral responsiveness to leptin, as well as normal postprandial values of adiponectin, insulin, glucose, triglycerides, and free-fatty acids (36).

Although overweight and obesity usually deal with alterations of vascular function, as observed in experimental models of DIO (37) and in ob/ob mice (Ref. 28 and Fig. 1A), an enhancement of vascular function in early DIO has been also described (38, 39). In the current study, despite increased visceral adiposity, we observed an enhancement of vasodilatory responses in the whole perfused MB. To characterize the underlying mechanisms, we first investigated in isolated mesenteric arteries of HF animals a pos-

**TABLE 3.**  

<table>
<thead>
<tr>
<th>Low-fat diet</th>
<th>High-fat diet</th>
<th>ob/ob mice</th>
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<tbody>
<tr>
<td><strong>Ach</strong></td>
<td><strong>Ach + L-NAME</strong></td>
<td><strong>Ach + L-NAME</strong></td>
</tr>
<tr>
<td>$E_{\text{max}}$ (nm)</td>
<td>$pD_2$ (M)</td>
<td>$E_{\text{max}}$ (nm)</td>
</tr>
<tr>
<td>$96.8 \pm 1.7^a$</td>
<td>$7.2 \pm 0.2$</td>
<td>$43.1 \pm 13.3^a$</td>
</tr>
<tr>
<td>$92.6 \pm 4.1^c$</td>
<td>$8.5 \pm 0.3^d$</td>
<td>$22.0 \pm 9.5^c$</td>
</tr>
<tr>
<td>$99.7 \pm 2.9^d$</td>
<td>$10.5 \pm 0.5^c$</td>
<td>$66.9 \pm 0.6^b$</td>
</tr>
<tr>
<td>$46.0 \pm 12.0$</td>
<td>$11.2 \pm 0.8^d$</td>
<td>$6.1 \pm 0.7^b$</td>
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</table>

$E_{\text{max}}$ is the maximal relaxation to acetylcholine in % of precontraction to noradrenaline; $pD_2$ is the negative logarithm of molar concentration of Ach causing half maximal responses. Data are expressed as mean ± SEM.

$^a P < 0.05$, and $^b P < 0.01$, compared with their corresponding matched control groups (Ach or SNP, respectively).

$^c P < 0.05$, $^d P < 0.01$, and $^e P < 0.001$ compared with the LF group.

$^f P < 0.05$, $^g P < 0.01$, and $^h P < 0.001$ compared with the HF group.
sible increase of endothelial NO availability. Because both DAF2-DA staining as well as Ach-induced relaxation were similar between groups, it is concluded that 8-wk HF diet does not affect endothelial NO release. Moreover, an increased sensitivity to the vasodilatory effect of NO, previously reported to occur in obesity (38, 39), can also be discarded as differences between groups in SNP-induced relaxation and in cGMP concentration were not detected. These results demonstrate that the sensitivity and/or activity of guanilylcyclase to NO are not modified because of HF.

The most relevant result in the current study is the overproduction of NO by mesenteric PVAT in HF mice. As illustrated in Fig. 2 and Table 3, shifting to the left of concentration-response curves for Ach and SNP in the whole perfused MB from HF suggests that PVAT might be an additional source of NO in HF mice. Such a possibility is supported by the finding that in absence of PVAT the response to these drugs in isolated mesenteric arteries is identical in both groups. Moreover, our result is consistent with previous studies showing that both inducible and endothelial isoforms of NO synthase are expressed in adipocytes (5, 6, 40) and appear to be up-regulated in obesity (3). An additional support for the role of PVAT-derived NO is yielded by the effect of l-NAME and ODQ in the whole MB. As these drugs are perfused intraluminally, they do not reach PVAT and thus appear to be unable to block adipose NO production and its effects. Therefore, it is NO from PVAT which accounts for the difference between LF and HF (Fig. 2). Interestingly, overproduction of NO is not linked to larger amounts of PVAT as the increase of NO observed in HF animals was not detected in age-matched ob/ob mice. From our results, we suggest that compensatory mechanisms in adipose tissue at initial steps of DIO or during temporary/seasonal overweight (i.e. hibernator mammals) aimed at preventing organ damage and lipotoxicity (29–31) might also include an adaptation of PVAT to protect, in this case, vascular function.

A main question raised by our data concerns the mechanism responsible for NO production in PVAT of HF mice. We detected a positive correlation between NO production by mesenteric PVAT and plasma leptin levels suggesting an effect of leptin in regulating NO production in perivascular adipocytes. Therefore, and aiming to elucidate the involvement of leptin in NO overproduction, we analyzed NO availability in mesenteric PVAT of ob/ob mice before and after leptin replacement. As expected, NO was almost absent in untreated ob/ob mice but significantly increased by leptin administration. Mehebik et al. (41) demonstrated that leptin induced a rapid increase of NO synthase activity in white adipocytes, which was mediated by phosphorylation of Ser1179 and Thr497 by JAK2, PKA, and MAPKs. Therefore, our results allow suggesting that NO overproduction in PVAT might be triggered by the moderate diet-induced hyperleptinemia in HF mice.

Hyperleptinemia has been considered a cause of cardiovascular and metabolic disorders linked to obesity. This concept, triggered by initial research showing that leptin inhibits both insulin secretion and lipogenic activity in white adipose tissue, has already been overcome, and hyperleptinemia is currently considered as an adaptive response to prevent metabolic alterations linked to overfeeding. Special attention has to be paid to a number of works by RH Unger (reviewed in Refs. 29–31), suggesting that fat-enriched diets trigger initial compensatory mechanisms in adipose tissue to avoid lipotoxicity in nonadipose tissues. Although some studies have linked chronic hyperleptinemia to endothelial dysfunction and damage

**FIG. 3.** Effect of HF on NO production in PVAT. Effect of leptin. A and B, Confocal projections showing *in situ* generation of NO, determined with DAF2-DA (10^{-5} M), in mesenteric adipocytes from LF animals (A) compared with HF animals (B) (n = 5). C, Fluorescence intensity in adipocytes from LF and HF animals. Results are expressed as mean ± SEM. ***, P < 0.001, compared with their corresponding matched control groups. D, Correlation between fluorescence intensity elicited by DAF-2 DA and plasma leptin concentrations, P < 0.001. E, Confocal projections showing *in situ* NO generation in mesenteric adipocytes determined with DAF2-DA (10^{-5} M) from ob/ob animals (n = 5). F, Confocal projections showing *in situ* NO generation in mesenteric adipocytes determined with DAF2-DA (10^{-5} M) from ob/ob animals after leptin administration (n = 5).
(42–44), here we suggest that hyperleptinemia evoked by the moderate increase of adiposity during early DIO accounts for NO overproduction in mesenteric PVAT leading to a paracrine improvement of vascular function. Therefore, we conclude that PVAT is integral to the general adaptation observed in adipose tissues at initial steps of HF feeding aimed, in this case, at preserving vascular function.

In conclusion, this is the first study demonstrating NO production in PVAT with a paracrine role on mesenteric vascular function. Moreover, we suggest that the rise in plasma leptin during early DIO might allow the adaptation of PVAT to prevent vascular damage.

Acknowledgments

We thank J. M. Garrido, J. Bravo, and I. Bordallo for skillful animal care during the experiment and Dolores Morales for her help with the confocal microscope.

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This work was supported by grants from Ministerio de Educación y Ciencia (SAF2006-02456, SAF2008-02703, SAF2008-02703), Fundación Universitaria San Pablo-Ceu, and Sociedad para el Estudio de la Alud Cardiometabólica. M.G.-O. and P.S. are supported by Ministerio de Educación y Ciencia. R.G.-R. is supported with a fellowship of 4.

Disclosure Summary: The authors have nothing to disclose.

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