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Early experimental obesity is associated with coronary endothelial dysfunction and oxidative stress

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Galili O, Versari D, Sattler KJ, Olson ML, Mannheim D, McConnell JP, Chade AR, Lerman LO, Lerman A. Early experimental obesity is associated with coronary endothelial dysfunction and oxidative stress. *Am J Physiol Heart Circ Physiol* 292: H904–H911, 2007. First published September 29, 2006; doi:10.1152/ajpheart.00628.2006.—Obesity is independently associated with increased cardiovascular risk. However, since established obesity clusters with various cardiovascular risk factors, configuring the metabolic syndrome, the early effects of obesity on vascular function are still poorly understood. The current study was designed to evaluate the effect of early obesity on coronary endothelial function in a new animal model of swine obesity. As to method, juvenile domestic crossbred pigs were randomized to either high-fat/high-calorie diet (HF) or normal chow diet for 12 wk. Coronary microvascular permeability and abdominal wall fat were determined by using electron beam computerized tomography. Epicardial endothelial function and oxidative stress were measured in vitro. Systemic oxidative stress, renin-angiotensin activity, leptin levels, and parameters of insulin sensitivity were evaluated. As a result, HF pigs were characterized by abdominal obesity, hypertension, and elevated plasma lysophosphatidylcholine and leptin in the presence of increased insulin sensitivity. Coronary endothelium-dependent vasorelaxation was reduced in HF pigs and myocardial microvascular permeability increased compared with those values in normal pigs. Systemic redox status in HF pigs was similar to that in normal pigs, whereas the coronary endothelium demonstrated higher content of superoxide anions, nitrotyrosine, and NADPH-oxidase subunits, indicating increased tissue oxidative stress. In conclusion, the current study shows that early obesity is characterized by increased vascular oxidative stress and endothelial dysfunction in association with increased levels of leptin and before the development of insulin resistance and systemic oxidative stress. Vascular dysfunction is therefore an early manifestation of obesity and might contribute to the increased cardiovascular risk, independently of insulin resistance.

endothelium; vasodilation; permeability; leptin

THE PREVALENCE OF BEING OVERWEIGHT and obesity is increasing in the Western world to epidemic proportions. Data from the Center for Disease Control and Prevention indicate the prevalence of being overweight and obesity to be ~60% and 30%, respectively, in the United States adult population. Childhood and adolescence obesity, also increasing (49), is associated with vascular dysfunction in otherwise healthy young children (57), as well as with increased cardiorespiratory morbidity (56). Importantly, the presence of obesity and other cardiovascular risk factors in childhood and adolescence tends to persist and progress clinically in early adulthood (14) with high-calorie intake being the predominant determinant of obesity in Western societies (7).

Obesity is well known to cosegregate with other cardiovascular and metabolic abnormalities, including hypertension, dyslipidemia, and glucose intolerance/Type 2 diabetes mellitus, in the so-called metabolic syndrome (22). The fundamental feature in the pathogenesis of the metabolic syndrome is considered insulin resistance (10, 38); in addition, endothelial dysfunction, an early manifestation of atherosclerosis and an independent predictor of cardiovascular events (20, 30, 52, 53), has also been consistently associated with the metabolic syndrome (15, 48) in a complex interplay with insulin resistance (10).

It has been previously demonstrated that obesity is an independent risk factor for coronary (1) and systemic (8) endothelial dysfunction. However, the mechanisms through which early obesity induces endothelial dysfunction are not clear. Obesity, in particular visceral obesity, is one of the main causes of the increased resistance to insulin. Therefore, the presence of endothelial dysfunction in obese subjects or animal models is likely influenced by the insulin-resistance state, which per se, and independently from dysglycemia, has been demonstrated to induce oxygen reactive species production, leading to nitric oxide (NO) breakdown and endothelial dysfunction (10). However, a possible effect of obesity in inducing endothelial dysfunction before the development of and independently from insulin resistance has been suggested in a rat model of diet-induced obesity (33). Various factors have been proposed to induce obesity-related endothelial dysfunction, including increased plasma levels of leptin (28) and free fatty acids (13).

The current study was designed to test the hypothesis that the initial cardiovascular manifestations of obesity, including endothelial dysfunction, might start early and before the establishment of the fully developed metabolic syndrome. For this purpose, in a new experimental model of large animal early obesity, we assessed coronary endothelial function, myocardial microvascular permeability, as well as parameters of oxidative stress and metabolic homeostasis.

METHODS

Animals

The Institutional Animal Care and Use Committee approved the study. Juvenile female domestic crossbred pigs (3 mo old, initial

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weight, 25–30 kg; Larson Products; Sargeant, MN) were placed on a high-fat/high-calorie diet (HF, $n = 6$; 20% lard, 4.31 kcal/g, TD.03358, Harlan Teklad, Madison, WI) or on a normal chow diet (N, $n = 6$, 0.81 kcal/g) for 12 wk. Content of carbohydrates, amino acids, minerals, and vitamins was identical in both diet regimens. After the completion of the diet period, fasting blood samples were drawn, animals were anesthetized and scanned by electron beam computerized tomography (EBCT) (39), and thereafter were euthanized with an overdose of pentobarbital sodium (10 mg/kg iv, Sleepaway, Fort Dodge Laboratories, Fort Dodge, IA). Coronary arteries were harvested immediately after euthanasia.

Systemic Measurements

At the end of the study period, body weight, abdominal wall fat thickness, and intra-abdomial fat (measured in EBCT images) (45, 46) were used as indicators of obesity. Blood pressure was recorded by an intra-arterial catheter during the EBCT study. High-sensitivity C-reactive protein (CRP) and plasma renin activity (PRA) were evaluated by standard procedures.

Metabolic parameters. Plasma lipid profile, glucose, and insulin were measured by standard methods. The glucose-to-insulin ratio and homeostasis model assessment (HOMA) index [(plasma glucose $\times$ insulin)/22.5] were used as indicators of insulin sensitivity. Plasma leptin was measured by Multi-Species Leptin RIA assay (LINCO Research, St. Charles, MO). Lysophosphatidylcholine (LPC) 16:0 and 18:0, highly atherogenic products of lipid metabolism (16, 29), were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS), using 17:0 Lyso-PC as internal standard, with an electrospray triple quadrupole MS (Sciex API 3000).

Systemic oxidative stress. Systemic oxidative stress was evaluated by plasma 8-isoprostanes (8-Isoprostane EIA Kit, Cayman Chemical, Ann Arbor, MI), a specific marker of free radical-induced damage (21) and oxidized LDL (OxLDL, ELISA, Mercodia AB, Uppsala, Sweden).

Plasma NO end products. Serum NO derivates were quantified by a two-step assay for the sum of both nitrites and nitrates using a commercially available kit (Nitric Oxide Quantitation Kit, Active Motif, Carlsbad, CA) following the manufacturer's instructions.

In Vivo Studies

Myocardial microvascular permeability by EBCT. Each animal was anesthetized with 0.5 g of intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg), intubated, and mechanically ventilated. Anesthesia was maintained with a mixture of ketamine (0.2 $mg \cdot kg^{-1} \cdot min^{-1}$) and xylazine (0.03 $mg \cdot kg^{-1} \cdot min^{-1}$) in saline. Under sterile conditions and fluoroscopic guidance, intravascular catheters were then positioned in the suprarenal aorta for measurement of arterial pressure and in the right atrium for injections of contrast medium (11, 39). Animals were transferred to the EBCT (Imatron C-150, Imatron South San Francisco, CA) scanning gantry and allowed a 30-min recovery, during which saline (5 ml/min) was administered and a blood sample collected from the central venous catheter. Non-contrast-enhanced scans at the umbilical levels were used for evaluation of the subcutaneous and visceral fat. Heart localization scans were performed to identify cross-sectional images at two adjacent mid-left ventricular levels. As previously described (6) for the assessment of coronary microvascular permeability, 40 consecutive ECG-triggered end-diastolic scans were obtained over the preselected levels at one to three heartbeat intervals after a bolus injection (0.3 ml/kg) of non-ionic, low-osmolar contrast agent iopamidol (Isovue-370, Squibb Diagnostics, Princeton, NJ) into the right atrium. The same acquisition sequence was repeated after intravenous infusion of adenosine (400 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and dobutamine (15 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in a randomized order.

EBCT data analysis. For the measurements of coronary microvascular permeability, a parameter of microvascular endothelial function in vivo, regions of interest were traced in the anterior left ventricular wall and chamber (31). Time-density curves were generated, and the intra- and extravascular transit of contrast medium was modeled, as previously described (31, 40). The area and first moment of each curve were calculated. Microvascular permeability (permeability index; in arbitrary units) was calculated as follows: $60 \times 1.05 \times$ (slope of extravascular curve $\times$ MTT)/area under input curve/BV, where slope is the maximal slope of the ascending arm of the extravascular curve that reflects vascular leakage of contrast medium (5), MTT represents the mean transit time, and BV is blood volume that is used as a surrogate for vascular surface area.

Intra-abdominal adipose tissue quantification by EBCT. For each pig, five EBCT-derived cross-sections (at the level of renal hilum) were analyzed to estimate the amount of intra-abdominal fat tissue (45, 46). With the use of image analysis software (Analyze, Biomedical Imaging Resource, Mayo Foundation, Rochester, MN), the density range of subcutaneous fat tissue was used to automatically threshold the adipose tissue in the entire image. Subsequently, a region of interest was traced internally to the muscular wall of the abdomen (Fig. 1). Intra-abdominal fat was expressed as a percentage of the selected area.

In Vitro Studies

Coronary endothelial function. Coronary endothelial function was evaluated by the organ chambers technique as previously described (5, 40). Briefly, arterial rings were precontracted with 10^{-7} mol/l endothelin-1 (Pheonix Pharmaceuticals, Mountain View, CA) and challenged by increasing doses of the endothelium-dependent vasodilator bradykinin (10^{-11}–10^{-6} mol/l, Sigma, St. Louis, MO), the nonreceptor-mediated endothelium-dependent vasodilator calcium ionophore A-23187 (10^{-11}–10^{-6} mol/l, Sigma), and the endothelium-independent vasodilator sodium nitroprusside (10^{-9}–10^{-4} mol/l, Sigma). Complete relaxation of each ring was tested by exposure to $10^{-3.5}$ mol/l papaverine, and the response was calculated as a percentage from complete relaxation. The effective dose required to reach 50% relaxation (ED_{50}) in each vessel was calculated and averaged.

Coronary oxidative stress. SUPEROXIDE ANIONS. Tissue superoxide anions production was evaluated by the oxidative-sensitive fluorescence dye dihydroethidium (DHE) (50). Unfixed frozen sections of coronary arteries were cut into 30-μm-thick slices and placed on a glass slide. After being incubated with DHE (10^{-6} mol/l) in a light-protected humidified chamber for 30 min at 37°C, tissue sections were imaged with a laser scanning microscope (Zeiss Laser Scanning Microscope 5 Pascal, version 3.2). An image analysis program (MetaMorph, Meta imaging series 4.6) was used to quantify the percentage of the positively stained area in a blinded fashion.

IMMUNOHISTOCHEMISTRY. Coronary artery tissue slices were stained for NADH/NADPH, the major source of superoxide production in vascular tissues, and nitrotyrosine, a marker of tissue protein oxidation. Briefly, coronary arterial slices were deparaffinized, rehydrated, and incubated with equimolar 3% H_2O_2 to block endogenous tissue peroxidase activity. Primary antibodies (nitrotyrosine 1:1,000, Sigma; NADPH oxidase subunits p67, 1:200; p47, 1:200; and gp91, 1:200; Santa Cruz Biotechnology; Santa Cruz, CA) were incubated overnight (4°C) and detected with the EnVision kit (Dako; Carpinteria, CA) in peroxidase-labeling technique with 3,3-diaminobenzidine tetra-hydrochloride as the chromogen (Vector; Burlingame, CA) to yield a brownish reaction product. Incubation with a nonspecific isotype antibody served as a negative control, and the sections were counterstained with hematoxylin. A computer-assisted light microscopy and image analysis program (MetaMorph, Meta imaging series 4.6) was used to semiautomatically quantify the immunohistochemistry results as percent area positively stained.

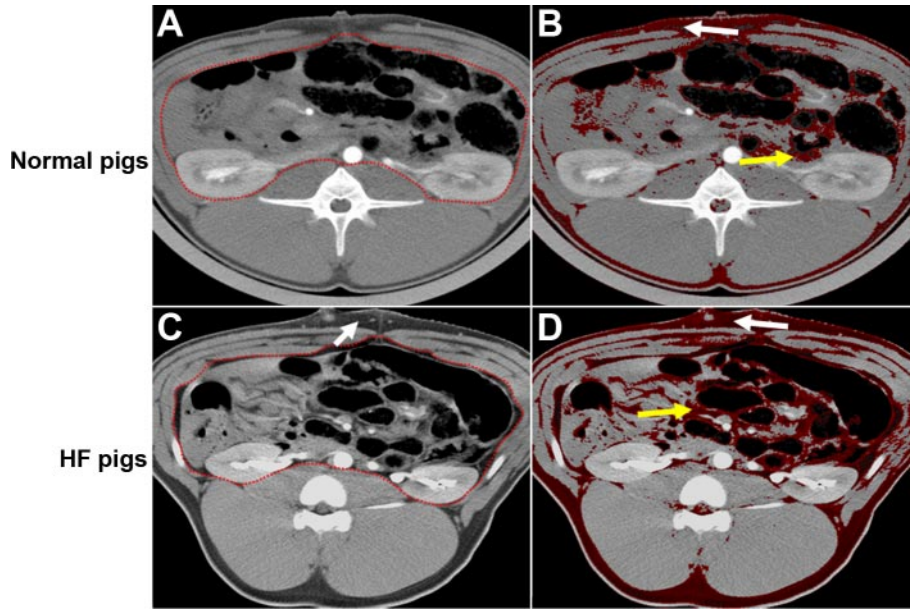

Fig. 1. Representative electron beam computerized tomography (EBCT) images at the umbilical level for the assessment of abdominal fat in normal diet (N; *A* and *B*) and high-fat/high-calorie diet (HF; *C* and *D*) pigs. *A* and *C*: EBCT slices used to trace the intra-abdominal cavity (dotted red line). *B* and *D*: the same slices after thresholding using the density range of the subcutaneous fat (red area). Intra-abdominal fat was calculated as the percentage of red within the dotted red line. White and yellow arrows indicate subcutaneous and intra-abdominal fat, respectively.

Histology for coronary fibrosis. Paraffin slides, 5-μm-thick cut sections, were stained with standard Masson's trichrome to evaluate perivascular fibrotic deposition in a similar fashion.

Statistical Analysis

Data are expressed as means ± SE or as percent change from baseline (in vitro endothelial function and EBCT permeability). Unpaired Student's *t*-test was used to compare groups. Statistical significance was accepted for a probability value of <0.05.

RESULTS

Systemic Measurements

As detailed in Table 1, pigs in the HF group were heavier, had significant accumulation of abdominal wall and intra-abdominal fat compared with N pigs (Table 1 and Fig. 1), and were hypertensive. In addition, HF pigs were characterized by a mild dyslipidemia, reflected by a significant increase in total and LDL cholesterol compared with N pigs, a tendency toward increased HDL levels and unchanged triglycerides (Table 1).

Table 1. *Systemic parameters in N and HF pigs*

	N	HF
n	6	6
Abdominal wall fat thickness, cm	1.35±0.13	1.78±0.05*
Intra-abdominal fat, %	6.1±1.0	10.4±1.7
Weight, kg	62.3±1.38	70.5±1.5*
Mean arterial pressure, mmHg	109±11.1	130±11.3*
Total cholesterol, mmol/l	1.96±0.21	2.63±0.17†
HDL cholesterol, mmol/l	0.93±0.12	1.20±0.12
LDL cholesterol, mmol/l	0.73±0.18	1.37±0.05†
Triglycerides, mmol/l	0.28±0.02	0.27±0.07
Glucose, mmol/l	7.6±0.9	5.4±0.7
Insulin, μU/ml	1.11±0.45	0.22±0.05
Glucose-to-insulin ratio, mg/μU	16.4±4.9	50.9±6.2*
HOMA index	0.43±0.23	0.06±0.02‡
Leptin, ng/ml	2.67±0.08	3.34±0.20*
8-Isoprostanes, pg/ml	200.8±14.3	200.4±48.5
Oxidized LDL, U/ml	10.45±0.9	10.97±1.2
Lysophosphatidylcholine 16:0, μmol/l	37.4±5.6	32.9±3.3
Lysophosphatidylcholine 18:0, μmol/l	17.0±2.7	28.7±3.1†
C-reactive protein, g/dl	0.04±0.012	0.02±0.001
Serum nitrites/nitrates, μmol/l	9.5±0.3	4.7±1*

Values are means ± SE; *n,* number of pigs. N, normal diet; HF, high-fat diet; HOMA, homeostasis model assessment. *$P < 0.01$; †$P < 0.05$; ‡$P = 0.057$.

Systemic leptin levels were higher in the HF group than in the N group (Table 1).

Systemic oxidative stress markers 8-isoprostanes and Ox-LDL, as well as CRP, were similar in N and HF pigs (Table 1). Plasma LPC 18:0 was significantly higher in HF pigs compared with N pigs, with no difference in the levels of LPC 16:0 (Table 1). In contrast, serum NO end-product levels were significantly lower in the HF group compared with the N group. PRA was similar in the two groups.

Pigs in the HF group were characterized by nonsignificantly lower values of both plasma glucose and insulin. Interestingly, systemic insulin sensitivity in HF pigs was increased, as demonstrated by the significantly higher glucose-to-insulin ratio and the tendency toward lower HOMA index values (Table 1).

Myocardial Microvascular Permeability by EBCT

Basal permeability was similar in the N and HF groups [1.5 ± 0.1 and 1.3 ± 0.4 arbitrary units, respectively; P = not significant (NS)]. As shown in Fig. 2, myocardial microvascular permeability did not change significantly in the N group in response to either adenosine (+6.1 ± 4.6%) or dobutamine (+18.8 ± 26.6%). On the contrary, in HF pigs myocardial permeability increased significantly after both adenosine (+56.2 ± 15.4%, $P < 0.05$ vs. N) and dobutamine (+177.8 ± 44.7%, $P < 0.05$ vs. N), hence suggesting coronary microvascular endothelial dysfunction in HF pigs.

Vascular Endothelial Function

The contraction to endothelin-1 was similar in the two groups. The maximal percent vasorelaxation to increasing doses of the endothelium-dependent vasodilator bradykinin was significantly attenuated in coronary vessels of HF (38.5 ±

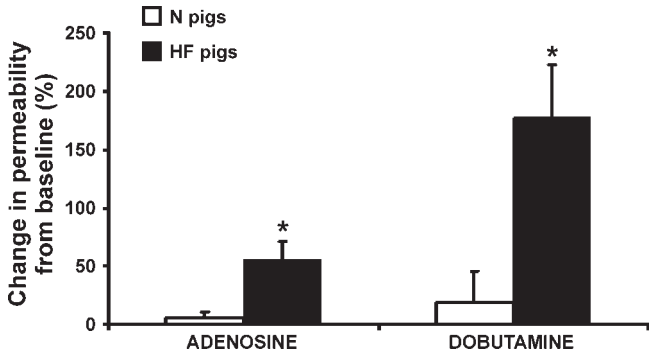

Fig. 2. Percent change in myocardial microvascular permeability in response to adenosine (*left*) and dobutamine (*right*) in N and HF pigs. Data are presented as percent change ± SE from baseline. *$P < 0.05$ compared with N.

5.3%) compared with N (90.5 ± 2.3%; $P < 0.001$; Fig. 3) pigs, and ED_{50} was significantly higher in HF pigs (logM −7.1 ± 0.2 and −8.4 ± 0.1, respectively, $P < 0.001$). Also, the maximal vasorelaxation to calcium ionophore was impaired in HF pigs (53.1 ± 8.2%) compared with N pigs (99.1 ± 0.6%; $P < 0.001$ vs. HF). Additionally, the dose-response curve to increasing doses of the endothelium-independent vasodilator sodium nitroprusside was mostly similar between the two groups, but, at the highest dose, HF pigs showed a significantly lower vasodilation (53.2 ± 4.6%) compared with that in N pigs (82.2 ± 4.1%; $P < 0.01$; Fig. 3). However, ED_{50} for vasorelaxation to sodium nitroprusside did not differ between HF and N pigs (logM −4.4 ± 0.2 and −5.0 ± 0.2, respectively; P = NS).

Vascular Tissue Measurements

A marked increase in DHE fluorescence was found throughout the vascular wall of HF (12.98 ± 0.66%) coronaries compared with N coronaries (5.94 ± 1.43%; $P < 0.05$), reflecting an increase in superoxide anion production (Fig. 4), which was localized mainly in the endothelial cells and to a lesser degree in the adventitia.

Coronaries from HF pigs showed higher expression of NADPH oxidase subunits p67 (N, 0.6 ± 0.2%; and HF, 3.7 ± 0.9%; $P < 0.05$; Fig. 5) and regulatory p47 (N, 0.4 ± 0.3; and HF, 4.2 ± 0.5; $P < 0.05$; Fig. 5), whereas no differences were observed between the groups in the expression of the catalytic subunit gp91 (N, 0.05 ± 0.01%; and HF, 0.07 ± 0.03%; P = NS; data not shown). HF pigs were also characterized by a higher rate of protein nitration as demonstrated by the immunostaining for nitrotyrosine (N, 0.8 ± 0.4%; and HF, 3.3 ± 0.1%; $P < 0.001$; Fig. 5), indicating an interaction between superoxide and NO.

However, no morphological changes were observed in the vascular structure of the HF coronary arteries, and they showed similar perivascular fibrotic deposition (N, 11.4 ± 0.7; and HF, 8.7 ± 1% area positively stained, P = NS).

DISCUSSION

The present study demonstrates that the early phases of abdominal obesity are characterized by coronary endothelial dysfunction in association with vascular oxidative stress, hypertension, and mild lipid profile abnormalities in the absence of a state of insulin resistance. These changes are accompanied by a systemic increase in leptin and LPC levels and decreased NO end products. In contrast, no systemic inflammation or oxidative stress was observed, suggesting that the early abnormalities induced by obesity are mainly localized at the vascular wall level.

The current study suggests that early obesity is associated with functional changes both in the epicardial arteries, as demonstrated by the impaired vasodilating response to the endothelium-dependent stimulus, as well as in the myocardial microcirculation, as demonstrated by the altered permeability response to cardiac challenge. These abnormalities may contribute to the progression of coronary atherosclerosis and cardiovascular events, before the development of insulin resistance, considered the center point of the metabolic syndrome (37).

The possible mechanisms involved in the pathogenesis of epicardial and microvascular coronary endothelial dysfunction are multifactorial and involve systemic and local factors.

NO Bioavailability

Obesity-associated vascular dysfunction is often related to impaired biological activity of NO (23, 34). In the present study, the reduced vasorelaxation to endothelium-dependent

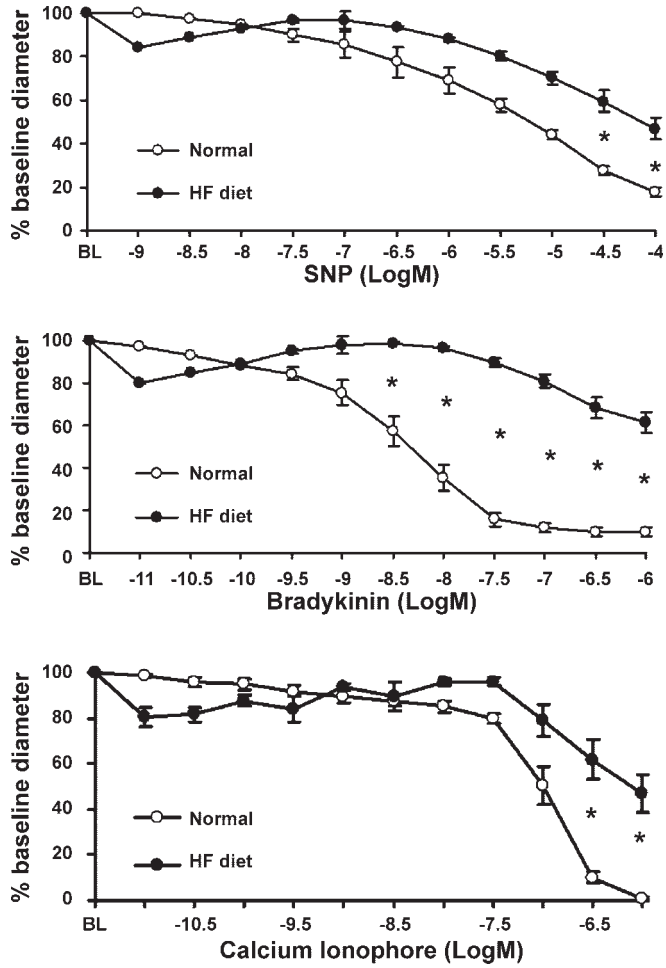

Fig. 3. Coronary artery vasorelaxation response to endothelium-independent stimulus sodium nitroprusside (SNP; *top*), endothelium-dependent stimuli bradykinin (*middle*), and calcium ionophore (*bottom*) in N and HF pigs. *$P <$ 0.001 compared with N.

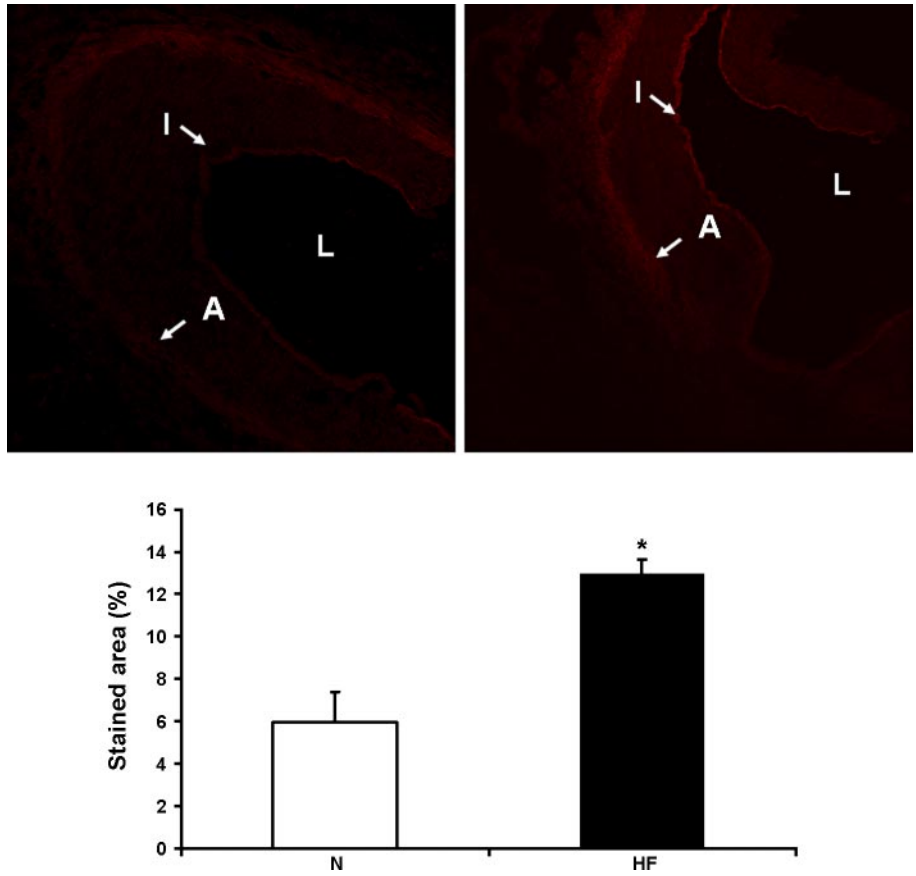

Fig. 4. *Top*: fluorescence photomicrograph showing in situ superoxide anions in N (*left*) and HF (*right*) pig coronaries (original magnification, ×10). A, adventitia; I, intima; L, lumen. *Bottom*: bar graph showing quantification of superoxide anions as percentage of the field that was positively stained. $*P = 0.002$ compared with N.

vasodilators in HF pigs confirms the altered bioavailability of NO. This was also supported by the lower plasma levels of nitrites/nitrates in HF pigs. Indeed, in the vessels of HF pigs, a significantly higher rate of tyrosine nitration is present compared with that in vessels of N pigs, as the result of the interaction between NO and superoxide to form the actively nitrating substance peroxynitrite. A previous study in hypertensive rats showed that the exposure to oxidant agents leads to a reduction in plasma levels of nitrites/nitrates, which are restored by antioxidant treatment (55). Conceivably, in HF pigs, the reduced levels of nitrites/nitrates might reflect the formation of other nitrated substances, such as nitrotyrosine, following the scavenging of NO by oxidative stress.

This is supported by the evidence of increased NADPH-oxidase subunits expression in the HF coronary endothelium. NADPH oxidase is the main source of superoxide anions in atherosclerosis (47), and, accordingly, we found increased levels of superoxide anions in HF endothelium. Since systemic levels of OxLDL and 8-isoprostane in the HF diet group were not increased compared with those in N pigs, these data implicate local vascular oxidative stress as a major determinant of early obesity-associated endothelial dysfunction.

Leptin and Toxic Lipid-Derived Products

Leptin, a hormone secreted by white adipose tissue, is elevated in obese individuals in proportion to the amount of adipose tissue (12). Evidence supports the involvement of leptin in the pathogenesis of obesity-induced cardiovascular risk, in particular, hypertension (32, 44). In a recent study, Beltowski et al. (3) demonstrated that pharmacological hyperleptinemia induces systemic and localized oxidative stress; decreases in NO bioavailability, possibly due to its degradation by reactive oxidative species; and renal sodium retention that may contribute to leptin induced hypertension. Although associative, the current study suggests the possibility that a lower nonpharmacological increase in endogenous circulating leptin levels, although not sufficient to raise systemic oxidative stress, might be associated with increased vascular oxidative stress and endothelial dysfunction, as well as hypertension. Moreover, since leptin has been associated with increased vascular wall stiffness (42), the increased leptin levels might account for the impaired vasodilating response to high-dose endothelium-independent stimulus sodium nitroprusside. It is to be noted that other adipokines not assayed in the present work, including adiponectin, have been demonstrated to play a role in the modulation of several cardiovascular functions and may therefore participate in inducing the abnormalities we observed.

Interestingly, HF pigs showed a significant increase in the plasma levels of LPC 18:0 compared with those in N pigs, with no difference in LPC 16:0 levels. LPC is a highly atherogenic phospholipid (16), and, in animals, its plasma levels are regulated principally by the activity of the enzyme lecithin-cholesterol acyltransferase (LCAT), which catalyzes the transfer of fatty acids from phosphatidylcholine to cholesterol and leads to the formation of cholesterol esters and LPC (18). Long-chain LPC (LPC $>$ 16:0) is known to increase endothelial permeability (25, 36, 59) and to induce endothelial dysfunction (17, 26, 41). Therefore, the increased levels of LPC 18:0 might

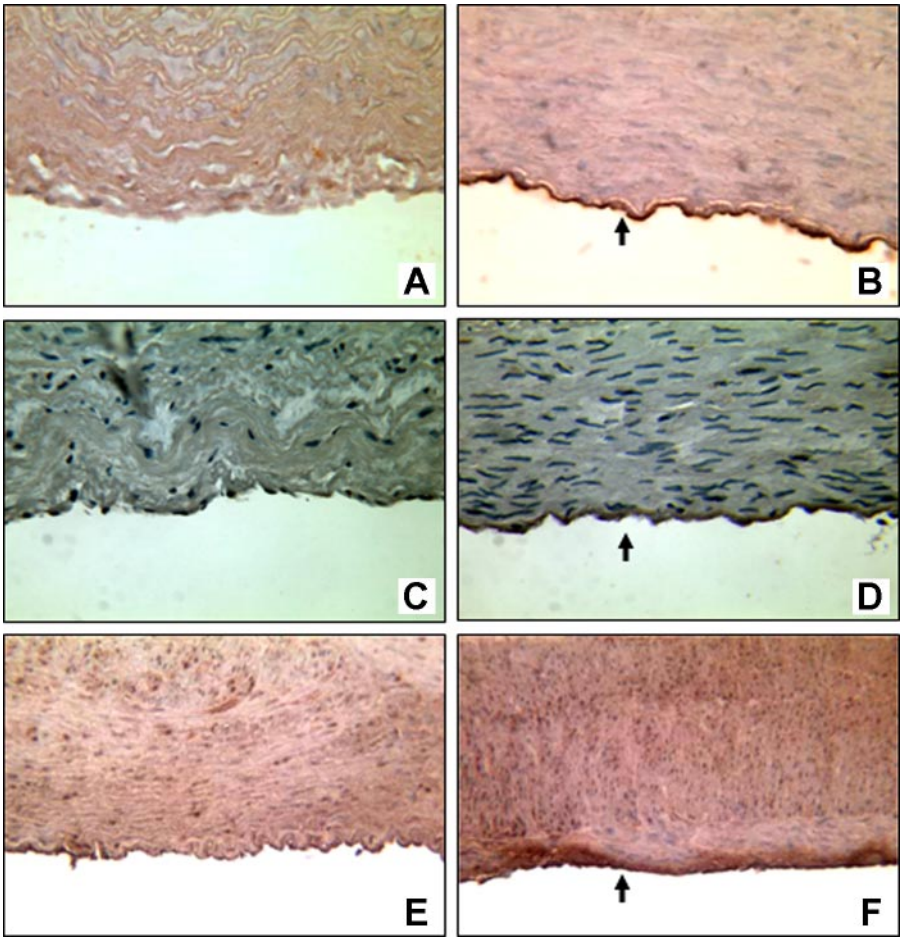

Fig. 5. Representative immunostaining for nitrotyrosine (*A* and *B*), NADPH oxidase p47 (*C* and *D*), and p67 (*E* and *F*) subunits in N (*A*, *C*, and *E*) and HF (*B*, *D*, and *F*) pig coronary arteries. Brown staining (arrow) represents positive staining. Original magnification, ×20.

account, at least partly, for the impairment in endothelial function and microvascular permeability, observed in the present study. Interestingly, in accordance with the results from our study, LPC was found to impair both endothelium-dependent and -independent vasorelaxation in porcine coronary arteries (41). Since LCAT plays an important role in the HDL-mediated transport of cholesterol from peripheral tissues to the liver (19), activation of the enzyme, possibly related to obesity (51) and/or high leptin levels (2), might lead, in these early phases of obesity, to an increase in the plasma levels of HDL cholesterol (as observed in the present study, although not reaching statistical significance) and contemporarily to overproduction of LPC, participating in the impairment of endothelium-dependent and, possibly, -independent vasorelaxation.

Insulin Resistance

Obesity is strongly associated with insulin resistance, and this latter is considered the main mechanisms inducing local and systemic abnormalities observed in the metabolic syndrome (37). A complex interplay between insulin resistance and endothelial function has been demonstrated (9), and studies have suggested that the endothelial dysfunction observed in patients with obesity is mediated by the reduced insulin-mediated NO release (48). However, the present study suggests that insulin resistance is unlikely to be the primary cause for the endothelial dysfunction in the early phases of obesity, since our porcine obesity model showed, in fact, increased insulin sensitivity. It might be speculated that the onset of insulin resistance in obesity is a later event and, as already proposed (9), can be induced or worsened by the presence of endothelial dysfunction, which on the contrary is an early feature of obesity.

Inflammation

The adipose tissue is not only a storage tissue but also an active endocrine organ and secretes numerous proinflammatory hormones and cytokines, such as interleukin-6 and tumor necrosis factor-α (27). Furthermore, macrophages reside in the adipose tissue and further secrete proinflammatory mediators and upregulate the secretory activity of the adipocytes (58). Hence, obesity is considered to be an inflammatory state that predisposes to atherogenesis in the long term. CRP, a highly sensitive marker of inflammation and an independent predictor of cardiovascular events, was not increased in HF pigs, arguing against the possibility that this early obesity model caused a systemic inflammatory reaction sufficient to explain the observed endothelial dysfunction. Moreover, whereas in obese subjects plasma leptin levels are correlated with inflammatory markers, particularly CRP (43), here we did not find such a relation. We might speculate that the increased CRP levels are rather an expression of more advanced stages of obesity in association with the onset of insulin resistance. A limitation of the present study is represented by the lack of adipose tissue biochemical and histological characterization, which, however,

has a limited direct impact of on the function of large and small coronary arteries.

Renin-Angiotensin System and Hypertension

Brook et al. (8) proposed that increased angiotensinogen levels derived directly from adipocytes secretion might be an important link between uncomplicated obesity and vascular endothelial dysfunction. Adipose angiotensinogen gene expression is increased in obesity (54), and the subsequent increase in angiotensin II at the vascular tissue level may stimulate vascular tissue production of superoxide (4), a common factor in the etiology of endothelial dysfunction. The development of hypertension in HF pigs may suggest activation of the renin-angiotensin system. However, systemic PRA was similar in the N and HF groups. Although, ruling out the systemic activation of the renin-angiotensin system, these data do not exclude an increased local tissue activity. Additionally, with the consideration of the early phase of obesity in our study, it is possible that the activation of the renin-angiotensin system establishes in subsequent stages, possibly in association with the onset of insulin resistance (35). Another possible cause of hypertension in our animal model might be represented by the activation of the sympathetic nervous system associated with obesity. In particular, this seems plausible considering the increased levels of leptin found in HF pigs and the well-established effect of leptin in inducing an overactivation of the sympathetic system (24).

The raised blood pressure levels observed as a part of the obesity syndrome might be partly responsible for the impairment in endothelial function. However, in a previous study from our group (40), coronary arteries from hypertensive pigs showed a milder reduction in the response to bradykinin and a normal response to calcium ionophore. Therefore, hypertension does not seem to explain completely the vascular alterations found in HF pigs.

Perspectives

The current study introduces a new model of experimental early obesity induced by HF diet. This model is associated with mild hyperleptinemia, increased vascular oxidative stress, and decreased NO bioavailability, leading to endothelial dysfunction and hypertension. Moreover, a possible contributor to the observed impairment in endothelial function and permeability might be represented by the increased plasma levels of the atherogenic phospholipid LPC. Although associative, these results show a clustering of metabolic and cardiovascular abnormalities in the early phases of atherosclerosis in the absence of systemic insulin resistance, oxidative stress, or inflammation. The results of the present study lead to possibly important clinical implications, since, in the initial phases of obesity, significant vascular functional alterations may occur, contributing to increase cardiovascular risk. The early intervention on obesity with dietetic and pharmacological approaches, as well as physical exercise, might prevent or correct these abnormalities and the later onset of insulin resistance, which, in turn, leads to the vicious cycle of the metabolic syndrome.

Importantly, these modifications are not associated with worsened insulin sensitivity, systemic inflammation, and systemic oxidative stress.

GRANTS

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