Leptin resistance extends to the coronary vasculature in prediabetic dogs and provides a protective adaptation against endothelial dysfunction

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Submitted 14 March 2005; accepted in final form 10 May 2005

Knudson, Jarrod D., Ü. Deniz Dincer, Gregory M. Dick, Haruki Shibata, Rie Akahane, Masayuki Saito, and Johnathan D. Tune. Leptin resistance extends to the coronary vasculature in prediabetic dogs and provides a protective adaptation against endothelial dysfunction. Am J Physiol Heart Circ Physiol 289: H1038–H1046, 2005. First published May 13, 2005; doi:10.1152/ajpheart.00244.2005.—Hyperleptinemia, universal in the human obese population (7), provides a protective adaptation against endothelial dysfunction. Whether this phenomenon persists in obesity and hyperleptinemia, conditions associated with leptin resistance, is unknown. The hypothesis that body adiposity is under homeostatic regulation has received long-standing support in medical and scientific communities and dates back nearly a century (9, 28). The hypothalamic control of food intake is no novel concept (13); however, it was not until the discovery of leptin (53) that the physiological mechanisms controlling food intake and energy expenditure began to surface (39). Mutations in the leptin axis lead to marked obesity in animals and humans (2, 5, 46); however, the majority of obese humans do not possess monogenetic perturbations, i.e., human obesity is a complex, multifactorial condition (48). As a result, the concept of leptin resistance as a mechanism of weight gain emerged (6).

An explanation for resistance to the central, satiety-producing effects of leptin is that the adipokine molecule crosses the blood-brain barrier via a saturable transport system, and thus effects on food intake wane as plasma concentration climbs with increasing adiposity (18). More recent studies indicate that there is selective resistance to leptin, i.e., resistance to central, appetite-suppressing effects without resistance to peripheral actions (25, 34). Leptin has been implicated as a mediator of many biological processes including growth and development, glucose metabolism, energy expenditure, and inflammation (24). Many of these processes require interplay between the central nervous system and peripheral target tissues; therefore, the concept of selective resistance requires further examination. As a result, the purpose of the present investigation was to test the direct effects of leptin on the coronary circulation; metabolic syndrome; obesity

HYPERLEPTINEMIA, universal in the human obese population (7), has recently been deemed an independent risk factor for cardiovascular disease (35) and, more specifically, a predictor of first myocardial infarction (43) and an independent risk factor for ischemic and hemorrhagic stroke (44). Since its discovery (53), leptin has been shown to promote platelet aggregation and thrombosis (3, 16, 17). Additionally, leptin is linked to the production of acute phase reactants (TNF-α, IL-6, and IL-12 (22), neointimal growth in mice (38), superoxide production in aortic endothelium (50), and calcification of vascular smooth muscle cells (30). The leptin receptor is a type I cytokine receptor, similar to gp130 and granulocyte colony-stimulating factor receptors (45, 51), and elevated plasma leptin levels occur concurrently with elevated IL-6 and C-reactive protein in human obesity-related conditions (19, 23, 27). Despite the superfluity of studies linking leptin to various atherogenic processes, few studies to date have examined the direct effects of leptin on the coronary circulation.

We (15) recently demonstrated that acutely raising coronary plasma leptin concentration to levels comparable with those observed in obese humans significantly attenuates acetylcholine (ACh)-induced coronary vasodilation in anesthetized, open-chest dogs and in isolated left circumflex coronary artery rings. That is, obese concentrations of leptin produce significant coronary endothelial dysfunction both in vivo and in vitro. Whether this phenomenon persists in obesity and hyperleptinemia, conditions associated with leptin resistance, is unknown.

The hypothesis that body adiposity is under homeostatic regulation has received long-standing support in medical and scientific communities and dates back nearly a century (9, 28). The hypothalamic control of food intake is no novel concept (13); however, it was not until the discovery of leptin (53) that the physiological mechanisms controlling food intake and energy expenditure began to surface (39). Mutations in the leptin axis lead to marked obesity in animals and humans (2, 5, 46); however, the majority of obese humans do not possess monogenetic perturbations, i.e., human obesity is a complex, multifactorial condition (48). As a result, the concept of leptin resistance as a mechanism of weight gain emerged (6).

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coronary circulation and coronary endothelial function in high-fat-fed dogs and thus to determine whether there is resistance to the coronary vascular effects of leptin in a model of prediabetes and chronic hyperleptinemia.

MATERIALS AND METHODS

This investigation was approved by the Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

Plasma leptin measurements. Venous blood samples were collected from control (n = 6) and high-fat-fed dogs (n = 6) in K$_3$ EDTA Vacutainer tubes and centrifuged at 4,300 rpm for 20 min at 4°C. The plasma supernatant was transferred to 1.5-ml microfuge tubes and stored at −80°C until analyses were performed. Plasma leptin was measured with a sandwich ELISA for canine leptin as previously described (11). Briefly, rabbit anti-canine leptin antibodies were produced using canine recombinant leptin as an antigen. Immunoreactivity with canine leptin was determined using Western blot analysis, and an ELISA assay was developed using standard biochemical techniques.

High-fat diet. Male mongrel dogs (n = 30) were fed either a standard laboratory dog chow (Teklad, −13% calories from fat, n = 10) or a high-fat diet (−60% calories from fat, n = 20). The high-fat diet was administered in the morning and afternoon for 5–6 wk (12, 40, 52). The morning feeding consisted of a homogenous mixture of canned dog food (Alpo, 748 g), dry dog food (Teklad, 227 g), and lard (Morrell, 57 g). The afternoon feeding consisted of a homogenous mixture of canned dog food (Alpo, 748 g), lard (Morrell, 454 g), and chicken or beef baby food (71 g).

Histology. After coronary flow studies, hearts were excised and immersed in cold (4°C) lactated Ringer solution (Baxter). Left circumflex coronary arteries were dissected from the epicardial surface of the heart (n = 4 control diet and n = 4 high-fat diet) and cleaned of periadventitial fat. Arteries were cut into 2-mm rings, mounted in 1.5-ml microfuge tubes and centrifuged at 4,300 rpm for 20 min at 4°C. The plasma supernatant was transferred to 1.5-ml microfuge tubes and stored at −80°C until analyses were performed. Plasma leptin was measured with a sandwich ELISA for canine leptin as previously described (11). Briefly, rabbit anti-canine leptin antibodies were produced using canine recombinant leptin as an antigen. Immunoreactivity with canine leptin was determined using Western blot analysis, and an ELISA assay was developed using standard biochemical techniques.

In vivo coronary dose-response experiments. Mongrel dogs (n = 30) were sedated with morphine (Baxter, 3 mg/kg sc), anesthetized with α-chloralose (Sigma, 100 mg/kg iv), and ventilated with room air supplemented with oxygen. The femoral arteries and a femoral vein were catheterized for aortic pressure measurement and for administration of supplemental fluids and anesthetics. Sodium bicarbonate (Sigma, 8.4%) was administered intravenously as needed, and the ventilatory rate was adjusted accordingly to maintain normal blood gas parameters. The left anterior descending coronary artery (LAD) was isolated distal to the first diagonal branch, and a stainless steel cannula was introduced. The LAD was perfused with blood from the left femoral artery at constant pressure (100 mmHg) with a servo-controlled, peristaltic pump (Ismatec). After a 20-min recovery period, various intracoronary dose-response experiments were performed as described below. Coronary blood flow was measured with an in-line Doppler flowprobe (Transonic Systems).

Leptin (0.1–30.0 μg/min) was infused into the coronary perfusion line (n = 5 high-fat diet). Each leptin dose was infused for 3–3 min, and data were recorded with IOX data-acquisition software (EMKA technologies) when coronary blood flow and hemodynamic parameters were stable.

To determine the effect of leptin on coronary endothelial function in high-fat-fed dogs, ACh (Sigma, 0.3–30.0 μg/min ic) was administered (each dose was infused for 2–3 min), and data were recorded when coronary blood flow and hemodynamic parameters were stable at each level of treatment (n = 6 high-fat diet). Ten minutes later, a continuous intracoronary infusion of leptin (3.0 μg/min) was initiated and continued throughout the remainder of the protocol. After 10 min of leptin administration, the ACh dose-response protocol was repeated. We (15) have previously performed time controls demonstrating that there is no tachyphylaxis to intracoronary ACh.

To compare coronary vascular smooth muscle sensitivity to nitrates between control and high-fat-fed dogs, sodium nitroprusside (SNP; Sigma, 1.0–100 μg/min ic) was administered (each dose was infused for 2–3 min). Data were recorded when coronary blood flow and hemodynamic parameters were stable at each treatment level (n = 6 control diet and n = 5 high-fat diet).

Venous blood samples were collected from control (n = 6) and high-fat-fed dogs (n = 6) in K$_3$ EDTA Vacutainer tubes and centrifuged at 4,300 rpm for 20 min at 4°C. The plasma supernatant was transferred to 1.5-ml microfuge tubes and stored at −80°C until analyses were performed. Plasma leptin was measured with a sandwich ELISA for canine leptin as previously described (11). Briefly, rabbit anti-canine leptin antibodies were produced using canine recombinant leptin as an antigen. Immunoreactivity with canine leptin was determined using Western blot analysis, and an ELISA assay was developed using standard biochemical techniques.

To determine the contribution of an endothelium-derived hyperpolarizing factor [EDHF(s)] to ACh-induced coronary vasodilation, additional ACh dose-response experiments were performed (n = 4 control diet and n = 4 high-fat diet). ACh (0.3–30.0 μg/min ic) was administered, and data were recorded as delineated above. Ten minutes later, indomethacin (Sigma, 10 mg/kg iv) was administered. Indomethacin was dissolved in 1 ml of equal parts of 95% ethanol, propylene glycol, and 1 N NaOH. The indomethacin mixture was added to the 9 ml of normal saline, pH was adjusted to 9.0 using 1 N NaOH, and the mixture was vortexed and sonicated with heat for 10 min. Five minutes after indomethacin administration, a continuous intracoronary infusion of N°-nitro-L-arginine methyl ester (L-NAME; Sigma, 150 μg/min) was started and continued throughout the remainder of the experiments. Fifteen minutes later, the ACh dose-response protocol was repeated as described above.

Functional assessment of isolated epicardial coronary rings. After coronary flow studies, hearts were excised and immersed in cold (4°C) lactated Ringer solution. Left circumflex coronary arteries were dissected from the heart and cleaned of periadventitial fat. Arteries were cut into 5-mm rings (n = 11 rings from 3 high-fat-fed dogs) and mounted in organ baths for isometric tension studies (Kent Scientific). Optimal length was found using 37.5 mM KCl. Passive tension was increased in gram increments until there was less than a 10% change in tension developed in response to KCl. Rings were precontracted using U-46619 (thromboxane A$_2$ mimetic, BioMol, 625 nmol/l). Graded concentrations of ACh (6.25 mmol/l–6.25 μmol/l) were added to the baths in a cumulative manner. Rings were then washed for 30 min. After the rings were washed, leptin (625 pmol/l–10 ng/ml) was added to the baths. Ten minutes later, rings were again precontracted with U-46619, and the ACh concentration-response protocol was repeated.

Isolation and quantitation of total RNA. Left circumflex coronary arteries from control (n = 4) and high-fat-fed dogs (n = 4) were cleanly dissected from the epicardial surface of the left ventricle, placed in liquid N$_2$, and stored at −80°C. Total RNA was extracted using the procedure provided with the SV Total RNA Isolation System (Promega). After isolation, RNA samples were dissolved in nuclease-free water (pH 7.5), and the optical density (OD) of each sample was measured using a UV-visible spectrophotometer (Bio-Rad) at wavelengths of 260 nm ($\lambda_{260}$) and 280 nm ($\lambda_{280}$). The amount of RNA in each sample was determined using the following formula: [RNA] = OD$_{260}$ dilution factor $\times$ 40 μg/ml. The OD$_{260}$/OD$_{280}$ ratio was used as a cursory estimate of RNA quality.

Preparation of first-strand cDNA via reverse transcriptase reactions. RNA samples were used as templates for synthesis of first-strand cDNAs as previously described (10). Briefly, 1 μl of oligo dT$_{15}$ primer (Promega) was added to equivalent amounts of total RNA obtained from coronary arteries isolated from control (n = 4) and high-fat-fed dogs (n = 4). The mixtures were then placed into a thermocycler (My Cycler, BioRad) and held at 70°C for 5 min. The samples were then transferred to an ice bath for 5 min to permit selective binding oligo dT$_{15}$ primers to the poly-A tails of the mRNA. First-strand cDNA was then synthesized using an ImProm-II Reverse Transcriptase kit (Promega).

Amplification of cDNA. Classical PCR was used to simultaneously amplify cDNAs encoding the canine long-form leptin receptor
Primers were designed based on published sequences in the National Center for Biotechnology Information GenBank database (http://www3.ncbi.nlm.nih.gov/entrez/) [ObRb, sense 289TGAGGAAGAGCAAGGGCTTA308 and antisense 453AAT-GGAAGGTGTGGCAAAAT434 (Accession No. AY 753649); /H9252-actin, sense 21GACATCCGCAAGGACCTCTA40 and antisense 176CA-CAGATCTTTGGCCTCAG157 (Accession No. U67202)]. PCR was performed using a Taq DNA Polymerase kit (Promega, 2.4 l/25 mmol/l MgCl in each 50-l reaction). PCR products were electrophoresed on a 1% agarose gel containing ethidium bromide (products: ObRb 165 bp, /H9252-actin 156 bp).

Real-time PCR. With the use of the primers described above, quantitative real-time PCR was also performed for ObRb and /H9252-actin with a custom-designed SYBR green mix [2.4 l of 25 mmol/l MgCl, 5 l of 1:10 000 dilution SYBR green I (Molecular Probes), and 5 l of 1 nmol/l fluorescein calibration dye (1 mmol/l in DMSO, Bio-Rad)] for 50 l total reaction using Taq DNA polymerase (Promega) and analyzed in triplicate for each animal with the iCycler IQ real-time PCR Detection system (Bio-Rad). Amplification was then carried out as follows: 45 s denaturation (95°C) followed by 45 s of annealing (58.5°C) and 2 min of extension (72°C), repeated for a total of 33 cycles. The \( 2^{-\Delta\Delta C_T} \) method (where \( C_T \) is threshold cycle) was used to analyze ObRb gene expression in high-fat-fed dogs relative to control dogs (21). The data were analyzed using the following \( \Delta\Delta C_T \) equation:

\[
\Delta\Delta C_T = (C_{T-ObRb} - C_{T-BA})_{\text{high-fat diet}} - (C_{T-ObRb} - C_{T-BA})_{\text{control}}
\]

The mean \( C_T \) values for both the target ObRb (T-ObRb) and internal control [target \( \beta \)-actin (T-BA)] genes were determined (control group, \( n = 4 \); high-fat diet, \( n = 4 \)). The fold change of each gene was normalized to \( \beta \)-actin, and expression relative to control was calculated for each triplicated sample using \( 2^{-\Delta\Delta C_T} \).

Results

High-fat feeding induces significant weight gain and hyperleptinemia. High-fat feeding significantly increased body weight (from 26.0 ± 0.9 to 31.3 ± 0.9 kg, \( P < 0.001, n = 20 \); Fig. 1A). Sandwich ELISA was used to measure canine leptin in blood plasma samples collected from control (\( n = 6 \)) and high-fat-fed (\( n = 6 \)) dogs. High-fat-fed dogs exhibited significantly higher plasma leptin concentrations than those fed the standard laboratory control diet (control diet, 5.75 ± 0.56 ng/ml).
ng/ml; high-fat diet, 13.30 ± 1.81 ng/ml, P = 0.003; Fig. 1B). Leptin concentrations measured in control dogs were similar to those found in nonobese humans (42). Moreover, the leptin concentrations measured in high-fat-fed dogs fell within the range reported in obese humans (8–90 ng/ml) (14, 41, 42).

Effects of high-fat feeding on coronary histomorphology. Analysis of H&E-stained left circumflex coronary arteries from control (n = 4) and high-fat-fed dogs (n = 4) revealed no histomorphological differences between groups. Representative H&E-stained arteries are displayed in Fig. 2. Slides were analyzed by a blinded anatomic pathologist in the LSUHSC pathology core, who determined that no appreciable changes associated with atherosclerosis (intimal thickening, inflammatory changes, fibromas, etc.) were present in either group. These findings clearly demonstrate that high-fat feeding for 5–6 wk does not induce atherosclerotic changes in large coronary arteries. Thus high-fat-fed dogs are a model of early prediabetes and do not possess overt histopathological coronary vascular changes.

Effects of intracoronary leptin on high-fat-fed, open-chest, anesthetized dogs. In a recent study (15) performed on normal control dogs, we demonstrated that leptin (0.1–30.0 μg/min ic) had no effect on coronary blood flow or myocardial oxygen consumption. As part of the present investigation, these studies were extended to a high-fat-fed canine model of the prediabetic metabolic syndrome. The direct effects of intracoronary leptin on coronary blood flow, mean aortic pressure, and heart rate in high-fat-fed dogs are shown in Fig. 3. The vertical bars in Fig. 3 represent infusion rates estimated to produce physiological and obese (pathophysiological) coronary plasma leptin concentrations. Coronary plasma leptin concentrations were calculated using the following equation:

\[
[\text{Leptin}]_{\text{plasma}} = \frac{\text{Infusion Rate} \times (1 - \text{HCT}) \times [\text{Leptin}]_{\text{infusate}}}{\text{Coronary Blood Flow}}
\]

where HCT is hematocrit. The estimated concentrations do not take endogenous leptin levels into account.

Leptin (0.1–30.0 μg/min ic) had no significant effect on coronary blood flow (Fig. 3A); however, there was a significant reduction in mean aortic pressure (P = 0.012; Fig. 3B) as well as a significant increase in heart rate (P < 0.001; Fig. 3C). A post hoc multiple-comparison test (SNK) detected no significant differences in aortic pressure between leptin treatment levels and baseline but did detect a dose-dependent increase in heart rate (Fig. 3C). These findings are consistent with other studies that demonstrate that leptin does not have direct vasodilator effects in vivo and that the hemodynamic actions of leptin are largely due to activation of the sympathetic nervous system (32, 33).

Coronary vascular leptin resistance in high-fat-fed dogs. A recent study (15) in our laboratory demonstrated that acutely raising leptin concentrations to those observed in obese humans (10–90 ng/ml) causes significant coronary endothelial dysfunction (marked attenuation of ACh-induced increase in coronary blood flow and coronary artery relaxation; Fig. 4, A and B). In the present investigation, these studies were extended to an experimental model of prediabetes and hyperleptinemia (high-fat-fed dogs). Interestingly, acute hyperleptinemia did not significantly alter ACh-mediated coronary vasodilation in open-chest, high-fat-fed dogs (Fig. 4C). Furthermore, leptin (625 pmol/l) did not significantly alter the maximum response to ACh in left circumflex coronary artery rings isolated from high-fat-fed dogs (Fig. 4D). These findings demonstrate that high-fat-fed, prediabetic, hyperleptinemic dogs are resistant to leptin-induced coronary endothelial dysfunction.
Potential functional mechanisms of leptin resistance. Resistance to leptin-induced coronary endothelial dysfunction in prediabetic dogs could be due to altered endothelium-dependent or -independent coronary vasodilation. As a result, we tested this hypothesis by performing intracoronary ACh and SNP dose-response experiments in control and high-fat-fed dogs. Responses to these agonists were not significantly different between control and high-fat-fed dogs (Fig. 5). These data demonstrate that prediabetic coronary vascular leptin resistance is not due to alterations in coronary endothelial function or changes in coronary vascular smooth muscle sensitivity to nitrates. High-fat-fed dogs do not exhibit coronary endothelial dysfunction (as assessed by the ACh dose response); therefore, this model of coronary vascular dysfunction may represent the early stages of the pathogenesis of coronary artery disease.

Given that ACh responses were very similar in control and high-fat-fed dogs (Fig. 5A), we hypothesized that high-fat feeding induces a switch to an EDHF as a significant mediator of endothelium-dependent coronary vasodilation. Furthermore, such a switch could explain the observed resistance to the effect of leptin on coronary responses to ACh. To test this hypothesis, additional ACh dose-response experiments were performed (control, n = 4; high-fat diet, n = 4) before and after combined nitric oxide synthase and cyclooxygenase inhibition with L-NAME and indomethacin (see MATERIALS AND METHODS). Coronary dilation to ACh was abolished by combined nitric oxide synthase and cyclooxygenase inhibition in both control (Fig. 6A) and high-fat-fed dogs (Fig. 6B). With combined blockade (L-NAME and indomethacin), there was a residual uprend in coronary blood flow at the higher doses of ACh in the high-fat-fed group (Fig. 6B); however, when this was statistically compared with the control group (L-NAME and indomethacin) by ANOVA, there was no difference (P = 0.316). Thus resistance to leptin-induced coronary endothelial dysfunction in high-fat-fed dogs is not mediated by a switch to an EDHF as a significant mediator of ACh-mediated coronary vasodilation.

Leptin receptor mRNA levels in left circumflex coronary arteries. Classical PCR was used to determine primer quality. A representative gel is shown in Fig. 7, left. Quantitative real-time PCR was used to determine the relative amount of left circumflex coronary artery ObRb transcripts in cDNA synthesized from total RNA obtained from control and high-fat-fed dogs. Quantitative assessment of left circumflex coronary artery ObRb mRNA levels via real-time PCR revealed no significant difference between control and high-fat-fed dogs (mean fold change in gene expression, 2−ΔCt = 1.09 ± 0.08; Fig. 7, right). Thus resistance to leptin-induced coronary endothelial dysfunction is not due to changes in ObRb transcript levels in coronary arteries. Whether there are changes in receptor protein density remains to be determined. At present, no anti-canine ObRb antibodies are commercially available.

DISCUSSION

The major new findings of this investigation were 1) high-fat feeding induces chronic hyperleptinemia in dogs (Fig. 1B), 2) leptin does not directly affect coronary blood flow in high-fat-fed dogs (Fig. 3A), and 3) high-fat-fed dogs are resistant to leptin-induced coronary endothelial dysfunction. In particular, intracoronary ACh responses are less affected by acute leptin administration in high-fat-fed animals than in controls (Fig. 4).

In summary, acute leptin administration causes coronary endothelial dysfunction in normal control dogs but not in...
According to the response to injury hypothesis, endothelial dysfunction is the inciting event in the pathogenesis of atherosclerotic vascular disease (36, 37); however, high-fat-fed dogs exhibit marked coronary circulatory dysfunction (40) in the absence of both overt coronary endothelial dysfunction (Fig. 5A) and histopathological change (Fig. 2). The histological sections clearly demonstrate that high-fat-fed dogs do not possess coronary atherosclerotic lesions. Nevertheless, as pointed out in High-fat feeding, prediabetes, and hyperleptinemia, high-fat-fed dogs exhibit many features of the prediabetic metabolic syndrome, a prevalent human condition (1, 26) associated with significantly increased cardiovascular morbidity and mortality (4, 29).

Direct effects of leptin on the coronary circulation. To our knowledge, no prior studies have examined the direct effects of leptin on coronary blood flow in animal models of obesity, non-insulin-dependent diabetes mellitus, or the prediabetic metabolic syndrome. These conditions are all associated with hyperleptinemia (7, 47, 49), an independent risk factor for cardiovascular disease (35). As a result, we tested the effects of intracoronarily administered leptin on coronary blood flow, aortic pressure, and heart rate in anesthetized, open-chest, high-fat-fed dogs.

Fig. 5. Resistance to leptin-induced coronary endothelial dysfunction in high-fat-fed dogs is not due to changes in coronary endothelial function or altered coronary vascular smooth muscle sensitivity to nitrates. Intracoronary dose responses to ACh (A) and sodium nitroprusside (B) do not differ between control and high-fat-fed dogs.

Fig. 6. Resistance to leptin-induced coronary endothelial dysfunction in high-fat-fed dogs is not mediated by an endothelium-derived hyperpolarizing factor (EDHF). Combined treatment with Nω-nitro-l-arginine methyl ester (L-NAME) and indomethacin abolished ACh-mediated coronary vasodilation in both control (A) and high-fat-fed dogs (B).

Histological analysis of epicardial coronary arteries. Histological analysis of left circumflex coronary arteries isolated from control and high-fat-fed dogs revealed no morphological differences between groups.
Consistent with previous studies in normal control animals (15, 32, 33), administration of leptin to high-fat-fed dogs did not produce vasodilation in vivo (Fig. 3A) but did produce an increase in heart rate (Fig. 3C).

**Leptin resistance extends to the coronary vasculature in prediabetes.** We (15) have previously demonstrated that acutely raising coronary plasma leptin concentrations to levels similar to those observed in human obesity and prediabetes significantly diminishes the coronary vasodilatory response to graded intracoronary doses of ACh in anesthetized, open-chest, normal control dogs (Fig. 4A). As mentioned above, endothelial dysfunction is taken to be the instigating event in the pathogenesis of coronary atherosclerosis (37), which is primarily a disease of large coronary arteries. Consequently, we also determined the effects of obese concentrations of leptin on isolated, large epicardial arteries. Acutely administered obese concentrations of leptin significantly impaired ACh-induced relaxation of left circumflex coronary artery rings isolated from normal control dogs (15) (Fig. 4B). Thus we concluded that acute hyperleptinemia (leptin concentrations in the obese range) induces significant coronary endothelial dysfunction. In the present investigation, as a logical subsequent step, we extended these studies to our experimental model of the prediabetic metabolic syndrome (high-fat-fed dogs) (40). The objective of the present investigation was to determine whether leptin resistance extends to the coronary vasculature in hyperleptinemic, prediabetic dogs.

Intracoronary infusion of obese concentrations of leptin did not impair coronary vasodilator responses to graded doses of ACh in prediabetic dogs (Fig. 4C). Additionally, obese concentrations of leptin did not attenuate the maximum ACh-mediated relaxation of left circumflex coronary artery rings isolated from prediabetic dogs (Fig. 4D). Thus prediabetic hyperleptinemic dogs are resistant to leptin-induced coronary endothelial dysfunction. On the surface, this finding seems perplexing because our canine model of prediabetes appears to be protected against coronary endothelial dysfunction induced with acute hyperleptinemia. However, it is possible that leptin resistance in early prediabetes provides a protective adaptation against the onset of coronary endothelial dysfunction. Alternatively, the effects of acute and chronic hyperleptinemia may be entirely different, i.e., leptin-induced coronary endothelial function may merely be an acute phenomenon. Another possibility is that the duration of high-fat feeding is short, such that the time of exposure to positive cardiovascular risk factors (e.g., dyslipidemia, insulin resistance, and hyperleptinemia) is not sufficient to produce a system analogous to that present in humans with long-standing (years) metabolic and cardiovascular risk factors.

Regardless of interpretation, the findings of the present study provide new information regarding the coronary vascular effects of leptin in obesity and chronic hyperleptinemia. To our knowledge, this investigation marks the first documentation of resistance to the direct effects of leptin on peripheral vascular targets. Selective leptin resistance (i.e., resistance to the central, appetite-suppressing effects of leptin without resistance at peripheral targets) is well documented (25, 34); however, the present investigation provides new information that indicates that leptin resistance may not be limited to centrally mediated effects.

**Potential mechanisms of coronary vascular leptin resistance.** Determining the precise mechanism(s) responsible for the observed resistance to leptin-induced coronary endothelial dysfunction in prediabetic dogs may prove difficult. This comes as no surprise given the elusive nature of biological phenomena of the like. A perfect example is the perpetual mystery surrounding the precise mechanisms of insulin resistance in the metabolic syndrome and non-insulin-dependent diabetes mellitus.

In the present investigation, several experiments were conducted in effort to determine the mechanism(s) of the observed leptin resistance in prediabetic dogs. Initially, we determined that high-fat-fed dogs do not exhibit coronary endothelial dysfunction (Fig. 5A) or alterations in coronary vascular smooth muscle sensitivity to nitrates (Fig. 5B). Thus differences in endothelium-dependent and -independent vasodilation can be ruled out as sources of the observed differences in the effects of leptin on coronary function between control and high-fat-fed dogs.

The results in Fig. 5 led us to the hypothesis that high-fat feeding induces a switch to an EDHF(s) as a major mediator of endothelium-dependent coronary vasodilation. Such a switch in the relative contribution of endothelium-derived molecules to ACh-mediated coronary vasodilation could explain the observed resistance to leptin-mediated endothelial dysfunction. To test the hypothesis, intracoronary ACh dose-response experiments were conducted before and after combined treatment with L-NAME and indomethacin. On the basis of our hypothesis, we predicted that L-NAME and indomethacin would produce less of an inhibitory effect on ACh dilation in high-fat-fed animals; however, our results indicated that there was not a switch to an EDHF(s) as a significant mediator of
muscarinic coronary dilation in high-fat-fed dogs (Fig. 6). In summary, resistance to leptin-mediated coronary endothelial dysfunction in prediabetic dogs is probably not the result of heightened release of an EDHF.

Quantitative real-time PCR was performed to determine whether coronary vascular leptin resistance is due to altered leptin receptor transcript levels in coronary arteries. Our results demonstrate that there is no significant difference in coronary artery ObRb transcript levels between prediabetic and control dogs. The present study offers no insight into the possibility that posttranscriptional modification in leptin receptor gene expression may be responsible for the observed resistance. Currently, there are no commercially available antibodies against the canine leptin receptor, making determination of protein levels difficult.

Recent studies in human brain capillaries (8) and human umbilical vein endothelial cells (31) demonstrate that endothelial cells possess two independent leptin-binding sites with different affinities for leptin. Downregulation of a high-affinity binding site and/or upregulation of a low-affinity binding site could explain vascular leptin resistance. Further studies are needed to examine this possibility. Given the nature of type I cytokine receptors (homodimer organization), there may be coexpression of two leptin receptor variants in endothelial cells. Speculatively, chronic inflammation associated with prediabetes and obesity may stimulate a significant change in the expression of receptor variants. Conceivably, the transcriptional or posttranscriptional regulation of the variants’ expression may differ.

In conclusion, our data demonstrate that high-fat feeding induces significant hyperleptinemia consistent with that observed in obese humans. Our results are consistent with other studies (noncoronary vascular beds) in control animals, i.e., leptin is not vasoactive in vivo, and its hemodynamic effects are likely mediated via increased sympathetic nervous activity. Additionally, the present study provides evidence that leptin resistance extends to the coronary circulation and provides an early protective adaptation against coronary endothelial dysfunction in prediabetic dogs.

**ACKNOWLEDGMENTS**

We thank Alberto Araiza for expert technical assistance and Dr. Samantha Huber for histological evaluation of coronary artery sections.

**GRANTS**

This study was supported by grants from the American Diabetes Association (to J. D. Tune) and National Institutes of Health Grants HL-67804 (to J. D. Tune) and P20-RR-018766 (to G. M. Dick).

**REFERENCES**


Coronary Leptin Resistance in Prediabetes


28. Neumann RO. Experimental contributions to the science of human daily nutritional needs with particular regard to the necessary amount of protein (author’s experiments). Arch Hgy Bakteriol 45: 69–70, 1902.


