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Endogenous adipose-derived factors diminish coronary endothelial function via inhibition of nitric oxide synthase

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Abstract

Adipocytokines may be the molecular link between obesity and vascular disease. However, the effects of these factors on coronary vascular function have not been discerned. Accordingly, the goal of this investigation was to delineate the mechanisms by which endogenous adipose-derived factors affect coronary vascular endothelial function. Both isolated canine coronary arteries and coronary blood flow in anesthetized dogs were studied with and without exposure to adipose tissue. Infusion of adipose-conditioned buffer directly into the coronary circulation did not change baseline hemodynamics; however, endothelial-dependent vasodilation to bradykinin was impaired both *in vitro* and *in vivo*. Coronary vasodilation to sodium nitroprusside was unaltered by adipose tissue. Oxygen radical formation did not cause the impairment because quantified dihydroethidium staining was decreased by adipose tissue and neither a superoxide dismutase mimetic nor catalase improved endothelial function. Inhibition of nitric oxide (NO) synthase with L-NAME diminished bradykinin-mediated relaxations and eliminated the subsequent vascular effects of adipose tissue. *In vitro* measurement of NO demonstrated that adipose tissue exposure quickly lowered baseline NO and abolished bradykinin-induced NO production. The results indicate that adipose tissue releases factor(s) that selectively impair endothelial-dependent dilation via inhibition of NO synthase-mediated NO production.

Keywords

coronary circulation; perivascular adipose tissue; vascular endothelium; adipocytokine; nitric oxide

INTRODUCTION

Adipose tissue is an active endocrine and paracrine organ that releases a variety of cytokines that influence many physiologic and pathophysiologic conditions (20). Recent studies have implicated perivascular adipose tissue in the pathogenesis of vascular dysfunction and disease (12;13;3;17). Adipocyte production of pathogenic adipocytokines and/or chemokines have been shown to stimulate chemotaxis (12), inflammation (15), smooth muscle proliferation (1) and activate key mediators of atherogenesis (20). These potentially harmful adipocytokines have been speculated to promote coronary atherogenesis via local paracrine and vasocrine pathways (30). In addition, perivascular adipose tissue has also been

shown to significantly attenuate contractile responses of rat aorta (6;10;21;34), rat mesenteric (9;36) and human internal thoracic arteries (11) to a variety of vasoconstrictor compounds. However, there are also abnormalities of vasodilation associated with adipocytokines. Our laboratory recently demonstrated that the adipocytokines leptin and resistin significantly impair canine coronary endothelial-dependent vasodilation both *in vivo* and *in vitro* (4;16) in normal animals. As might be expected, we have also demonstrated that obesity and insulin resistance alter the control of coronary blood flow and significantly impair the balance between oxygen delivery and myocardial metabolism (32). This vascular dysfunction is related to sensitization of key coronary vasoconstrictor pathways (5;17;38), some of which could be influenced by factors released from adipose tissue.

The goal of the present investigation was to delineate the mechanisms by which endogenous adipose-derived factors affect coronary vascular endothelial production of nitric oxide (NO) at the level of both microvessels and conduit arteries. Potential mechanisms were examined by *in vitro* studies in isolated canine coronary arteries with or without perivascular adipose tissue as well as *in vivo* experiments in open-chest anesthetized dogs to evaluate microvascular resistance regulation by endothelial vasodilators before and during treatment with adipose-conditioned buffer. This approach was used to document the effects of adipose tissue on coronary vascular reactivity in large arteries *in vitro* where coronary disease predominantly occurs as well as coronary flow responses *in vivo* which reflect alterations in function of microvascular resistance vessels.

MATERIALS AND METHODS

This investigation was approved by the Institutional Animal Care and Use Committee in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85–23, Revised 1996). All dogs studied were lean mongrel dogs weighing between 20 and 30 kg.

Functional assessment of isolated epicardial coronary rings

Isolated coronary artery studies were performed as previously described (4;16). Briefly, left circumflex coronary arteries from lean dogs were dissected from the heart with or without the naturally occurring perivascular adipose tissue surrounding the conduit artery. Representative coronary arteries with or without perivascular adipose tissue were stained with Sudan IV and are shown in Fig 1. The arteries were cut into 3 mm rings and mounted in organ baths for isometric tension studies. Perivascular adipose tissue was either rigorously removed from the arterial rings or allowed to remain intact (approximately 0.25 g adipose per ring). Optimal length was found by assessing contraction to 60 mM KCl. Passive tension was increased in gram increments until there was < 10% change in active KCl contractions.

Endothelial function was assessed by the addition of graded concentrations of bradykinin (0.1 nM/L–10 μ M/L, n = 5) or sodium nitroprusside (1.0 nM/L–0.1mM/L, n = 3) to the tissue bath. In additional studies, bradykinin concentration responses were conducted in the presence of the NO synthase inhibitor N^o-nitro-L-arginine methyl ester (L-NAME, 300 μ M/L, n = 7), the superoxide dismutase mimetic tempol (10 μ M/L, n = 3), and the H₂O₂ degrading enzyme catalase (1000 U/ml, n = 4). All results obtained during bradykinin and sodium nitroprusside dose response experiments are reported as the percent relaxation for each animal (Fig 3, 4, 5 and 6). 100 percent relaxation is defined as a return to the level of tension prior to U46619 contraction.

Coronary blood flow evaluation of microvascular performance

Dogs were initially sedated with morphine (3 mg/kg, sc) and anesthetized with α -chloralose (100 mg/kg, iv). The animals were then intubated and mechanically ventilated (Harvard respirator) with room air supplemented with oxygen. A catheter was placed in the right femoral vein for intravenous administration of supplemental anesthetic and sodium bicarbonate. The femoral artery was then cannulated to supply blood to an extracorporeal perfusion system which subsequently perfused the left anterior descending coronary artery (LAD) at a controlled pressure. A left lateral thoractomy was performed to expose the heart, and the LAD was isolated distal to its first major diagonal branch. After the administration of heparin (500 U/kg, iv) the LAD was cannulated with a stainless steel cannula (3 mm external diameter, 2.2 mm internal diameter). Coronary perfusion pressure was measured through a saline filled catheter advanced to the orifice of the LAD cannula. The pressure of the perfusion system was held constant at 100 mmHg by a servo-controlled roller pump. Similarly, an in line Transonic Systems flow transducer (Ithaca, NY) was used in the perfusion system to measure coronary blood flow. Data were continuously recorded on IOX data acquisition software from Emka Technologies (Falls Church, VA). The preparation was allowed a ~30 minute recovery time before data were recorded for analysis.

In order to test the effects of endogenous adipose-derived factors on coronary microvascular endothelial function *in vivo*, bradykinin was infused (0.3–3.0 μ g/min) in the absence and presence of adipose conditioned buffer (n = 6). The adipose-conditioned buffer was prepared in phosphate buffered saline that was allowed to shake and mix with parietal pericardial adipose tissue (3 g/ml) for 30 min at 37°C in a shaking water bath. The conditioned buffer was then filtered (0.2 μ m) and infused directly into the coronary circulation via the perfusion system (0.3 ml/min). In additional studies, bradykinin was simultaneously infused with either L-NAME (150 μ g/min, n = 5) or tempol (10 mg/min, n = 5). Lastly, studies were also conducted to determine if adipose-conditioned buffer had any direct effect on coronary hemodynamics. During these studies, adipose-conditioned buffer was infused at various rates (0.3–3.0 ml/min, n = 3) without the administration of bradykinin.

Nitric oxide measurements

NO concentration was measured in isolated coronary arteries (n = 3) before and during exposure to 400 nM bradykinin with or without the addition of perivascular adipose tissue. This concentration of bradykinin was a supramaximal concentration for NO production by isolated arterial segments. NO was evaluated by a polarographic technique, using a carbon fiber, recessed-tip glass microelectrodes as previously described (37). The microelectrodes had a sharpened outer tip diameter of 7–10 μ m and were polarized at +0.7 or +0.9 V relative to either a World Precision Instruments carbon fiber reference electrode (Sarasota, FL) or a simple silver-silver chloride electrode. The currents generated ranged from 0–20 pA. A calibration curve was established by measurement of the microelectrode current at NO concentrations of 0, 600, and 1,200 nM. These concentrations were based on the composition of the NO-N₂ precision calibration gases in saline at 37.5°C. The working resolution of the microelectrodes is typically <10 nM, allowing for random noise and current drift. The microelectrodes are completely insensitive to oxygen when positively polarized.

Rings of vessel were placed in a perfused bath (5 ml/min, 5 ml bath volume) of media similar to that used for tension studies and equilibrated with 95% oxygen and 5% carbon dioxide. The tip of the NO microelectrode was placed in the lumen of the vessel and touched an endothelial surface. The baseline concentration of NO was measured followed by the response to bradykinin (400 nM). Thereafter, the adipose tissue removed from the arterial ring was placed upstream of the ring in the flowing media for 30 minutes before repeating the NO measurements at rest and during bradykinin exposure.

Dihydroethidium staining

Dihydroethidium (DHE) staining for superoxide (O_2^-) was carried out as described previously (4). Left circumflex coronary arteries with ($n = 5$) and without ($n = 5$) perivascular adipose tissue were incubated with $10 \mu\text{M}$ DHE at 37°C for 30 min with or without tempol ($10 \mu\text{M/L}$) administration. The arteries were embedded in OCT and flash frozen in liquid nitrogen. Tissue sections ($10 \mu\text{m}$) were then prepared using a cryostat on thaw-mounted slides. Ethidium fluorescence was assessed with 508 nm excitation and 615 nm emissions. Scion Image for Windows was used to perform histogram analysis of brightness in images of DHE-stained arteries.

Adipokine content in adipose tissue extract

A multiplexed biomarker immunoassay from Linco Laboratories was used to measure the concentration of key adipokines in the adipose-conditioned buffer (Table 2). The assay was conducted according to the manufacturer's specifications. Briefly, the assay functions by using antibody coated beads, which are selective for specific human adipokines. Adipose-conditioned buffer samples used during the *in vivo* studies were collected, filtered ($0.2 \mu\text{m}$) and individually measured in triplicate on a 96 well plate. The antibody coated beads were incubated with each individual sample and allowed to bind overnight. Biotinylated detection antibodies were added to each well, and streptavidin-phycoerythrin was subsequently added in order to detect the fluorescence on each bead. A Luminex Instrument was used to both identify which adipokines were expressed and to quantify their relative concentrations.

Statistical analyses

Data are presented as mean \pm standard error. For both *in vitro* and *in vivo* studies, a two-way repeated measures ANOVA was used to test the effects of the presence or absence of adipose tissue or conditioned buffer (Factor A) and various doses of sodium nitroprusside or bradykinin (Factor B) on coronary physical or chemical responses (Sigma Stat 3.0 Software). Identical statistics were performed for studies conducted in the presence of L-NAME, tempol and catalase. For the *in vitro* experiments, data were analyzed per animal. When statistical differences were found by ANOVA, a Student-Newman-Keuls multiple comparison test was performed. The criterion for statistical significance was $P < 0.05$ in all tests.

RESULTS

Adipose tissue and baseline hemodynamics

Data shown in Figure 2 demonstrate that increases in the intracoronary infusion rate of the adipose-conditioned buffer ($0.3\text{--}3.0 \text{ ml/min}$) did not significantly affect baseline coronary blood flow ($P = 0.68$). Effects of adipose-conditioned buffer, L-NAME and tempol on baseline coronary blood flow, mean aortic pressure and heart rate are given in Table 1. Intracoronary infusion of adipose-conditioned buffer (0.3 mL/min) did not significantly affect coronary blood flow ($P = 0.58$), mean aortic pressure ($P = 0.96$) or heart rate ($P = 0.89$). Baseline coronary hemodynamics were also unaffected by adipose-conditioned buffer in the presence of L-NAME ($P = 0.58$) or the superoxide dismutase mimetic tempol ($P = 0.19$). The average concentrations of adipokines in adipose-conditioned buffer are presented in Table 2. Notably, the concentration of resistin was substantially greater than the other adipokines.

Adipose tissue and coronary endothelial function

Adipose-conditioned buffer exposure significantly attenuated coronary endothelial-dependent vasodilation, as judged by blood flow responses, to the higher doses of

bradykinin (1–3 $\mu\text{g}/\text{min}$; ~ 10 nM bradykinin) in open-chest anesthetized dogs (Fig 3A; $P < 0.01$). In isolated coronary arteries, arterial relaxation to bradykinin was also decreased by perivascular adipose tissue (Fig 3B; 1–100 nM; $P < 0.01$). In contrast, adipose tissue did not significantly affect endothelial-independent relaxation to sodium nitroprusside in isolated arteries (Fig 4).

Adipose tissue and coronary reactive oxygen species

DHE staining for coronary O_2 showed a significant decrease ($67 \pm 7\%$ of control) in tempol-sensitive fluorescence between arteries with perivascular adipose tissue relative to arteries fully cleaned of adipose tissue (Fig 5A and 5B). Therefore, vessel exposure to adipose tissue did not increase O_2^- formation. Additional studies demonstrated that the administration of tempol (10 μM) to scavenge oxygen radicals did not improve reactivity of arteries with perivascular adipose tissue to bradykinin (Fig 5D). Instead, tempol significantly impaired relaxation of arteries, with or without adipose tissue, to bradykinin (Fig 5C and 5D, $P < 0.001$). These findings were confirmed by additional *in vivo* studies in Figure 5E which found that tempol failed to reverse the adipose tissue-induced impairment of bradykinin-mediated coronary vasodilation in open-chest dogs (Fig 5E, $P < 0.001$). To test for H_2O_2 effects, catalase was applied prior to *in vitro* relaxation studies. Enzymatic degradation of H_2O_2 with catalase diminished the relaxation to bradykinin at 1 nM to 30 nM (Fig 5F) in comparison to the control relaxation shown in Figure 5C. However, arteries with perivascular adipose tissue continued to display further impairment ($P < 0.01$) to bradykinin.

Adipose tissue and nitric oxide production

Inhibition of NO synthase with L-NAME eliminated the relaxation differences between coronary artery rings with or without perivascular adipose tissue (Fig 6A, $P = 0.32$). Also note that compared to responses of untreated vessels in Figure 3B, L-NAME caused a right shift of the dose-relaxation curve indicating major suppression of endothelial dependent relaxation. These initial observations were further supported by *in vivo* studies demonstrating that pretreatment with L-NAME abolished the effect of adipose-conditioned buffer on bradykinin-mediated coronary vasodilation (Fig 6B; $P = 0.24$). Again, L-NAME caused a significant attenuation of the dose-blood flow response curve indicating major suppression of endothelial dependent relaxation in the *in vivo* coronary microvasculature (Fig. 3A vs. Fig 6B). Importantly, measures of coronary NO concentration with an NO sensitive microelectrode showed that adipose tissue both markedly lowered basal NO production by 60% and eliminated coronary endothelial NO production in response to bradykinin (Fig 6C, $P < 0.05$).

DISCUSSION

The present investigation was designed to examine the mechanisms by which endogenous adipose-derived factors might impair coronary endothelial function within both the coronary microcirculation and conduit arteries. The major new findings from this study are that factor(s) released from adipose tissue: 1) do not affect baseline coronary blood flow or systemic hemodynamics; 2) significantly impair endothelial-dependent vasodilation to bradykinin in both *in vivo* preparations and isolated coronary arteries; 3) do not alter coronary endothelial-independent vasodilation to sodium nitroprusside, i.e. vascular smooth muscle response to NO; 4) do not significantly increase coronary O_2^- production or H_2O_2 -mediated vasodilation; and 5) markedly attenuate coronary artery endothelial production of NO in isolated vessels. Taken together, these data indicate that adipose tissue releases factor(s) that selectively impair endothelial-dependent dilation via inhibition of NO synthase-mediated NO production. This impairment is independent of alterations in

coronary vascular smooth muscle response to NO, O_2^- mediated decreases in NO-bioavailability or H_2O_2 -mediated coronary vasodilation.

Adipose tissue and baseline coronary hemodynamics

Before trying to interpret any effect of adipose tissue, it was necessary to test the direct effect of adipose-derived factors on baseline coronary blood flow, arterial pressure and heart rate (Fig. 2 and Table 1). Our results indicate that factors released by adipose tissue do not directly affect overall coronary microvascular resistance under normal resting conditions. Importantly, the present findings are consistent with earlier studies and the current L-NAME studies (Table 1) which demonstrated that blockade of NO synthase-mediated NO production has little effect on baseline coronary blood flow (7;35).

Effects of adipose tissue on coronary endothelial function

Data from this study are the first to document that endogenous factors released from adipose tissue selectively inhibit coronary endothelial-dependent dilation to bradykinin judged both by *in vivo* measures of coronary blood flow (regulated predominantly by microvessels; Figs 3, 5, and 6), by relaxation of isolated coronary arteries (where atherosclerotic disease predominantly occurs; Fig 3) and by *in vitro* measurement of NO (Fig 6C). Our findings indicate that the adipose-induced impairment of coronary endothelial function is mediated by a selective impairment of NO synthase mediated NO production because the effect of adipose tissue on bradykinin-mediated dilation was reversed by L-NAME (Fig 6A–B) and direct measures of NO demonstrated a decline in both basal NO and bradykinin stimulated NO production (Fig 6C). We propose that the impaired relaxation is specific to NO generation as we did not detect any effect of adipose tissue on relaxations to sodium nitroprusside (Fig 4) or to bradykinin in the presence of L-NAME (Fig 6), suggesting that adipose tissue does not affect dilation to prostacyclin or endothelial-derived hyperpolarizing factors.

The findings documented in this investigation are potentially marred by a few concerns. First, there was a potential that the measured endothelial impairment was simply an artifact of decreasing responses to bradykinin after repeated doses (i.e. tachyphylaxis). However, a recent study from our laboratory demonstrated no tachyphylaxis to repeated treatments of bradykinin both *in vivo* in open-chest anesthetized dogs and *in vitro* in isolated coronary arteries (4). Hence any measured experimental differences in bradykinin-mediated dilation are not an artifact of repeated bradykinin dose-response curves. Second, the isometric tension and NO production measurements were done under circumstances where there was at most a trivial amount of hemoglobin, oxyhemoglobin or myoglobin. Therefore, these iron containing organ compounds were highly unlikely to suppress NO bioavailability (13;14;39). This conclusion is further supported by our *in vivo* coronary flow data.

Adipose tissue and coronary reactive oxygen species

Recently, many investigations have focused on the role of reactive oxygen species in regulating endothelial function (2;29). Specifically, O_2^- has been shown to decrease NO bioavailability by reacting to produce peroxynitrite (22). Additional studies have also shown that H_2O_2 acts as an endothelium-derived hyperpolarizing factor and metabolic vasodilator of the coronary circulation (25;28;29;31). Together, both O_2^- and H_2O_2 are potential direct and/or indirect mediators of the observed endothelial dysfunction caused by adipose tissue. The fact that DHE staining of arteries with perivascular adipose tissue showed a significant decrease in fluorescence (Fig 5A–B) argues that adipose tissue does not impair coronary endothelial-dependent vasodilation via increases in O_2^- production. This is supported by additional *in vitro* and *in vivo* studies with tempol that showed this O_2^- dismutase mimetic did not significantly improve the adipose tissue induced impairment of bradykinin-mediated

coronary vasodilation (Fig 5C, 5D, 5E). In fact, administration of tempol significantly decreased bradykinin-mediated relaxation suggesting that O_2^- may function as a vasodilator, a hypothesis supported by other recent investigations (18;19;24;33). Enzymatic degradation of H_2O_2 with catalase also failed to reverse adipose-induced endothelial impairment (Fig 5F). Taken together these important data argue against O_2^- or H_2O_2 as a mechanism of adipose-induced coronary endothelial dysfunction.

Identity of adipose tissue derived vasoactive factor(s)

We performed a “targeted” immunoassay to measure the expression of key adipocytokines in adipose-conditioned buffer (Table 2). This assay was in no way exhaustive of all potential adipose-derived mediators; however this approach allowed us to identify candidate adipokines that could contribute to the adipose-induced endothelial impairment. It is important to point out that the use of anti-human antibodies for this canine adipose tissue assay was an unavoidable limitation of this assay. However, it should be pointed out that there was no clear correlation between the % homology of human vs. canine adipokine protein sequences (ranged from 63–92%) and the measured adipokine concentrations (Table 2) which suggests that antibody specificity alone was not the reason for the low concentrations measured.

Using this targeted immunoassay we found that resistin levels were markedly high relative to the other measured adipokines (5.9 ± 1.4 ng/ml, Table 2). This resistin concentration results in an estimated average plasma concentration of 0.19 ± 0.09 ng/ml, which is significantly lower than concentrations reported in human plasma (23;27) and the 10 ng/ml that our laboratory (4) as well as others (17;33) previously demonstrated to impair coronary endothelial-dependent vasodilation. Additionally, the present study found that L-NAME reversed the effect of adipose tissue on bradykinin-mediated dilation (Fig 6A and 6B) but an early study from our laboratory showed that L-NAME failed to inhibit resistin-induced suppression of bradykinin dilation (4). These findings do not support resistin as the primary mechanism of adipose-induced coronary endothelial dysfunction.

Other adipokines could potentially contribute the observed coronary effects of adipose tissue. Recently, our laboratory found that leptin induces significant impairment of coronary endothelial-dependent vasodilation (16). However, the measured concentration of leptin in the adipose-conditioned buffer (2.40 ± 0.20 pg/ml) is substantially lower than the ~10 ng/ml previously found to be necessary for leptin-induced coronary endothelial dysfunction (16). Therefore, as was the case for resistin, the functional concentration for leptin was below the pathologic concentration that negatively influenced endothelial-dependent dilation. Alternatively, TNF- α and long chain fatty acids have both been shown to attenuate endothelial-dependent vasodilation, albeit via mechanisms that elevate oxidative stress/ O_2^- production (3;8;26) which is inconsistent with our findings with adipose tissue (Fig 5). Therefore, at present it is difficult to attribute the adipose-induced impairment to any known adipocytokine. However, it is plausible that several different adipose-derived factors could act in an additive and/or synergistic manner to diminish coronary NO production. Clearly future studies are needed to identify the exact adipose-derived factor(s) and cellular/molecular mechanisms involved.

In conclusion, results from this study are the first to demonstrate that endogenous adipose-derived factors diminish production of coronary endothelial NO as judged by pharmacological challenges and direct measurements of endothelial NO. The overall findings implicate that adipocytokines have the ability to rapidly depress coronary microvascular and arterial NO generation. It is important to note that during *in vivo* circumstances, perivascular adipose tissue has little or no access to the endothelial cells of either arteries or arterioles. We are only demonstrating that adipose tissue contains factors

that when released and circulated throughout the heart cause potentially dire consequences for endothelial mediated dilation. These results provide us with a new potential explanation of how alterations in adipokine expression in obesity may contribute to the development of vascular dysfunction and coronary atherosclerosis.

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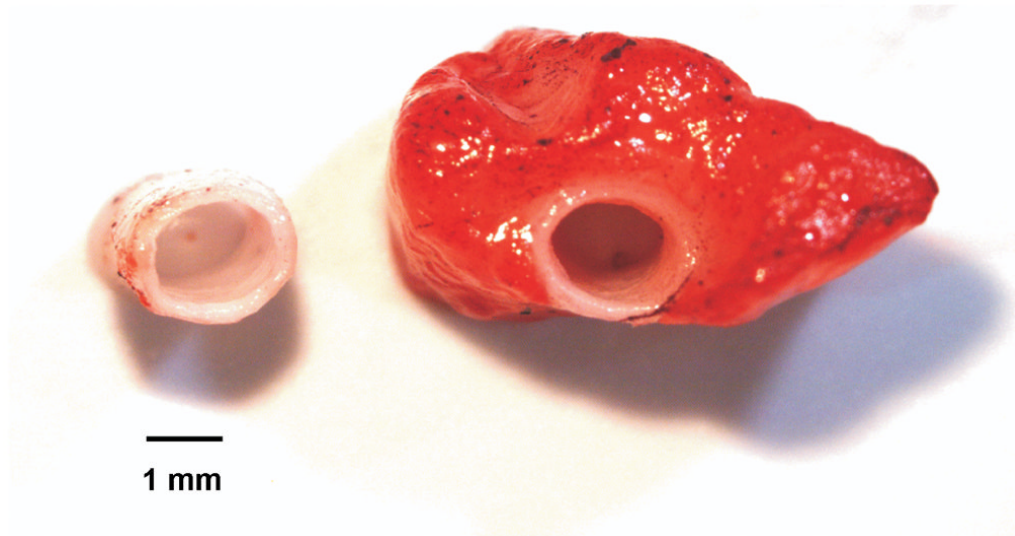


Fig 1. Representative isolated left circumflex coronary arteries with or without perivascular adipose tissue stained with Sudan IV (adipose tissue staining red).

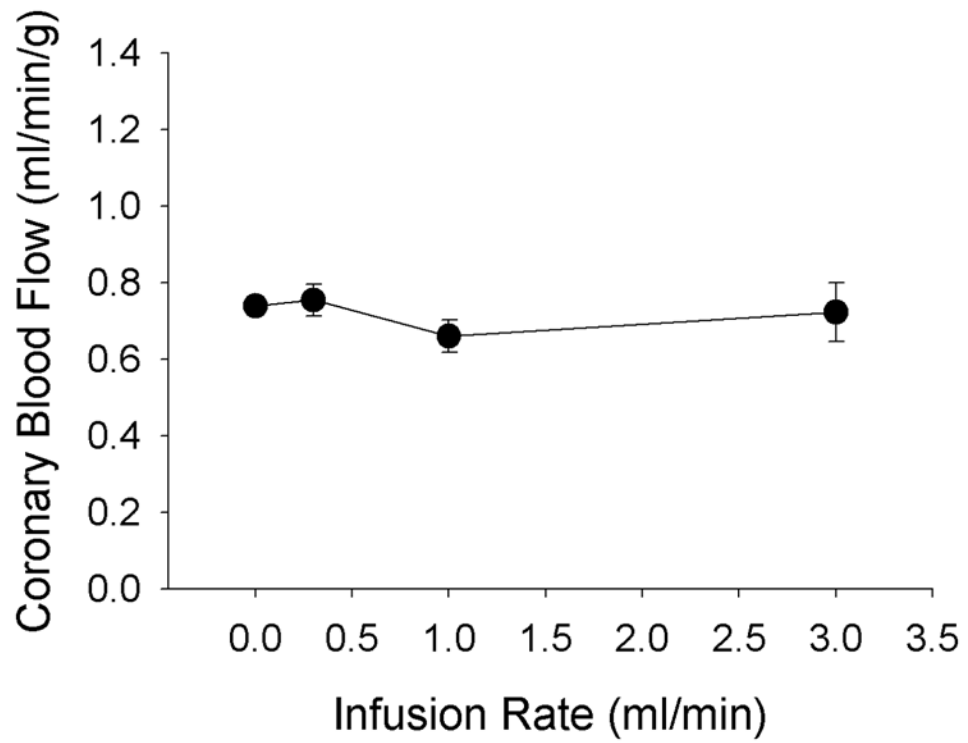


Fig 2. Direct infusion of endogenous adipose-derived factors from adipose-conditioned buffer into canine coronary circulation has no effect on baseline coronary blood flow (n = 3).

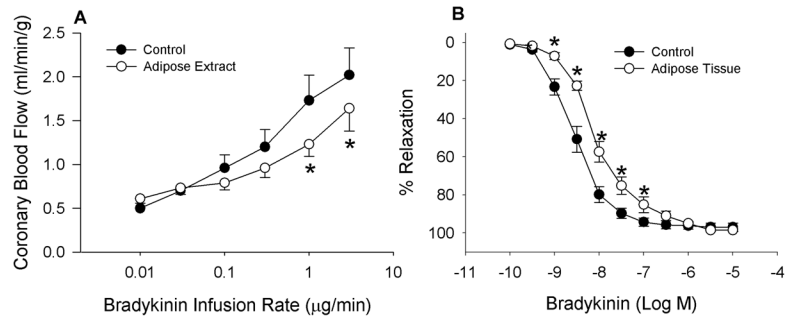


Fig 3. Adipose tissue significantly attenuates coronary endothelial-dependent vasodilation to bradykinin *in vivo* (3A, n = 6) and in isolated coronary arteries (3B, n = 5). * = $P < 0.01$.

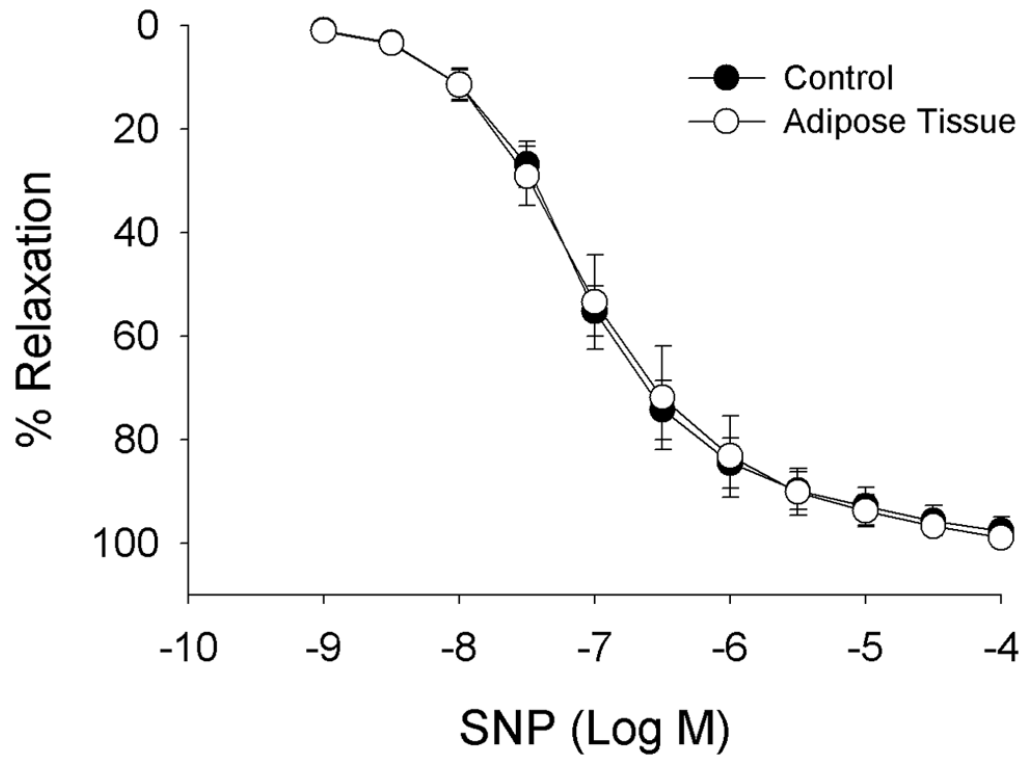


Fig 4. Adipose tissue has no significant effect on coronary endothelial-independent vasodilation to sodium nitroprusside (SNP) $n = 3$.

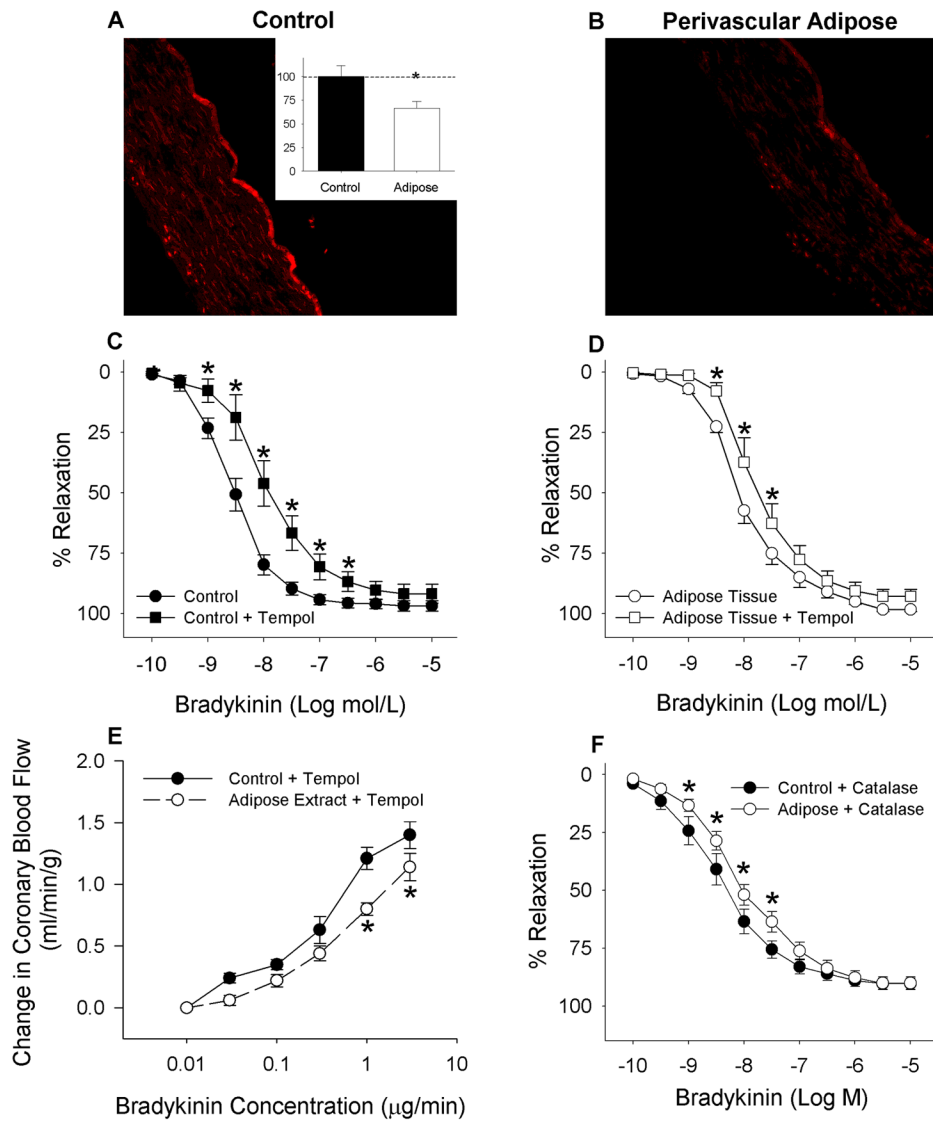


Fig 5. DHE staining showed a significant decrease in fluorescence in coronary arteries with perivascular adipose tissue (Representative pictures A and B; inset shows average DHE fluorescence). The superoxide dismutase mimetic tempol did not improve reactivity of coronary arteries with perivascular adipose tissue (D); rather it significantly impaired reactivity of arteries without (C) and with (D) adipose tissue (n = 3). In addition, tempol also failed to improve reactivity *in vivo* (E, n = 5). * $P < 0.001$. Enzymatic degradation of peroxide (H_2O_2) with catalase also did not reverse the endothelial impairment * $P < 0.01$ (F, n = 4).

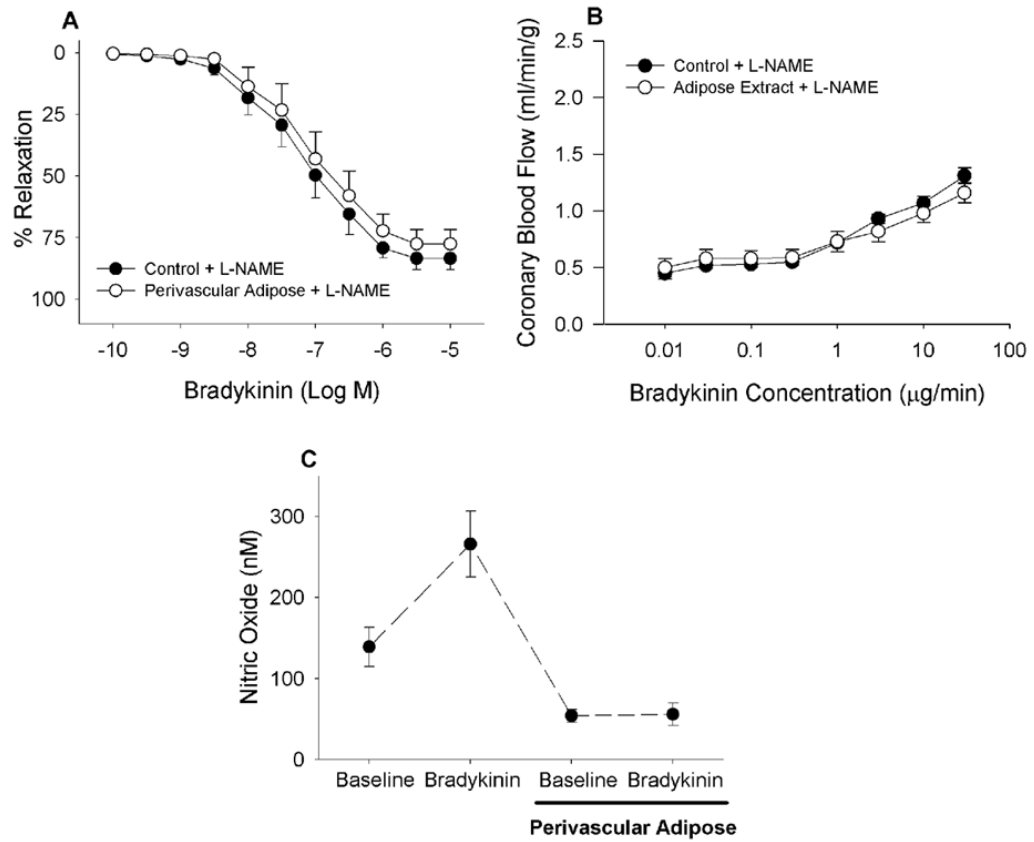


Fig 6. Inhibition of nitric oxide synthase with L-NAME reversed the effect of adipose tissue on bradykinin-mediated vasodilation in isolated coronary arteries (A, n = 7) and in open-chest anesthetized dogs (B, n = 5). Perivascular adipose tissue markedly impaired bradykinin (400 nM)-mediated increases in NO production (C, n = 3).

Table 1

Effects of adipose-conditioned buffer, L-NAME and tempol on baseline hemodynamic data in anesthetized, open-chest dogs.

	Coronary Blood Flow (ml/min/g)	Aortic Pressure (mmHg)	Heart Rate (beats/min)
Untreated			
Control (n = 6)	0.50 ± 0.01	103 ± 6	87 ± 13
Adipose-conditioned buffer (n = 6)	0.61 ± 0.05	96 ± 9	110 ± 24
L-NAME			
Control (n = 5)	0.45 ± 0.05	105 ± 8	72 ± 9
Adipose-conditioned buffer (n = 5)	0.50 ± 0.08	103 ± 9	90 ± 11
Tempol			
Control (n = 5)	0.69 ± 0.03	102 ± 9	77 ± 10
Adipose-conditioned buffer (n = 5)	0.94 ± 0.15	98 ± 12	94 ± 8

Values are mean ± SE. n = number of dogs.

Table 2

Adipokine expression in adipose-conditioned buffer. A multiplexed biomarker immunoassay was conducted in order to measure adipokine concentrations present within the adipose-conditioned buffer.

Adipokines	Concentration in Adipose-conditioned buffer (pg/ml)
TNF- α	0.04 \pm 0.01
IL-1 β	0.24 \pm 0.02
PAI-1(active)	0.42 \pm 0.08
MCP-1	0.45 \pm 0.01
HGF	1.64 \pm 0.20
Leptin	2.40 \pm 0.20
Resistin	5338.00 \pm 1032.00

Values are mean \pm SE for all measured samples (n = 10). IL-1 β –interleukin 1 β , PAI-1 -plasminogen activator inhibitor 1, MCP-1 - monocyte chemoattractant protein-1, HGF -hepatocyte growth factor.