

# Regional Heterogeneity of Functional Changes in Conduit Arteries After High-fat Diet

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**Objective:** To determine effects of dietary fat content on vascular responses in different conduit arteries in mice.

**Methods and Procedures:** Vascular responses to reactive oxygen species (ROS)/hydroxyl radical ( $\cdot\text{OH}$ ), acetylcholine (ACh), endothelin-1 (ET-1), and angiotensin II (Ang II) were determined in carotid and femoral arteries of C57BL/6J mice fed with diets varying in fat content (low fat (LF), 12.3%; high fat (HF), 41%; and very high fat (VHF), 58% (kcal from fat)) for 15 weeks, beginning at 4 weeks of age.

**Results:** In precontracted rings of carotid and femoral artery, ROS/ $\cdot\text{OH}$ -induced a rapid, transient vasodilation. In the carotid, but not in femoral artery, ROS/ $\cdot\text{OH}$ -induced dilation increased with increasing dietary fat intake ( $P < 0.05$  vs. LF diet), while contractile responses to ROS/ $\cdot\text{OH}$  remained unaffected. In femoral arteries, ROS/ $\cdot\text{OH}$ -induced contractions were reversed into relaxations after both HF and VHF diet ( $P < 0.05$  vs. LF diet). Both ET-1 and Ang II induced strong contractions in the femoral artery that were unaffected by dietary fat intake. In contrast, in the carotid artery Ang II-induced contraction was attenuated after HF and VHF diets ( $P < 0.005$  vs. LF diet), whereas ET-1-induced vasoconstriction was significantly increased ( $P < 0.05$  VHF vs. LF and HF). Treatment with VHF diet enhanced ACh-mediated endothelium-dependent relaxation only in the femoral artery ( $P < 0.05$  vs. HF).

**Discussion:** These findings demonstrate that dietary fat content has regional and distinct effects on vascular function in different vascular beds. The data also suggest the possibility that in selected conduit arteries ROS-dependent vasodilator mechanisms become activated in response to increased dietary fat intake.

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## INTRODUCTION

Modern urban lifestyle patterns involve easy accessibility to a variety of foods with high-fat (HF) content. The associated increased risk for developing obesity is linked with numerous comorbidities such as insulin resistance, type 2 diabetes, hypertension, coronary artery disease, and cancer (1). Dietary habits across the globe are changing rapidly, with increased consumption of saturated fat and refined carbohydrates (2). Overweight and obesity are now recognized to accelerate the development of atherosclerosis in children, juveniles, and adults (3–7).

Experimental and clinical evidence suggests that obesity and its associated clinical conditions such as insulin resistance and diabetes result in abnormal vascular function and oxidative stress (8–11). In experimental models of diet-induced obesity, vascular production of reactive oxygen species (ROS) and vasoconstrictor prostanoids contribute to abnormal endothelial cell function (12,13), and, accordingly, adhering to low-caloric diet improves endothelium-dependent vasodilation in obese patients (14). The hydroxyl radical ( $\cdot\text{OH}$ ), a short-lived ROS, which affects vascular function (15,16), is generated under physiological conditions by the interaction between

$\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  in the presence of catalytic metal ions (17). We have recently reported that ROS contribute to the maintenance of vascular function in a model of monogenetic obesity (18), as well as in diet-induced obesity (19).

The influence of dietary fat intake on vascular responsiveness to ROS/ $\cdot\text{OH}$  or to vasoactive agonists and effects of fat diet in anatomically distinct vascular beds within the same organism have not been previously studied. In this work, we, therefore, investigated the effects of diets with defined fat content on vascular functional changes in healthy C57BL/6J mice. Specifically, vascular responses to ROS/ $\cdot\text{OH}$ , as well as vaso-reactivity to acetylcholine (ACh), endothelin-1 (ET-1), and angiotensin II (Ang II) in carotid and femoral conduit arteries, were determined in isolated vascular rings.

## METHODS AND PROCEDURES

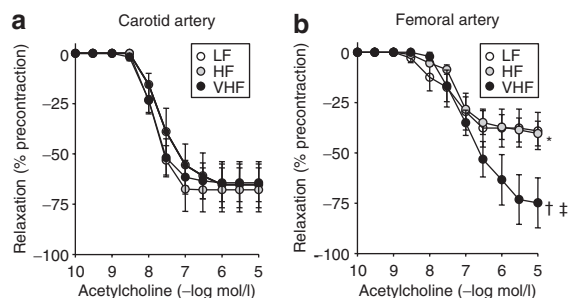
### Animals and dietary treatments

Healthy male mice (C57BL/6J; Charles River, Sulzfeld, Germany; 4 weeks of age) were treated for 15 weeks with diets varying in fat content. Control animals were fed with standard rodent chow with low-fat (LF) content (12.3% of total kcal from fat; Kliba Nafag 3430, Kaiseraugst, Switzerland). The other groups were fed with either HF

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**Figure 1** Endothelium-dependent relaxation to acetylcholine. Rings of (a) carotid and (b) femoral artery of control animals on LF diet, and animals receiving HF, or VHF diet were exposed to acetylcholine causing endothelium-dependent relaxation in a concentration-dependent manner. Data are expressed as percent of precontraction (carotid artery:  $n = 6-10$ /group; femoral artery:  $n = 7-9$ /group). \* $P < 0.05$  vs. carotid artery, † $P = 0.052$  vs. LF and ‡ $P = 0.016$  vs. HF.

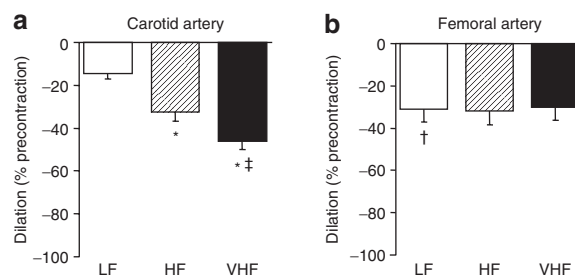
diet (HF, 41% of total kcal from fat; Research Diets, D12079B, New Brunswick, NJ) or with very high-fat (VHF) diet (58% of total kcal from fat; Research Diets, D12331). All animals were housed in the institutional animal facilities with a 12:12-h light–dark cycle and had free access to water. Animals were killed at 19 weeks of age, anesthetized by IP injection (xylazine: 100 mg/kg body weight (BW); ketamine: 23 mg/kg BW; and acepromazine: 3.0 mg/kg BW), and exsanguinated via cardiac puncture. Housing facilities and experimental protocols were approved by the local authorities for animal research (Kommission für Tierversuche des Kantons Zürich, Switzerland) and conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

### Glucose tolerance studies

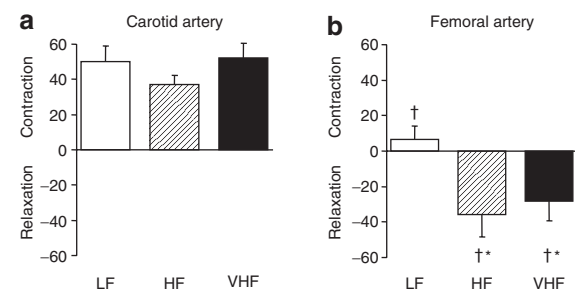
Mice were fasted 14 h before the experiment. From each animal, venous blood was obtained from the tail vein for baseline glucose measurements (0 min) and subsequently upon glucose injection (IP; 2 mg/g BW). Blood was collected at 5, 10, 15, 30, 45, 60, 90, and 120 min after glucose injection, and blood glucose was determined with an Accu-Chek Advantage glucose meter (Roche Diagnostics, Basel, Switzerland). Glucose tolerance was determined by calculating the area under the curve from 0 to 120 min and given in arbitrary units (AU), as described (19).

### Vascular function studies

Carotid and femoral arteries were isolated and placed in cold Krebs Ringer bicarbonate solution (in mmol/l: NaCl 118.6; KCl 4.7; CaCl<sub>2</sub> 2.5; MgSO<sub>4</sub> 1.2; KH<sub>2</sub>PO<sub>4</sub> 1.2; NaHCO<sub>3</sub> 25.1; EDTA<sub>Na2Ca</sub> 0.026; glucose 10.1), dissected free of connective tissue under a microscope (Olympus SZX9, Volketswil, Switzerland), and cut into rings 3 mm in length. Special care was taken not to damage the endothelium during this procedure. Experiments were performed, as previously described (13). Briefly, vessel segments were mounted onto two 100 μm or 50 μm tungsten wires for the carotid and femoral artery, respectively, transferred to water-jacketed organ baths containing Krebs solution (95% O<sub>2</sub>, 5% CO<sub>2</sub> at 37 °C, pH 7.4), and connected to force transducers (Hugo Sachs Elektronik, March-Hugstetten, Germany). Vascular rings were allowed to equilibrate for 30 min in the organ chamber and were passively stretched. Thereafter, resting tension was gradually increased to the optimal level (carotid artery: 1.25 g; femoral artery: 1.0 g), as described (20). Before starting the experimental protocol, vessels were repeatedly exposed to 100 mmol/l KCl until a stable response was achieved (20). To normalize the effects of vascular reactivity, calculations of contractile or relaxant effects of agonists were related to KCl- or phenylephrine-induced contractions, as previously described (19).



**Figure 2** Vasodilator responses to reactive oxygen species/hydroxyl radical. (a) Carotid and (b) femoral artery rings of control animals fed a LF diet and animals receiving HF or VHF diet were pre-treated for 30 min with L-NAME (300 μmol/l) and then exposed to ROS/·OH. Data are expressed as percent of precontraction (carotid artery:  $n = 7-11$ /group; femoral artery:  $n = 6-7$ /group). \* $P < 0.05$  vs. LF; † $P < 0.05$  vs. carotid artery; ‡ $P < 0.05$  vs. HF.



**Figure 3** Contractions to reactive oxygen species/hydroxyl radical. Rings of (a) carotid and (b) femoral artery of control animals on LF diet and animals on HF or VHF diet were pretreated with L-NAME (300 μmol/l) for 30 min and exposed to ROS-generating agents, as described in Methods. Data are expressed as contraction or dilation as percentage of precontraction (carotid artery:  $n = 7-11$ /group; femoral artery:  $n = 6-7$ /group). \* $P < 0.05$  vs. LF; † $P < 0.05$  vs. carotid artery.

### Effects of exogenous ROS/·OH on precontracted arteries

Prior to exposure to ROS/·OH, rings were precontracted with phenylephrine to 50% of the KCl-induced contraction, as described (13). Responses to exogenously generated ROS/·OH produced by ascorbic acid (100 μmol/l) and Fe<sup>2+</sup> (100 μmol/l) (17) were investigated in vascular rings from each treatment group. To exclude any effects of endogenous nitric oxide (NO), all experiments were performed in the presence of the NO synthase inhibitor N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME; 300 μmol/l, 30 min of preincubation).

### Endothelium-dependent and -independent vasodilator responses

Endothelium-dependent vasodilation to ACh (0.1 nmol/l to 10 μmol/l) was investigated in precontracted vascular rings from each treatment group, as described (13). Endothelium-independent vasodilation was investigated using the NO donor sodium nitroprusside (10 μmol/l) (13).

### Vascular responses to ET-1 and Ang II

Vascular rings were either exposed to cumulative concentrations of ET-1 (0.01–300 nmol/l) (20) or to a single concentration of Ang II (100 nmol/l) as described (21). To exclude effects of endogenous NO, all experiments were performed in the presence of L-NAME (300 μmol/l), as described (13).

## Materials

ET-1 and L-NAME were bought from Alexis Corp (Lausanne, Switzerland). All other chemicals were purchased from Sigma Chemicals (Buchs, Switzerland).

## Statistical analyses

Data are given as means  $\pm$  s.e.m. Contraction is expressed as a percentage of contraction to 100 mmol/l KCl, and dilation is given as a percentage of precontraction to phenylephrine. Data were analyzed using ANOVA for repeated measurements with Bonferroni correction, the unpaired Student's *t*-test, or the Mann-Whitney *U* test, when appropriate. A *P* value  $<0.05$  was considered significant.

## RESULTS

### Animal weight gain and glucose tolerance

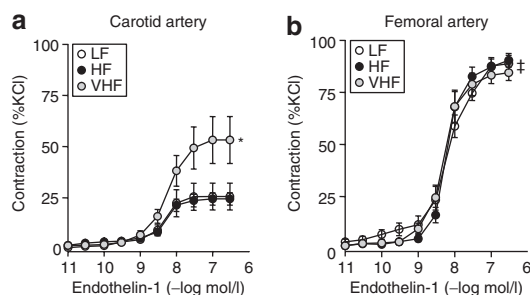
After the 15-week treatment protocol, control mice fed the normal LF chow diet had gained  $11 \pm 1$  g in BW. Mice fed a HF diet had gained  $17 \pm 1$  g ( $P < 0.05$  vs. LF), and mice fed a VHF diet had gained  $21 \pm 1$  g ( $P < 0.05$  vs. LF and HF diets). Glucose tolerance was similarly impaired in mice fed on either of the HF diets (LF diet:  $1300 \pm 144$  AU; HF diet:  $1964 \pm 105$  AU; VHF diet:  $2034 \pm 147$  AU; both HF and VHF diets  $P < 0.05$  vs. LF diet).

### Endothelium-dependent and -independent relaxation

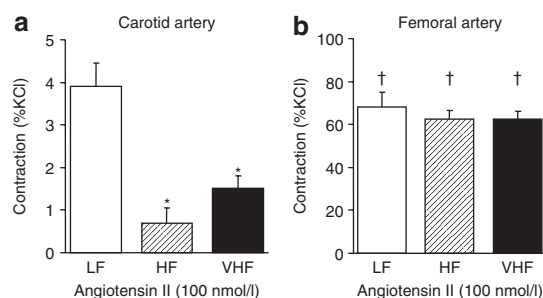
In control mice on an LF diet, endothelium-dependent relaxation to ACh was greater in the carotid than in the femoral artery ( $60 \pm 10\%$  vs.  $39 \pm 9\%$ ;  $P < 0.05$ ; **Figure 1**). Increasing dietary fat intake had no effect on endothelium-dependent responses in the carotid artery (**Figure 1a**). In femoral arteries, VHF but not HF diet increased ACh-induced relaxation ( $P = 0.052$  vs. LF diet and  $P = 0.016$  vs. HF diet; **Figure 1b**), with relaxation reaching levels comparable to those seen in the carotid artery. No difference in endothelium-independent vasodilation in response to sodium nitroprusside between the groups was observed (data not shown).

### Vasodilator responses to ROS/OH

In control mice on an LF diet, vasodilator responses to ROS/OH in precontracted vascular rings were two-fold greater in the femoral than in the carotid artery ( $31 \pm 6\%$  vs.  $15 \pm 2\%$ ;  $P < 0.05$ ; **Figure 2**). Treatment with either HF or VHF diet increased relaxation in the carotid artery and the change in relaxant responses was proportional to the dietary fat intake (LF diet:  $15 \pm 2\%$ ; HF diet:  $32 \pm 4\%$ ; VHF diet:  $46 \pm 3\%$ ;  $P < 0.05$  vs. LF diet and  $P < 0.05$  vs. HF diet; **Figure 2a**). In contrast, ROS/OH-induced relaxation in the femoral artery remained unaffected by increasing dietary fat intake (**Figure 2b**).



**Figure 4** Endothelin-1-mediated vasoconstriction. Rings of (a) carotid and (b) femoral artery of control animals on LF diet and animal fed with HF or VHF diet were pretreated with L-NAME ( $300 \mu\text{mol/l}$ ) for 30 min and then exposed to ET-1. Data (carotid artery:  $n = 9$ –12/group; femoral artery:  $n = 6$ –12/group) are expressed as percentage of KCl (100 mmol/l). \* $P < 0.05$  vs. LF and HF;  $^{\dagger}P < 0.001$  vs. carotid artery.



**Figure 5** Contractions to angiotensin II. Rings of (a) carotid and (b) femoral artery of control mice fed an LF diet, HF, or VHF diet were pretreated with L-NAME ( $300 \mu\text{mol/l}$ ) for 30 min and subsequently exposed to a single concentration of Ang II (100 nmol/l). Data (carotid artery:  $n = 7$ –11/group; femoral artery:  $n = 10$ –12/group) are expressed as percentage of KCl (100 mmol/l). Note different scales of y-axis, 20-fold greater for the femoral artery. \* $P < 0.005$  vs. LF;  $^{\dagger}P < 0.002$  vs. carotid artery.

### ROS/OH-induced contractions

Contractile responses on exposure to ROS/OH were eight-fold greater in carotid than femoral artery of control mice ( $50 \pm 9\%$  vs.  $6 \pm 7\%$ ,  $P < 0.05$ ; **Figure 3**). HF or VHF diet had no effect on vascular responses in the carotid artery (**Figure 3a**), whereas in femoral arteries, ROS/OH-induced contraction was reversed into a pronounced relaxation in animals treated with either HF or VHF diet (HF diet:  $-36 \pm 12\%$ ; VHF diet:  $-28 \pm 11\%$ ;  $P < 0.05$  vs. LF diet; **Figure 3b**).

### ET-1-mediated contractions

Contractile responses to ET-1 were stronger in the femoral than in the carotid artery of control mice ( $P < 0.001$ ; **Figure 4**). In the carotid artery, contractions increased in mice fed on a VHF but not on an HF diet ( $P < 0.05$  vs. LF and HF diets; **Figure 4a**). In contrast, increasing dietary fat intake had no effect on ET-1-induced contractions in the femoral artery (**Figure 4b**).

### Ang II-induced contractions

Contractile response to Ang II were 17-fold more potent in femoral than in carotid arteries ( $68 \pm 7\%$  vs.  $4 \pm 1\%$ ;  $P < 0.002$ ; **Figure 5**). Increasing dietary fat intake reduced Ang II-induced contractions in the carotid artery (HF diet:  $0.7 \pm 0.3\%$ ,  $P < 0.001$  vs. LF diet; VHF diet:  $1.5 \pm 0.3\%$ ,  $P < 0.002$  vs. LF diet; **Figure 5a**) but had no effect on reactivity in the femoral artery (**Figure 5b**).

## DISCUSSION

This study demonstrates that high dietary fat intake has differential effects on vascular function in different conduit arteries in mice. Increasing fat intake selectively altered angiotensin- or endothelin-mediated vasoreactivity, depending on the anatomical localization of the artery investigated. Our findings further suggest that high dietary fat intake selectively activates mechanisms that result in improved vasodilation and/or attenuation or reversal of contraction that appear to involve ROS.

The vessels investigated in this study, namely, carotid and femoral arteries are susceptible to the development of atherosclerosis in humans (22,23), and they both display increased intima-media thickness after high intake of saturated fatty acids (24). Despite this pathogenetic similarities both vessels display marked anatomical differences. While the carotid artery can be defined as an elastic artery, the femoral artery belongs to the group of muscular arteries (25). Contraction of muscular

arteries is dependent on the organization of its structural components, particularly smooth muscle cells (26). Moreover, both vimentin and desmin represent the major smooth muscle cell filament protein expressed in muscular arteries, whereas in the elastic arteries only vimentin is the major filament protein. It has been suggested that differences in smooth muscle cell architecture, arrangement and tissue protein constitution contribute to the contractile differences between arteries (27–30). Given that the vessels investigated in this study display different smooth muscle cell density, rings of ~3 mm in length for either vessel were used to normalize the effect of length of the vascular rings studied.

$\cdot\text{OH}$  is a highly reactive oxygen intermediate which is formed *in vivo* via the Fenton or Haber–Weiss reactions (31). In the present study,  $\cdot\text{OH}$  was generated *ex vivo* by the reaction between ascorbic acid and  $\text{Fe}^{2+}$ , which provides a reliable source of  $\cdot\text{OH}$  for *in vitro* investigations (17,18). Formation of  $\cdot\text{OH}$  was confirmed by using terephthalic acid (TPA), which is a sensitive and highly specific probe for detection of  $\cdot\text{OH}$  (18,32,33). The experiments of this study demonstrate that the vascular effects mediated by ROS/ $\cdot\text{OH}$  not only depend on fat intake but also on the anatomical localization of the vascular bed investigated. Carotid and femoral arteries of animals on an LF diet showed a marked dilator response to ROS/ $\cdot\text{OH}$ . However, after HF diet, the dilator response was augmented only in the carotid artery indicating that anatomical factors importantly determine changes in vascular reactivity. Furthermore, we found unexpectedly that after increasing dietary fat intake, ROS/ $\cdot\text{OH}$ -induced contractility in the femoral artery was completely abrogated and, in fact, reversed into relaxation. Previous studies have suggested either dilatory or contractile activity of  $\cdot\text{OH}$  in small mouse cerebral arterioles (34), as well as in the aorta of rabbits (16) and rats (15). Moreover, Noronha and colleagues have shown that in experimental diet-induced obesity endothelium-dependent relaxation to ACh is reduced in the presence of catalase, supporting the concept of vasodilator function of ROS (35). Indeed, ROS other than  $\cdot\text{OH}$  such as hydrogen peroxide and nitroxyl anion have been implicated in vasodilation (36,37). Our observation that in control animals ROS/ $\cdot\text{OH}$  caused a dilatory effect, and only a relatively small contraction in the femoral compared to the carotid artery, suggests the involvement of mechanisms that mask the contractile responses to ROS/ $\cdot\text{OH}$  in the femoral artery, possibly due to suppression of cellular calcium mobilization (38) or structural differences of vascular smooth muscle cells (39). It is unlikely that the relaxation response to ROS/ $\cdot\text{OH}$  observed in this study involves the NO-guanylyl cyclase pathway (40), since all experiments were performed in the presence of a NO synthase inhibitor. Taken together, the present data are compatible with the notion that ROS/ $\cdot\text{OH}$  provides vasodilator activity even during decreased NO bioavailability in the early stages of diet-induced obesity.

The renin–angiotensin system and the endothelin system have been implicated in the pathogenesis of cardiovascular diseases associated with obesity (41). In the present study, the

vasoactive peptides ET-1 and Ang II induced strong contractions in the femoral artery that remained unaffected even after high dietary fat intake. In line with earlier studies, we also observed that ET-1 and Ang II are more potent vasoconstrictors in mouse femoral than in the carotid artery (20,42–44). After high dietary fat intake, Ang II-mediated contractions in the carotid artery were reduced while at the same time ET-1-induced contractions were increased. To the best of our knowledge, this is the first study to report opposing effects of fat diet on ET-1 or Ang II-mediated contractility in the same vascular bed. The attenuation of Ang II-mediated contraction in the carotid artery after an HF diet may correspond with an indirect “vasoprotective” role of HF diet in this vascular bed that may be time-dependent. Indeed, prolonged exposure to HF diet for 30 weeks had no effect on Ang II mediated contractility in the carotid artery (44). Possibly, suppression of Ang II-mediated responses may also be linked to a local rise in ROS formation in response to HF intake (12) leading to downregulation of Ang II receptors (45), alterations in intracellular  $\text{Ca}^{2+}$  signaling (46), altered expression and/or affinity of vasoconstrictor receptors (16), as well as changes in expression and/or signaling of cyclooxygenases (47) or thromboxane receptors (13), which play an important role for Ang II-mediated contractions in the mouse vasculature (21).

Endothelium-dependent relaxation to ACh has been reported to be impaired in obese conditions in some but not in all studies (13,48). Interestingly, we found that in the femoral artery impaired relaxation to ACh was augmented in animals treated with very HF diet, increasing the maximum response to a level comparable to that found in the carotid artery. Preliminary data from our group indicate that similar changes occur in leptin-deficient mice, a monogenetic model of severe obesity, where ACh-mediated endothelium-dependent relaxation in femoral artery was significantly greater than in lean mice (unpublished data). Thus, increases in body fat and/or even obesity per se may activate endothelium-dependent “vasoprotective” effects in some but not all vascular beds.

Endothelium-dependent relaxation to ACh is greater in the carotid than in the femoral artery (25), and relaxation responses are mediated predominantly by NO in the carotid but not in the femoral artery (49). In the present study, endothelium-dependent relaxation in the carotid artery was independent of cyclooxygenase-derived prostanoids, since a nonselective COX inhibitor (meclofenamate;  $10\ \mu\text{mol/l}$ ) had no effect on the response (unpublished data). Indeed, in mouse femoral arteries, a part of the relaxation is mediated by a nonprostanoid, non-NO factor causing hyperpolarization (49), and it has also been suggested that endothelium-derived hyperpolarizing factor is also a predominant vasodilator in rat femoral arteries (50). It is, therefore, possible that in the present study high dietary fat intake enhanced endothelium-derived hyperpolarization in the femoral artery.

Obesity and insulin-resistance accelerate the development of atherosclerosis and other cardiovascular disorders causing vascular dysfunction and, as a consequence, impair blood flow by promoting vasoconstriction. C57BL/6J mice used in this study

are genetically susceptible to develop obesity and diabetes when fed on an HF, high-sucrose diet (51–53), and indeed, feeding of HF diet significantly impaired glucose tolerance of the study animals. Interestingly, though a similar degree of glucose intolerance was observed with either of the fat diets, the extent on functional changes were highly different between diets, indicating the involvement of other factors. Intriguingly, in our study we observed that feeding of HF diet in mice might exert “protective” vasodilator effects in certain vascular beds, by enhancing relaxation to ACh or relaxant responses to ROS. As these effects were observed in otherwise healthy young animals at 19 weeks of age, it would be of interest to determine whether these effects are affected by risk factors such as aging or in atherosclerosis. Compared to life expectancies in humans, 19-week-old animals correspond to juveniles, ~10–15 years of age, based on the assumption that the average life spans are 3 and 80 years for mice and humans, respectively. The intake of high amounts of fat in the experimental animals for 15 weeks would correspond to living on HF diet in humans beginning already at 2 years of age for ~8–10 years. This study shows that intake of high dietary fat causes insulin resistance but no manifestation of atherosclerotic plaque formation, as previously described (13). Young C57BL/6J mice are normally resistant to atherosclerotic plaque formation even after intake of high amounts of dietary fat (13), however in apolipoprotein E-deficient C57BL/6J mice, high dietary fat intake has been reported to accelerate the development of atherosclerosis (54–56).

In conclusion, the data presented here demonstrate marked regional differences in changes of vascular reactivity to vasoconstrictors and ROS/OH in conduit arteries in response to increasing dietary fat intake. High dietary fat intake not only selectively affects vasoconstriction but also activates vasodilator effects suggesting a previously unnoticed, beneficial role of dietary fat intake on vascular reactivity. These findings may be important for explaining the heterogeneity of atherosclerosis seen in patients with obesity (6,7,57,58) and suggest a direct role of dietary fat modulating the risk of vascular disease in selected regions of the arterial tree.

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#### DISCLOSURE

The authors declared no conflict of interest.

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