Restoration of coronary endothelial function in obese Zucker rats by a low-carbohydrate diet

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Submitted 1 November 2006; accepted in final form 8 January 2007

Focardi M, Dick GM, Picchi A, Zhang C, Chilian WM. Restoration of coronary endothelial function in obese Zucker rats by a low-carbohydrate diet. Am J Physiol Heart Circ Physiol 292: H2093–H2099, 2007. First published January 12, 2007; doi:10.1152/ajpheart.01202.2006.—A popular diet used for weight reduction is the low-carbohydrate diet, which has many calories derived from fat and protein, but effects of this dietary regimen on coronary vascular function have not been identified. We tested the hypothesis that obesity-induced impairment in coronary endothelial function is reversed by a low-carbohydrate diet. We used four groups of male Zucker rats: lean and obese on normal and low-carbohydrate diets. Rats were fed ad libitum for 3 wk; total caloric intake and weight gain were similar in both diets. To assess endothelial and vascular function, coronary arterioles were cannulated and pressurized for diameter measurements during administration of acetylcholine or sodium nitroprusside or during flow. When compared with lean rats, endothelium-dependent acetylcholine-induced vasodilation was impaired by ~50% in obese rats (normal diet), but it was restored to normal by the low-carbohydrate diet. When the normal diet was fed, flow-induced dilation (FID) was impaired by >50% in obese compared with lean rats. Similar to acetylcholine, responses to FID were restored to normal by a low-carbohydrate diet. Nω-nitro-l-arginine methyl ester (10 μM), an inhibitor of nitric oxide (NO) synthase, inhibited acetylcholine- and flow-induced dilation in lean rats, but it had no effect on acetylcholine- or flow-induced vasodilation in obese rats on a low-carbohydrate diet. Tetrathylammonium, a nonspecific K+ channel antagonist, blocked flow-dependent dilation in the obese rats, suggesting that the improvement in function was mediated by a hyperpolarizing factor independent of NO. In conclusion, obesity-induced impairment in endothelium-dependent vasodilation of coronary arterioles can be dramatically improved with a low-carbohydrate diet most likely through the production of a hyperpolarizing factor independent of NO.

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A GROWING BODY OF EVIDENCE suggests that endothelial dysfunction is associated with cardiovascular events (28, 37). The endothelium acts to maintain vascular homeostasis through complex interactions with cells in the vessel wall and lumen. The endothelium regulates vascular tone by balancing production of vasodilators, including nitric oxide (NO) (23, 31, 48), prostacyclin (29), several hyperpolarizing factors (9, 13, 43), and vasoconstrictors (endothelin-1) (61). The presence of risk factors for coronary heart disease (CHD) is thought to initiate a chronic inflammatory response that is accompanied by a loss of vasodilator and antithrombotic factors and an increase in vasoconstrictor and prothrombotic products (20, 59). Smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, and a family history of premature atherosclerosis disease are associated with an attenuation of endothelium-dependent vasodilation (26, 51, 65). Recently, also the presence of obesity was associated with endothelial dysfunction independent of other risk factors (1a, 8, 36).

Although several pharmacological strategies have been demonstrated to improve endothelial function [3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), angiotensin-converting enzyme inhibitors, estrogens, and angiotensin-II receptor antagonists], nonpharmacological interventions, such as physical activity, smoking cessation, and nutritional factors, also can be effective. In recent years, it was demonstrated that endothelial function can be modulated by some dietary components. Low-carbohydrate diet was first characterized by William Banting in the 1860s (3), but this type of diet has received much attention due to the publication of Dr. Atkins’ New Diet Revolution (1). This diet recommended limiting complex and simple sugars, which cause the body to oxidize fat to meet energy requirements. Low-carbohydrate diets also reduce plasma levels of triglycerides and increase HDL cholesterol; LDL cholesterol levels are increased in the first 3 mo, but these differences are not significantly different at a longer follow-up (19, 33, 66). However, it is not clear yet whether this beneficial effect on lipid profile also reflects an improvement in endothelial function in obesity.

The obese Zucker rat (OZR) is an animal model of genetic obesity that results from an inactivating mutation in leptin receptor gene. Homozygous Zucker rats (fa/fa) exhibit most of the metabolic picture seen in human obesity, including hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia, and proteinuria. In OZR, previous studies (25, 67, 68) have indicated that intestinal and mesenteric microvessel dilation to NO-dependent stimuli was significantly reduced compared with responses in lean Zucker rat (LZR) control dilation. The aim of this study is to test the hypothesis that the impairment in NO-mediated dilation in coronary arterioles of OZR compared with those in LZR can be restored by feeding a low-carbohydrate diet.

METHODS

Animals. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center. All experiments used 12- to 13-wk-old male LZR (fa/fa) and OZR (fa/fa) fed standard rat chow or low-carbohydrate chow and tap water ad libitum. Rats were divided into...
four groups: OZR fed with low-carbohydrate diet (n = 7); OZR fed with standard chow (n = 7); LZR fed with low-carbohydrate diet (n = 4); and LZR fed with standard chow (n = 5). After 3 wk, rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg ip).

**Diet composition.** Caloric content of low-carbohydrate and normal diets was 30/10/60% and 28/59/13% protein/carbohydrate/fat, respectively.

**Isolated coronary arterioles and study protocol.** Single arterioles were dissected from the left ventricle as previously described (32). A portion of the left ventricle was removed, and several arterioles of the appropriate size were located under a dissecting microscope. Each arteriole with surrounding ventricular muscle was excised, transferred to a temperature-controlled dissection dish (4°C) containing physiological saline solution, and dissected free of the muscle tissue. Side branches were ligated using 11-0 suture. The vessel was transferred to a lucite chamber and cannulated at both ends by using micropipettes with matched resistances. The arterioles were tied to each pipette by using 11-0 suture. The cannulated microvessel/perfusion chamber was positioned on the stage of an inverted microscope, and temperature in the bath was maintained at 37°C via heat exchangers around the chamber. Physiological (flow-dependent responses) and pharmacological studies were performed. For flow-induced dilation (FID), the two reservoirs were initially set at the same hydrostatic level (i.e., no pressure drop between the two reservoirs and no flow). Flow was initiated by simultaneously moving the reservoirs in opposite directions to generate pressure difference between the inflow and outflow reservoirs (ΔP), which is linearly related to flow. Because the resistances of both pipettes are equivalent, movement of the reservoirs in equal and opposite directions does not change midpoint intraluminal pressure. Thus the vessel was perfused at different flow rates without intraluminal pressure being changed. Diameters were measured using a video caliper at magnifications of ×400 and ×640.

To establish the relationship between dilation and flow (endothelium-dependent response), we measured diameter at several ΔP. To evaluate agonist-mediated, endothelium-dependent dilation, we administered acetylcholine to the microvessels (10⁻⁶–10⁻⁵ M) before and after N⁶-nitro-l-arginine methyl ester (l-NAME; 10⁻³ M for 20 min). To evaluate endothelium-independent dilation, we administered sodium nitroprusside to microvessels at different concentrations (10⁻⁶–10⁻³ M). To evaluate the role of K channels and hyperpolarizing factors in endothelium-dependent dilation, we incubated the arterioles with 10 mM tetrathylammonium (TEA), a nonselective K⁺ channel antagonist.

**Plasma chemistry.** Plasma total cholesterol, LDL cholesterol, triglycerides, and glucose levels were assessed in the laboratory core of Charity Hospital in New Orleans. Plasma insulin was determined using a rat insulin ELISA assay (Crystal Chem). Insulin resistance was estimated as homeostasis model assessment of insulin resistance (HOMA-IR) by using the mathematical approximation HOMA-IR = insulin (in mU/ml) × glucose (in mmol/l) /225 (2, 21).

**Statistics.** All data are expressed as means ± SE. Percent dilation was calculated by establishing the maximal diameter (10⁻³ M sodium nitroprusside), and the fractional dilation produced by flow or acetylcholine was then calculated as a percentage of the total dilation. Comparisons between groups were performed by two-way ANOVA (reactivity curves with dose and condition as variables) and two-way repeated-measures ANOVA using Bonferroni’s post hoc test for comparisons. All analyses were performed with SigmaStat software (Systat Software). All results are presented as means ± SE, and significance was accepted at P < 0.05.

**RESULTS**

**Body weight and food intake.** OZR had a significantly higher body weight compared with LZR at basal conditions and during the 3 wk of follow-up (Fig. 1). Although there was an initial weight loss with the low-carbohydrate diet, after 3 wk, the weight gain in either the OZR or lean animals was no different from those fed the normal chow (Fig. 1). Daily food intake throughout the study was greater in OZR than in their lean littermates fed the same diet (P < 0.05). Caloric intake of the two groups of lean rats and the two groups of obese rats did not differ (Fig. 2).

**Plasma biochemistry.** Total cholesterol, HDL cholesterol, triglycerides, insulin levels, and insulin resistance were significantly higher in OZR compared with LZR. Low-carbohydrate diet reduced significantly plasma triglyceride levels in OZR but did not affect the other variables (Table 1).

**Coronary arteriolar characteristics.** Basal coronary arteriolar diameters of the four groups of study were as follows: obese low/carb, 102.57 ± 29.3 μm; obese normal diet, 121.3 ± 22.5 μm; lean low/carb, 113.7 ± 25.9 μm; and lean normal diet, 100 ± 19.7 μm.

**In vitro vascular function.** Endothelium-dependent dilation to flow and to acetylcholine was not affected by diet in the lean animals (Fig. 3). When fed a normal diet, responses to flow and acetylcholine were lower than those observed in lean animals. In contrast, the low-carbohydrate diet in obese animals increased dilation to flow and to acetylcholine (Fig. 3) to levels comparable with the lean animals. In lean animals on either diet, l-NAME significantly reduced the responses to flow (Fig. 4) and to acetylcholine (Fig. 5). In contrast, l-NAME was without effect in the obese animals (Figs. 4 and 5). The improvement in endothelial dilation to flow with the low-carbohydrate diet (Fig. 4) was not affected by l-NAME. Likewise, the responses to acetylcholine with the low-carbohydrate diet were not influenced by l-NAME (Fig. 5). TEA (10 mM), an antagonist of K⁺ channels, blocked flow-dependent dilation in the obese animals (n = 3) on either normal or low-carbohydrate diet. The maximal effects of flow on dilation of arterioles from obese rats on the low-carbohydrate diet and normal diets were 55% and 28%, respectively; this was reduced to 19% and 16% dilation after TEA. In contrast, as shown in Fig. 4, l-NAME did not affect flow-dependent dilation in the obese rats. These observations suggest that endothelium-dependent dilation in obese animals is mediated by the production of a hyperpolarizing factor and not NO and that the improvement in endothelial function in obese animals on the low-carbohydrate diet is mediated by this hyperpolarizing factor. Endothelium-independent vasodilation to sodium nitroprusside did not differ among the lean and obese animals regardless of the diet (Fig. 6).
DISCUSSION

The major findings of this study are 1) endothelial function of coronary arterioles is impaired in obese rats, 2) a low-carbohydrate diet improves endothelial function in obese rats, and 3) this beneficial effect is not related to NO production.

Endothelial function in obesity. The initial observation of the present study was that dilator responses of coronary arterioles of OZR to endothelium-dependent (acetylcholine and FID) agonist or stimuli were severely impaired when compared with responses determined in LZR control animals. The normal endothelium regulates vascular tone, releasing numerous dilator and constrictor substances. NO is a pivotal endothelium-derived substance; a defect in NO production or activity has been proposed as a major mechanism of endothelial dysfunction and a contributor to atherosclerosis. Several studies have shown a correlation between endothelial dysfunction and the presence of coronary risk factors in subjects with no clinical evidence of coronary disease. Many of the traditional risk factors, including obesity, are associated with endothelial dysfunction. The interaction between CHD and obesity has been confirmed by the PROCAM Study (52); the association between obesity and CHD becomes more evident when the presence of coronary risk factors in subjects with no clinical evidence of coronary disease.

The authors hypothesized that the mechanism underlying this dysfunction was a reduced bioavailability of NO, potentially due to increased scavenging by oxidative free radicals (16). These animals presented also insulin resistance, which is often associated with obesity and other components of metabolic syndrome. Insulin resistance is defined as a decreased sensitivity and/or responsiveness to metabolic actions of insulin; this is also a prominent component of cardiovascular disorders such as hypertension, coronary artery disease, and atherosclerosis, which are characterized by endothelial dysfunction (49). Conversely, endothelial dysfunction is present in diabetes, obesity, and dyslipidemias (39). In addition to its essential metabolic actions, insulin has important vascular actions that involve stimulation of the production of NO from endothelium, leading to vasodilation, increased blood flow, and augmentation of glucose disposal in skeletal muscle (4). Mechanisms contributing to insulin resistance and endothelial dysfunction include glucotoxicity, lipotoxicity, and inflammation (27).

![Fig. 2. Caloric intake (per day) in obese Zucker rats on normal and low-carbohydrate diets.](image)

**Table 1. Plasma chemistry**

<table>
<thead>
<tr>
<th></th>
<th>Obese Low Carb</th>
<th>Obese Normal</th>
<th>Lean Low Carb</th>
<th>Lean Normal</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>139.3±41.6</td>
<td>166.6±78.6</td>
<td>72.5±6.3</td>
<td>60.3±10.9</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>111±25.5</td>
<td>97.1±12.4</td>
<td>59.5±4.9</td>
<td>49.3±8.3</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>16.3±8.2</td>
<td>17.1±15.8</td>
<td>15±1.0</td>
<td>8.6±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>314.1±93.8</td>
<td>910±873.6</td>
<td>85.5±20.5</td>
<td>82±44.9</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>Glucose</td>
<td>219.3±62.1</td>
<td>199.1±28.4</td>
<td>165±25.4</td>
<td>187±30.5</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.3±0.4</td>
<td>1.2±0.4</td>
<td>0.7±0.1</td>
<td>0.4±0.1</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>1.6±0.5</td>
<td>1.4±0.4</td>
<td>0.8±0.2</td>
<td>0.5±0.1</td>
<td>&lt;0.05**</td>
</tr>
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Values are means ± SE. *Obese low carbohydrate (carb), obese normal vs. lean low carb, lean normal; *Obese low carb vs. lean low carb, obese normal vs. lean normal; *Obese low carb vs. lean low carb, obese normal vs. lean low carb, lean normal; *Obese low carb, obese normal vs. lean low carb, lean normal; *Obese low carb, obese normal vs. lean low carb, lean normal. NS, not significant.
Low-carbohydrate diet improves endothelial function in obese rats. Dietary advice regarding cardiovascular disease prevention is complex; the American Heart Association has recommended a low-fat diet of 55% of total calories from carbohydrates, 30% from fat, and 15% from protein, with cholesterol restricted to <300 mg/day (30). Owing to the increasing prevalence of obesity, despite low-fat recommendations, many new popular diets have emerged; the Atkins’ diet has become the most popular in the United States. It recommends 2 wk of extreme carbohydrate restriction, followed by gradually increasing carbohydrates to 35 g/day. Low-carbohydrate diets, in general, limit the intake of complex and simple sugars, causing the body to oxidize fat to meet energy requirements. A drastic reduction in carbohydrates also leads to an overall decrease in caloric intake (5); four randomized controlled clinical trials have compared low-carbohydrate diets with low-fat diets (6, 15, 50, 56, 69); all these studies found an average of 4- to 6-kg greater weight loss in the low-carbohydrate diet group at 6 mo; however, the two studies followed to one year showed no significant weight difference. These trials also demonstrated a greater increase in HDL cholesterol and a decrease in triglycerides in the low-carbohydrate group. Our results showed that low-carbohydrate diet significantly decreased triglyceride levels in OZR without affecting the other blood lipid levels or the glucose metabolism.

Studies on low-carbohydrate diet demonstrated that subjects stop feeling hungry, and this provokes a weight reduction; in this study, the body weight was not reduced in the OZR on low-carbohydrate diet, and this is probably due to the leptin receptor mutation that alters the appetite control of these animals; in fact, they did not satisfy their appetite and therefore did not stop eating. We were intrigued by the observation that endothelial function improved in the obese rats without weight

Fig. 4. Flow-mediated dilation in all groups before and after N^G-nitro-L-arginine methyl ester (L-NAME). \textit{Top}: in lean rats, L-NAME inhibited flow-mediated dilation on either diet. *\textit{P} < 0.05 responses without vs. with L-NAME. \textit{Bottom}: in obese rats, L-NAME had no effect on flow-mediated dilation in rats on low-carbohydrate diet. \#\textit{P} < 0.05 low-carbohydrate vs. normal diet.

Fig. 5. ACh dose-response curve in all four groups before and after L-NAME. \textit{Top}: effects of L-NAME on vasodilation in lean rats on normal and low-carbohydrate diet. L-NAME attenuated dilation of lean rats on either diet. *\textit{P} < 0.05 responses without vs. with L-NAME. \textit{Bottom}: effects of L-NAME on vasodilation in obese rats on normal and low-carbohydrate diet. L-NAME did not affect dilation of obese rats on normal or low-carbohydrate diet. \#\textit{P} < 0.05 low-carbohydrate vs. normal diet.

Fig. 6. Sodium nitroprusside (SNP) dose-response curve in lean and obese rats on normal and low-carbohydrate diets. Responses to SNP were similar in all groups.
Endothelial function improvement is not related to NO production. The most important observation in this study is that a low-carbohydrate diet restored endothelial function in obesity almost to normal. Although we did not elucidate the precise mechanisms, we know that the restoration of endothelium-dependent dilation was independent of NO. Interestingly, endothelial function in normal rats, regardless of diet, was dependent on NO. l-NAME reduced flow-mediated and acetylcholine-induced dilation in lean animals on either normal chow or the low-carbohydrate diet. l-NAME did not effect the same responses in OZR. These data suggest that mitigation of NO-dependent dilation is likely the cause of abrogated endothelial dilation in obesity. We have to admit that our results and experimental design did not enable us to distinguish among various possible mechanisms causing reduced NO-dependent dilation in the obese rats. However, the literature is replete with many possible mechanisms, including reduced NO bioavailability because of elevated ROS (16, 47), activation of PKC that could inhibit activation of Akt and thus endothelial NO synthase (eNOS) (45), reduced expression of eNOS (34, 47), although this point is controversial (17), or even alterations in levels of critical cofactors for eNOS (11, 41, 54). Interestingly, the partial restoration of endothelium-dependent dilation by the low-carbohydrate diet was not through restoration of NO-mediated functions, rather, it was via other endothelium-dependent dilators. We state this because l-NAME did not attenuate either flow- or acetylcholine-dependent dilation in the obese animals on the low-carbohydrate diet. Although we do not know precisely which other factor is responsible for endothelium-dependent dilation in obese animals on the low-carbohydrate diet, there are many endothelium-derived vasodilator substances, such as epoxyeicosatrienoic acids (58) and hydrogen peroxide (14), that play an important role in the presence of coronary risk factors when NO bioavailability is reduced.

Endothelium-derived hyperpolarizing factor (EDHF) dilates arteries by opening potassium channels in the vascular smooth muscle, although the precise K+ channel opened is still in question. Several endothelium-derived substances are candidates for EDHF (62). Most prominent among these are epoxyeicosatrienoic acids, which are derived from arachidonic acid metabolism through cytochrome P-450 epoxygenase (18, 40). Recently, hydrogen peroxide was shown to act as an EDHF in multiple arteries, including human coronary vessels (24, 38, 42). The role of each of these vasodilators in modulating coronary vascular tone depends on the species studied and the presence or absence of disease. For example, FID is mediated by NO in normal rat coronary arteries (64); in dogs, EDHF probably mediates steady-state coronary conduit dilation to changes in flow, but NO is critical in the additional dilation produced by pulsatility superimposed on flow (10). In humans with coronary artery disease (CAD), flow-induced dilation may be mediated by factors other than NO (55). In the microcirculation, NO plays a small role in subjects with CAD or its risk factors. Instead EDHF plays a major role, especially in subjects with CAD (44). We speculate that low-carbohydrate diet improves the endothelial function through the increased production of other vasodilators substances, in particular, EDHFs, since TEA blocked endothelium-dependent dilation in obese rats on low-carbohydrate diet. Although this may be somewhat premature to conclude unequivocally, in four obese animals, TEA blocked dilation to flow and to acetylcholine, suggesting that, in obese animals, an EDHF was responsible for dilation to these stimuli.

We would be remiss not to discuss other mechanisms that could have contributed to the restoration of endothelial function in the obese animals by the low-carbohydrate diet. Within this context, prostaglandins appear to play a role in maintaining endothelial function when eNOS has been genetically knocked out in mice (57); however, we are compelled to mention that the majority of studies suggest prostaglandins make little or no contribution to acetylcholine- and flow-induced dilation in rat coronary arteries from normal rats (46, 64) or rats with endothelial dysfunction (7, 60, 63). Thus prostaglandins would appear to be an unlikely mechanism to explain the improvements in coronary dilation that we observed.

Another possible mechanism of restored dilation in the obese rats on a high-fat diet could be related to increased levels of reactive oxygen species produced during flow. Specifically, it was reported that the reactive oxygen species, hydrogen peroxide, mediated flow-induced dilation of coronary arterioles from patients with ischemic heart disease (35). Because high-fat diets are associated with enhanced production of reactive oxygen species (53), it is possible that the high-fat/low-carbohydrate diet restored endothelial function via production of these species.

We conclude that endothelium-dependent dilation is markedly impaired in obese Zucker rats and that a low-carbohydrate diet can improve endothelial function in obesity. Furthermore, the endothelial dilation improved by the low-carbohydrate diet is independent of NO, but the exact nature of this compensation remains unknown.

GRANTS
This study was supported by National Institutes of Health Grants HL-32788, HL-73755, and Center of Biomedical Research Excellence (COBRE) RR-018766.

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