Effects of long-term high-saturated and unsaturated fatty acid diets on relaxation and contraction of renal arteries in insulin resistant rats

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Abstract: The present study was designed to investigate the effects of high-saturated and high-unsaturated fatty acid diets on relaxation and contraction of the renal arteries in insulin resistance (IR) rats. Wistar rats were fed normal chow diet (control), high-saturated fatty acid diet or high-unsaturated fatty acid diet for 6 months (n=14 in each group). IR was evaluated by glucose infusion rate (GIR) of hyperinsulinemic euglycemic clamp. Blood pressure was measured via the tail-cuff method. Body weight (BW), plasma total triglyceride (TG), free fatty acid (FFA), insulin, fasting blood glucose (FBG) and nitric oxide metabolite (NO\textsubscript{2}/NO\textsubscript{3}) were compared among the three groups. The rats were sacrificed and the renal arterial rings were placed in the physiological tissue baths for measurement of vascular response to various agents. After the arterial rings were constricted with 3 mmol/L noradrenaline (NA), endothelium-dependent vasorelaxation to acetylcholine (ACh) and endothelium-independent vasorelaxation to sodium nitroprusside (NTP) were measured. Endothelium-dependent vasorelaxation to ACh was also observed in renal arterial rings incubated with L-arginine (L-Arg), N\textsuperscript{ω}-nitro-L-arginine (L-NNA) and methylene blue (MB), respectively. Arterial contractility was evaluated from concentration-response curves to 10 nmol/L-100 µmol/L NA. Saturated or unsaturated fatty acids led to moderate rises in blood pressure (P<0.05). It was associated with higher levels of plasma lipids and lower whole body insulin sensitivity (P<0.01). There were no significant differences in BW, FBG, TG, insulin and FFA between saturated and unsaturated fatty acid-fed rats. A decrease in endothelium-dependent vasorelaxation of the renal arteries in saturated and unsaturated fatty acid-fed rats was observed (P<0.01), but there was no marked difference between the two high-fatty acid diet groups. Endothelium-dependent vasorelaxation was increased when the arteries were incubated with L-Arg and decreased when incubated with L-NNA and MB in both high-fatty acid diet groups (P<0.05, P<0.01). But no difference was found before and after incubation with L-Arg, L-NNA and MB in the control rats. In the mean time, endothelium-independent maximal vasorelaxation response of renal arteries to NTP and renal arterial contractile responses to cumulative dose of NA were assayed, and there was no difference among the three groups (P>0.05). Endothelium-dependent vasorelaxation was negatively correlated with systolic blood pressure and TG, and positively correlated with NO\textsubscript{2}/NO\textsubscript{3} and GIR. There was a significantly negative correlation between FFA and NO\textsubscript{2}/NO\textsubscript{3}. The present study suggests that both high-saturated and unsaturated fatty acid diets result in hypertension associated with significantly decreased endothelium-dependent vasorelaxation, dyslipidemia and IR, and that decreased endothelium-dependent vasorelaxation induced by high fatty acid diets is associated with impaired L-Arg-NO-cGMP pathways.

Key words: endothelium; vasorelaxation; vasoconstriction; nitric oxide; diet; hypertension; insulin resistance

长期高饱和、高不饱和脂肪酸饮食诱导胰岛素抵抗大鼠肾动脉舒张和收缩功能的变化

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摘 要: 本文旨在探讨长期高饱和、高不饱和脂肪酸饮食诱导胰岛素抵抗(insulin resistance, IR)大鼠肾动脉舒张和收缩功能的变化。成年 Wistar 大鼠随机分为对照组、高饱和脂肪酸组和高不饱和脂肪酸组，每组 14 只。喂养 6 个月后，用高胰岛素正常葡萄糖钳夹技术的葡萄糖输注率(glucose infusion rate, GIR)评价 IR；用尾套法测定大鼠血压，同时比较三组大鼠的体重、...
A group of syndromes including hyperglycemia, hypertension, hyperlipidemia and obesity have been termed "metabolic syndrome", because of the extremely high atherogenic profile it creates. It has been suggested that impaired vasorelaxation may lead to hypertension, while endothelial vasomotor dysfunction may predict long-term atherosclerotic disease progression and the rate of cardiovascular events[1,2].

The vascular endothelium plays a significant regulatory role in many physiological processes in the body, including the regulation of vasomotor tone via a range of bioactive substances. Nitric oxide (NO) and endothelin-1 (ET-1) are two important factors derived from the endothelium and they exert opposite influences on vascular tone[3]. Previous work in our laboratory has shown that synthesis of NO and ET-1 is inhibited in human umbilical vein endothelial cells (HUVECs) incubated with different kinds of free fatty acids (FFAs). In the present study, to explore the possible mechanism of vascular complication in metabolic syndrome, male Wistar rats fed with high-saturated and unsaturated fatty acid diets were used to observe the influence of different diets on relaxation and contraction of renal arteries, and the in relation with altered lipidemia and insulin sensitivity.

1 MATERIALS AND METHODS

1.1 Animals
Male Wistar rats supplied by the Experimental Animal Center of Hebei Medical University were conditioned at (22±0.5)°C in 12-hour light/12-hour dark cycle for one week in communal cages. Animals were fed ad libitum a standard chow diet for at least a week before experimental diets. After habituation, rats (250-300 g) were randomized to three matched groups (n=14 in each group). (1) Control group: continued to be fed ad libitum a low fatty acid diet, containing 10% fat, 24% protein and 66% carbohydrate as percentage of total calories. (2) High-saturated fatty acid diet (HSF) group: fed regular diets mixed with 20% butter, containing 41% fat (total saturated fatty acid 28% and unsaturated fatty acid 13% mainly containing palmitic acid), 16% protein and 43% carbohydrate as percentage of total calories. (3) High-unsaturated fatty acid diet (HUF) group: fed regular diets mixed with 20% soybean oil, containing 41% fat (total saturated fatty acid 6.6% and unsaturated fatty acid 34.4% mainly containing oleate), 16% protein and 43% carbohydrate as percentage of total calories. The soybean oil and butter were obtained from the Experimental Animal Center of Hebei Medical University. The systolic blood pressure was recorded by using the tail-cuff plethysmographic method under conscious conditions.

1.2 Hyperinsulimemic euglycemic clamp
Experiment was conducted between 9:00 and 12:00 in 12-hour fasted animals after 6 months. After being weighed, rats were anesthetized with pentobarbitone (40 mg/kg, i.p.). The left femoral vein was exposed and a catheter was inserted for infusion of glucose and insulin. Another catheter was inserted into the femoral artery for blood sampling. A 30-minute basal period was followed to reach steady state. Then a 120-minute hyperinsulinemic euglycemic clamp was performed according to Kraegen et al[5]. In brief, human insulin (Actrapid, Novo-Nordisk, China) was infused at a constant rate of 1.67 mU/kg per minute and the arterial blood glucose concentration was clamped at the basal fasting level by infusing glucose at variable rates. Under the hyperinsulinemic conditions, the steady
glucose infusion rate (GIR) required to maintain euglycemia (usually calculated between 60-120 min) is a standard measure of the whole-body insulin sensitivity.

1.3 Analytical methods

Blood glucose levels were measured with a quick glucose analyzer (Roche, Germany). Plasma immunoactive insulin concentration was assayed by radioimmunoassay. Plasma triglyceride was determined on an automatic biosynthesis analyzer (Beckman X20, America). Serum-FFA were incubated with copper coloration and nitrate reductase kits, respectively (Jiancheng Biological Corporation, Nanjing).

1.4 Assessment of vascular function

Four third-order renal arteries (300-500 μm diameter, 1.5 mm) were carefully dissected from each animal. Each artery was free of fat and connective tissue and mounted on a 40-μm diameter steel wires through the lumen on an automated Mulvany myograph (Model 610M, J.P. Trading I/S, Denmark). One wire was fixed to a displacement transducer. Four arteries at a time were incubated in 5 mL organ bath containing physiological salt solution (PSS) (in mmol/L): NaCl 119, KCl 4.7, CaCl2 2.5, MgSO4·7H2O 1.17, NaHCO3 25, KH2PO4 1.18, EDTA 0.027, glucose 5.5), gassed with 95% O2 and 5% CO2 at 37 °C.

After an equilibration period of 30 min, the tissue was normalized to 90% of the inner circumference that corresponds to 100 mmHg blood pressure[6], using a non-linear curve-fitting programme developed by McPherson et al[7]. The setting represents a resting tension of 2-3 mN under the present experimental condition. The arteries were allowed a further 30 min to equilibrate before being depolarized twice with high-potassium PSS (KPSS), in which NaCl in the normal PSS was replaced by an equimolar concentration of KCl (final [K+]′=125 mmol/L). Any artery failing to reach its predetermined target tension in response to KPSS was discarded.

Arterial contractility was evaluated from concentration-response curves to 10 nmol/L-100 µmol/L noradrenaline (NA). Arterial relaxation was measured in NA-precontracted arteries following exposure to endothelium-dependent vasorelaxant acetylcholine (ACh), and endothelium-independent vasodilator sodium nitroprusside (NTP). Arterial ring segments were contracted with 3 mmol/L NA. When contraction reached a plateau (after 2 min), concentration-response curves to ACh and NTP over the range of 10 nmol/L-100 µmol/L were carried out. Arterial ring segments were incubated with L-arginine (L-Arg, substrate of nitric oxide synthase), Nω-nitro-L-arginine (L-NNA, inhibitor of nitric oxide synthase) and methylene blue (MB, inhibitor of guanylate cyclase), respectively, for 20 min, then the arterial relaxation response to ACh was recorded. During each experiment, PSS was changed every 15-20 min.

1.5 Statistics

Contractile responses to NA were expressed as absolute tension generated and the tension of vessel was measured in mN/mm, while relaxation response to each concentration of ACh and NTP was calculated as the percentage of reduction from the maximal tension induced by 3 mmol/L NA. All data were expressed as means±SEM. Analyses of variance (ANOVA) were used to assess the changes from the baseline and the differences among groups when appropriate. Student’s t-test was used to evaluate data between two comparisons. P<0.05 was considered statistically significant.

2 RESULTS

2.1 Metabolic data

Body weight, fasting blood glucose, plasma triglyceride, insulin and FFA in HSF and HUF groups were significantly higher than that in the control group, whereas the levels of NO2−/NO3− were significantly lower than that in the control group (P<0.01). There was no difference in the above-mentioned parameters between HSF and HUF groups except that the level of NO2−/NO3− in HSF group was lower than that in HUF group (P<0.05)(Table 1).

2.2 Systolic blood pressure

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>FBG (mmol/L)</th>
<th>TG (mg/dL)</th>
<th>INS (µU/mL)</th>
<th>NO2−/NO3− (µmol/L)</th>
<th>FFA (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal chow diet</td>
<td>450.00±60.39</td>
<td>5.26±0.34</td>
<td>0.95±0.19</td>
<td>20.81±1.79</td>
<td>64.79±15.46</td>
<td>0.78±0.07</td>
</tr>
<tr>
<td>High-saturated fatty acid</td>
<td>31.52±5.93</td>
<td>6.76±0.45</td>
<td>1.69±0.17</td>
<td>38.75±6.83</td>
<td>17.40±7.29</td>
<td>1.33±0.35</td>
</tr>
<tr>
<td>High-unsaturated fatty acid</td>
<td>306.42±36.59</td>
<td>6.62±0.66</td>
<td>1.27±0.04</td>
<td>37.75±8.57</td>
<td>31.52±5.93</td>
<td>1.22±0.40</td>
</tr>
</tbody>
</table>

BW, body weight; FBG, fasting blood glucose; TG, plasma triglyceride; INS, serum insulin; FFA, free fatty acid. means±SEM, n=8.

P<0.01 vs normal chow diet group, P<0.05 vs high-saturated fatty acid diet group.
The systolic blood pressure in HSF group [mean (131.57±6.40) mmHg] and in HUF group [mean (128.00±4.65) mmHg] was significantly elevated compared with that in the control group [mean (100.83±18.00) mmHg] (P<0.01).

2.3 Assessment of insulin resistance (IR)
The whole-body insulin sensitivity was assessed by GIR. GIR in HSF and HUF groups was (5.75±0.84) and (5.95±1.50) mg/kg per minute, respectively, significantly lower than that in the control group [(9.25±0.84) mg/kg per minute], clearly indicating the presence of IR. There was no significant difference in the degree of IR between HSF and HUF groups.

2.4 Contractile responses of renal arteries
There was no significant difference in the maximal contraction to NA among the three groups [control group: (1.20±0.27) mN/mm; HSF group: (1.04±0.21) mN/mm; HUF group: (1.17±0.23) mN/mm]. There was no difference in the renal arterial contractility to NA (10 nmol/L-100 µmol/L), either (Fig.1).

2.5 Endothelium-independent relaxation
Renal arterial ring segments were tested for endothelium-independent relaxation response to NTP. In all groups the arteries relaxed in response to NTP in a dose-dependent manner. At the maximal concentration of NTP (100 µmol/L), the renal arterial ring in HSF group relaxed to (88.3±6.4)%, that in HUF group relaxed to (86.8±4.7)%, and that in the control group relaxed to (92.2±10.1)%. Despite blunted vasorelaxation at lower dose (i.e., 1×10⁻⁸-1×10⁻⁶ mol/L), the maximal NTP-induced relaxation remained unchanged in both HSF and HUF groups compared with that in the control group (Fig.2).

2.6 Endothelium-dependent relaxation
As shown in Fig.3, in both HSF and HUF groups, the sustained vasorelaxation was substantially impaired at all concentrations of ACh tested (P<0.01). The maximal relaxation induced by ACh was (43.1±1.8)% and (47.8±2.1)% in HSF and HUF groups, respectively, approximately half of that in the control group (80.5±8.1)%. However there was no statistic difference in the relaxation between HSF and HUF groups. Endothelium-dependent maximal relaxation to ACh was increased significantly by L-Arg in HSF group.

![Fig.1. Vasocontraction of isolated renal arteries to increasing doses of noradrenalin. Control, normal chow diet; HSF, high-saturated fatty acid diet; HUF, high-unsaturated fatty acid diet. means±SEM, n=6.](image1)

![Fig.2. Vasorelaxation of preconstricted isolated renal arteries to sodium nitroprusside. Control, normal chow diet; HSF, high-saturated fatty acid diet; HUF, high-unsaturated fatty acid diet. means±SEM, n=6. #P<0.01 vs control.](image2)

![Fig.3. Vasorelaxation of preconstricted isolated renal arteries to acetylcholine. Control, normal chow diet; HSF, high-saturated fatty acid diet; HUF, high-unsaturated fatty acid diet. means±SEM. *P<0.01 vs control.](image3)
was significant negative correlation between FFA and NO2
(\(P<0.05\)). L-NNA and MB reduced the maximal relaxation induced by ACh in HSF and HUF groups (\(P<0.01\), \(P<0.05\)) (Fig.4).

2.7 Correlation analysis

We examined the relationships between endothelium-dependent vasorelaxation and systolic blood pressure, plasma triglyceride, NO2/NO3, GIR. There was a negative correlation between plasma triglyceride (\(r = -0.4570, P=0.0166\)) and systolic blood pressure (\(r = -0.4076, P=0.0348\)) and ACh-induced maximal vasorelaxation. There was a significant positive correlation between NO2/NO3 (\(r = 0.4353, P=0.028\)) and systolic blood pressure (\(r = 0.4079, P=0.0347\)) and ACh-induced vasorelaxation. There was significant negative correlation between FFA and NO2/NO3 (\(r = -0.4085, P=0.0344\)).

3 DISCUSSION

Not only epidemiologic survey but also empirical study suggests that IR is associated with obesity or excessive energy intake. In the present experiment, the effects of saturated and unsaturated fatty acids-enriched diet on developed hypertension, hypertriglyceridemia, hyperinsulinemia, obesity and induced IR were studied. It has been suggested that unsaturated fatty acids provided 30% calories bene-fited glucose and lipid metabolism. But it was found that long-term diet rich in high-unsaturated fatty acids brought the same risks as that rich in high-saturated fatty acids in our study.

Normal endothelial function is important in the control of vascular tone and subsequent regulation of blood pressure and blood flow to organs and tissues. The present study investigated the effect of diet rich in saturated and unsaturated fatty acids on vascular tension and the relation with impaired endothelium-dependent vasorelaxation but normal vascular contractile function. While still largely responding to NTP, the isolated renal arteries from these high fatty acids-fed rats were not sensitive to ACh, indicating a link of impaired endothelial vasodilation of the arteries and the elevated systemic blood pressure.

As one of the chief endothelium-derived relaxant factors, NO is important in regularizing vascular tone. NO is a kind of unstable gas and it can easily diffuse through the cell membrane to dilate vascular smooth muscle. NO is synthesized from L-Arg and oxygen by nitric oxide synthase. The endothelial nitric oxide synthase plays a pivotal role in controlling NO synthesis and NO is released from endothelial cells and diffuses to vascular smooth muscle binding guanylate cyclase on cell membrane and stimulates cGMP which exerts relaxation in vascular smooth muscle. Our results showed that inhibition of nitric oxide synthase with L-NNA or inhibition of guanylate cyclase with MB reduced ACh-induced relaxation and substrate of nitric oxide synthase L-Arg increased ACh-induced relaxation significantly in HSF and HUF groups. This suggests endothelium-derived NO is involved in impaired endothelial function and the dysfunction may be associated with impaired L-Arg-NO-cGMP pathways by saturated or unsaturated fatty acid diets. Stulak et al. found the similar results from aorta endothelium in rats fed high-cholesterol diets. Further studies are required to elucidate the detailed mechanism.

The cellular mechanisms behind endothelial dysfunction due to high-fat diets remain undefined. In the present research, rats fed saturated and unsaturated fatty acid diets increased plasma triglyceride levels. Some researches showed that the elevated triglyceride is also associated with decreased endothelium-dependent vasorelaxation. Endothelial dysfunction related to hyperlipidemia and atherosclerosis has been postulated to be the result of increased oxidant stress, leading to superoxide and oxygen free radical production, and free radicals may then inactive NO upon contact. It may be a mechanism for the decreased endothelial responsiveness shown in HSF and HUF groups. In recent studies, FFA elevation is regarded to play an important role in endothelial dysfunction. FFA elevation may cause vascular endothelial dysfunction either indirectly.
via increased release of vasoconstrictor substances such as ET-1 and/or through a direct impairment of basal NO production. Davda et al.\(^{[14]}\) and Gupta et al.\(^{[15]}\) demonstrated that in endothelial cell cultures FFA elevation inhibits NO production by decreasing endothelial nitric oxide synthase activity because endothelial nitric oxide synthase protein content was not altered by FFA elevation. Our research found plasma FFA increased and NO\(_2^-\)/NO\(_3^-\) decreased in the rats fed high saturated and unsaturated fatty acids. With a significant correlation between NO\(_2^-\)/NO\(_3^-\), GIR and FFA in HSF and HUF groups, we assume that the results may be attributable to decreased endothelium-dependent vasorelaxation and IR.

In summary, we have shown that rats fed two different high fat diets rich in saturated and unsaturated fatty acids develop obesity, hypertension, hypertriglyceridemia, hyperinsulinemia, and there is no difference between them in the present study. It appears to be a direct correlation between high-fatty acid diets and the development of endothelial dysfunction (reduction of endothelium-dependent vasorelaxation response to ACh), and may lead to the vascular complication in patients with type 2 diabetes mellitus.

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