Effects of dietary composition on postprandial endothelial function and adiponectin concentrations in healthy humans: a crossover controlled study¹,²

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ABSTRACT

Background: Abnormalities during the postprandial state contribute to the development of atherosclerosis. Reportedly, postprandial hyperglycemia, hypertriglyceridemia, and hyperlipidemia independently cause postprandial cytokine activation. However, it is not clear which dietary composition preferentially affects postprandial endothelial function in healthy subjects.

Objective: We aimed to examine the associations of dietary composition and postprandial endothelial function in healthy subjects.

Design: The effects of a single ingestion of a high-carbohydrate meal (300 kcal, 100% carbohydrate), a high-fat meal (30 g fat/m², 35% fat), or a standard test meal (478 kcal; 16.4% protein, 32.7% fat, 50.4% carbohydrate) on postprandial plasma concentrations of adiponectin and forearm blood flow (FBF) during reactive hyperemia were studied in healthy subjects.

Results: The peak FBF response and the total reactive hyperemic flow (flow debt repayment; FDR), indexes of resistance artery endothelial function, were unchanged after ingestion of a high-carbohydrate and standard test meal but decreased 120 and 240 min after a high-fat meal. After a high-fat meal, decreases in peak FBF and FDR were well correlated with an increase in plasma free fatty acids (FFA) concentrations but not with the other biochemical variables, including triacylglycerol, insulin, glucose, total cholesterol, HDL cholesterol, and adiponectin.

Conclusions: Postprandial endothelial function was impaired only after the high-fat diet and not after the high-carbohydrate or standard test meal in healthy subjects. Because such endothelial dysfunction after a high-fat meal was closely correlated with FFA concentrations, postprandial state could be hazardous, mostly through acute hyperlipidemia in healthy subjects.

KEY WORDS Free fatty acids, endothelial function, adiponectin, postprandial state, hyperlipidemia, hyperglycemia

INTRODUCTION

The abnormalities of the postprandial state are important contributing factors to the development of atherosclerosis. There is evidence that postprandial hypertriglyceridemia is a risk factor for cardiovascular diseases (CVD) (1), whereas in diabetic subjects, postprandial hyperglycemia has been proposed as an independent risk factor for CVD (2).

The risk of coronary artery disease is increased by consumption of a diet rich in saturated fatty acids (3). In both healthy subjects and in diabetic patients, a single high-fat meal induces endothelial activation, which is associated with increased inflammatory cytokine production (4). Meanwhile, postprandial hypertriglyceridemia (4–6) and hyperglycemia (7) can elicit endothelial dysfunction via an independent and a cumulative increase in oxidative stress. Free fatty acids (FFAs) elevated by intravenous lipid or heparin infusion directly impair the vasodilatory response to acetylcholine in healthy humans, which may be pathophysiologically relevant to the development of postprandial endothelial dysfunction in patients with obesity and insulin resistance (8–10).

Adiponectin is an adipocyte-derived plasma protein (adipocytokine) that accumulates in injured arteries and has potential antiatherogenic properties. Maintenance of adiponectin concentrations is closely associated with normal endothelial function in humans (11, 12); therefore, postprandial changes in plasma adiponectin concentrations (13–15) could be related to postprandial endothelial dysfunction. However, such a relation has not been evaluated. We investigated effects of diet composition on postprandial plasma concentrations of adiponectin and endothelial function in healthy subjects.

SUBJECTS AND METHODS

Subjects

Twelve healthy subjects (6 men and 6 women) aged 30–42 y (± SD: 36 ± 1 y) participated in this study. The subjects had a mean (±SD) body weight of 62 ± 4 kg, body mass index (in kg/m²) of 23.3 ± 0.9, systolic blood pressure of 121 ± 5 mm Hg, diastolic blood pressure of 72 ± 3 mm Hg, and heart rate of 63 ± 2 beats/min. All subjects gave their written informed consent before the study began. The study protocol was approved by the Ethical Committee of the University of Ryukyus and was carried out from April 24, 2007, to May 11, 2007.
out in accordance with the principles of the Declaration of Helsinki as revised in 2000. None of the subjects had an acute or chronic illness, had experienced recent body weight changes, or were taking medication regularly. All subjects abstained from alcohol, tobacco, and strenuous physical activity for 24 h and caffeine-containing drinks overnight.

**Endothelial function**

On 3 separate mornings ≥7 d apart, participants ingested a high-carbohydrate meal (300 kcal, 100% carbohydrate) (16), a high-fat meal (30 g fat/m², 35% fat, 342 kcal/100 g), or a standard test meal (478 kcal, 16.4% protein, 32.7% fat, 50.4% carbohydrate; proposed by a working group of the Japan Diabetes Society) after fasting overnight (17). The order for meal ingestion was randomized in a crossover design. Endothelial function was measured by using forearm blood flow (FBF) before and 120 and 240 min after the ingestion of each meal. Blood samples were obtained at 0, 30, 60, 120, and 240 min. FBF was measured with the use of a mercury-filled silastic strain-gauge plethysmograph (EC-5R; DE Hokanson Inc, Issaquah, WA) as described (11) below:

A representative tracing of an FBF curve is shown in Figure 1. FBF was calculated from gradient of tracing (ΔmV/s) with a mercury-filled strain-gauge plethysmograph at baseline and after 5 min of upper arm cuff occlusion.

**Blood flow debt (mL) = control flow rate (mL/s) × duration of occlusion (s) (1)**

**Reactive hyperemic flow (mL)**

\[ = [\text{total flow during reactive hyperemia (mL)}] - [\text{control flow rate (mL/s)} \times \text{duration of reactive hyperemia (s)}] \] (2)

**Blood flow debt repayment (%)**

\[ = \frac{\text{reactive hyperemic flow/blood flow debt}}{100} \] (3)

Before and after release of a 5-min upper arm cuff occlusion at 200 mm Hg (reactive hyperemia) and after a single sublingual administration of 0.3 mg nitroglycerin (Nihonkayaku Co, Tokyo, Japan). In the preliminary study, we confirmed the reproducibility of reactive hyperemia and sublingual nitroglycerin-induced vasodilatation on 2 separate occasions in healthy male subjects (11).

**Biochemical measurements**

Before and after meal ingestion, venous blood was collected from subjects by using a vacuum tube with serum separation reagents or heparin sodium and then stored frozen until used. Preliminary studies for blood sampling conditions, including serum separation reagents, heparin sodium, and EDTA-Na₂, on adiponectin measurements were compared. There was a good correlation between adiponectin concentration and serum and heparin-treated plasma (r = 0.997; regression line y = 0.96x + 0.05) and also between serum and EDTA-treated plasma (r = 0.999, regression line y = 0.93x + 0.08).

Plasma glucose concentration was measured with a glucose oxidase method, and insulin was measured by enzyme-linked immunosorbent assay (ELISA). Serum concentrations of total cholesterol, HDL cholesterol, and triacylglycerols were measured by routine enzymatic methods. Plasma and serum adiponectin concentrations were determined with a latex-particle-enhanced turbidimetric immunoassay (LTIA) (Human Adiponectin Assay Kit; Mitsubishi Kagaku Iatron Inc, Chiba, Japan) with an automated analyzer (Hitachi H7170) (18). The LTIA results were well correlated with the commercially available ELISA assay (18).

**Statistical analysis**

Values are expressed as means ± SEMs. Multigroup comparisons were made by one-factor repeated-measures analysis of variance (ANOVA). Multigroup comparisons of time course curves were first analyzed by two-factor repeated-measures ANOVA. If the multigroup difference was significant, intragroup comparisons were made by one-factor repeated-measures ANOVA and followed by Tukey’s post hoc test. For comparisons between metabolic variables and endothelial function during meal loading, the analysis was adjusted by analysis of covariance (ANCOVA). Probabilities were considered to be significant if <0.05. The data were processed by using the GRAPHPAD INSTAT 3 for Macintosh (GraphPad Software Inc, San Diego, CA) software package.
RESULTS

The meals were well tolerated by all patients, and no adverse events were observed during the study. Systemic hemodynamic and metabolic variables before test meal ingestion were comparable on the 3 study mornings.

The effects of the high-carbohydrate, high-fat, and standard test meals on plasma glucose, plasma insulin, serum lipid, and serum adiponectin concentrations are shown in Figure 2. After the high-carbohydrate and standard test meals, plasma glucose concentrations increased from baseline by \(\Delta 50\) mmol/L at 60 min and returned to baseline at 240 min (Figure 2). Plasma glucose concentrations did not change after the high-fat meal. After the high-carbohydrate and standard test meals, plasma insulin concentrations increased from baseline by \(\Delta 50\) pmol/L to a peak of 540–590 pmol/L at 60 min. The plasma insulin concentration did not change after the high-fat meal. After the high-carbohydrate and standard test meals, plasma insulin concentrations increased from baseline by \(\Delta 50\) pmol/L to a peak of 540–590 pmol/L at 60 min. The plasma insulin concentration did not change after the high-fat meal. Serum FFA concentrations decreased during the 120 min after the high-carbohydrate and standard test meals and returned to baseline at 240 min. Serum FFA concentrations increased to 0.87 ± 0.06 mmol/L at 240 min after the high-fat meal. Serum triacylglycerol concentrations increased to 2.77 ± 0.83 mmol/L at 240 min after the high-fat meal, but did not change after the high-carbohydrate and standard test meals. Serum concentrations of total and HDL cholesterol and adiponectin did not change during 240 min after either test meal.

The effects of the high-carbohydrate, high-fat, and standard test meals on FBF are shown in Figure 3, A, B, and C. The peak FBF was unchanged before and after the high-carbohydrate and standard test meals, but decreased significantly 120 and 240 min after the high-fat meal (Figure 3, A and B). The total reactive hyperemic flows (FDR), indexes of resistance artery endothelial function, also decreased 240 min after the high-fat meal (Figure 3, C). After the high-fat meal, changes from baseline in peak FBF (\(\Delta FBF\)) and FDR (\(\Delta FDR\)) were inversely well correlated with the change in plasma FFA concentration (\(\Delta FFA\)) (Figure 4) but not with the other biochemical variables, including triacylglycerol, insulin, glucose, total cholesterol, HDL cholesterol, and adiponectin (data not shown).

DISCUSSION

In this study, we investigated the effects of diet composition on postprandial plasma concentrations of adiponectin and endothelial function in healthy subjects. Effects of the high-carbohydrate, high-fat, and standard test meals on plasma
glucose, insulin, and serum lipid concentrations were different, and postprandial forearm endothelial function was impaired after high-fat diet but not after the high-carbohydrate and standard test meals.

Postprandial hyperlipidemia (4) and postprandial hyperglycemia independently produce endothelial dysfunction and both are now largely recognized as potential underlying mechanisms of macrovascular events in subjects with normal or impaired glucose tolerance (1, 2). Postprandial state is a complex dynamic phase. Hyperlipidemia, hyperglycemia, or changes in the bloodstream of various humoral factors are simultaneously present in the postprandial phase. Thus, the specific molecule mostly responsible for endothelial dysfunction has not been determined.

We therefore examined the effects of diet composition on postprandial biochemical variables and endothelial function. The study was done on 3 separate mornings, and the order in which the 3 types of meals were ingested was randomized in a crossover design to minimize the other confounding factors.

FIGURE 3. Effects of dietary composition (high-carbohydrate, high-fat, or standard test meal) on forearm blood flow (FBF) and total reactive hyperemic flow (flow debt repayment; FDR) in 12 healthy subjects (6 men and 6 women). A: Measurements were taken at baseline and during reactive hyperemia 120 and 240 min after the meals. B: Peak FBF was measured during reactive hyperemia before (0 min) and 120 and 240 min after the meals. C: Total reactive hyperemic flow (FDR) was measured during reactive hyperemia. FDR (%) = (reactive hyperemic flow/blood flow debt) × 100. Data are presented as means ± SEMs. One-factor ANOVA with Tukey’s post hoc test was used to compare intragroup means. *Significantly different from 0 min, \( P < 0.01 \).
infusion of lipid and heparin in healthy subjects (9, 10). Previous findings support the notion that acute hyperlipidemia directly impairs endothelial function, which was observed in this study. The mechanism for such FFA-related endothelial dysfunction could not be drawn from this study. One can assume that hypertriglyceridemia, a cumulative effect of endothelial activation, or both may be associated with increased serum concentrations of inflammatory cytokines such as tumor necrosis factor-α, interleukin-6, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 (13).

Another possible explanation is the production of reactive oxygen species (ROS) by FFAs. Ceriello (7) reported that increased production of ROS, determined by nitrotyrosine, was closely associated with endothelial dysfunction after consumption of a high-fat meal. We confirmed that FFA-induced endothelial dysfunction could be protected by co-infusion of antioxidant, vitamin C, in healthy humans (10). Experimentally, we observed that FFA directly inhibited vascular response to acetycholine in stripped aorta isolated form normal animal models (19). Also, we found that FFA directly enhanced the production of vascular ROS via up-regulation of NADPH oxidase subunit mRNA, and the inhibition of ROS production by N-acetylcysteine (a general antioxidant), diphenyle nidium, and apocynin (NADPH oxidase inhibitors) can prevent FFA-induced endothelial dysfunction (19).

In summary, we investigated the effects of diet composition on postprandial plasma concentrations of adiponectin and endothelial function in healthy subjects. Postprandial endothelial function was impaired only after ingestion of the high-fat diet and not after consumption of the high-carbohydrate and standard test meals. Because such endothelial dysfunction after a high-fat meal was closely correlated with FFA concentrations, the postprandial state could be hazardous, mostly through acute hyperlipidemia in healthy subjects.

None of the authors had a conflict of interest.

REFERENCES
8. Steinberg HB, Chaker H, Leaming R, Johnson A, BrechTEL G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction:

![Graph](https://example.com/graph.png)

FIGURE 4. Changes from baseline in peak forearm blood flow (Δpeak FBF) and flow debt repayment (ΔFDR) and changes in serum free fatty acids (ΔFFA) and triacylglycerols 120 and 240 min after high-fat meal loading in 12 healthy subjects. After adjustment by ANCOVA with the use of time point after loading as covariance, Pearson’s correlation coefficients (r) and P values were calculated.

(13) reported that adiponectin concentrations decreased from baseline after the high-fat meal, but not after the high-carbohydrate meal. Although the reason for the discrepancy between their study and ours is unknown, the difference in the percentage of energy as fat of the diets could be one reason (60% compared with 32.9%).

We reported that the high-carbohydrate and standard test meals did not produce endothelial dysfunction in healthy subjects with normal glucose tolerance (16, 17). However, it has been reported that the high-fat meal could impair endothelial function, even in healthy subjects (4–7). Our results confirm the previous findings that endothelial function is impaired after ingestion of a high-fat meal but not after ingestion of a high-carbohydrate or standard test meal.

As shown in Figure 4, decreases in peak FBF and FDR after a high-fat meal were well correlated with increases in serum FFA concentrations, but not with the other biochemical variables, including triacylglycerol, insulin, glucose, total cholesterol, HDL cholesterol, or adiponectin. Because adiponectin concentrations did not change after the meals, adiponectin oscillation is unrelated to postprandial changes in acute vascular reactivity. Our previous study showed that plasma adiponectin concentrations were well correlated with baseline (fasting) endothelial function in nondiabetic subjects (11). We postulated that there are 2 possible mechanisms by which hypoadiponectinemia decreases endothelial function: 1) hypoadiponectinemia can cause endothelial dysfunction by decreasing insulin sensitivity, and 2) hypoadiponectinemia may be directly linked to early atherosclerotic vascular damage and subsequent endothelial dysfunction.

In the present study, we showed that the FFA concentration, rather than the plasma adiponectin concentration, is mainly associated with postprandial endothelial dysfunction in healthy subjects. Taken together, hypoadiponectinemia could be linked to chronic vascular injury but not to acute postprandial vascular injury. It has been shown that the FBF response to the intraarterial infusion of acetylcholine was impaired acutely after systemic