Effects of Aerobic Exercise on Postprandial Carbohydrate and Lipoprotein Metabolism Following Cookie Ingestion in Healthy Young Women

Sayuki HASHIMOTOF2, Erika MIZUTANIF2, Maiko SUZUKIF2, Akihiro YOSHIDAF2 and Michitaka NAITOF1,*

1 Division of Nutrition & Health, School & Graduate School of Life Studies, Sugiyama Jogakuen University,
17–3, Hoshijouka-motomachi, Chikusa-ku, Nagoya 464–8662, Japan
2 Department of Food and Nutritional Environment, College of Human Life and Environment,
Kinjo Gakuin University, 2–1723, Omori, Moriyama-ku, Nagoya 463–8521, Japan
3 Department of Clinical Laboratory, Nakatsugawa Municipal General Hospital,
Nakatsugawa 508–8502, Japan
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Summary We examined the acute effects of postprandial aerobic exercise on glucose and lipid metabolism following cookie ingestion. Fifteen healthy young women with a sedentary lifestyle, normal weight and apolipoprotein E3/3 participated. After a 12-h overnight fast, each subject ingested a cookie (1.53 g/kg, Meal Test C) and then performed two trials, one with postprandial exercise (E trial) and one without exercise (C trial), in a randomized crossover design. A single 30-min bout of walking exercise was performed 20 min after the cookie intake. Venous blood samples were drawn before (0 h) and 20 min and 1, 2, 4, and 6 h after cookie ingestion. The Δglucose concentration was not significantly different between the two trials, but the Δinsulin concentration at 1 h and the incremental area under the curve (IAUC) (0–2 h)-insulin in the E trial were significantly lower than in the C trial. The ratio of glucose/insulin at 1 h was significantly higher in the E trial than in the C trial. The ΔTG, ΔRLP-TG, ΔapoB48 and ΔRemL-C concentrations at 1 h in the E trial were significantly higher than in the C trial. The IAUC (0–2 h)-apoB48 in the E trial was significantly larger than in the C trial. Postprandial exercise showed an insulin-sparing effect following the cookie ingestion by increasing insulin sensitivity. However, postprandial exercise transiently stimulated the secretion of exogenous apoB48-containing lipoprotein during the early period, and no further effects were observed. These results suggest that postprandial aerobic exercise is effective for the promotion of postprandial carbohydrate metabolism, but not lipidemia.

Key Words triglyceride, remnant, apolipoprotein B-48, insulin, walking

There has been increasing interest in postprandial lipemia and glycemia, especially increased remnant particles, as a risk factor for atherosclerosis (1). Zilversmit (2) first proposed that atherogenesis is a postprandial phenomenon, and there have been many studies of postprandial dyslipidemia since then. Postprandial glycemia has been studied more extensively than postprandial lipemia. Postprandial hyperglycemia, i.e., impaired glucose tolerance, has been reported to be more important than fasting hyperglycemia, i.e., impaired fasting glucose (3). For the evaluation of both glycemia and diabetes mellitus, the 75 g oral glucose tolerance test (75gOGTT) is widely used. In the 75gOGTT, blood samples are drawn at 0 (fasting), 0.5, 1 and 2 h following the ingestion of glucose.

However, methods to evaluate postprandial lipemia have not been established, although several types of fat tolerance tests have been used to estimate postprandial lipemia (4). An oral cookie tolerance test (the Cookie Test) was developed for the simultaneous evaluation of carbohydrate and lipid metabolism (5). In the Cookie Test, a subject ingests the standardized cookie and the blood is drawn at fasting, and 0.5, 1 and 2 h; the serum glucose, insulin and triglyceride (TG) responses are evaluated. However, for the evaluation of postprandial lipid metabolism it is necessary to collect blood samples until 6 or 8 h after fat ingestion, because postprandial lipid and lipoprotein metabolism continues much longer than that for carbohydrate (6, 7). Another disadvantage of the Cookie Test is that the amount of cookie ingested is fixed (i.e., 115 g total comprised of 75 g carbohydrate, 28.5 g fat and 8 g protein), and the test does not take into account the physique of the subjects. In the present study, we attempted to accommodate these disadvantages of the Cookie Test.

In our previous study using oral fat tolerance test (OFTT) cream, which contains essentially no carbohydrate, we found that postprandial aerobic exercise was more effective for promoting postprandial lipid metabo-
lism than immediately preprandial exercise in healthy but sedentary young Japanese women (6). However, because normal meals usually contain a substantial amount of carbohydrate along with fat, we next examined the effects of postprandial aerobic exercise on glucose and lipid metabolism after the ingestion of glucose supplemented or not with the fat cream (7). We observed that the postprandial aerobic exercise alleviated the glycemc peak at 1 h that is associated with insulin ‘sparking.’ However, the effects of exercise on lipid metabolism were transient, enhancing the secretion of exogenous lipoproteins at an early phase without further significant effects.

In the present study, we examined the acute effects of postprandial aerobic exercise on glucose and lipid metabolism following the ingestion of cookie containing the three major nutrients in healthy but sedentary young Japanese women.

**METHODS**

**Subjects.** Fifteen healthy young Japanese female subjects with a sedentary lifestyle, normal weight (body mass index [BMI]: 18.5 to <25), a normal ovarian cycle, and the apolipoprotein (apo) E3/3 phenotype were enrolled in this study. We chose the apoE3/3 phenotype to minimize the individual variability. All subjects were nonsmokers and were not taking any medication or dietary supplements or suffering from any apparent acute or chronic illness. This study was approved by the Institutional Review Board of the Sugiyama Jogakuen University School of Life Studies in accord with the Helsinki Declaration, and the subjects provided informed consent.

**Anthropometric and body composition measurement.** Body weight, height, and waist and hip circumference were measured by standard methods. The waist circumference was assessed as the abdominal girth at the level of the umbilicus, and the hip circumference was measured at the level of the greater trochanters. The waist-to-hip ratio (W/H) was calculated. Body composition including visceral fat area (VFA) was analyzed using an eight-polar bioelectrical impedance method, using the InBody720 analyzer (BioSpace, Tokyo, Japan).

**Exercise.** An incremental exercise protocol was designed to increase walking speed on a treadmill, aiming for an intensity of 50%VO₂ max as described (6). Following a 3–5 min warm-up, the initial speed was set at 3.7 km/h, and the speed was then increased by 0.3 km/h every 3 min for the first 15 min, and then by 0.2 km/h every 3 min for another 15 min. The aerobic exercise lasted for a total of 30 min, and the last speed attained was 5.9 km/h. Heart rate was determined every 3 min with a cardiotachometer (HR-40, Japan Precision Instruments, Shibukawa, Japan), and blood pressure was recorded before and after the exercise. HR max, %HR max, and %VO₂ max were calculated as described (6).

**Test meal.** The cookie (Meal Test C, Saraya, Osaka, Japan) in a carton (115 g) consisted of 75 g carbohydrate (flour starch and maltose), 28.5 g butter fat, and 8 g protein for a total of 592 kcal. We determined the cholesterol content as 2.5 mg/100 g by a colorimetric method. The cookie was used at 1.53 g/kg body weight (1 g/kg carbohydrate, 0.38 g/kg fat, and 0.11 g/kg protein).

**Experimental design.** The subjects fasted for 12 h overnight and then ingested the cookie. During the 12 h overnight fast, the subjects were requested to abstain from ingesting caffeine and alcohol. At our laboratory, the subjects were instructed to ingest the cookie with water (150 mL), and they were encouraged to consume the cookie within 10 min. The venous blood was drawn before (0 h) and 20 min and 1, 2, 4 and 6 h after ingestion; the time count began 10 min after the start of cookie ingestion. All of the blood samples were taken in the supine position. The subjects were allowed to drink water ad libitum and were restricted from exercise during the test period except for the designated exercise testing.

The protocol of the experiments is shown in Fig. 1. Each subject performed two trials: the control (C; without exercise) and exercise (E; with exercise) trials, in a randomized crossover design. The experiments were performed at least 4 wk apart between the test days to minimize the confounding effects of menstrual status on lipid metabolism. The subjects were instructed to maintain their sedentary lifestyle during the interval between the study sessions.

**Biochemical analysis.** The serum samples were immediately refrigerated (4°C) or frozen (−80°C) until
analysis. The concentrations of TG, remnant-like particle (RLP)-TG, apolipoprotein B-48 (apoB48), free fatty acid (FFA), glucose, insulin and lactate were measured at 0, 1, 2, 4 and 6 h after the cookie ingestion, and the concentrations of glucose and insulin were also measured at 20 min after ingestion. The concentrations of TG (Sekisui Medical, Tokyo), lactate (Kyowa Medex, Tokyo), remnant lipoprotein-cholesterol (RemL-C) (8) (MetaboRead, Kyowa Medex), and FFA (Eiken Chemical, Tokyo) were measured enzymatically. RLP-TG was measured by an immunosorbent assay (9) (Otsuka Pharmaceutical, Tokyo). ApoB was measured by immunonephelometry (Sekisui Medical). The concentration of apoB48 was measured by a chemiluminescent enzyme immunoassay (10) (FujiRebio, Tokyo). The small dense low-density lipoprotein (sdLDL) was measured by a homogeneous assay (11) (Denka Seiken, Tokyo). The concentration of glucose was measured by the mutarotase GOD method (Wako, Osaka). The concentration of insulin was measured by a chemiluminescent enzyme immunoassay (FujiRebio).

The following items were measured only in the fasting state (0 h). The concentration of total cholesterol (TC) was measured enzymatically (Sysmex, Hyogo, Japan). High-density lipoprotein-cholesterol (HDL-C) was measured by a direct method (Fujirebio). Low-density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald formula (12). Hemoglobin A1c (HbA1c) and lipoprotein(a) (Lp(a)) were measured by a latex agglutination method (Phenotyping ApoE IEF System, Joko, Tokyo). ApoE phenotype was measured using the isometric electrophoresis method (Phenotyping ApoE IEF System, Joko, Tokyo). Insulin resistance was evaluated using homeostasis model assessment-insulin resistance (HOMA-IR) (13).

Quantification of postprandial metabolism. We quantified the postprandial metabolism by calculating the incremental area under the curve (IAUC) as described (6). Postprandial changes of glucose, insulin, FFA, TC, TG, RLP-TG, RemL-C and apoB48 were calculated as differences (Δ) from the baseline mean value (as 0 at 0 h).

Statistics. All data were expressed as the mean±SE. We used StatView ver. 5.0 software (SAS Institute, Cary, NC) for all statistical analyses. We analyzed differences between the two trials by conducting paired t-tests and differences in the time-course changes from the initial values by a repeated measure one-way ANOVA, followed by Fisher’s PLSD as a post-hoc test. The difference between the value in the C trial and that in the E trial at each time point was assessed by a paired t-test. Values of p<0.05 were considered significant in all analyses.

RESULTS

The physical characteristics and fasting blood chemi-
cal data of the 15 subjects are shown in Table 1. Other than slight but significant differences in weight, BMI, HDL-C and LPL mass, there were no significant differences in the physical characteristics of fasting blood values between the two trials. No subjects had nausea, vomiting or diarrhea during or after the experiments.

Exercise

During the E trial, the pulse rate slowly increased. The average heart rate during exercise was 127.8±2.9 beats/min. The %HRmax and %VO2max were calculated as 64.5±1.4% and 51.9±1.9%, respectively. None of the subjects appeared to be in poor physical condition during the experiments, and their blood pressure remained within the normal range before and after the exercise (data not shown).

Glucose, insulin, FFA and lactate

The concentrations of serum fasting and postprandial glucose, insulin, FFA and lactate are shown in Table 2. The Δglucose, Δinsulin and ΔFFA are illustrated in Fig. 2A–C as differences from baseline. In both the C and E trials, the concentration of serum glucose was increased at 20 min, 1 h and 2 h compared to baseline (0 h) and returned to baseline at 4 h, and in the E trial, it was lower than baseline at 6 h. However, there was no significant difference in the Δglucose between the two trials (Fig. 2A).

The Δinsulin in the C trial peaked at 1 h, and in the E trial, it plateaued from 20 min to 1 h, and returned to baseline at 4 h in both trials. The Δinsulin at 1 h in the E trial was significantly lower than the corresponding value in the C trial (p<0.05) (Fig. 2B). The glucose/insulin ratio at 1 h was significantly higher in the E trial.

| Table 1. Anthropometric and clinical characteristics. |
|--------------------------|--------------------------|
|                          | Control                  | Exercise                  |
| Age (y)                  | 21.6±0.6                 | —                         |
| Height (cm)              | 159.5±1.5                | —                         |
| Weight (kg)              | 50.4±1.0                 | 50.0±0.9**                |
| BMI (kg/m²)              | 19.9±0.4                 | 19.7±0.4**                |
| Waist (cm)               | 69.7±0.9                 | 68.5±0.6                 |
| W/H                      | 0.77±0.0                 | 0.76±0.0                 |
| VFA (cm³)                | 26.9±2.9                 | 28.6±2.7                 |
| TC (mg/dL)               | 167.9±6.6                | 171.4±4.5                |
| HDL-C (mg/dL)            | 62.5±2.2                 | 66.5±2.6**                |
| LDL-C (mg/dL)            | 95.6±6.0                 | 94.7±4.6                 |
| sdLDL (mg/dL)            | 21.8±2.0                 | 22.6±2.4                 |
| Lp(a) (mg/dL)            | 20.5±3.6                 | —                         |
| LPL mass (ng/mL)         | 50.4±5.6                 | 42.8±3.0*                 |
| ApoA-I (mg/dL)           | 151.8±4.3                | 159.0±4.8                |
| ApoA-II (mg/dL)          | 25.6±0.6                 | 26.1±0.8                 |
| ApoC-II (mg/dL)          | 2.4±0.2                  | 2.4±0.2                  |
| ApoC-III (mg/dL)         | 6.7±0.3                  | 6.9±0.2                  |
| ApoE (mg/dL)             | 4.1±0.2                  | 4.0±0.2                  |
| HbA1c (%) (NGSP)         | 5.2±0.1                  | 5.2±0.1                  |
| HOMA-IR                  | 1.3±0.2                  | 1.6±0.1                  |

All values were obtained in the fasting state. All values are presented as the mean±SE. *p<0.05, **p<0.01.
The serum TG concentration increased at 1 h, peaked at 2 h in both trials, and returned to baseline at 6 h in both trials. The serum apoB48 at 1 h was significantly higher than that in the C trial (p<0.01) (Table 2).

The concentration of serum FFA decreased at 1 and 2 h, returned to baseline at 4 h, and increased at 6 h compared to baseline in both trials. The ΔFFA at 1 h was significantly higher in the E trial than in the C trial (p<0.01) (Fig. 2C). The IAUC (0–2 h)-FFA at 1 h in the E trial was significantly higher than that in the C trial (p<0.01) (Fig. 2F).

RLP-TG concentration in the E trial at 1 h was significantly higher than that in the C trial (p<0.01). In both trials, the concentration of RemL-C increased at 1 h, then plateaued, and did not return to baseline until 6 h. The ΔRemL-C at 1 h in the E trial was significantly higher than in the C trial (p<0.01) (Fig. 2G). ApoB and ApoB48

The serum concentrations of fasting and postprandial apoB and apoB48 are presented in Table 2, and the time course of ΔapoB48 is shown in Fig. 2G.

The concentration of serum apoB was decreased at 1–4 h in the C trial compared to baseline, but in the E trial it was decreased at 2 and 4 h compared to baseline, and it returned to baseline at 6 h in both trials. The serum apoB48 concentration peaked at 1 h and did not return to baseline levels at 6 h in either trial. The ΔapoB48 at 1 h was significantly higher in the E trial than in the C trial (p<0.01) (Fig. 2G). IAUC (0–2 h)-apoB48 was significantly larger in the E trial than in the C trial (p<0.01) (Table 3).

**DISCUSSION**

The major finding in this study is that the effects of postprandial aerobic exercise on postprandial carbohy-
Effects of Exercise on Postprandial Metabolism Following Cookie Ingestion

Carbohydrate and lipid metabolism following the ingestion of a solid meal (cookie) are similar to those following the ingestion of a liquid meal (OFTT cream plus glucose solution) in our previous study (7). Here we observed that the postprandial aerobic exercise mitigated the postprandial glucose metabolism by sparing insulin. However, the postprandial aerobic exercise transiently stimulated the secretion of exogenous apoB48-containing lipoprotein during the early period with no later effects, suggesting that postprandial aerobic exercise is useful for the enhancement of postprandial glucose metabolism, but not of lipid and lipoprotein metabolism.

Although the amounts of carbohydrate (1 g/kg) and fat (0.38 g/kg) in the cookie used in the present study are similar to those in the glucose (1 g/kg)+OFTT cream (0.35 g/kg as fat) used in our earlier study (7), the cookie also contains a small amount of protein derived from butter and egg (0.11 g/kg). It was reported that postprandial lipemia was reduced when protein (50 g sodium caseinate) was added to fat food (14); however,
the rise of TG and RLP-TG in the present study was comparable to that observed in our previous study (7). This may be because the amount of protein administered in the present study was small.

In this study, the sharp rise of the Δinsulin was significantly alleviated by aerobic exercise, indicating its insulin-sparing effect, probably mediated by the activation of α-adrenergic receptors on pancreatic β cells, from the increase in sympathetic activity (15). The glucose/insulin ratio, an index of insulin sensitivity, at 1 h was significantly higher in the E trial compared to that in the C trial, suggesting again the enhancement of insulin sensitivity. The postprandial exercise also transiently mitigated the suppression of FFA after the ingestion of the cookie. It is known that insulin acutely suppresses the lipoprotein production from both endogenous and exogenous sources, which may be caused by an insulin-mediated suppression of circulating FFAs and also the direct effects of insulin (16, 17).

It was also reported that an acute elevation of plasma FFA stimulates not only endogenous but also exogenous lipoprotein particle production in the fed state (18). In the present study, the ΔapoB48 at 1 h and the IAUC (0–2 h)-apoB48 were significantly higher/larger in the E trial compared to the C trial, suggesting that the synthesis and/or secretion of apoB48-containing exogenous lipoproteins were promoted in the early phase. Postprandial exercise stimulated the transient secretion of apoB48-containing lipoproteins with a rapid rise of serum apoB48, TG, RLP-TG and RemL-C, but the subsequent course of lipidemia was not changed. The postprandial exercise may have stimulated the release of apoB48-containing lipoproteins transiently by alleviating the rise of insulin levels. A transient release of apoB48-containing lipoproteins due to the increase of intestinal lymphatic flow following exercise may also be a candidate (7). In the present study, serum total TG and RLP-TG, but not serum apoB48 or RemL-C, returned to baseline at 6 h in both trials, suggesting that postprandial exogenous lipoprotein metabolism was not finished even at the end of the experiment, irrespective of exercise.

Most guidelines recommend postprandial moderate aerobic exercise for the prevention of glucose intolerance or diabetes mellitus (19). Consistent with these guidelines, the present results showed that postprandial exercise was beneficial for the promotion of postprandial glucose metabolism in healthy young women. However, postprandial exercise transiently exaggerated the postprandial rise of exogenous lipoproteins at an early phase. We thus cannot recommend postprandial exercise for the purpose of promoting postprandial lipoprotein metabolism. However, because of the relatively small number of subjects examined in this study (n=15), the results must be interpreted with caution. It should also be pointed out that the present conclusion in healthy young women may not be generalized for men, the elderly, or individuals with health problems.

**CONCLUSION**

In healthy but sedentary young Japanese women, moderate-intensity postprandial aerobic exercise mitigated the peak of the serum insulin concentration and increased the glucose/insulin ratio after the ingestion of the cookie. However, the exercise transiently stimulated the secretion of exogenous apoB48-containing lipoproteins at an early phase. Based on these results, we recommend postprandial aerobic exercise for the mitigation of postprandial glycemia, but not lipidemia.

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