Effects of Evening-Only Low-Carbohydrate Meal on Healthy Volunteers

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Summary We performed a pre/post-interventional study with participants as self-controls to evaluate the effects of consuming an evening-only low-carbohydrate meal (LCM) at 1800 h on biochemical measures of glucose and lipid metabolism. Study participants comprised 14 healthy men (age range, 20–29 y) who, consumed standard test meals (STMs) or LCM at 1800 h. Blood samples were collected at fasting, and at 60-, 120-, and 240 min after the start of the meals. The 60-min postprandial levels and the area under the curve (AUC) 0–120 min for plasma glucose were significantly lower after the LCM than after the STMs. The 60- and 120-min postprandial levels and the AUC 0–240 min for plasma insulin were significantly lower after the LCM than after the STMs (p<0.01). Postprandial triglyceride (TG) levels at 120- and 240 min and the AUC 0–240 min were significantly higher after the LCM than after the STMs (p<0.05, p<0.01, and p<0.05, respectively). The interleukin-6 levels were significantly higher 240 min after the STMs than before the meals (p<0.05), but not after the LCM. In these healthy volunteers, consuming an LCM at 1800 h suppressed postprandial hyperglycemia and insulin secretion; however, postprandial TG increased. Consuming an LCM at 1800 h was beneficial as it inhibited elevation of blood glucose; however, it may also increase the risk of arteriosclerosis through increasing TG levels.

Key Words low-carbohydrate diet, postprandial glucose, insulin, triglyceride, healthy participants

Since the publication of “Dr Atkins’ Diet Revolution” in 1972 (1), low-carbohydrate diets (LCDs) have polarized medical opinion. One study reported that an LCD had been used to effectively treat type 2 diabetes mellitus and obesity (2), while another study considered this form of diet to be simply a fad in conflict with current globally accepted dietary guidelines that advocate low-fat diets to reduce the risk of cardiovascular disease (3).

According to Feinman et al. (2), LCDs are categorized into three groups: very low-carbohydrate ketogenic diet (carbohydrate, 20–50 g/d or <10% of the 2,000 kcal/d diet); low-carbohydrate diet (carbohydrate, <130 g/d or <26% of the total energy), and moderate-carbohydrate diet (carbohydrate, 26–45%).

LCDs have not generally been recommended as adherence rates have been reported to be low. One study reported that an LCD was the most effective diet for weight loss (4); however, adherence rates at 2 y were 78.0% in the LCD group, 90.4% in the low-fat diet group, and 85.3% in the Mediterranean diet group. Moreover, LCDs were more effective in HbA1C and body weight reduction in the short-term compared to other diets, whereas no superiority was observed in the long-term (5, 6). Based on these findings, because a LCD was not able to be continued, an LCD appears to be less effective long-term. In addition, blood glucose levels have been shown to have a greater increase in the evening than in the morning (7), and an increasing carbohydrate intake at the expense of fat in the morning has been shown to be protective against the development of diabetes (8) and metabolic syndrome (9). Thus, consuming an evening-only low-carbohydrate meal (LCM) may be effective for reducing blood glucose levels. However, because cholesterol (10) and triglyceride (TG) (11) are likely to rise at night, consuming an evening-only LCM may increase the risk of arteriosclerosis. Therefore, we performed a pre/post-interventional study with participants serving as self-controls to evaluate the effects of consuming an evening-only LCM on biochemical measures of glucose and lipid metabolism.

MATERIALS AND METHODS

Participants. We recruited 14 healthy males from a university in Sapporo, Japan, via a bulletin board between February 1 and 28, 2018. Eligibility criteria comprised non-smoking participants aged between 20 and 29 y. Exclusion criteria comprised individuals who worked late at night, who had a BMI ≥25 kg/m², who routinely took supplements, who had been previously diagnosed with a metabolic disease, who had a familial history of hypercholesterolemia, or who took corticoste-
roid medication. Participants who performed high intensity exercise on a daily basis were not excluded. However, soft lean mass and fat-free mass of all participants were within 2-times the standard deviation, with no considerable difference. The study was undertaken after written informed consent had been obtained from all eligible participants, and approval was obtained from the Ethics Committee of Tenshi College (reference number 2017-20) and was carried out in accordance with the Declaration of Helsinki (1997). This trial was registered in the UMIN Clinical Trials Registry as UMIN000035855 (http://www.umin.ac.jp/ctr/index-j.htm).

**Study protocol.** This study was undertaken at Tenshi College between March 1 and March 10, 2018. Figure 1 shows an outline of the study. Participants were required to participate in the study for three consecutive days. On the first day, after overnight fasting, morning blood samples were collected, and measurements of height, weight, body composition, and blood pressure were obtained. Subsequently, participants consumed a standard test meal (STM) at 1800 h. Afterwards, we distributed STMs to be consumed at 0800 and 1300 h on the second day at home, along with two 500 mL bottles of water. On the second day, the participants consumed their STMs at 0800 and 1300 h at their homes, and arrived at Tenshi College at 1700 h to consume another STM at 1800 h. Blood samples were collected at fasting and 60, 120, 240 min after the start of the meal at 1800 h. At 2220 h, we distributed the STMs to be consumed at 0800 h and at 1300 h on the third day at home, along with three 500 mL bottles of water. On the third day, the dietary and blood sampling protocols were the same, except that the participants were required to consume an LCM at 1800 h rather than an STM. Prior to the study, the participants had been provided with the following instructions: for 7 d they were requested not to consume nutritional supplements, they were to continue with their regular eating habits, and they were not to significantly alter their weight and physical activity levels. From 1800 h on the first day to 2230 h on the third day, the study participants were instructed to eat only the meals that had been distributed at the specified times, to avoid eating between meals, to undertake the same level of physical activity while avoiding strenuous exercise, and to avoid the consumption of alcohol, caffeine, and sugary drinks. On the first day, we clearly set out the importance of adhering to a balanced lifestyle. We contacted the participants by email before the meal start time at home, and we reminded the participants to adhere to the instructions that had been highlighted in the email, to prompt participants to follow the instructions. Moreover, to determine whether participants conformed to the instructions, we distributed a paper on which participants recorded meal start time and meal end time, and the residual contents of the meal. If the participants did not complete the test meal, they were instructed to take a picture of the remaining meal and to note the contents of the remaining meal. We confirmed these details on the recording paper on the second and third days at 1700 h and collected the paper at the end of the study. On the paper, the instructions for the research period were stated.

**Test meals.** Two meal types were prescribed. The LCM consisted of 115 g steamed chicken meat (sarada-chikin 115 g, LAWSON, Japan), 15 g mayonnaise (pyu-aserekuto® mayoneizu 15 g, Ajinomoto, Japan), 80 g canned tuna (raitotunahureiku maguro daizuyuzuke 80 g, LAWSON), 52 g boiled egg (ajituketamago, LAWSON), and a frozen box lunch (bento); the STM consisted of 80 g steamed chicken meat (saradachikin 115 g, LAWSON), 12 g mayonnaise (pyuaserekuto® mayoneizu 12 g, Ajinomoto), 250 g white rice (oosoroshibarikogoh, LAWSON), and a frozen box lunch (bento). A registered dietitian supervised the preparation of the frozen box lunch (bento) (Healthy Network Corporation, Tokyo, Japan). We obtained frozen box lunches (bento) by accessing “https://www.healthynetwork.co.jp/” and used “the broiled chicken meat balls with 5 kinds of side dishes” on the first day at 1800 h, “simmered flounder with 5 kinds of side dishes” on the second and third days at 0800 h, “hamburger steak, cheese, and demi-glace sauce with 5 kinds of side dishes”at 1200 h, and “sweet and sour chicken” at 1800 h. The LCM consisted of 10.8% carbohydrate (21.0 g), 30.8% protein (59.9 g), 58.4% fat (50.5 g), and fiber (4.3 g). According to the definition by Feinman et al. (2), this meal constituted a low-carbohydrate diet (carbohydrate, <130 g/d or <26% of the total energy). The STM to be consumed on the second day at 1800 h consisted of 51.3% carbohydrate (100.0 g), 20.4% protein (39.8 g), 28.3% fat (24.5 g), and fiber (4.8 g). Both the LCM and the STM that was to be consumed on the second day at
1800 h were matched for energy (779 kcal, Table 1). All the STMs during the study were similar meals other than the frozen box lunch (bento). The STM prepared for consumption on the first day at 1800 h consisted of 801 kcal, 48.7% carbohydrate (99.9 g), 20.2% protein (40.5 g), 31.1% fat (27.7 g), and fiber (5.8 g). The STMs prepared for consumption on the second and third days at 1300 h consisted of 789 kcal, 49.7% carbohydrate (99.6 g), 20.2% protein (40.5 g), 31.1% fat (27.7 g), and fiber (5.7 g). The STMs prepared for consumption on the second and third days at 0800 h consisted of 789 kcal, 49.7% carbohydrate (100.5 g), 20.6% protein (40.6 g), 29.7% fat (26.1 g), and fiber (5.7 g). The STMs prepared for consumption on the second and third days at 1300 h consisted of 801 kcal, 48.7% carbohydrate (99.9 g), 20.2% protein (40.5 g), 31.1% fat (27.7 g), and fiber (5.8 g). The participants consumed the same meals at the same time on the second and third days, and we took account of the second-meal phenomenon (12) not occurring at dinnertime on biochemical measures of glucose and lipid metabolism. The participants were asked to consume the steamed chicken first, and to complete the test meals within 15 min.

Biochemical analysis. On the first day, the following parameters were measured for the screening tests and to obtain the general characteristics of the participants: fasting blood glucose, hemoglobin A1c, insulin, triglycerides (TG), serum total cholesterol (TC), low-density lipoprotein cholesterol, HDL-C, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, blood urea nitrogen, creatinine, estimated glomerular filtration rate, and uric acid. On the second and third days, we measured glucose, insulin, active glucagon-like peptide-1 (GLP-1), active glucose-dependent insulino-metotropic polypeptide (GIP), glucagon, TG, and non-HDL-C in all blood samples. Interleukin-6 (IL-6) and high sensitivity C-reactive protein (Hs-CRP) were measured at fasting and at 240 min after the start of the meals consumed at 1800 h. Non-HDL-C was determined using the following equation: total cholesterol minus HDL-C. For active GLP-1, active GIP, and glucagon, the blood samples were collected in EDTA-2Na tubes with aprotinin and DPP-IV inhibitor was added immediately following blood collection. The samples were then centrifuged after being mixed. The separated plasma was then stored at −30°C or lower, until analysis. Plasma concentrations of active GLP-1 were measured using a sandwich Enzyme Immuno Assay (EIA) kit (GLP-1, Active form Assay Kit-IBL, Immuno-Biological Laboratories, Gunma, Japan), active GIP were measured using a sandwich ELISA kit (Human GIP, Active form Assay Kit-IBL, Immuno-Biological Laboratories), and IL-6 levels were measured using a sandwich ELISA kit (Human IL-6 ELISA Kit, Thermo Fisher Scientific, USA), according to the manufacturers’ instructions. Plasma glucagon concentration conducted by the blood analysis company SRL Inc (Tokyo, Japan), which were measured using the specific dual-antibody sandwich ELISA (Mercodia AB, Uppsala, Sweden/Cosmic Corporation Co., Ltd., Tokyo, Japan), according to the manufacturers’ instructions. Other serum analyses were conducted by the blood analysis company SRL Inc using standardized techniques.

Anthropometric measurements and dietary assessment. Height, weight, and waist circumference measurements were obtained for all participants using standardized techniques, and BMI was calculated as weight (kg)/height² (m²). Body composition was determined using a bioelectrical impedance device (Inbody 770, Inbody Japan Corporation, Tokyo, Japan). Blood pressure was measured using a digital automatic blood pressure measurement device (HEM-7051-HP, Omron, Kyoto, Japan). Information concerning dietary intake throughout the month preceding the study was assessed using a validated, self-administered, brief diet history questionnaire (BDHQ) (13).

Calculations and statistical analysis. Data are shown as mean ± SD, and a p value <0.05 was regarded as statistically significant. For postprandial glucose, insulin, active GLP-1, active GIP, glucagon, TG, and non-HDL-C, the area under the curve (AUC) of each measurement was calculated above zero according to the trapezoidal rule. All AUCs were calculated using a computer spreadsheet (Microsoft Office Excel 2010). The Shapiro-Wilk statistic was performed for testing normality. When parametric analysis was possible, Student’s t-test was used for paired comparisons. If parametric analysis was not possible, the Wilcoxon signed rank test was used. All statistical analyses were performed using SPSS for Windows (version 24.0; SPSS, Chicago, IL).

RESULTS

Participant characteristics

The mean values ± SD for age and BMI were 21.1 ± 2.4 y and 21.8 ± 2.2 kg/m², respectively. Other study participant characteristics are shown in Table 2. As all the participants completed the test meal and ate according to the prescribed regimens, all of their results were analyzed.

Postprandial glucose and insulin responses

Glucose. There were no statistical differences in
AUC 0–240 min for plasma glucose between meals. The 60-min postprandial level and AUC 0–120 min for plasma glucose were significantly lower after the LCM than after the STMs (p=0.008, p<0.012); however, the 120-min postprandial plasma glucose was significantly higher after the LCM than after the STMs (p=0.030) (Fig. 2, Table 3).

**Insulin.** The 60-min postprandial and 120-min postprandial levels and AUC 0–240 min for plasma insulin were significantly lower after the LCM than after the STMs (p<0.001, p<0.001, and p<0.001, respectively) (Fig. 3, Table 3).

**Active GLP-1, active GIP, and glucagon responses**

**Active GLP-1.** The postprandial levels of GLP-1 at 60, 120, and 240 min and AUC 0–240 min were significantly higher after the LCM than after the STMs (p<0.001, p<0.001, p<0.002, and p<0.001, respectively) (Fig. 3, Table 3).

**Active GIP.** The 120-min postprandial levels and AUC 0–240 min for GIP were significantly lower after the LCM than after the STMs (p<0.001, p<0.001, p=0.002, and p=0.001, respectively) (Fig. 3, Table 3).

**Glucagon.** The postprandial levels of glucagon at 60, 120, and 240 min and AUC 0–240 min were significantly higher after the LCM than after the STMs (p=0.008 and p=0.029, respectively) (Fig. 3, Table 3).

Fig. 2. Effects of the LCM and the STM at 1800 h on biochemical measures of glucose. LCM, low-carbohydrate meal; STM, standard test meal. *p<0.05, **p<0.01.

Table 2. Study participant characteristics.

| Characteristic                                      | Total (n=14)       |
|-----------------------------------------------------|--------------------|
| Age (y)                                             | 21.1±2.4           |
| Weight (kg)                                         | 65.3±9.4           |
| BMI (kg/m²)                                         | 21.8±2.2           |
| Underweight (n)                                     | 2                  |
| Normal (n)                                          | 12                 |
| Waist circumference (cm)                            | 77.6±7.1           |
| Baseline fasting blood glucose (mg/dL)              | 85.4±3.4           |
| Baseline hemoglobin A1c (NGSP) (%)                  | 5.2±0.3            |
| Baseline insulin (µIU/mL)                           | 5.5±1.6            |
| Baseline fasting TG (mg/dL)                         | 73.1±33.0          |
| Baseline TC (mg/dL)                                 | 167.7±26.9         |
| Baseline low-density lipoprotein cholesterol (mg/dL)| 93.8±21.3          |
| Baseline HDL-C (mg/dL)                              | 61.4±13.0          |
| Baseline aspartate aminotransferase (U/L)           | 18.1±3.3           |
| Baseline alanine aminotransferase (U/L)             | 16.4±6.0           |
| Baseline gamma-glutamyl transpeptidase (U/L)        | 19.3±4.2           |
| Baseline blood urea nitrogen (mg/dL)                | 13.9±2.8           |
| Baseline creatinine (mg/dL)                         | 0.8±0.1            |
| Baseline estimated glomerular filtration rate (mL/min/1.73 m²) | 101.0±11.0         |
| Baseline uric acid (mg/dL)                          | 5.9±1.4            |
| Body composition (derived using BIA)                |                    |
| Fat (%)                                             | 17.7±5.1           |
| Muscle mass (kg)                                    | 50.4±5.7           |
| Fat-free mass (kg)                                  | 53.4±6.1           |
| Dietary intake (derived from an FFQ)                |                    |
| Energy (kcal)                                       | 2,078.4±637.9      |
| Protein (g)                                         | 70.4±25.2          |
| Fat (g)                                             | 61.2±23.3          |
| Carbohydrate (g)                                    | 291.8±96.4         |
| Dietary Fiber (g)                                   | 11.1±5.1           |

Values are means±SD. SD, standard deviation; TG, triglyceride; TC, total cholesterol; BIA, bioelectrical impedance analysis.
Table 3. Effects of the LCM and the STM at 1800 h on biochemical measures of glucose, insulin, active GLP-1, active GIP, glucagon, TG, non-HDL-C, IL-6, and Hs-CRP.

| Glucose (mg/dL) | LCM | STM | p value (between meals) |
|----------------|-----|-----|-------------------------|
| 0 min          | 85.1±9.1 | 83.6±4.5 | 0.579 |
| 60 min         | 78.9±7.6 | 91.1±13.5 | 0.008** |
| 120 min        | 86.0±7.7 | 89.2±11.7 | 0.432 |
| 240 min        | 88.9±5.5 | 83.8±8.2 | 0.030* |
| AUC_{0-120 min} | 9,866±556 | 10,650±1,002 | 0.012* |
| AUC_{0-240 min} | 20,361±1,148 | 21,030±1,819 | 0.193 |

| Insulin (µIU/mL) | LCM | STM | p value (between meals) |
|-----------------|-----|-----|-------------------------|
| 0 min           | 11.8±18.7 | 9.5±6.9 | 0.683 |
| 60 min          | 13.0±8.4 | 32.6±15.3 | 0.001** |
| 120 min         | 12.1±5.5 | 25.0±7.8 | <0.001** |
| 240 min         | 10.4±4.9 | 13.7±8.6 | 0.135 |
| AUC_{0-240 min} | 2,837±1,680 | 5,319±1,780 | 0.001** |

| Active GLP-1 (pg/mL) | LCM | STM | p value (between meals) |
|----------------------|-----|-----|-------------------------|
| 0 min                | 57.6±31.0 | 68.6±62.9 | 0.365 |
| 60 min               | 75.5±35.3 | 47.8±17.3 | 0.001** |
| 120 min              | 77.3±28.0 | 49.8±19.9 | <0.001** |
| 240 min              | 66.2±24.6 | 49.6±23.4 | 0.002** |
| AUC_{0-240 min}      | 17,186±6,527 | 12,387±5,711 | 0.001** |

| Active GIP (pg/mL)   | LCM | STM | p value (between meals) |
|---------------------|-----|-----|-------------------------|
| 0 min               | 32.5±31.2 | 42.3±17.5 | 0.084 |
| 60 min              | 84.1±30.9 | 89.2±28.7 | 0.429 |
| 120 min             | 71.6±27.1 | 91.0±29.4 | <0.001** |
| 240 min             | 46.2±21.5 | 53.9±17.9 | 0.348 |
| AUC_{0-240 min}     | 15,243±5,617 | 18,042±4,821 | 0.029* |

| Glucagon (pg/mL)    | LCM | STM | p value (between meals) |
|---------------------|-----|-----|-------------------------|
| 0 min               | 51.5±29.7 | 53.2±20.9 | 0.510 |
| 60 min              | 93.3±31.2 | 38.7±15.3 | <0.001** |
| 120 min             | 119.4±25.4 | 41.9±15.5 | <0.001** |
| 240 min             | 105.0±41.0 | 63.5±34.8 | 0.006** |
| AUC_{0-240 min}     | 23,635±7,033 | 11,506±3,895 | <0.001** |

| TG (mg/dL)          | LCM | STM | p value (between meals) |
|---------------------|-----|-----|-------------------------|
| 0 min               | 70.7±30.0 | 77.0±28.2 | 0.168 |
| 60 min              | 86.3±28.6 | 95.4±42.3 | 0.230 |
| 120 min             | 123.9±37.7 | 95.8±37.0 | 0.019* |
| 240 min             | 126.7±48.5 | 84.6±32.6 | <0.001** |
| AUC_{0-240 min}     | 26,055±7,914 | 21,735±8,044 | 0.016* |

| Non-HDL-C (mg/dL)   | LCM | STM | p value (between meals) |
|---------------------|-----|-----|-------------------------|
| 0 min               | 107.4±20.9 | 108.5±20.8 | 0.386 |
| 60 min              | 107.1±20.2 | 108.0±20.0 | 0.343 |
| 120 min             | 107.1±19.6 | 108.8±20.0 | 0.051 |
| 240 min             | 108.6±20.4 | 107.9±20.0 | 0.266 |
| AUC_{0-240 min}     | 25,798±4,812 | 26,001±4,800 | 0.140 |

| IL-6 (pg/mL)        | LCM | STM | p value (between meals) |
|---------------------|-----|-----|-------------------------|
| 0 min               | 1.8±2.0 | 1.9±2.4 | 0.445 |
| 240 min             | 2.9±2.9 | 4.0±4.9 | 0.477 |

| Hs-CRP (ng/mL)      | LCM | STM | p value (within meal) |
|---------------------|-----|-----|-----------------------|
| 0 min               | 642.0±1,274.9 | 435.6±468.9 | 0.826 |
| 240 min             | 640.9±1,310.6 | 504.9±600.0 | 0.300 |

Values are means±SD.

GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; Hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; LCM, low-carbohydrate meal; STM, standard test meal; TG, triglycerides.

* p<0.05, ** p<0.01 (between meals).
† p<0.05 (within meals).
240 min and AUC<sub>0-240 min</sub> were significantly higher after the LCM than after the STMs (p<0.019, p<0.001, and p=0.016, respectively) (Fig. 3, Table 3).

**Non-HDL-C.** There were no statistical differences when we compared non-HDL-C levels between meals and within meals (Table 3).

**Postprandial IL-6 and Hs-CRP**

IL-6. The IL-6 level was significantly higher at 240 min after the STMs than at fasting (p=0.041), but not after the LCM (Table 3).

Hs-CRP. There were no statistical differences when we compared the Hs-CRP levels between meals and within meals (Table 3).

**DISCUSSION**

Consuming an evening-only LCM at 1800 h was advantageous in that postprandial glucose, insulin, and active GIP levels were suppressed, and the LCM promoted active GLP-1; however, TG levels increased. The 60-min postprandial levels and AUC<sub>0-120 min</sub> for plasma glucose, and the 60-min postprandial and 120-min postprandial levels and AUC<sub>0-240 min</sub> for plasma insulin were significantly lower after the LCM than after the STMs. Therefore, consuming an evening-only LCM at 1800 h suppressed postprandial hyperglycemia and insulin secretion. This effect is considered to be due to the low carbohydrates in LCM.

However, the 240-min postprandial plasma glucose was significantly higher after the LCM than after the STMs consumed at 1800 h. This effect is considered to be due to the promotion of glucagon secretion that acts to raise the blood glucose level. A previous study reported that consuming three LCD meals a day increased the postprandial blood glucose level the following day (14), and we consider that consuming an evening-only LCM at 1800 h had a similar effect.

The AUC<sub>0-240 min</sub> for GLP-1 was significantly higher after consuming the LCM than after consuming STMs at 1800 h, but the GIP levels were significantly lower. GLP-1 has been reported to exert antiatherogenic effects through various mechanisms (15), in contrast the GIP exacerbates fatty liver disease (16) and obesity (17). Thus, as the GLP-1 and GIP results indicate, consuming an evening-only LCM at 1800 h may have a good effect in relation to these factors.

According to the study by Yamane et al. (18), GIP was higher in a standard diet containing fat than in a test diet containing only carbohydrates. According to the study by Shibue et al. (19), GIP is reported that the fat content was higher than that of carbohydrate. In contrast, our study showed that GIP was significantly lower after the LCM than after the STM. Based on the combination of these results (18, 19) with the results of our study, we believe that GIP is affected not only by the amount of fat but also by the amounts of carbohydrate and protein. Therefore, it is thought that different results will be obtained depending on the ratio and intake of these nutrients.

The postprandial levels of TG at 120 and 240 min and the AUC<sub>0-240 min</sub> were significantly higher after the LCM than after the STMs. Our results concur with LCD effects after breakfast and LCD effects after three meals (breakfast, lunch, dinner) (20). Thus, our study revealed that consuming an evening-only LCM at 1800 h produced high post-prandial TG levels in line with the effects of an LCD after breakfast, and the effects of an LCD after three meals (breakfast, lunch, dinner). Also, at 240 min after meals, the TG values were 126.7 mg/dL in the LCM and 84.6 mg/dL in the STMs. A total of 115 mg/dL or more of non-fasting TG has been shown to increase...
the risk of developing coronary artery disease in Japan (21). Thus, in our study, consuming an evening-only LCM at 1800 h may increase the risk of arteriosclerosis due to increased TG levels. However, in previous studies (21), the participants measured non-fasting TG in varying periods, such as <2 h, 2 h, 3 to 7 h, or ≥8 h after meals, and the method of postprandial blood sampling differed from that used in the present study. In addition, in Japan reference values for non-fasting TG have not been reported, and in Europe the optimal diagnostic threshold for cardiovascular disease onset is 175 mg/dL in a non-fasting state (22, 23). Therefore, from the TG results in our study, we were not able to conclude that consuming an evening-only LCM at 1800 h raised the risk of developing coronary artery disease.

The IL-6 levels were significantly higher at 240 min after the STMs than after fasting, but not after the LCM. This result differed from a previous study (24) that showed that an LCD in healthy volunteers elevated IL-6 after meals. There may be two reasons for this. Firstly, in our study, the fat-to-energy ratio was 58.4%, whereas it was higher (76.7%) in the previous study (24). Secondly, in our study, participants consumed an evening-only LCM at 1800 h, whereas participants consumed an LCD after breakfast in the previous study (25).

Since LCM is high fat diet, it may be necessary to reduce the number of saturated fatty acids and increase the proportion of polyunsaturated fatty acids in order to apply it to dietary therapy for diabetics.

There are several limitations in this study. First, during the research phase, because we were unable to ensure 24-h monitoring, there was a possibility that participants did not proceed as instructed. However, we clearly set out the importance of adhering to a balanced lifestyle, we contacted the participants by email before the meal start time at home, and we reminded the participants to adhere to the instructions that had been highlighted in the email to prompt participants to keep to those instructions. Second, we did not consider the quality of the carbohydrates, proteins, and fats. For example, our results may have been influenced by the amount of saturated fatty acids in the meals. Third, as we investigated the effects over a relatively short timeframe in this study, the long-term effects remain unknown. Fourth, our study population comprised healthy males aged between 20 and 29 y, and our results may not be applicable to other population age groups.

To our knowledge, this is the first study to examine the effects of consuming an evening-only LCM at 1800 h. Further studies are needed to better determine the effects of consuming an evening-only LCM at 1800 h in relation to diabetes, dyslipidemia, in participants ≥30 y old, and with a longer follow-up time.

In conclusion, consuming an evening-only LCM at 1800 h in healthy volunteers suppressed postprandial hyperglycemia and insulin secretion; however, postprandial TG increased. Consuming an evening-only LCM at 1800 h was also beneficial in inhibiting the elevation of blood glucose; however, it may increase the risk of arteriosclerosis through increasing TG levels.

**Authorship**

Research conception and design: AY and JS; experiments: AY and JS; statistical analysis of the data: AY and JS; interpretation of the data: AY and JS; writing of the manuscript: AY.

**Disclosure of state of COI**

No conflicts of interest to be declared.

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