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# Effects of Ingesting Amino Acids Simultaneously with Glucose and Fat on Postchallenge Metabolism in Healthy Young Women

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**Abstract:** Aim: To investigate the effects of ingesting amino acids simultaneously with glucose and fat on postchallenge glycemia and lipidemia. Methods: The subjects were eleven healthy young Japanese women (age 21.1 ± 0.1 y). In each session, the subjects ingested one of three beverages in a randomized crossover design. Materials used were an amino acid mixture (Amizet B™, 0.6 g/kg of body weight, 60 mg/kg as amino acids), glucose (1 g/kg of body weight), and fat cream (OFTT™ cream, 1 g/kg of body weight, 0.35 g/kg as fat). The three beverages were as follows: an amino acid mixture (A trial), glucose and fat cream (GF trial), or a mixture of glucose, fat cream, and amino acids (GFA trial). Venous blood samples were obtained before (0) and 0.5, 1, 2, and 3 h after ingestion in the A trial and 0, 0.5, 1, 2, 4, and 6 h after ingestion in the GF and GFA trials. Results: The ingestion of amino acids alone (without glucose) slightly stimulated the secretion of insulin, and both insulin and glucagon levels significantly increased without changes in the glucose level. Co-ingestion of fat with glucose reduced the glucose rise, and the addition of amino acids suppressed the reduction in the blood glucose rise. There were no significant effects of the ingestion of amino acids simultaneously with glucose and fat on the lipid or lipoprotein metabolism as compared to the ingestion of glucose and fat. Conclusion: Co-ingestion of amino acids with glucose and fat increased the glucose level compared to the ingestion of glucose and fat, probably by suppressing insulinotropic and glucagon-inhibitory effects. We conclude that the ingestion of amino acids simultaneously with glucose and fat inhibits postchallenge glucose metabolism but does not significantly influence lipid and lipoprotein metabolism as compared to the ingestion of glucose and fat.

**Keywords:** Amino Acids, Postchallenge Glycemia, Postchallenge Lipidemia, Young Women, Insulin, Glucagon, GIP, GLP-1

## 1. Introduction

We previously showed that, even in healthy young women, simultaneous ingestion of fructose and fat markedly exacerbates and delays postprandial lipid metabolism [1], and postprandial hyperglycemia and early morning hypoglycemia are often observed [2]. However, the effects of proteins or amino acids ingested with carbohydrates and fat in a normal diet are not well known. Although there have been many studies on diabetic patients, there are few studies on the effects of amino acids, components of proteins, on postprandial metabolism in healthy subjects. Therefore, we also studied the effects of the co-ingestion of amino acids and fat (but not glucose) on postchallenge lipidemia and glycemia in young women [3]. In the present study, we evaluated the effects of ingesting amino acids simultaneously with glucose and fat on postchallenge glycemia and lipidemia in healthy young women.

## 2. Subjects and Methods

### *2.1. Subjects*

Eleven healthy young Japanese women (age 21.1 ± 0.1 y) with a normal ovarian cycle were examined. They were non-smokers, had no apparent acute or chronic illness, and

were not taking any medications or dietary supplements. This study was approved by the Institutional Review Board of Sugiyama Jogakuen University School of Life Studies (No. 2019-23), and each subject provided written informed consent for participation. The procedures were conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983.

### *2.2. Anthropometric and Body Composition Measurements*

Body height was measured using a standard method. Waist circumference was assessed as the abdominal girth at the level of the umbilicus, and hip circumference was measured at the level of the greater trochanters. The waist-to-hip (W/H) ratio was calculated. Body weight and composition, including the visceral fat area (VFA), were analyzed using an 8-polar bioelectrical impedance method (InBody 720, Biospace, Tokyo, Japan).

### *2.3. Test Beverages*

Each subject underwent three test trials, and they ingested one of three beverages: (1) for the amino acid (A) trial, 0.6 g/kg of body weight of the amino acid mixture (Amizet B, Terumo, Tokyo, containing 60 mg/kg as amino acids); (2) for the glucose and fat (GF) trial, 1 g/kg of body weight of glucose (Marugo, Saitama, Japan) + 1 g/kg of body weight of fat cream (OFTT cream, Jomo, Takasaki, Japan, containing 0.35 g/kg as fat); or (3) for the glucose, fat, and amino acid (GFA) trial, glucose + fat cream + amino acids. We added 20 mg of sucralose (San-Ei Gen FFI, Osaka, Japan) to the beverage for the A trial to improve the taste. Water was added to each beverage, for a total volume of 250 mL. The OFTT cream and Amizet B formulas are shown in Tables 1 and 2, respectively.

### *2.4. Experimental Design*

The subjects abstained from consuming caffeine or alcohol on the day before the experiment. Each subject was studied on three occasions in a randomized single-blinded crossover design, and each ingested a beverage after a 12 h overnight fast.

Venous blood samples were collected while the subject was in a supine position, before (0) and 0.5, 1, 2, and 3 h after ingesting the beverage in the A trial and at 0, 0.5, 1, 2, 4, and 6 h after ingestion in the GF and GFA trials. During the test period, the subjects avoided exercise and eating but had free access to water from 1 h after ingestion. There was a 4-week interval between the trials.

***Table 1.*** *Formula of OFTT cream.*

| | |
|---|---|
| Energy value (kcal/100g) | 342 |
| Composition (%) | |
| Butter fat | 35.0 |
| Casein Na | 1.5 |
| Sucrose esters of fatty acids | 0.5 |
| Lecithin | 0.4 |
| Glycerin fatty acid ester | 0.25 |
| Stevia sweetener | 0.02 |
| Moisture | 62.4 |
| Proportion in the fat (%) | |
| Saturated fatty acids | 64.3 |
| Monounsaturated fatty acids | 32.2 |
| Polyunsaturated fatty acids | 3.5 |

***Table 2.*** *Formula of Amizet B.*

| | | |
|---|---|---|
| Ingredients (mg/dL) | L-Isoleucine | 850 |
| | L-Leucine | 1350 |
| | Lysine malate (L-Lysine) | 1216 (800) |
| | L-Methionine | 390 |
| | L-Phenylalanine | 770 |
| | L-Threonine | 480 |
| | L-Tryptophan | 160 |
| | L-Valine | 900 |
| | Cysteine malate (L-Cysteine) | 155 (100) |
| | L-Tyrosine | 50 |
| | L-Arginine | 1110 |
| | L-Histidine | 470 |
| | L-Alanine | 860 |
| | L-Aspartic acid | 50 |
| | L-Glutamic acid | 50 |
| | Glycine | 550 |
| | L-Proline | 640 |
| | L-Serine | 420 |
| Additive | Succinic acid | Appropiate amount |
| Composition | Total amino acids (mg/dL) | 10,000 |
| | Branched chain amino acids (%) | 31 |
| | Total nitrogen (mg/dL) | 1,560 |
| | Essential amino acids / non-essential amino acids | 1.33 |

### *2.5. Biochemical Analysis*

The serum or plasma samples were immediately refrigerated (4°C) or frozen (−20°C or −80°C) until analysis. The serum glucose level was measured using a mutarotase–glucose oxidase method (Wako, Osaka). The serum insulin was measured using a chemiluminescent enzyme immunoassay (CLEIA) (Fujirebio, Tokyo). The plasma glucagon level was measured using an enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden). The insulin/glucagon (Ins/Glg) ratio was calculated. The plasma glucose-dependent insulinotropic polypeptide (GIP) (active form) and glucagon-like peptide-1 (GLP-1) (active form) levels were measured using an ELISA (Immuno-Biological Laboratories, Gunma, Japan). The hemoglobin A1c (HbA1c) level was measured using an enzymatic method (Minaris Medical, Tokyo). The serum C-peptide immunoreactivity (CPR) level was measured using a CLEIA (Fujirebio). The level of free fatty acids (FFA) was measured using an acyl-CoA synthetase–acyl-CoA oxidase method (Wako). The total cholesterol (TC) level was measured using a cholesterol oxidase–peroxidase method (Sekisui Medical, Tokyo). The level of high-density lipoprotein-cholesterol (HDL-C) and that of low-density lipoprotein-cholesterol (LDL-C) were measured using a direct method (Sekisui Medical). The small, dense LDL (sdLDL) level was measured using a direct method (Denka, Tokyo). The TG level was enzymatically measured (Sekisui Medical). The level of remnant lipoprotein-cholesterol (RLP-C) was measured using an enzymatic method (Minaris Medical). The serum lipoprotein(a) [Lp(a)] level was

measured using a latex agglutination method (Sekisui Medical). Apolipoprotein (Apo) levels A-I, A-II, B, C-II, C-III, and E were measured using an immunoturbidimetric method (Sekisui Medical). The ApoB48 values were determined by a CLEIA (Fujirebio). The ApoB100 level was calculated by subtracting the value of ApoB48 from the value of ApoB. The total ketone bodies (TKB), β-hydroxybutyrate (β-HB), and acetoacetate (AA) were measured using an enzymatic method (Kainos Laboratories, Tokyo). The apoE phenotype was examined using the isoelectric electrophoresis method (Joko, Tokyo).

### *2.6. Quantification of the Postchallenge Metabolism*

The postchallenge changes in the concentrations of glucose, insulin, glucagon, GIP, GLP-1, TG, RLP-C, ApoB48, FFA, and TKB were calculated as the difference from the baseline mean value (as 0 at 0 h) and are shown as *Δ*glucose, *Δ*insulin, *Δ*glucagon, *Δ*GIP, *Δ*GLP-1, *Δ*TG, *Δ*RLP-C, *Δ*ApoB48, *Δ*FFA, and *Δ*TKB, respectively. We quantified the postchallenge metabolism by calculating the incremental area under the curve (*Δ*AUC), which was defined as the area determined by the trapezoidal method minus the area below the baseline (0 h) from 0 to 2 h (glucose, insulin, glucagon, GIP, and GLP-1) or from 0 to 6 h (TG, RLP-C, ApoB48, FFA, and TKB).

### *2.7. Statistical Analysis*

All data are expressed as the mean ± standard error of the mean (SEM). The statistical analyses were performed using SPSS ver. 28 (IBM, Tokyo). Normal distributions of data were verified using the Shapiro–Wilk test. Non-normally distributed data were examined for normal distribution when logarithmically transformed and were analyzed using parametric statistics. The differences in the measured values at each time point in the three trials were assessed using a repeated measures analysis of variance followed by the Bonferroni test. For all data, $p<0.05$ was considered significant.

## 3. Results

All subjects completed the trials and ingested the test beverages without problems. The physical characteristics and fasting blood chemical data of the subjects are summarized in Table 3. There were no significant differences in any of these data among the three trials (data not shown). The fasting and postchallenge chemical data in the three trials are presented in Table 4 (glucose, insulin, glucagon, Ins/Glg ratio, GIP, and GLP-1) and Table 5 (TG, RLP-C, ApoB48, FFA, TKB, AA, and β-HB). The time courses of *Δ*glucose, *Δ*insulin, *Δ*glucagon, Ins/Glg ratio, *Δ*GIP, and *Δ*GLP-1 are shown in Figure 1, and those of *Δ*TG, *Δ*RLP-C, *Δ*ApoB48, *Δ*FFA, and *Δ*TKB are shown in Figure 2. The *Δ*AUC of glucose, insulin, glucagon, GIP, GLP-1, TG, RLP-C, ApoB48, FFA, and TKB are also shown in the figure insets.

***Table 3.*** *Physical characteristics and fasting blood chemical data of the subjects.*

| | |
|---|---|
| Height (cm) | 157.8 ± 1.2 |
| Weight (kg) | 50.0 ± 1.2 |
| BMI (kg/m$^2$) | 20.1 ± 0.4 |
| %Body fat (%) | 24.9 ± 1.3 |
| Waist (cm) | 69.3 ± 1.5 |
| Hip (cm) | 91.5 ± 1.0 |
| W/H | 0.76 ± 0.01 |
| VFA (cm$^2$) | 22.8 ± 2.7 |
| SBP (mmHg) | 104.3 ± 2.9 |
| DBP (mmHg) | 64.5 ± 1.6 |
| PR (beats/min) | 71.7 ± 2.6 |
| CPR (ng/mL) | 1.3 ± 0.1 |
| HOMA-IR | 1.3 ± 0.1 |
| HbA1c (%) | 5.2 ± 0.1 |
| TC (mg/dL) | 171.7 ± 5.9 |
| HDL-C (mg/dL) | 68.5 ± 3.2 |
| LDL-C (mg/dL) | 92.5 ± 6.2 |
| sdLDL (mg/dL) | 17.1 ± 1.2 |
| Lp (a) (mg/dL) | 17.7 ± 5.5 |
| ApoA-I (mg/dL) | 155.7 ± 7.0 |
| ApoA-II (mg/dL) | 26.4 ± 1.7 |
| ApoB100 (mg/dL) | 61.8 ± 3.5 |
| ApoC-II (mg/dL) | 2.2 ± 0.2 |
| ApoC-III (mg/dL) | 7.1 ± 0.5 |
| ApoE (mg/dL) | 3.4 ± 0.3 |

Values are the mean ± SEM. SBP: systolic blood pressure, DBP: diastolic blood pressure, PR: pulse rate.

### *3.1. Glucose, Insulin, Glucagon, and Ins/Glg Ratio*

The serum glucose concentration peaked at 0.5 h in the GF trial and at 1 h in the GFA trial (Table 4, Figure 1A). In the A trial, it did not rise significantly. In the GFA trial, the peak was higher than in the GF trial, but not significantly. The *Δ*AUC (0–2 h) for glucose tended to be greater in the GFA trial than in the GF trial, but not significantly.

The serum insulin concentration in the A trial was slightly but significantly increased at 0.5 h (Table 4, Figure 1B). It peaked at 0.5 h in the GF trial and at 1 h in the GFA trial. In the GF and GFA trials, the *Δ*AUC (0–2 h) for insulin was significantly greater than that in the A trial.

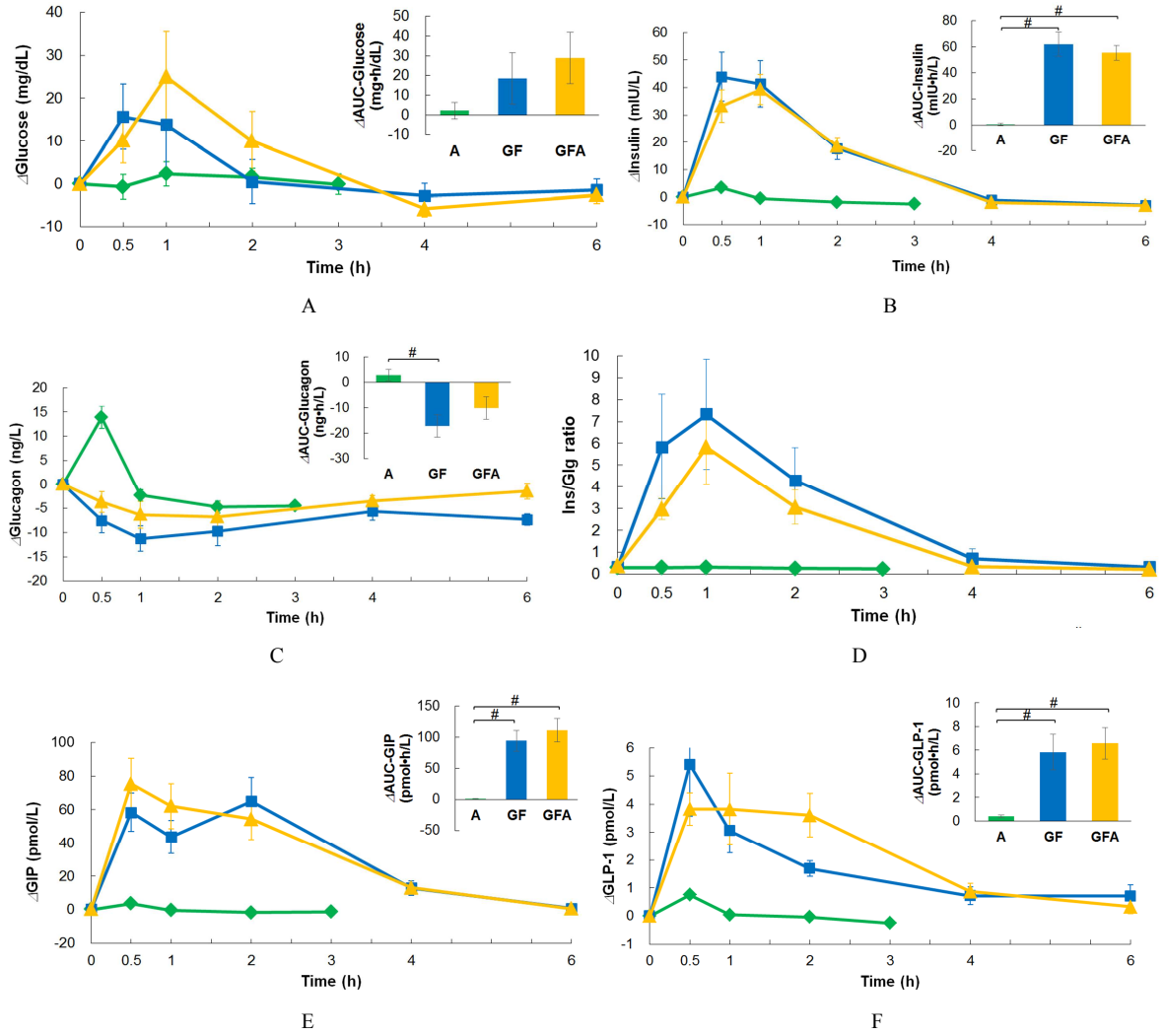
The plasma glucagon concentration in the A trial was significantly increased at 0.5 h, returned to baseline at 1 h, and then decreased to below baseline at 2 and 3 h (Table 4, Figure 1C). In the GF trial, it decreased at 0.5 h, was lowest at 1 h, and did not return to baseline until the end of the experiment (6 h). In the GFA trial, it slightly but not significantly decreased compared to the baseline. In the A trial, the *Δ*AUC (0–2 h) for glucagon was significantly greater than that in the GF trial, and in the GFA trial, it was slightly greater than in the GF trial, but not significantly.

The Ins/Glg ratio was significantly increased at 1 h in the GF and GFA trials, and in the GF trial, it tended to be higher than in the GFA trial during the experiment, but not significantly (Table 4, Figure 1D). In the A trial, it did not change significantly.

***Table 4.*** *Fasting and postchallenge concentrations of glucose, insulin, glucagon, the ratio of Ins/Glg, GIP, and GLP-1.*

| | | 0 | 0.5 | | 1 | | 2 | | 3 | 4 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Glucose (mg/dL) | A | 87.7 ± 1.6 | 87.0 ± 1.6 | ]# | 90.0 ± 1.6 | | 89.3 ± 1.2 | | 87.6 ± 1.2 | | |
| | GF | 90.2 ± 2.3 | 105.9 ± 7.0* | | 104.0 ± 10.8 | | 90.7 ± 5.7 | | | 87.5 ± 1.5 | 88.8 ± 2.8 |
| | GFA | 88.2 ± 0.9 | 99.5 ± 5.4 | | 114.2 ± 10.7* | | 99.1 ± 6.9 | | | 83.4 ± 1.9* | 86.6 ± 2.1 |
| Insulin (mIU/L) | A | 6.0 ± 0.4 | 9.4 ± 0.8* | ]# | 5.4 ± 0.4 | ]# | 4.2 ± 0.5* | ]# | 3.5 ± 0.5* | | |
| | GF | 6.2 ± 0.6 | 50.1 ± 8.9* | ]# | 47.6 ± 8.6* | ]# | 24.0 ± 4.2 | ]# | | 5.1 ± 1.3 | 3.3 ± 0.3 |
| | GFA | 6.1 ± 0.6 | 39.3 ± 5.6* | | 45.3 ± 5.6* | | 24.7 ± 3.2* | | | 4.0 ± 0.4 | 3.0 ± 0.3 |
| Glucagon (ng/L) | A | 20.3 ± 1.7 | 34.2 ± 3.3* | ]# | 18.0 ± 1.8 | ]# | 15.5 ± 1.7* | | 15.8 ± 1.4* | | |
| | GF | 20.7 ± 3.0 | 13.1 ± 2.1* | ]# | 9.4 ± 1.5* | | 10.9 ± 2.7* | | | 15.0 ± 3.0* | 13.3 ± 2.5* |
| | GFA | 18.5 ± 2.6 | 14.8 ± 1.5 | | 12.2 ± 1.9 | | 11.7 ± 2.0 | | | 15.0 ± 2.8 | 17.1 ± 3.5 |
| Ins/Glg ratio | A | 0.31 ± 0.03 | 0.29 ± 0.03 | ]# | 0.32 ± 0.03 | ]# | 0.27 ± 0.02 | ]# | 0.24 ± 0.03 | | |
| | GF | 0.35 ± 0.05 | 5.84 ± 2.39 | | 7.32 ± 2.52* | | 4.30 ± 1.50 | | | 0.72 ± 0.42 | 0.32 ± 0.06 |
| | GFA | 0.37 ± 0.05 | 2.99 ± 0.48 | | 5.83 ± 1.70* | | 3.07 ± 0.78* | | | 0.35 ± 0.08 | 0.23 ± 0.03 |
| GIP (pmol/L) | A | 4.5 ± 0.6 | 8.1 ± 0.7* | ]# | 4.0 ± 0.6 | ]# | 2.9 ± 0.5* | ]# | 3.0 ± 0.5* | | |
| | GF | 4.6 ± 0.7 | 63.0 ± 11.9* | ]# | 48.3 ± 10.0* | ]# | 69.4 ± 14.8* | ]# | | 17.5 ± 4.2* | 5.3 ± 1.2 |
| | GFA | 4.0 ± 0.7 | 79.4 ± 15.2* | | 66.1 ± 13.2* | | 58.4 ± 12.3* | | | 17.1 ± 3.0* | 4.6 ± 0.6 |
| GLP-1 (pmol/L) | A | 1.4 ± 0.2 | 2.1 ± 0.2* | ]# | 1.4 ± 0.2 | ]# | 1.3 ± 0.2 | ]# | 1.1 ± 0.2 | | |
| | GF | 1.3 ± 0.2 | 6.7 ± 1.9* | | 4.4 ± 0.8* | | 3.0 ± 0.2 | | | 2.0 ± 0.3 | 2.0 ± 0.4 |
| | GFA | 1.3 ± 0.1 | 5.1 ± 0.7* | | 5.1 ± 1.3* | | 4.9 ± 0.8* | | | 2.1 ± 0.3 | 1.6 ± 0.2 |

Values are the mean ± SEM. *$p$<0.05 vs. the fasting value. #$p$<0.05 between the trials.




The ΔAUC (0–2 h) for each parameter is shown in the inset. A trial (◆), GF trial (■), GFA trial (▲). Values are the mean ± SEM. #$p$<0.05 between the trials.

***Figure 1.*** *Postchallenge changes for Δglucose (A), Δinsulin (B), Δglucagon (C), the Ins/Glg ratio (D), ΔGIP (E), and ΔGLP-1 (F).*

### *3.2. GIP and GLP-1*

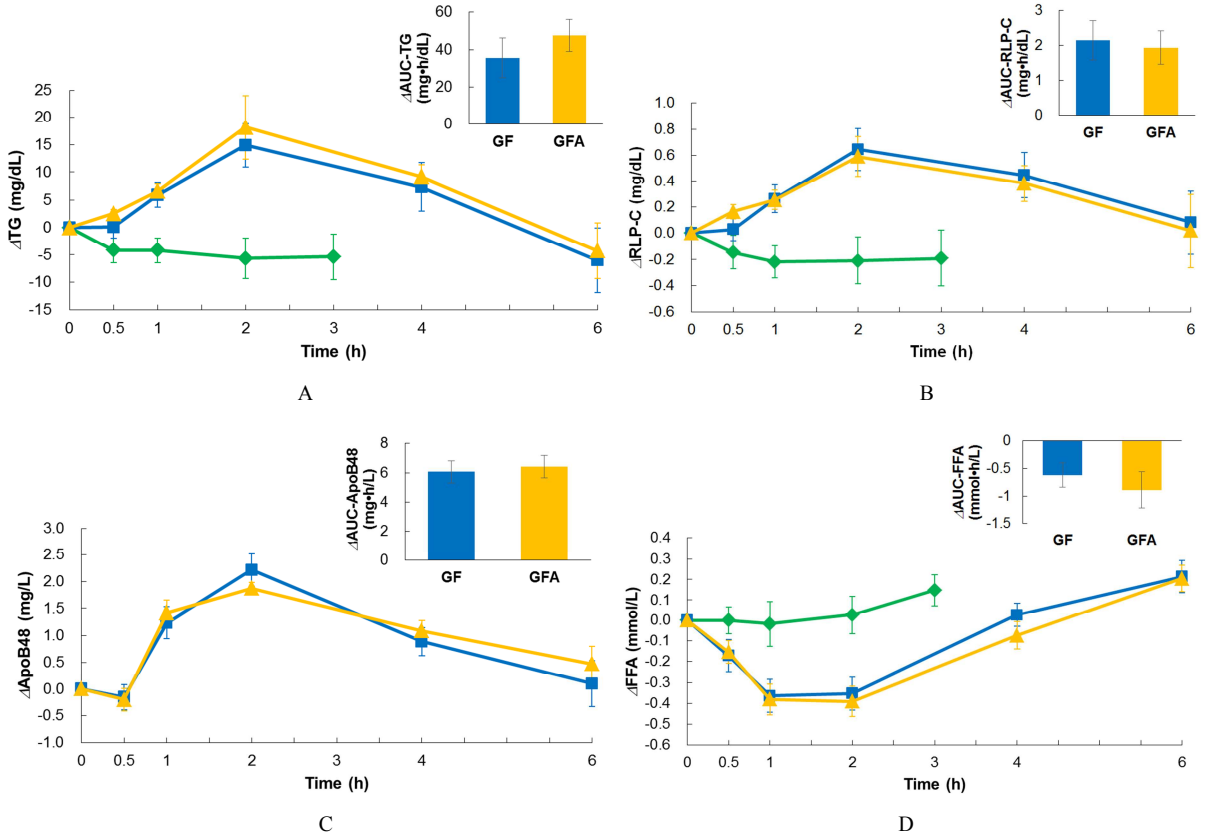
In the A trial, the plasma GIP concentration was increased slightly but significantly at 0.5 h. In the GFA trial, it was increased and peaked at 0.5 h (Table 4, Figure 1E). In the GF trial, the peaks at 0.5 h and 2 h looked diphasic. At 0.5–2 h in the GF and GFA trials, it was significantly higher than in the A trial. In the GF and GFA trials, the *Δ*AUC (0–2 h) for GIP was significantly greater than in the A trial.

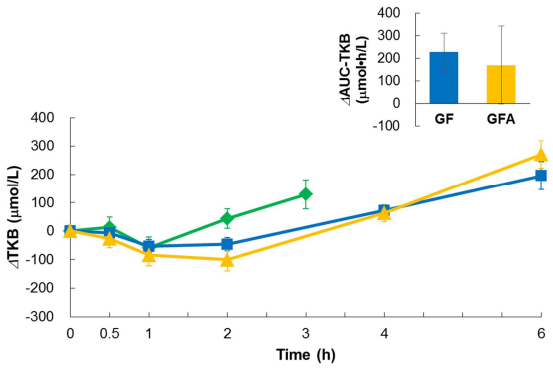
The plasma GLP-1 concentration was slightly but significantly increased at 0.5 h in the A trial (Table 4, Figure 1F). In the GF and GFA trials, it was significantly increased at 0.5–2 h and was significantly higher than in the A trial. In the GF and GFA trials, the *Δ*AUC (0–2 h) for GLP-1 was significantly greater than in the A trial.

***Table 5.*** *Fasting and postchallenge concentrations of TG, RLP-C, ApoB48, FFA, TKB, AA, and β-HB.*

| | | 0 | 0.5 | 1 | 2 | 3 | 4 | 6 |
|---|---|---|---|---|---|---|---|---|
| TG (mg/dL) | A | 69.8 ± 12.2 | 65.6 ± 10.2 | 65.6 ± 10.4 | 64.2 ± 9.3 | 64.5 ± 8.9 | | |
| | GF | 67.0 ± 8.7 | 67.1 ± 7.7 | 72.9 ± 9.7* | 81.9 ± 11.3* | | 74.4 ± 8.2 | 61.0 ± 5.8 |
| | GFA | 66.0 ± 11.6 | 68.6 ± 12.2* | 72.6 ± 11.8* | 84.2 ± 16.9* | | 75.2 ± 10.8* | 61.7 ± 7.7 |
| RLP-C (mg/dL) | A | 3.3 ± 0.5 | 3.1 ± 0.4 | 3.1 ± 0.3 | 3.1 ± 0.3 | 3.1 ± 0.3 | | |
| | GF | 3.2 ± 0.3 | 3.2 ± 0.3 | 3.5 ± 0.4* | 3.8 ± 0.4* | | 3.6 ± 0.3* | 3.3 ± 0.2 |
| | GFA | 3.3 ± 0.5 | 3.5 ± 0.5* | 3.6 ± 0.5* | 3.9 ± 0.6* | | 3.7 ± 0.4* | 3.3 ± 0.3 |
| ApoB48 (mg/dL) | GF | 2.8 ± 0.3 | 2.6 ± 0.3 | 4.0 ± 0.4* | 5.0 ± 0.5* | | 3.6 ± 0.3 | 2.9 ± 0.3 |
| | GFA | 2.2 ± 0.3 | 2.0 ± 0.2 | 3.7 ± 0.3* | 4.1 ± 0.3* | | 3.3 ± 0.2* | 2.7 ± 0.1 |
| FFA (mmol/L) | A | 0.42 ± 0.07 | 0.42 ± 0.05 | 0.40 ± 0.09 | 0.45 ± 0.07 | 0.57 ± 0.07 | | |
| | GF | 0.49 ± 0.08 | 0.32 ± 0.02 | 0.13 ± 0.02* | 0.14 ± 0.01* | | 0.52 ± 0.11 | 0.71 ± 0.13 |
| | GFA | 0.55 ± 0.07 | 0.39 ± 0.04 | 0.17 ± 0.02* | 0.16 ± 0.02* | | 0.48 ± 0.05 | 0.75 ± 0.08* |
| TKB (μmol/L) | A | 126.6 ± 43.4 | 139.4 ± 36.1 | 67.8 ± 9.2 | 170.0 ± 32.1 | 255.5 ± 52.8* | | |
| | GF | 90.8 ± 24.5 | 83.7 ± 11.4 | 37.5 ± 2.6 | 44.6 ± 6.3 | | 163.6 ± 35.1 | 286.6 ± 58.3* |
| | GFA | 138.7 ± 39.1 | 111.9 ± 16.5 | 54.4 ± 6.8 | 38.6 ± 3.9* | | 201.6 ± 32.3 | 410.2 ± 53.5* |
| AA (μmol/L) | A | 35.8 ± 10.3 | 44.3 ± 11.1 | 22.6 ± 3.2 | 46.6 ± 8.4 | 59.4 ± 12.0* | | |
| | GF | 24.9 ± 5.2 | 25.1 ± 2.9 | 14.4 ± 1.1 | 19.6 ± 2.9 | | 40.6 ± 7.2 | 59.8 ± 10.2* |
| | GFA | 38.1 ± 9.5 | 33.9 ± 5.9 | 23.4 ± 3.5 | 18.6 ± 1.8 | | 54.1 ± 8.1 | 87.4 ± 12.2* |
| β-HB (μmol/L) | A | 90.8 ± 33.3 | 95.1 ± 25.2 | 45.3 ± 6.4 | 123.5 ± 24.5 | 196.1 ± 41.0* | | |
| | GF | 65.9 ± 19.5 | 58.6 ± 8.7 | 23.1 ± 2.1 | 25.0 ± 3.7 | | 123.0 ± 28.1 | 226.8 ± 48.4* |
| | GFA | 100.6 ± 29.8 | 78.0 ± 11.0 | 31.0 ± 3.5 | 20.0 ± 2.8* | | 147.6 ± 25.8 | 322.8 ± 42.1* |

Values are the mean ± SEM. *$p$<0.05 vs. the fasting value. #$p$<0.05 between the trials.



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The *Δ*AUC (0–6 h) for each parameter is shown in the inset. A trial (◆), GF trial (■), GFA trial (▲). Values are the mean ± SEM.

***Figure 2.*** *Postchallenge changes for ΔTG (A), ΔRLP-C (B), ΔApoB48 (C), ΔFFA (D), and ΔTKB (E).*

### *3.3. TG, RLP-C, ApoB48, and FFA*

In the GF trial, the serum TG concentration significantly increased at 1 and 2 h, and the RLP-C increased at 1–4 h (Table 5, Figure 2A, B). In the GFA trial, both the TG and RLP-C concentrations were significantly increased at 0.5–4 h. There were no differences in the *Δ*AUC (0–6 h) for TG or the *Δ*AUC (0–6 h) for RLP-C between the two trials. The serum ApoB48 concentration was significantly increased at 1 and 2 h in the GF trial and at 1–4 h in the GFA trials (Table 5, Figure 2C). There were no significant differences in the *Δ*AUC (0–6 h) for ApoB48 between the two trials. There were no increases in the TG or RLP-C concentrations in the A trial. The serum FFA concentration was significantly decreased at 1 and 2 h and gradually increased toward the end of experiment in the GF and GFA trials (Table 5, Figure 2D). There was no significant change in the A trial until 3 h.

### *3.4. TKB*

The serum TKB concentration was slightly but not significantly decreased at 1 h and increased at 3 h in the A trial (Table 5, Figure 2E). In the GF and GFA trials, it was decreased at 0.5–2 h and increased thereafter. It was significantly higher at 6 h in the GFA trial compared to the GF trial. The β-HB and AA concentrations showed patterns similar to that of TKB.

## 4. Discussion

The major findings of this study are as follows. Ingestion of amino acids only (without glucose and fat) stimulated the secretion of insulin. There were also significant increases in insulin and glucagon levels without significant changes in the serum glucose level, probably due to the stimulation of insulin secretion mediated by incretins (GIP and GLP-1) and the direct effect of amino acids on pancreatic β cells. The absence of serum glucose fluctuations was considered to be the result of simultaneous coordinated secretion of insulin and glucagon. Additional ingestion of amino acids caused a higher and delayed peak in the glucose level compared to glucose and fat ingestion. In our previous study, simultaneous ingestion of fat with glucose reduced blood glucose elevation. However, the addition of amino acids may inhibit the reduction in blood glucose elevation by fat ingestion. No significant differences were found for postchallenge lipid and lipoprotein metabolism when amino acids were added to glucose and fat.

It has long been thought that insulin secretion is stimulated but glucagon secretion is suppressed postprandially, but now it is shown that both insulin and glucagon are secreted postprandially [4]. The insulin secretion in the A trial with no ingestion of glucose suggests the involvement of incretins. Incretin levels increased significantly at 0.5 h after ingestion of the beverage, and several amino acids (leucine [5], isoleucine [6], lysine, methionine, tyrosine [7], glutamine [8], arginine, and alanine [9]) in the amino acid mixture used in this study have been reported to stimulate incretin secretion. Incretins have an insulinotropic effect, so we assumed the insulin level would increase after the incretin increase; but in fact, both increased simultaneously at 0.5 h. In this study, blood levels were measured only from 0.5 h, and thus the possibility that insulin increased after the increase in incretins cannot be excluded. In addition to incretin-mediated effects, another possible pathway for insulin secretion is through direct stimulation of pancreatic β cells by amino acids. In fact, amino acids, such as phenylalanine [10], have been reported to stimulate insulin secretion by directly stimulating pancreatic β cells, as discussed in our previous study.

In the GFA trial, the glucose peak was higher and delayed compared to the GF trial. In our previous study, with the ingestion of glucose (1 g/kg of body weight) in healthy young women, the glucose peak reached 137.9 ± 9.8 mg/dL 1 h after ingestion [11]. In the present study, the glucose peaks were lower than in the previous study, with a peak of 105.9 ± 7.0 mg/dL at 0.5 h after ingestion in the GF trial and 114.2 ± 0.7 mg/dL at 1 h in the GFA trial. This suggests that the ingestion of fat with glucose inhibits blood glucose elevation and that the addition of amino acids mitigates the inhibition of blood glucose elevation.

The insulin peak was slightly higher and earlier in the GF trial than in the GFA trial. The glucagon level was less suppressed in the GFA trial than in the GF trial. This may be due to the glucagon-secreting effect of amino acids [12]. The

Ins/Glg ratio tended to be higher in the GF trial than in the GFA trial, confirming that simultaneous ingestion of glucose and fat resulted in more insulin and less glucagon secretion than when amino acids were added. This could also be due to higher insulin secretion, as the *Δ*AUC (0–2 h) for insulin tended to be greater in the GF trial than in the GFA trial (not significant). In the GF trial, the insulinotropic effect of fat [13] may have resulted in a higher insulin peak and a greater *Δ*AUC. However, in the GFA trial, the addition of amino acids might have weakened the effect of fat, although amino acids themselves slightly but significantly stimulated the insulin secretion, as in the A trial.

Incretins are known to be secreted primarily by nutrient stimuli, and their blood levels increase within minutes of food ingestion [14]. Moreover, incretins are secretagogues of insulin, and GLP-1 is more potent in insulin secretion than GIP [14, 15]. In addition, although GIP inhibits glucagon secretion [15], GLP-1 stimulates it [16]. In the GFA trial, the GIP peak was higher and the GLP-1 peak lower than in the GF trial.

Regarding postprandial lipid and lipoprotein metabolism, no significant differences were found between the GF and GFA trials, suggesting that the ingestion of amino acids simultaneously with glucose and fat did not affect lipid or lipoprotein metabolism significantly. In our previous study [3], the ingestion of amino acids simultaneously with fat resulted in a slightly (but not significantly) higher increase in TG, RLP-C, and ApoB48 compared to the ingestion of fat alone. All these peaks after the ingestion of amino acids with fat (without glucose) were higher compared to those in the present GF and GFA trials. Therefore, the addition of glucose to fat and amino acids may have mitigated postchallenge lipid and lipoprotein metabolism. We also showed that the simultaneous ingestion of glucose and fat resulted in a slightly lower peak of TG and lower elevations of RLP-C compared to the ingestion of fat alone [17].

This study has some limitations. We used an amino acid mixture and did not examine the effect of individual amino acids. However, individual amino acids probably have different effects on carbohydrate and lipid metabolism. Moreover, the effects of proteins may be different from the effects of amino acids. Finally, because the number of subjects was small, and only healthy young Japanese women were studied, the present results may not be applicable generally and should be interpreted with caution.

# 5. Conclusion

The ingestion of amino acids simultaneously with glucose and fat exacerbated the postchallenge glucose level compared to the ingestion of glucose and fat, probably by inhibiting both the insulinotropic effect of fat and the suppression of glucagon secretion. The addition of amino acids had no significant effect on the postchallenge lipid or lipoprotein metabolism compared to the ingestion of glucose and fat. Based on these results, we conclude that the ingestion of amino acids with glucose and fat can influence postchallenge glucose metabolism, but not lipid or lipoprotein metabolism, compared to the ingestion of glucose and fat. The effects observed in this study may provide valid information for the dietary prevention of postprandial dysmetabolism.

# Conflicts of Interest

The authors declare that they have no competing interests.

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