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Original Research

Metabolic Effects of Short-Term High-Fat Intake Vary Depending on Dietary Amino Acid Composition

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A B S T R A C T

Background: It is generally accepted that excessive fat intake has undesirable effects on the energy metabolism of our body. Dietary amino acid composition is also critical to the regulation of lipid metabolism.
Objectives: This study aimed to investigate whether high-fat diets (HFDs) with different amino acid deficiencies lead to different metabolic outcomes.
Methods: Six-wk-old male Wistar rats were fed either a control diet (CN; 3.7 kcal/g, 12% calories from fat) or HFDs (5.1 kcal/g, 60% calories from fat) with 7 different amino acid compositions [control or methionine, arginine, histidine, lysine, threonine, or branched-chain amino acids (BCAAs) deficient], for 7 d. Tissue weights and lipid accumulation in the liver, skeletal muscle, and adipose tissue were measured, and serum biochemical parameters were analyzed.
Results: Although the food intake of the HFD groups was a little less than that of the CN group, the total calorie intakes were comparable among the groups, except for histidine-deficient and BCAA-deficient groups. In rats fed am HFD with a control amino acid composition (HFCN), dramatic increase in triglyceride (TG) accumulation in the liver and serum LDL cholesterol concentration were observed compared with the CN group. However, when the arginine content in the diet was reduced, liver TG accumulation was completely inhibited, with no apparent effects on serum lipoprotein-cholesterol concentrations. Meanwhile, deficiency of the other amino acids, such as threonine, reversed HFD-induced upregulation of serum LDL cholesterol.
Conclusions: It is observed that although the rats ingested an excessive amount of fat, neither ectopic fat accumulation nor dyslipidemia were always induced at least in the short term; hence, the consequent metabolic change was dependent on the dietary amino acid composition. These findings introduce an important perspective regarding HFD regimens in both scientific and clinical contexts.

Keywords: high-fat diet, dietary amino acid composition, fatty liver, dyslipidemia, triglyceride, cholesterol, HDL cholesterol, LDL cholesterol

Introduction

Globally, the prevalence of obesity and related metabolic dysfunctions has been increasing with around 20%–40% of the global population estimated to be affected by metabolic syndrome [1–3]. Metabolic syndrome is a complex pathologic condition that comprises overweight, insulin resistance, hypertension, dyslipidemia, and metabolic abnormalities, including hepatic steatosis and type 2 diabetes mellitus [1,4]. For decades, enormous efforts have been made to understand the pathophysiology of this syndrome to address this global issue, and numerous studies have been reported; however, to the best of our knowledge, no radical methods to treat this epidemic have been found to date.

In most studies, diet-induced obese rodent models, as they share many functional similarities with humans, and high-fat

Abbreviations: BCAA, branched-chain amino acid; BW, body weight; HFD, high-fat diet; NEFA, nonesterified fatty acid; TG, triglyceride.

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diets (HFDs) are often used. When rodents are fed HFD, they gain weight, exhibit increased fat deposition in the liver, muscles, and adipose tissue, show upregulation of circulating LDL cholesterol, and eventually develop cardiovascular disease and diabetes [4–6]. Hence, it is widely accepted that excessive fat intake is one of the major risk factors for the disturbance of systemic lipid metabolism and consequently a series of dietary guidelines have been made following these observations. However, in recent years, the large-scale human cohort studies of several independent research groups have suggested that no apparent detrimental effects of high-fat consumption, such as cardiovascular disease, cancer, obesity, and mortality, can be observed; in contrast, it sometimes seems beneficial [7,8]. These results suggest that the conventional attribution of metabolic health problems solely to fat consumption may be questionable.

We previously reported that the quantity of protein intake or composition of dietary amino acids has a significant impact on systemic lipid metabolism. When young rats were fed a low-protein/amino acid diet, marked neutral lipid accumulation in the liver and skeletal muscle, and increased adiposity were observed [9–11]. Arginine deficiency caused lipid accumulation only in the liver, whereas lysine (Lys) deficiency induced it in skeletal muscle and adipose tissue but not in the liver [10,11]. Interestingly, Arg-deficient diet-induced hepatic steatosis was completely inhibited by the simultaneous shortage of dietary methionine and branched-chain amino acids (BCAAs; leucine, valine, and isoleucine) [12]. Given that the effect of dietary amino acids on tissue lipid concentrations could be observed much faster than that of dietary fats, within a few days and over several weeks, respectively [10,12], we hypothesized that body lipid accumulation was more affected by dietary amino acids than dietary fat, especially in the acute phase of feeding.

Therefore, in this study, we prepared HFDs with different amino acid compositions to feed rats for 1 wk and identified whether the metabolic effects of excessive fat consumption varied depending on the dietary amino acid composition.

Methods

Materials

For the rat experimental diets, vitamin mixture (OYC formula), mineral mixture (OYC formula), cellulose powder, corn starch, dextrin, and lard were purchased from Oriental Yeast; soybean oil and sucrose were purchased from Nacalai Tesque; and lactose was purchased from LOHAStyle The other reagent grade chemicals used in this study are commercially available.

Diets

All experimental diets used in this study were self-made, by mixing all ingredients evenly (Table 1). All diets contained an amino acid mixture as a nitrogen source, the formula of which was determined according to that of bovine casein [10]. The control diet (CN) comprised 15.3% (w/w) amino acids, 64.8% carbohydrates, and 5.0% lipids, with a total energy of 3.7 kcal/g. The HFD with a control amino acid mixture (HFCN) comprised 15.3% (w/w) amino acids, 35.8% carbohydrates, and 34.0% lipids, with a total energy of 5.1 kcal/g. The HFDs that were

TABLE 1
The compositions of experimental diets[1]

	CN	HFCN	HF_ΔMet	HF_ΔArg	HF_ΔHis	HF_ΔLys	HF_ΔThr	HF_ΔBCAA
L-isoleucine	7.1	7.1	7.1	7.1	7.1	7.1	7.1	2.4
L-leucine	13	13	13	13	13	13	13	4.3
L-lysine·HCl	14.1	14.1	14.1	14.1	14.1	4.7	14.1	14.1
DL-methionine	6.4	6.4	2.1	6.4	6.4	6.4	6.4	6.4
L-cystine	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
L-phenylalanine	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
L-tyrosine	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
L-threonine	6.1	6.1	6.1	6.1	6.1	6.1	2.0	6.1
L-tryptophan	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
L-valine	9.2	9.2	9.2	9.2	9.2	9.2	9.2	3.1
L-histidine	4.1	4.1	4.1	4.1	1.4	4.1	4.1	4.1
L-arginine	5.2	5.2	5.2	1.7	5.2	5.2	5.2	5.2
L-alanine	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
L-aspartic acid	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
L-asparagine·H_2O	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
L-glutamic acid	14.6	14.6	14.6	14.6	14.6	14.6	14.6	14.6
Glycine	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
L-proline	15	15	15	15	15	15	15	15
L-serine	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
L-glutamine	14.6	14.6	14.6	14.6	14.6	14.6	14.6	14.6
Cellulose	100	100	100	100	100	100	100	100
Vitamin mix	10	10	10	10	10	10	10	10
Mineral mix	40	40	40	40	40	40	40	40
Soybean oil	50	180	180	180	180	180	180	180
Lard	0	160	160	160	160	160	160	160
Corn starch	428.2	138.2	142.5	141.7	140.9	147.6	142.3	157.7
Dextrin	82.5	82.5	82.5	82.5	82.5	82.5	82.5	82.5
Lactose	69.3	69.3	69.3	69.3	69.3	69.3	69.3	69.3
Sucrose	67.5	67.5	67.5	67.5	67.5	67.5	67.5	67.5
Total	1000	1000	1000	1000	1000	1000	1000	1000

[1] The units are in grams.

deficient in Met, Arg, His, Lys, Thr, or BCAAs were produced based on the HFCN by reducing the corresponding amino acid(s) to one-third. The loss of amino acid(s) was compensated by adding the same amount of starch. As we have previously shown that dietary Met, Arg, Lys, and BCAAs were related to the body lipid accumulation in rats [9–12], we focused on these amino acids in this study. Considering that Met, Lys, and BCAAs are essential amino acids and Arg and Lys are basic amino acids, we chose Thr and His deficiency for comparison as they are essential and basic amino acids, respectively. These diets were referred to as HF_ΔMet, HF_ΔArg, HF_ΔHis, HF_ΔLys, HF_ΔThr, and HF_ΔBCAA, respectively. The proportion of the other components was even among all experimental diets. All diets were prepared just before the beginning of the experiment and stored at −30 °C until use.

Rats

Five-wk-old male Wistar rats were purchased from the Jackson Laboratory. The rats were caged individually and maintained at 24 ± 1 °C with 50%–60% humidity and a 12-h light/dark cycle (8:00–20:00/20:00–8:00). They were allowed free access to food and water throughout the experiment.

On arrival, the rats were reared on a normal feed pellets diet (CE-2; CLEA Japan) for ≥3 d. Then, they were fed the CN diet for the next 4 d as an acclimation period. After that, the rats were divided into 8 experimental groups (5 rats in each), as the average and variance of body weight (BW) was approximately even among all groups (initial BW of each rat: 178–184 g). Each group was given 1 of the 8 experimental diets and kept for another 7 d. Throughout the experimental period, the BW and food intake of all rats was measured at 10:00 every day. Food was refreshed every day. On the morning on the seventh day, the rats were deprived of food for 1 h, anesthetized with isoflurane (DS Pharma Animal Health), and decapitated. Blood samples were collected from the carotid arteries, and the liver, skeletal muscles, and adipose tissues were isolated. The blood samples were kept on ice for 1–2 h to make clots, after which they were centrifuged at 1200 × *g* for 15 min at 4°C to collect sera. The isolated tissues were weighed and immediately frozen in liquid nitrogen. All samples were stored at −80°C until use.

The experiment was conducted twice independently, and reproducibility of the presented results has been confirmed. All rat care and experiments conformed to the Guidelines for Animal Experiments of The University of Tokyo and were approved by the Animal Research Committee of the University of Tokyo.

Measurement of liver and muscle triglyceride

Total lipids in the liver and skeletal muscles were extracted according to Folch method with small modifications [13]. The frozen tissue pieces (weighed in advance) were homogenized in a methanol:chloroform solution (1:2, vol:vol), followed by the addition of 20% volume of 0.8% KCl solution, and centrifugation at 13,000 × *g* for 10 min at 4°C. Subsequently, the organic (chloroform) layer was collected, the solvent was evaporated, and the remaining lipids were reconstituted in isopropanol. The triglyceride (TG) content in the lipid extract was measured using Triglyceride E-test Wako (Fujifilm Wako Pure Chemical).

Serum amino acid analysis

For serum amino acid extraction, 50 μL of serum samples were mixed on ice with 120 μL of methanol containing internal control substances: 25 μM of 2-morpholinoethanesulfonic acid and 100 μM of methionine sulfone. After centrifugation (16,000 × *g*; 10 min; 4°C), 130 μL of supernatant was mixed with 250 μL of ultrapure water and subjected to ultrafiltration using 3-kDa cutoff filters (Amicon Ultra 3K device; Merck), followed by 30 min of evaporation and 6 h of lyophilization.

The lyophilized metabolite samples were reconstituted in 200 μL of ultrapure water, further diluted if necessary, and subjected to LC-MS/MS (LCMS-8030; Shimadzu) analysis using the Method Package for Primary Metabolites ver. 2 (Shimadzu) according to the manufacturer's protocol. The standard solution, consisting of a fixed concentration of amino acid mixture, was simultaneously subjected to analysis, and absolute amounts of amino acids in each specimen were calculated.

Blood parameters

The assays of blood parameters other than amino acids were performed by using the following commercial kits: cholesterol E-test Wako (Fujifilm Wako Pure Chemical); Triglyceride E-test Wako (Fujifilm Wako Pure Chemical); HDL cholesterol E-test Wako (Fujifilm Wako Pure Chemical); Cholestest LDL (SEKISUI Medical); nonesterified fatty acid (NEFA)-HR (Fujifilm Wako Pure Chemical); L-type Wako phospholipids (Fujifilm Wako Pure Chemical); total protein-HR II (Fujifilm Wako Pure Chemical); albumin II HA-test Wako (Fujifilm Wako Pure Chemical); GLU-HK (Shino-Test); Urease GLDH kit (Oriental Yeast); L-type Wako CRE.M (Fujifilm Wako Pure Chemical); L-type Wako AST.J2 (Fujifilm Wako Pure Chemical); L-type Wako ALT.J2 (Fujifilm Wako Pure Chemical); L-type Wako ALP IFCC (Fujifilm Wako Pure Chemical); L-type Wako CK (Fujifilm Wako Pure Chemical); Aqua-auto Kainos TBA kit (Kainos); and Auto-Wako total ketone body (Fujifilm Wako Pure Chemical). All the assays were conducted according to the manufacturer's protocols.

Statistical analysis

Data are expressed as the mean ± SEM. Comparisons among the experimental groups were carried out by 1-way analysis of variance, and if the *P* value was <0.05, the Tukey–Kramer post hoc test were performed. A *P* value of <0.05 was considered statistically significant. All statistical calculations were carried out using JMP Pro 17 (SAS Institute).

Results

Neither fat nor calorie intake correlated with body growth, but dietary amino acid composition was critical

After the acclimation period, 6-wk-old young male Wistar rats were given CN, HFCN, HF_ΔMet, HF_ΔArg, HF_ΔHis, HF_ΔLys, HF_ΔThr, or HF_ΔBCAA for 7 d. Although the food intake of rats taking HFDs decreased by ∼20% compared with those taking the CN, the total calorie intake was comparable, except for the HF_ΔHis and HF_ΔBCAA groups with significant suppression of food intake (Figure 1C–H). In contrast, BW gain during the experimental period significantly differed among the groups (Figure 1A, B). Although the BW of rats in the HF_ΔHis, HF_ΔThr,

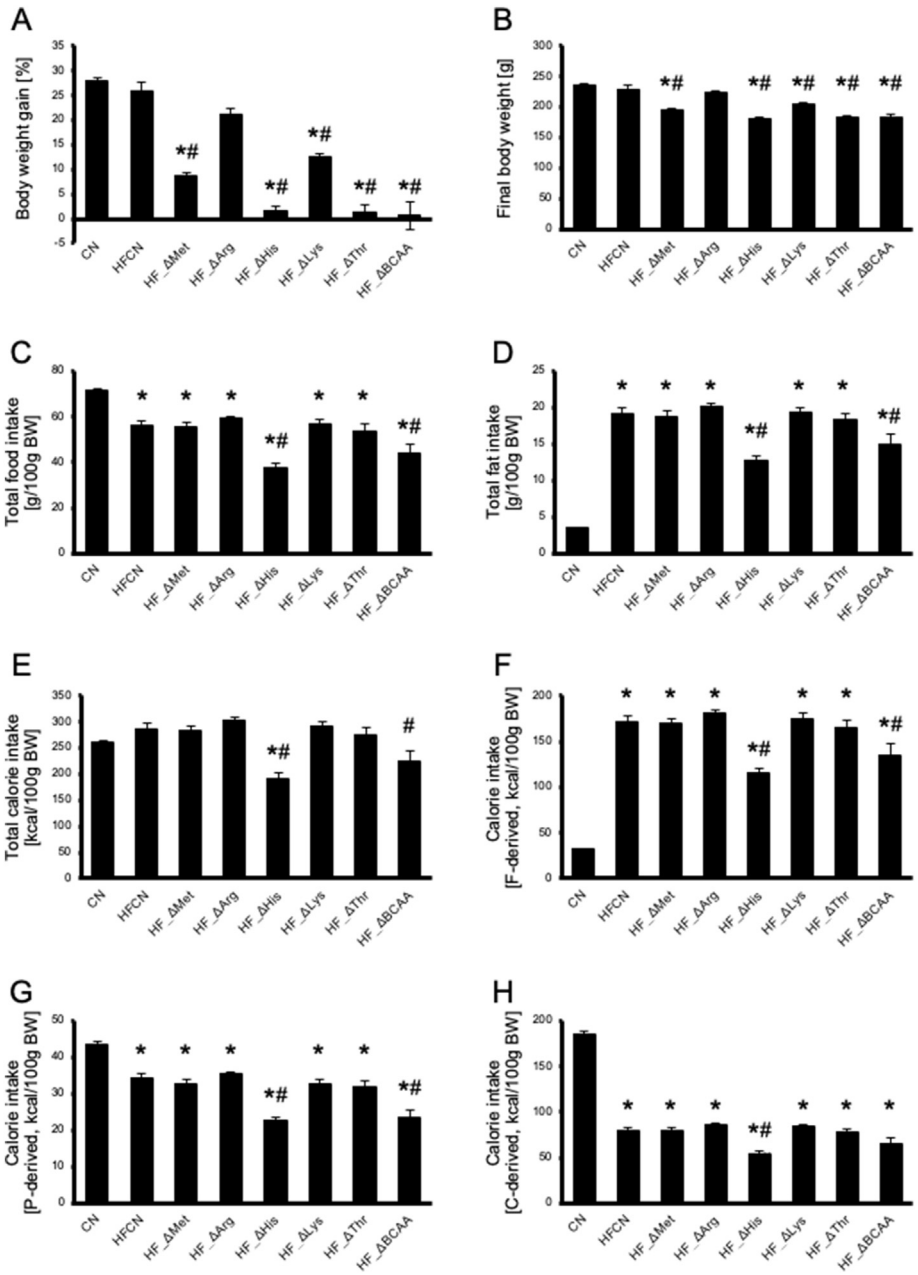

FIGURE 1. Body weight change, food intake, and calorie intake of the rats fed an amino acid-restricted HFD. Six-wk-old male Wistar rats were reared on a control, low-fat diet (CN) containing 15% amino acid mixture as a sole nitrogen source and 5% fat; a high-fat diet (HFD) with the same amount of amino acids as the CN diet and 34% fat (HFCN); or HFDs with only the indicated amino acid(s) being reduced (HF_Δ—), for 7 d. (**A, B**) Body weight change during the experimental diet feeding and final body weight. (**C, D**) Total food and fat intake during the experimental diet feeding. (**E, H**) Total calorie intake during the experimental diet feeding. The calorie intake was divided based on calorie sources, and fat-derived (**F**), protein-derived (**G**), and carbohydrate-derived (**H**) calories are presented separately. Bar: mean ± SEM; *$P < 0.05$ (vs. CN), #$P < 0.05$ (vs. HFCN); $n = 5$.

and HF_ΔBCAA groups were almost unchanged from the beginning, rats in the HF_ΔMet and HF_ΔLys groups gained roughly a half of BW compared with that by the CN group and HFCN group. The HF_ΔArg group showed just a slight tendency of decreased BW gain compared with the CN group and HFCN group. Given that the BW gain and adipose tissue weight of the CN group and HFCN group were comparable (Figures 1A and 2C, D), the difference in BW except for the HF_ΔHis and HF_ΔBCAA groups was unlikely to be due to massive adiposity during the study period.

Tissue lipidation by HFD was considerably affected by dietary amino acid composition

Although the liver weight was slightly decreased in most HFD groups with amino acid-deficiency, it was increased only in the HF_ΔArg group compared with HFCN group (Figure 2A). Significant TG accumulation in the liver was observed in the HFD groups; however, the extent unexpectedly varied (Figure 3A). The HFCN, HF_ΔMet, HF_ΔHis, and HF_ΔBCAA groups exhibited similar significant hepatic TG accumulation, whereas the

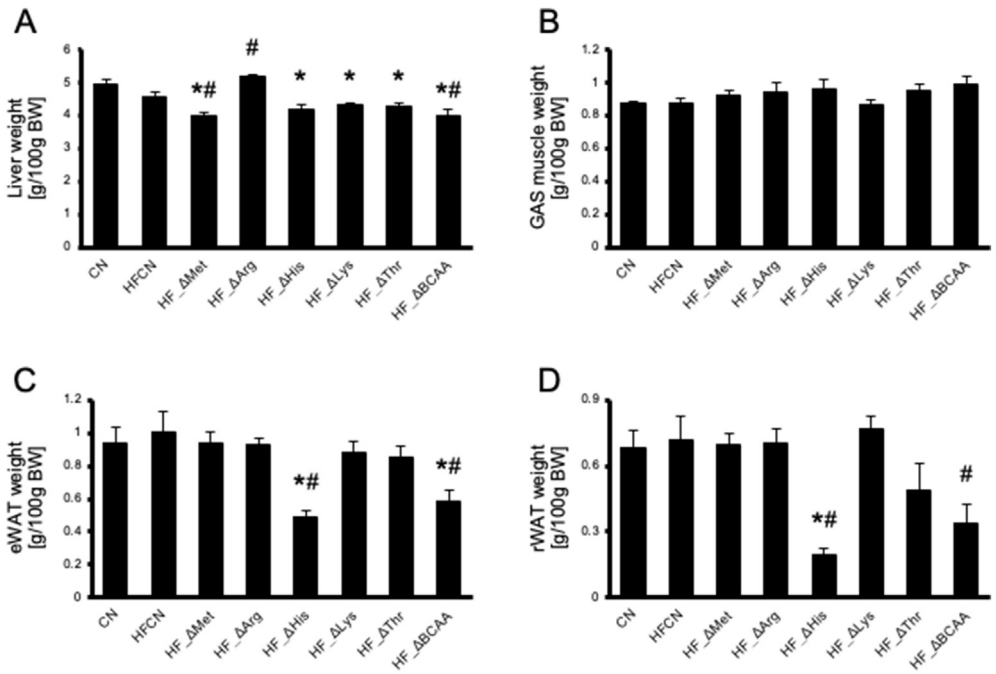

FIGURE 2. Tissue weights of the rats fed an amino acid-restricted high-fat diet (HFD). (**A–D**) Wistar rats were fed the CN, HFCN, or HF_Δ— diet for 7 d, as shown in Figure 1. Whole weights of the liver (**A**), gastrocnemius muscle (**B**), epididymal white adipose tissue (WAT) (**C**), and retroperitoneal WAT (**D**) are shown. Bar: mean ± SEM; $*P < 0.05$ (vs. CN), $\#P < 0.05$ (vs. HFCN); $n = 5$.

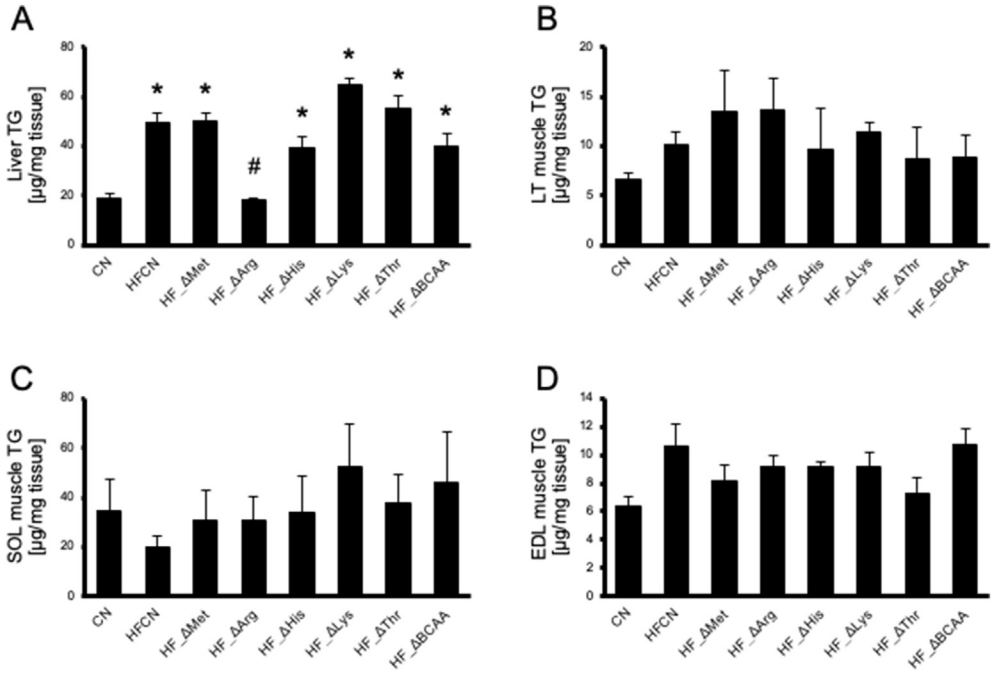

FIGURE 3. TG accumulation in the liver and skeletal muscles of the rats fed an amino acid-restricted high-fat diet (HFD). **(A–D)** Wistar rats were fed the CN, HFCN, or HF_Δ— diet for 7 d, as shown in Figure 1. Total lipids were extracted from the preweighed liver (**A**), longissimus thoracis muscle (**B**), soleus muscle (**C**), and extensor digitorum longus muscle (**D**), and triglyceride (TG) content was measured. Bar: mean ± SEM; $*P < 0.05$ (vs. CN), $\#P < 0.05$ (vs. HFCN); $n = 5$.

HF_ΔLys and HF_ΔThr groups showed slightly enhanced accumulation compared with the HFCN group, although the difference was not statistically significant. Surprisingly, Arg deficiency completely inhibited HFD-induced hepatic TG accumulation, although body growth, food and calorie intake, and fat consumption of the HF_ΔArg group were equal to those of the HFCN group (Figures 1 and 3A).

The skeletal muscle and adipose tissue weight were almost comparable among all groups; however, the HF_ΔHis and HF_ΔBCAA groups had a significantly small mass of white

adipose tissue, presumably because of the marked reduction of food and energy intake (Figure 2B–D). The variation of TG content in the skeletal muscles between the groups was not statistically significant; however, a tendency was observed (Figure 3B–D). HFCN enhanced lipid accumulation in some, but not all, of the skeletal muscles compared with the CN. Moreover, a deficiency of different amino acids in the diet might have a specific effect on the different muscles, implying a tissue-specific effect of amino acids or certain amino acid deficiencies.

HFD-induced serum atherogenic lipid profile was partially improved by certain amino acid deficiencies

Consistent with previous reports [14,15], HFCN feeding caused an increase in serum LDL cholesterol and a tendency to decrease in HDL cholesterol compared with CN feeding, whereas the total cholesterol and NEFA concentrations were unchanged, and TG concentrations were decreased during the study period (Table 2). Remarkably, dietary deficiency in Arg, His, and Lys increased serum HDL cholesterol concentrations, and deficiency in Met, His, Lys, Thr, and BCAAs decreased LDL cholesterol concentrations, although the restoration was partial and some of them did not reach statistical significance. Meanwhile, non-HDL cholesterol concentrations, which were calculated by subtracting HDL cholesterol concentrations from total cholesterol concentrations, increased by HFCN feeding and reversed totally (or tended to be reversed) by Met, His, Lys, Thr, and BCAA deficiency.

Moreover, regarding other serum parameters, high-fat consumption significantly decreased serum BCAA concentrations and tended to decrease phenylalanine, His, and Thr concentrations (Table 2). Serum protein concentrations were slightly affected by Me-, Lys, and Thr deficiency, but not by HFCN only. Dysregulation of sugar metabolism or insulin resistance might not have been induced during the study period, as evidenced by the comparable serum glucose concentrations. Nitrogen metabolism was significantly downregulated by HFCN, but it was rescued by Met, His, Lys, Thr, and BCAA deficiency. The liver might not have been damaged by HFCN considering the normal AST and ALT concentrations in the serum; however, the HF_ΔMet, HF_ΔLys, and HF_ΔThr diets might possibly be involved in liver damage. The HF_ΔHis, HF_ΔThr, and HF_ΔBCAA diets tended to increase serum creatine kinase activities, implying they might have damaged the muscles, although HFCN might not.

Discussion

In most studies handling rodent models of HFD-induced obesity or metabolic syndrome, rats are usually reared on a HFD for more than a few weeks, sometimes ≤10 mo [16]. Actually, it is known that 4 wk of HFD feeding are required to cause a statistically significant increase in BW and fat mass in Wistar rats compared with their low-fat counterparts [17,18]. In contrast, inactivation of ligand-responsive insulin-receptor phosphorylation in the liver, skeletal muscle, and adipose tissue can be observed after 14 d of HFD feeding in Sprague-Dawley rats, and insulin-sensitivity of hepatic sugar metabolic control is impaired within only 3 d after initiation of HFD feeding in Wistar rats [19–21]. In this study, the metabolic effects of the HFCN on hepatic steatosis and atherogenic serum lipoprotein profile were observed within 7 d, with no change of BW and fat mass (Table 2, Figure 3). Although susceptibility to an HFD is known to be highly varied among individuals and strains [22,23], these results indicate that, before developing substantial obesity, HFD ingestion considerably affects rats metabolically within the early period. Meanwhile, we previously demonstrated that a low-protein and low-Arg diet-induced massive liver TG accumulation in Wistar rats within 7 d [10], suggesting that dietary proteins or amino acids and dietary fats may cooperatively contribute to the acute-phase reaction of rat lipid metabolism.

In a normal (low)-fat regimen, Arg deficiency causes marked hepatic steatosis in rats [10]. However, this study showed that a high-fat regimen with Arg deficiency had the opposite effect, inhibiting HFD-induced hepatic steatosis (Figure 3A). Similarly, there have been several conflicting reports on Met restriction. Some claim that Met restriction exacerbates fatty liver, whereas the others claim that Met restriction prevents dietary-induced or genetically induced fatty liver [24,25]. Besides, our results showed no effects of Met restriction on HFD-induced fatty liver (Figure 3A). For another example, the greater proportion of dietary protein/amino acids, or addition of some essential amino acids to the standard diet reportedly caused hypercholesterolemia even in a low-fat regimen [26–29], whereas serum TG concentrations decreased significantly by the HFCN diet feeding compared with CN diet in our study (Table 2). Whereas these results are surprising and confusing at first glance, they further support the idea that the functional interaction of dietary proteins or amino acids and dietary fats have a great impact on the diet-induced lipid metabolic reaction, where one's metabolic effect appears to be dependent on the context of the other. More specifically, severity of Met restriction, comprehensive amino acid composition, fat content, and fat type were more or less different among those studies, which might lead to seemingly conflicting results. Moreover, food, calorie, and fat intakes are certainly important parameters. In this respect, careful attention should be paid for HF_ΔHis and HF_ΔBCAA groups, because food restriction and consequent loss of calorie intake were observed in those groups and, thus, potential effects of them on their phenotypes, such as significant reduction of adipose tissue weight, cannot be disregarded (Figures 1 and 2). Meanwhile, another possible explanation linking the effects of dietary amino acids and fats is the serum amino acid profile. We have previously illustrated that a serum amino acid profile could act as a metabolic code to determine hepatic lipid accumulation. For example, high concentrations of serum Met and His plus low concentrations of serum tyrosine (Tyr), which were observed in rats fed a low-Arg diet, represented a "lipid-inducing cue," whereas high concentrations of serum Met and His plus low concentrations of serum Tyr, Val, Leu, and Ile, which were observed in rats fed a low-Arg/BCAA diet, represented a "lipid-reducing cue" [12]. Consequently, the serum amino acid profile of rats in the HF_ΔArg group had high concentrations of Met and His plus low concentrations of serum Tyr, Val, Leu, and Ile compared with that of rats in the HFCN group. As the serum amino acid profile of the HF_ΔArg group was similar to that of the low-Arg/BCAA diet-fed rats, TGs may hardly have accumulated in the liver, although the food intake, energy intake, fat intake, and BW gain were comparable with those of the HFCN group (Figures 1 and 3,

TABLE 2
Blood parameters[1]

	CN	HFCN	HF_ΔMet	HF_ΔArg	HF_ΔHis	HF_ΔLys	HF_ΔThr	HF_ΔBCAA
Lipids								
Total cholesterol (mg/dL)	78.7 ± 5.9	82.2 ± 1.5	68.7 ± 1.5	88.9 ± 5.8	74.9 ± 1.7	71.5 ± 2.7	53.8 ± 3.5[2,3]	64.2 ± 2.1
Triglyceride (mg/dL)	143.3 ± 12.9	99.6 ± 9.1[2]	48.8 ± 3.7[2,3]	80.0 ± 5.9[2]	57.5 ± 6.2[2,3]	51.6 ± 9.2[2,3]	54.7 ± 8.3[2,3]	48.2 ± 6.2[2,3]
HDL-C (mg/dL)	47.0 ± 3.2	36.3 ± 0.9	34.8 ± 2.5[2]	41.8 ± 2.2	42.3 ± 2.0	42.4 ± 1.3	29.7 ± 2.0[2]	37.1 ± 2.9
LDL-C (mg/dL)	7.2 ± 0.8	21.0 ± 0.9[2]	17.8 ± 1.0[2]	21.0 ± 2.7[2]	17.2 ± 0.7[2]	14.4 ± 0.8[2]	11.0 ± 0.7[3]	12.6 ± 1.4[3]
Non–HDL-C (mg/dL)	31.7 ± 2.8	45.9 ± 1.5[2]	33.9 ± 1.5	47.1 ± 4.9[2]	32.6 ± 1.5[3]	29.1 ± 2.4[3]	24.1 ± 1.6[3]	27.1 ± 1.7[3]
NEFA (μEq/L)	369.4 ± 31.6	452.4 ± 47.2	312.8 ± 48.7	432.0 ± 67.5	343.4 ± 53.9	362.2 ± 33.0	512.0 ± 37.0	596.6 ± 58.3
Phospholipids (mg/dL)	173.0 ± 7.1	158.4 ± 4.7	125.4 ± 2.7[2]	151.0 ± 6.7	139.2 ± 3.1[2]	138.0 ± 5.4[2]	117.4 ± 10.2[2,3]	121.0 ± 8.1[2,3]
Amino acids								
Alanine (μM)	697.9 ± 18.6	644.4 ± 33.2	718.4 ± 94.1	780.8 ± 34.2	796.9 ± 66.1	605.7 ± 26.1	487.0 ± 45.3	681.8 ± 76.0
Cystine (μM)	61.6 ± 4.3	53.1 ± 6.9	23.8 ± 3.9[2,3]	52.0 ± 2.8	47.4 ± 3.2	56.0 ± 2.6	47.0 ± 4.7	43.9 ± 3.6
Aspartic acid (μM)	55.6 ± 1.9	64.5 ± 4.3	54.1 ± 2.1	61.9 ± 2.8	61.3 ± 2.5	57.7 ± 1.7	56.1 ± 1.1	55.0 ± 1.5
Glutamic acid (μM)	178.7 ± 8.5	185.3 ± 12.1	163.2 ± 4.6	201.7 ± 14.5	166.9 ± 12.9	175.2 ± 5.8	132.6 ± 4.3[3]	155.8 ± 8.5
Phenylalanine (μM)	56.9 ± 2.3	47.4 ± 1.0	55.6 ± 2.4	40.6 ± 3.4[2]	53.2 ± 1.8	58.0 ± 2.8	49.5 ± 2.4	40.7 ± 2.9[2]
Glycine (μM)	309.5 ± 23.7	268.7 ± 16.2	354.9 ± 28.5	305.0 ± 29.3	435.1 ± 15.2[3]	355.1 ± 21.0	404.0 ± 44.8	373.5 ± 37.1
Histidine (μM)	35.3 ± 1.0	26.6 ± 1.2	41.3 ± 3.5[3]	36.6 ± 0.8[3]	8.3 ± 0.3[2,3]	29.3 ± 1.0	28.1 ± 1.7	32.6 ± 2.1
Isoleucine (μM)	81.6 ± 3.5	57.3 ± 0.9[2]	60.6 ± 1.8[2]	52.6 ± 4.3[2]	50.9 ± 2.5[2]	62.1 ± 4.6[2]	41.7 ± 2.7[2]	27.6 ± 5.0[2,3]
Lysine (μM)	487.5 ± 14.9	441.4 ± 24.6	538.8 ± 45.8	444.2 ± 21.3	402.0 ± 11.9	101.8 ± 15.9[2,3]	448.4 ± 15.0	433.6 ± 28.0
Leucine (μM)	133.6 ± 6.1	92.7 ± 1.7[2]	105.2 ± 7.3	88.4 ± 3.4[2]	87.7 ± 3.1[2]	101.8 ± 6.9[2]	74.3 ± 4.2[2]	45.2 ± 8.2[2,3]
Methionine (μM)	104.5 ± 12.2	100.9 ± 11.0	52.7 ± 4.5[2,3]	120.8 ± 10.9	74.0 ± 3.8	57.8 ± 10.1[2]	48.4 ± 2.7[2,3]	59.5 ± 8.6[2]
Asparagine (μM)	135.6 ± 4.1	116.8 ± 3.5	111.5 ± 5.6	115.1 ± 6.9	124.2 ± 6.6	99.9 ± 6.5[2]	89.3 ± 5.1[2]	112.5 ± 3.5
Proline (μM)	230.9 ± 42.6	205.7 ± 21.3	227.4 ± 62.8	193.7 ± 19.2	156.7 ± 5.9	147.4 ± 14.9	136.1 ± 3.0	181.1 ± 18.7
Glutamine (μM)	729.4 ± 47.6	866.0 ± 30.6	892.1 ± 22.3	1222.7 ± 75.8[2,3]	970.6 ± 32.9[2]	855.8 ± 42.6	817.5 ± 27.9	1052.8 ± 58.7[2]
Arginine (μM)	78.2 ± 4.7	71.6 ± 3.2	87.5 ± 6.7	67.4 ± 6.5	84.8 ± 1.9	85.4 ± 4.2	90.9 ± 12.5	90.8 ± 8.5
Serine (μM)	421.3 ± 13.1	423.2 ± 21.5	866.7 ± 55.3[2,3]	473.6 ± 19.3	593.9 ± 4.9[2,3]	582.5 ± 7.3[2,3]	636.0 ± 38.7[2,3]	594.6 ± 20.9[2,3]
Threonine (μM)	671.6 ± 43.5	477.4 ± 34.0	794.5 ± 88.0[3]	453.4 ± 17.8	520.8 ± 36.6	788.8 ± 41.3[3]	85.0 ± 8.6[2,3]	800.1 ± 52.3[3]
Valine (μM)	198.7 ± 7.8	142.0 ± 2.7[2]	150.8 ± 12.5[2]	127.7 ± 7.1[2]	114.0 ± 4.0[2]	138.4 ± 6.5[2]	93.4 ± 5.5[2,3]	61.3 ± 7.2[2,3]
Tryptophan (μM)	98.8 ± 4.3	82.1 ± 5.4	76.8 ± 6.4	59.5 ± 5.1[2]	84.8 ± 2.5	84.6 ± 3.4	72.5 ± 3.4[2]	74.3 ± 6.5
Tyrosine (μM)	123.2 ± 6.7	127.3 ± 6.9	81.1 ± 12.3[2,3]	57.3 ± 4.0[2,3]	104.6 ± 5.3	70.2 ± 3.5[2,3]	55.8 ± 4.3[2,3]	86.3 ± 11.2[3]
Others								
Total protein (g/dL)	5.4 ± 0.09	5.4 ± 0.08	4.9 ± 0.08[2,3]	5.1 ± 0.07	5.5 ± 0.09	4.8 ± 0.11[2,3]	4.7 ± 0.14[2,3]	5.2 ± 0.1
Albumin (g/dL)	4.0 ± 0.06	4.0 ± 0.05	3.5 ± 0.04[2,3]	3.8 ± 0.06	4.4 ± 0.06[3]	3.6 ± 0.08[2,3]	3.5 ± 0.1[2,3]	4.2 ± 0.08
Glucose (mg/dL)	194.4 ± 9.6	193.8 ± 3.1	177.8 ± 5.9	200.8 ± 3.3	183.2 ± 7.1	191.2 ± 7.9	175.8 ± 4.9	182.0 ± 5.4
BUN (mg/dL)	8.8 ± 0.4	4.4 ± 0.6	8.9 ± 1.4	3.9 ± 0.3	7.3 ± 1.0	7.9 ± 1.8	8.9 ± 0.6	6.4 ± 0.8
Creatinine (mg/dL)	0.202 ± 0.003	0.206 ± 0.006	0.234 ± 0.006	0.204 ± 0.004	0.232 ± 0.002	0.216 ± 0.011	0.244 ± 0.010[2,3]	0.246 ± 0.011[2,3]
BUN:creatinine (ratio)	43.6 ± 2.2	21.3 ± 3.0[2]	37.9 ± 5.7	18.9 ± 1.4[2]	31.8 ± 4.3	35.0 ± 6.4	37.2 ± 3.6	26.3 ± 3.3
AST (IU/L)	111.6 ± 6.5	109.8 ± 6.4	154.4 ± 14.5	124.6 ± 7.2	140.2 ± 15.5	125.4 ± 8.6	170 ± 11.9[2,3]	130.2 ± 12.4
ALT (IU/L)	28.4 ± 2.1	40.0 ± 2.3	86.8 ± 9.2[2,3]	48.6 ± 4.9	50.8 ± 5.3	62.0 ± 4.3[2]	69.8 ± 6.8[2,3]	51.2 ± 7.7
ALP (IU/L)	268.2 ± 13.3	423.8 ± 26.7[2]	557.2 ± 27.3[2]	360.8 ± 36.2	350.4 ± 19.9	487.2 ± 22.3[2]	411.4 ± 37.3[2]	245.6 ± 22.3[3]
CK (IU/L)	4388.6 ± 286.7	3617.8 ± 383.9	3870.0 ± 441.6	4046.4 ± 394.8	6349.8 ± 1199.8	3668.8 ± 580.2	5495.8 ± 789.1	5762.8 ± 1077.2
Total bile acids (μM)	13.4 ± 3.5	18.8 ± 2.0	32.0 ± 8.3	23.4 ± 4.4	62.2 ± 13.9	15.0 ± 2.3	62.8 ± 22.4	26.6 ± 3.9
Total ketone body (μM)	130.8 ± 14.1	483.4 ± 62.5	385.6 ± 103.3	564.8 ± 169.6	552.8 ± 207.3	384.6 ± 39.3	676.4 ± 119.9	884.8 ± 235.3[2]

Abbreviations: ALP, alkaline phosphatase; ALT, alanine amino transferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase.
[1] Data are presented as means ± SEM.
[2] $P < 0.05$ vs. CN.
[3] $P < 0.05$ vs. HFCN.

Table 2). It is notable that Arg deficiency inhibited HFD-induced liver TG accumulation, whereas Lys deficiency tended to further enhance it (Figure 3A) ($P = 0.16$ compared with HFCN). Considering that Arg and Lys absorption compete with each other on the cationic amino acid transport system [30,31], the opposite effects shown by HF_ΔArg and HF_ΔLys feeding are worth mentioning, further supporting the significance of amino acids absorbed into the circulation.

The chronic effects of dietary amino acid composition in combination with an HFD are still unclear. Owing to the relatively short duration of feeding, no significant increase in total cholesterol, TG, NEFA, aspartate transaminase, and alanine transaminase concentrations in the serum of HFCN rats were observed in comparison with CN rats, indicating that the HFCN rats harbored a simple fatty liver, with no apparent liver injury or cirrhosis during the tested period (Table 2). In addition, although lard was used to make the HFD, it is known that HFDs consisting of different types of fat sources (e.g., saturated compared with unsaturated fatty acids, or animal-derived compared with plant-derived fat/oil) have different effects on animal metabolism [6]. To comprehend the total effect of dietary amino acids on metabolic syndromes such as obesity, nonalcoholic fatty liver disease, and cardiovascular disease, further investigations with long-term feeding and different sources of protein and fat in a diet are warranted.

In summary, we demonstrated that ectopic fat accumulation and dyslipidemia were not always induced by HFDs at least in the short term, although the rats ingested an excessive amount of fat and energy. The metabolic outcome of high-fat consumption was crucially dependent on the protein or amino acid intake or dietary amino acid composition. As HFDs have been widely used in the basic research of metabolic syndrome and many epidemiologic studies have assumed the relationship between fat consumption (or fat:sugar ratio) and metabolic syndrome, our findings may give rise to an important perspective on HFD regimens in both scientific and clinical contexts. Our findings may help researchers and clinicians interpret the related data by providing a new measure: dietary amino acids.

Author contributions

The authors' responsibilities were as follows – HN, DY, FH: designed the study; HN, YG, RO, DY, RI, RM, FH: performed the experiments; DY: contributed to LC-MS analysis; S-IT, KI, FH: supervised the study; HN, DY, FH: wrote the manuscript; and all authors: have read and approved the final version of the manuscript.

Conflicts of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript and analytic code will be made available on request pending. Further information and requests should be directed to and will be fulfilled by the Lead Contact, Fumihiko Hakuno (hakuno@g.ecc.u-tokyo.ac.jp).

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