Randomized trial of amino acid mixture combined with physical activity promotion for abdominal fat reduction in overweight adults

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Purpose: The purpose of this study was to test the efficacy of arginine, alanine, and phenylalanine mixture (A-mix) ingestion at 1,500 mg/day in combination with the promotion of physical activity for abdominal fat reduction in overweight adults.

Methods: A placebo-controlled, double-blind, parallel-group, randomized trial for 12 weeks combined with a 4-week follow-up period was conducted at a single center in Minato-ku, Tokyo, Japan, between December 2016 and May 2017. Data were analyzed between June and August 2017. The study participants were 200 overweight adults within the age range of 20–64 years. The participants were randomly assigned to the A-mix group (n=100) or a placebo group (n=100) and were administered 500 mL of test beverage containing 1,500 or 0 mg of A-mix, respectively, for 12 weeks. All participants maintained a physically active lifestyle between week 0 and week 12 through monthly sessions of physical activity. The primary outcomes were the 12-week changes in the abdominal total, subcutaneous, and visceral fat areas, as assessed by computed tomography.

Results: Of the 200 enrolled participants, 199 (99%) accomplished the 12-week intervention and 4-week follow-up period. The per-protocol-based analysis for 194 participants demonstrated that the abdominal total fat area decreased significantly in the A-mix group compared with that in the placebo group (difference, 10.0 cm²; 95% confidence interval [CI]: 0.4–19.6 cm²; P=0.041). Comparable outcomes were obtained for the abdominal subcutaneous fat area (difference, 7.4 cm²; 95% CI: 0.1–14.7 cm²; P=0.047). No study-related unfavorable events occurred.

Conclusion: A-mix supplementation in combination with physical activity promotion facilitated abdominal fat reduction in overweight adults.

Keywords: amino acid supplementation, physical activity promotion, abdominal fat, randomized controlled trial

Introduction
Obesity, which is a common medical issue worldwide,1 is a major risk factor for type 2 diabetes, insulin resistance, atherosclerosis, hypertension, stroke, and several types of cancer.2 The condition results from a chronic imbalance in energy metabolism. To avoid or alleviate the risks associated with obesity, weight reduction by dietary restriction and a general exercise program is viewed as the best, accessible, nonpharmacologic, and nonsurgical treatment system.3

The additive effects or synergistic impact of eating routines and exercise have recently been thoroughly studied.4,5 Our research group has considered the impact of amino acid supplementation, in combination with exercise, on fat oxidation for over two decades.5–15 Recently, we concentrated on the amino acids arginine, alanine, and...
phenylalanine, which are involved in glucagon synthesis and secretion when delivered acutely. We also established that a single session of aerobic exercise in conjunction with oral administration of a mixture of arginine, alanine, and phenylalanine (A-mix) increased blood ketone body levels compared with placebo in humans.13,14 These conclusions indicated a lipolytic effect during exercise that was likely a result of A-mix ingestion. Moreover, we recently conducted a pilot randomized controlled trial to evaluate the long-term efficacy of A-mix consumption in conjunction with physical activity promotion for overweight individuals, and this trial helped to estimate the effective dose of the mixture. The pilot trial suggested that a dose of 1,500 mg/day of A-mix facilitated abdominal fat reduction in overweight adults.16

Therefore, we conducted a randomized controlled trial to confirm the efficacy of the combination of 1,500 mg/day A-mix ingestion with physical activity promotion in abdominal fat reduction in overweight adults between the age of 20 and 64 years.

Methods

Trial design
The study had a double-blind, placebo-controlled, parallel-group, and randomized design. The trial was a single-center study organized at a site in Minato-ku, Tokyo, Japan, and was conducted between December 2016 and May 2017. The data were analyzed between June and August 2017. The target number of participants was set at 200 (i.e., 100 participants per group), as a sample size of 100 participants in each group enabled us to perceive a group difference effect size (Cohen’s $d$) of 0.4 with a significance level of 5% and a power of 80%. Given the assumption that some individuals would refuse to participate in the trial or fail to meet the inclusion criteria, a target number of 340 adults was set for the introductory session. The investigation protocol was approved by the Institutional Review Board of the University of Tsukuba Faculty of Medicine, the Meiji Institutional Review Board, and the Ethical Committee of Nihonbashi Cardiology Clinic. Written informed consent was obtained from each participant before randomization. The study protocol was enrolled in the UMIN Clinical Trials Registry (UMIN000025186) on December 10, 2016. This article completely conforms to the Consolidated Standards of Reporting Trials 2010 rules.17 A data monitoring group guaranteed the accuracy of information collected and the sources of information.

Participants
Participants were recruited from a volunteer database associated with a contract research organization. The included participants had to be overweight (25 kg/m² ≤ body mass index [BMI] < 30 kg/m²) adults between 20 and 64 years of age. Individuals were excluded if they 1) continually used oral medication that might conceivably influence the body weight and lipid metabolism; 2) had food allergies or cardiovascular, severe hepatic, respiratory, metabolic, or endocrine disorders; 3) could not avoid the use of health food products or supplements that might conceivably influence the body weight and lipid metabolism; 4) had a current or previous history of drug or alcohol dependence; 5) could not abstain from alcohol consumption for 2 days before each measurement visit; 6) had metal installed in their abdominal site; 7) had a cardiac pacemaker or an implanted defibrillator; 8) were diagnosed with hyperphenylalaninemia or phenylketonuria; 9) had a history of familial hyperlipidemia; 10) were currently pregnant, nursing, or intended to become pregnant during the study period; 11) had extremely irregular dietary propensities or lifestyle, for example, midnight working; 12) participated or were eager to participate in clinical trials of other foods, medications, or beauty products; 13) had a fear of confined places; and 14) were judged as inappropriate for the study by the principal investigator owing to abnormal blood or urine parameters, or other reasons.

Randomization and blinding
After arrangement by sex and intervention waves, the participants who met the inclusion criteria were randomized to the A-mix or placebo group with a 1:1 allocation ratio. An investigator who had no contact with the participants and staff individuals created a random number sequence by using an approved computer program. The random sequence was kept by an assigned individual who was accountable for shipping the test beverages to the participants but was not involved in the plan, enrollment, evaluation, intervention, or analysis. The participants, investigators, and all staff members involved in the trial were blinded to the group allocation. The randomization code was opened after the study information was checked, collated, and finalized.

Interventions
Test beverage
The test preparation for the A-mix and placebo groups consisted of a 500 mL polyethylene terephthalate bottle beverage that contained 1,500 and 0 mg of A-mix, respectively. The
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Test beverages had an identical appearance and comparable tangible attributes. The ingredients of the A-mix were as follows: 42% mol/mol of phenylalanine, 38% mol/mol of alanine, and 20% mol/mol of arginine. Each bottle of the test beverage contained 20 and 13 kcal for the A-mix and the placebo groups, respectively. Neither of the test beverages contained caffeine. The assigned individual transported the test beverages to the participants based on group allocation. The participants were instructed to keep the test beverages away from high humidity, high temperature, and direct sunlight and to consume one bottle per day before and during physical activity or exercise under free-living conditions.

Physical activity promotion
Regardless of group allocation, the participants followed a monthly physical activity promotion session throughout the 12-week intervention period (four sessions in total). The intervention program consisted of a group-based exercise class with personalized advice. Participants were urged to add 1,000 steps/day or more from their benchmark step targets suggested by the current Japanese physical activity recommendations.18,19 The group-based exercise class started with a 30 min lecture and a 60 min group-based workout session that included walking, muscle strengthening, and stretching. Trained professionals conducted the personalized advice sessions in conjunction with the analysis of participants’ recent step counts, body weight, test beverage ingestion, physical condition, medication use, dietary behaviors, daily step counts measured by pedometer (FB-736; Tanita Corporation, Tokyo, Japan), and exercise, all of which were recorded in diaries. To avoid the confounding effects of lifestyle changes, participants were instructed to consume well-balanced meals three times per day in accordance with the Japanese Food Guide Spinning Top20 and to abstain from excessive strenuous exercise, gorging, serious activity restrictions, ingestion of healthful supplements, functional foods, and over-the-counter medications that could possibly influence the results.

Measurements
All study outcomes, except for abdominal fat area, were assessed at baseline and at each visit after 4 weeks by experienced staff members who were blinded to the group allocation. The abdominal fat areas were scanned by using computed tomography (CT) at the baseline and 12-week visits. The primary outcome measures were the 12-week changes in the abdominal fat areas. The secondary outcomes comprised changes in body weight, percent fat mass, waist and hip circumferences, and metabolic risk factors such as lipid profiles, blood glucose parameters, and blood pressure. To monitor adherence to the intervention, dietary intake was measured every 4 weeks and physical activity was measured at baseline and week 12.

Anthropometrics and body composition
Body weight (to the nearest 0.1 kg) and percent fat mass (to the nearest 0.1%) were measured by using a multi-frequency bioelectrical impedance device (InBody 430; Biospace, Seoul, Korea).21 Height was measured to the nearest 0.1 cm by utilizing a compact stadiometer (DSN-90; Muratec-KDS Corp., Kyoto, Japan) at baseline. BMI was estimated from participants’ weight in kilograms divided by their height in meters squared. Waist and hip circumference were measured twice to the nearest 0.1 cm at the umbilicus and maximum circumference of buttocks, respectively in the standing position by using a flexible plastic tape. The average value of the two measurements was used for analysis. Waist to hip ratio was calculated from participants’ waist circumference divided by their hip circumference.

Abdominal fat areas
The abdominal fat areas were analyzed at the L4 level by using CT (Supria; Hitachi Ltd, Tokyo, Japan) and computed by a commercial program (Fat Scan Ver. 5.0.; e-JAPAN IT Co., Ltd, Ibaraki, Japan).22

Blood pressure readings
Systolic and diastolic blood pressure readings were measured with a mechanized sphygmomanometer (Digital Automatic Blood Pressure Monitor HEM-907; Omron Healthcare, Kyoto, Japan). The measurements were taken on the arm of seated participants who had rested for no less than 5 min with the arm supported at the heart level. An average value of both readings was used for data analysis.

Blood biochemistry
A blood sample was drawn from each participant after an overnight (≥12 h) fast. Venous blood was assayed by an independent laboratory (LSI Medience Corporation, Tokyo, Japan). The levels of serum total cholesterol (IatoroLQ T-CHO(A) II; LSI Medience Corporation) and triglycerides (IatoroLQ TG II; LSI Medience Corporation) were determined enzymatically. Serum high- and low-density lipoprotein cholesterol levels were measured by the selective inhibition method (MetaboLead HDL-C and MetaboLead LDL-C, respectively; Kyowa Medex Co., Ltd, Tokyo, Japan). Blood glucose was assayed by an enzymatic method.
(IatoroLQ GLU; Unitika, Aichi, Japan) and insulin by a chemiluminescent immunoassay (Architect Insulin; Abbott Japan, Tokyo, Japan). Glycated hemoglobin A (HbA1c) was determined enzymatically (CinQ HbA1c; ARKRAY Inc., Kyoto, Japan). Homeostasis model assessment of insulin resistance (HOMA-R) was calculated using the following formula: Blood glucose (mg/dL) × insulin (μU/mL) / 405.

Dietary intake
The macronutrient intake in grams and the total energy intake in kilocalories were evaluated from records of food intake over 3 days. Participants were instructed to record their total food consumption for 3 days, including two weekdays and one weekend day. Food quantities were measured by using standard measuring glasses, spoons, and digital scales.

Physical activity
Physical activity was measured by using a triaxial accelerometer (Active style Pro HJA-750C; Omron Healthcare) worn at the waist for 7 days. The accelerometer counted steps and computed the intensity of physical activity (expressed as metabolic equivalents) from a published algorithm.23,24 The devices were not worn during sleeping, water-based activities (e.g., washing or swimming), or participating in certain exercises for safety reasons (e.g., contact sports). Records obtained were characterized as substantial when a device was worn for no less than 10 h/day.25 When substantial records were gathered for over 1 day, step counts and time spent in moderate to vigorous physical activity (≥3 metabolic equivalents) were calculated for each participant.

Safety assessment
Hematology tests, blood biochemical tests, and urinalyses were performed at every visit after 4 weeks. At the same visit, a medical staff checked the health status of the participants by means of a medical interview and consultation with their diaries.

Adverse events
Data on adverse events were subjectively obtained through the participants’ diaries and were independently analyzed by using pulse rate measurements and blood hematologic and biochemical tests. In this trial, an adverse event was characterized as any negative and unintended sign, manifestation,

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**Figure 1** Flowchart of trial participants.

**Abbreviations:** BMI, body mass index; PPB, per-protocol-based.
or malady. The study physician then judged whether a given unfavorable event was serious or nonserious, and if it was study related.

**Statistical analysis**

All statistical analyses were performed in accordance with the analysis plan in the study protocol using IBM SPSS Statistics Ver. 24 (IBM Japan, Ltd, Tokyo, Japan). Values of *P*<0.05 were considered statistically significant. The baseline participant characteristics were presented as the mean and standard deviation for continuous variables or as frequencies and percentages for categorical variables. Our primary analysis followed a per-protocol-based (PPB) principle, in which we excluded participants who met no less than one of the accompanying predetermined criteria as follows: 1) missed the 12-week visit; 2) low compliance rate (<80%) for consumption of the test beverages; 3) absent or inadequate diary logs; 4) announced ineligible after randomization; and 5) serious infringement of study consistence, as judged by the principal investigator.

An unpaired Student’s *t*-test was used to compare the 12-week changes in the primary, secondary, and compliance outcomes. A stratified analysis was conducted: those with step counts increased by fewer than 1,000 steps during the 12-week intervention period. In the safety assessment, the adverse events and deviations identified by fasting blood tests were compared between the two groups by using chi-square tests for categorical variables and unpaired *t*-tests for continuous variables. In addition, paired *t*-tests were used to compare the data before and after the intervention within each group.

**Results**

The complete flow chart of the study participants is displayed in Figure 1. A total of 377 candidates attended the introductory session; written informed consent was obtained from 370 participants and the baseline examination was completed by 340 participants. Finally, 200 individuals met the inclusion criteria and were assigned randomly to the A-mix group (n=100) or to the placebo group (n=100).

The characteristics of the participants measured at baseline are presented in Table 1. There were no significant differences in any study or compliance outcomes between the two groups. Among the 200 participants, 199 (99%) completed the 12-week intervention and 4-week follow-up period; of the 199 who completed the trial, the median rate of test beverage intake was 100% with no between-group difference (*P*=0.42; Mann–Whitney *U*-test). After the exclusion of five additional participants (1 missed the 12-week visit, 1 was ineligible after randomization, and 3 seriously violated the study compliance requirements), 194 participants were included in the PPB analysis.

The results of the PPB analyses of the primary and secondary outcomes are presented in Table 2 and Figure 2, and those of the compliance outcomes are summarized in Table 3. The abdominal total fat area decreased significantly in the A-mix group compared with that in the placebo group (difference, 10.0 cm²; 95% confidence interval [CI]: 0.4–19.6 cm²; *P*=0.041). Similar results were obtained for the abdominal subcutaneous fat area measurements (difference, 7.4 cm²; 95% CI: 0.1–14.7 cm²; *P*=0.047). No noteworthy

| Table 1 Baseline characteristics of the participants (n=200) |
|-----------------|-----------------|-----------------|
| **Characteristic** | **A-mix group** (n=100) | **Placebo group** (n=100) |
| **Female, n (%)** | 50 (50) | 50 (50) |
| **Age, years** | 43.5 (9.9) | 43.5 (9.7) |
| **Height, cm** | 164.9 (9.2) | 164.3 (8.2) |
| **Weight, kg** | 73.5 (9.1) | 73.4 (7.8) |
| **Body mass index, kg/m²** | 27.0 (1.2) | 27.1 (1.3) |
| **Percent fat mass, %** | 32.1 (6.9) | 32.4 (6.9) |
| **Waist circumference, cm** | 93.8 (5.8) | 93.6 (4.9) |
| **Hip circumference, cm** | 101.2 (4.2) | 100.5 (3.8) |
| **Waist to hip ratio** | 0.93 (0.05) | 0.93 (0.05) |
| **Abdominal fat area, cm²** |  |  |
| **Total** | 339.2 (73.4) | 327.6 (71.1) |
| **Subcutaneous** | 244.0 (66.2) | 229.7 (70.4) |
| **Visceral** | 95.2 (35.9) | 98.0 (38.9) |
| **Blood pressure, mm Hg** |  |  |
| **Systolic** | 129.8 (13.6) | 129.4 (12.5) |
| **Diastolic** | 77.6 (10.4) | 77.5 (10.6) |
| **Blood biochemical parameters** |  |  |
| **Triglycerides, mg/dL** | 103.1 (53.8) | 113.4 (55.5) |
| **Total cholesterol, mg/dL** | 208.3 (33.5) | 215.0 (30.2) |
| **HDL-C, mg/dL** | 126.1 (30.6) | 133.6 (27.1) |
| **LDL-C, mg/dL** | 58.3 (13.8) | 56.4 (11.3) |
| **Fasting blood glucose, mg/dL** | 85.1 (6.2) | 85.3 (7.4) |
| **Glycated hemoglobin A1c, %** | 5.5 (0.3) | 5.5 (0.3) |
| **Fasting insulin, μU/mL** | 5.6 (2.7) | 5.8 (2.4) |
| **HOMA-R** | 1.18 (0.61) | 1.22 (0.53) |
| **Dietary intake** |  |  |
| **Total energy, kcal/day** | 1,934 (471) | 1,850 (393) |
| **Protein, g/day** | 72.4 (21.0) | 68.1 (16.0) |
| **Fat, g/day** | 67.1 (22.0) | 63.0 (17.0) |
| **Carbohydrate, g/day** | 243.8 (62.0) | 238.5 (57.1) |
| **Physical activity** |  |  |
| **Step counts, steps/day** | 7,565 (3,308) | 8,454 (3,598) |
| **MVPA, min/day** | 65.5 (32.7) | 70.2 (37.2) |

**Notes:** Data shown as mean (standard deviation) unless specified. *Data were available for 198 participants (100 in the A-mix and the placebo groups, respectively).

**Abbreviations:** HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-R, homeostasis model assessment of insulin resistance; MVPA, moderate-to-vigorous physical activity.
difference between groups was found for the other primary and secondary outcomes. The daily total energy intake was decreased by 131 kcal in the A-mix group and by 77 kcal in the placebo group. The daily step count was increased by 1,779 in the A-mix group and by 1,629 in the placebo group. No significant between-group differences were found in the consistency of the outcome measurements.

In the stratified analysis (Table 4) among the participants whose step counts increased by more than 1,000 steps during the 12-week intervention period, the A-mix group showed a significantly larger reduction in the abdominal total and subcutaneous fat areas compared with the placebo group ($P$=0.019 and 0.011, respectively). However, no significant between-group differences were observed in the participants whose step counts increased by fewer than 1,000 steps during the intervention.

In the safety assessment, no serious, unfavorable events occurred in the trial. A total of 133 nonserious unfavorable events were accounted for through participants’ diaries and medical interviews (Table S1). These occasions included fever, migraine, toothache, arthralgia, and common symptoms, for example, cold or sensitivity to dust. Although there were significant differences between the groups (e.g., allergy to pollen and allergic rhinitis), none of these were judged by the study physician to be related to the consumption of the study beverage. Moreover, a total of 456 deviations from the reference ranges were identified through blood analysis (Table S2). Although there were significant differences between groups, such as increased number of red blood cells, elevated levels of hemoglobin, decreased levels of aspartate amino transferase, decreased levels of alanine aminotransferase, elevated levels of total bilirubin, elevated levels of blood urea nitrogen, elevated levels of calcium, and elevated levels of serum iron, there were no clinically significant variations from the norm or discoveries, as judged by the study physician, due to the consumption of the test beverage on the basis of the physical assessments, blood biochemical analyses, and hematologic parameters. The results of the blood analyses and urinalyses are summarized in Tables S3–S6.

### Discussion

The aim of this study was to test the efficacy of A-mix in conjunction with the promotion of physical activity for the reduction of abdominal fat in overweight adults. We found significantly larger reduction in abdominal total and subcutaneous fat areas in the A-mix group compared with the placebo group. From a safety perspective, no serious,
study-related, adverse events were identified throughout the examination. Although common symptoms, such as cold or allergy to pollen, and some abnormal lab tests of blood biochemistry were identified, these unfavorable events were independent of group assignment or were judged by the study physician to be not related to the consumption of the test beverage.

The larger reduction in abdominal fat in the A-mix group could be explained by the following physiological mechanisms described previously by our group. In brief, a previous study showed that pre-exercise ingestion of the A-mix was associated with increased blood concentrations of adrenaline and glucagon during and after exercise. Gannon and Nuttall reported that specific amino acids such as arginine, glycine, phenylalanine, and alanine can stimulate glucagon secretion. Therefore, the stimulation of glucagon secretion by the A-mix is characteristic of these amino acids. In this way, the reduction in blood glucose by exercise and a transient increase in blood amino acid levels by ingestion may stimulate pancreatic alpha cells and promote glucagon secretion. Additionally, past studies have noticed that some of the wide-ranging effects of adrenaline and glucagon appear to be facilitated by a common effector, cyclic adenosine 3',5'-monophosphate. Therefore, cyclic adenosine 3',5'-monophosphate-dependent lipolysis and fat oxidation appears to increase after a combination of the acute administration of A-mix and exercise, and the accumulated effects reduced abdominal fat in this study. However, the pathway described above is one of the mechanisms for the enhanced lipolysis and fat oxidation associated with A-mix supplementation combined with exercise. Moreover,
Table 3 Per-protocol-based analyses for compliance outcomes (n=194)

| Outcome                          | A-mix group (n=95) | Placebo group (n=99) | P-value |
|----------------------------------|--------------------|----------------------|---------|
|                                  | Week 0 | Week 4 | Week 8 | Week 12 | Week 16 | 12-week change | Week 0 | Week 4 | Week 8 | Week 12 | Week 16 | 12-week change |
| Dietary intake                  |        |        |        |        |        |               |        |        |        |        |        |               |
| Total energy, kcal/day           | 1928   | 1842   | 1829   | 1798   | 1766   | −131          | 1853   | 1820   | 1853   | 1776   | 1860   | −77           | 0.29   |
| (433)                            | (438)  | (427)  | (386)  | (409)  | (−201.−61) |               | (394)  | (392)  | (377)  | (359)  | (390)  | (−150.−4)    |
| Protein, g/day                  | 71.9   | 69.9   | 69.7   | 68.9   | 66.4   | −3.0          | 68.2   | 69.3   | 70.3   | 68.6   | 70.6   | 0.4           | 0.16   |
| (18.9)                          | (18.9) | (17.7) | (17.9) | (17.0) | (−6.5, 0.5) |               | (15.9) | (15.9) | (16.7) | (16.7) | (15.6) | (−2.9, 3.7)  |
| Fat, g/day                      | 67.0   | 64.9   | 63.5   | 62.4   | 61.2   | −4.6          | 63.1   | 63.3   | 63.3   | 61.6   | 65.0   | 1.5           | 0.25   |
| (20.9)                          | (23.0) | (21.4) | (19.5) | (19.2) | (−8.1, 1.0) |               | (18.7) | (17.2) | (18.3) | (18.3) | (18.5) | (−5.5, 2.5)  |
| Carbohydrate, g/day             | 243.3  | 233.1  | 232.5  | 228.2  | 226.3  | −15.0         | 238.8  | 231.3  | 238.4  | 224.4  | 236.1  | −14.3         | 0.93   |
| (58.1)                          | (55.3) | (55.3) | (50.6) | (59.6) | (−25.7, −4.3)|               | (53.5) | (53.3) | (47.1) | (55.4) | (−24.9, −3.8)|       |
| Physical activity               |        |        |        |        |        |               |        |        |        |        |        |               |
| Step counts, steps/day*         | 7,702  | NA     | 9,481  | 1,779  | 10,117 | −110          | 8,488  | NA     | 10,117 | NA     | 1,629  | −288          | 0.73   |
| (3,302)                         | NA     | (3,909)| (1,175,2,383)|            | (4,460) | (1,023,2,235)|       | NA     | (1,023,2,235)|       |
| MVPA, min/day*                  | 66.9   | NA     | 86.1   | 19.3   | 70.3   | −110          | 70.3   | 137.4  | 85.4   | NA     | 15.1   | −24.3         | 0.29   |
| (32.6)                          | NA     | (40.2) | (14.1, 24.4)|            | (47.8)  | (9.1, 21.0)   |       | NA     | (47.8)  |       |

Notes: Data at weeks 0, 4, 8, 12, and 16 are presented as mean (standard deviation). Changes from baseline to week 12 are presented as mean (95% confidence interval). P-value is a result of unpaired t-test for 12-week change between groups. *Eligible data were available for 192 participants (95 and 97 in the A-mix and the placebo groups, respectively).

Abbreviations: MVPA, moderate-to-vigorous physical activity; NA, not assessed.
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Table 4 Stratified analyses: participants whose step counts increased by 1,000 steps or more (n=109) and increased by fewer than 1,000 steps (n=83) during the 12-week intervention period

| Participants whose step counts increased by more than 1,000 steps in 12 weeks | Week 0 | Week 12 | 12-week change | Week 0 | Week 12 | 12-week change | P-value |
|---|---|---|---|---|---|---|---|
| A-mix group (n=61) | | | | | | | |
| Abdominal total fat area, cm² | 330.1 (79.1) | 319.5 (69.4) | −7.6 (−15.4, −0.3) | 304.6 (80.2) | 311.1 (69.8) | −6.5 (−15.4, −0.4) | 0.019 |
| Abdominal subcutaneous fat area, cm² | 211.7 (68.8) | 219.2 (68.7) | −7.5 (−15.4, −0.4) | 217.7 (68.8) | 218.6 (67.9) | −0.6 (−15.4, −0.4) | 0.011 |
| Abdominal visceral fat area, cm² | 98.8 (31.4) | 100.3 (38.0) | 1.5 (−15.4, −0.4) | 86.9 (34.2) | 92.5 (39.0) | −5.7 (−15.4, −0.4) | 0.23 |
| Weight, kg | 74.6 (9.0) | 74.7 (9.0) | 0.1 (−15.4, −0.4) | 72.9 (8.7) | 72.5 (7.9) | −0.4 (−15.4, −0.4) | 0.51 |
| Body mass index, kg/m² | 93.9 (5.1) | 94.1 (5.1) | 0.2 (−15.4, −0.4) | 92.7 (5.1) | 92.7 (5.1) | 0.0 (−15.4, −0.4) | 0.97 |
| Hip circumference, cm | 100.2 (4.7) | 99.8 (3.8) | −0.4 (−15.4, −0.4) | 99.3 (4.2) | 99.2 (3.8) | −0.6 (−15.4, −0.4) | 0.42 |
| Waist to hip ratio | 0.94 (0.04) | 0.94 (0.04) | 0.0 (−15.4, −0.4) | 0.93 (0.05) | 0.94 (0.04) | −0.1 (−15.4, −0.4) | 0.57 |
| Placebo group (n=48) | | | | | | | |

| Participants whose step counts increased by fewer than 1,000 steps in 12 weeks | Week 0 | Week 12 | 12-week change | Week 0 | Week 12 | 12-week change | P-value |
|---|---|---|---|---|---|---|---|
| A-mix group (n=34) | | | | | | | |
| Abdominal total fat area, cm² | 354.4 (60.8) | 332.5 (71.8) | −21.9 (−15.4, −0.4) | 348.9 (59.1) | 323.8 (74.3) | −25.1 (−15.4, −0.4) | 0.60 |
| Abdominal subcutaneous fat area, cm² | 270.1 (54.4) | 236.9 (71.1) | −33.2 (−15.4, −0.4) | 269.7 (52.9) | 233.9 (71.4) | −35.8 (−15.4, −0.4) | 0.64 |
| Abdominal visceral fat area, cm² | 84.2 (30.9) | 95.6 (40.9) | −11.4 (−15.4, −0.4) | 79.2 (33.6) | 89.9 (39.6) | −10.7 (−15.4, −0.4) | 0.80 |
| Weight, kg | 71.8 (8.7) | 72.7 (7.6) | 0.9 (−15.4, −0.4) | 71.5 (8.9) | 71.7 (7.6) | −0.2 (−15.4, −0.4) | 0.17 |
| Body mass index, kg/m² | 27.3 (1.3) | 27.2 (1.4) | −0.1 (−15.4, −0.4) | 27.1 (1.3) | 26.9 (1.5) | −0.2 (−15.4, −0.4) | 0.21 |
| Percent fat mass, % | 34.4 (6.6) | 32.3 (7.0) | −2.1 (−15.4, −0.4) | 33.8 (6.6) | 31.2 (7.2) | −2.6 (−15.4, −0.4) | 0.24 |
| Waist circumference, cm | 94.0 (4.2) | 93.9 (5.8) | 0.1 (−15.4, −0.4) | 93.4 (4.4) | 92.8 (6.0) | −0.6 (−15.4, −0.4) | 0.24 |
| Hip circumference, cm | 101.8 (3.4) | 100.4 (3.5) | −1.4 (−15.4, −0.4) | 101.2 (3.4) | 99.9 (3.6) | −1.3 (−15.4, −0.4) | 0.78 |
| Waist to hip ratio | 0.92 (0.04) | 0.94 (0.04) | 0.0 (−15.4, −0.4) | 0.92 (0.04) | 0.93 (0.05) | −0.01 (−15.4, −0.4) | 0.25 |
| Placebo group (n=49) | | | | | | | |

Notes: Data at weeks 0 and 12 are presented as mean (standard deviation). Changes from baseline to week 12 are presented as mean (95% confidence interval). P-value is a result of unpaired t-test for 12-week change between groups.

The trial conducted in this study has some important strengths. First, this trial had a double-blind, placebo-controlled, and parallel-group randomized research design. Therefore, the findings are highly reliable. Second, the abdominal fat areas, as primary outcomes, were measured by CT, which is considered to provide the highest quality assessment of abdominal fat. Third, the study included the promotion of physical activity by approximately 1,000 daily steps. This type of mild intervention, as opposed to an entirely regulated exercise program, can be easily adopted by the general population. Finally, the retention rate of the study was almost perfect (99%).

There are also a few limitations of the study. First, we targeted overweight adults (25 kg/m²≤BMI<30 kg/m²). Therefore, the effect of A-mix combined with physical activity promotion for obese adults (BMI≥30 kg/m²) was not investigated. Second, the changes in compliance outcomes, such as total and macronutrient intake, were different between the two groups; however, these between-group differences were not significant. Moreover, the energy intake of the test beverage is not included in this result. If it had been included, the difference of energy intake between the two groups would have been smaller.

Conclusion

The combination of A-mix supplementation and physical activity promotion facilitated abdominal fat reduction in overweight adults.
Author contributions
Study concept and design: KU, HS, CS, SI, and YN. Intervention: TT and YN. Statistical analysis: NS. Study physician: SS. Safety committee: HK. Interpretation: KU, HS, SS, and YN. Writing the first draft: KU. Overall supervision as a principal investigator: YN. All authors contributed toward revising the paper and agree to be accountable for all aspects of the work.

Disclosure
KU, CS, and SI are employees of Meiji Co., Ltd. The other authors HS, TT, HK, NS, SS, and YN report no conflicts of interest in this work.

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