*Original Article*

# Metabolic Changes Induced by Dapagliflozin, an SGLT2 Inhibitor, in Japanese Patients with Type 2 Diabetes Treated by Oral Anti-diabetic Agents: A Randomized, Clinical Trial

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## ABSTRACT

**Aim:** We aimed to determine whether SGLT2 inhibitor dapagliflozin treatment affects body composition and amino acid (AA) metabolism.

**Methods:** Fifty-two overweight patients treated by oral antidiabetic agents were randomly assigned to dapagliflozin (Dapa) or a standard treatment (Con) and followed for 24 weeks. The primary outcome was the change in body mass (BM) between baseline and week 24. Body composition, intrahepatic triglyceride (IHTG) content, and plasma AA concentrations were examined as secondary outcomes.

**Results:** The change in BM was significantly larger in the Dapa than in the Con group, with a difference in the mean change of −1.72 kg (95%CI: −2.85, −0.59; $P = 0.004$) between the groups. Total fat mass was reduced by dapagliflozin treatment, but fat-free mass was maintained. IHTG content was significantly reduced in the Dapa than in the Con ($P = 0.033$). Changes in AAs showed small differences between the groups, but only serine concentrations were significantly reduced in the Dapa. Intra-group analysis showed that positive associations were observed between changes in branched chain AA concentrations and body composition only in the Dapa.

**Conclusions:** Dapagliflozin treatment causes a reduction in BM mainly by reducing fat mass. AA metabolism shows subtle changes with dapagliflozin treatment.



**Keywords**: sodium glucose cotransport, fat mass, muscle mass, non-alcoholic fatty liver disease, nuclear magnetic resonance spectroscopy, amino acid


**Abbreviations**:

| | |
|---|---|
| BM | body mass |
| TBM | total body mass |

| | | |
|---|---|---|
| 26 | IHTG | intrahepatic triglyceride |
| 27 | SGLT | sodium-dependent glucose cotransporter |
| 28 | MRI | magnetic resonance imaging |
| 29 | MRS | magnetic resonance spectroscopy |
| 30 | NAFLD | non-alcoholic fatty liver disease |
| 31 | DEXA | dual energy X-ray absorptiometry |
| 32 | BIA | bioelectrical impedance analysis |
| 33 | OAD | oral anti-diabetic drugs |
| 34 | BCAA | branched chain amino acid |

## 1. Introduction

Sodium-dependent glucose cotransporter 2 (SGLT2) inhibitors improve glycemic control and induce a reduction in body mass (BM)[1, 2]. Previous studies have shown a reduction in BM of approximately 2 kg after 24 weeks of administration of SGLT2 inhibitors[3]. This reduction in BM is thought to be the result of a reduction in fat and lean mass[4]. The metabolic changes induced by SGLT2 inhibitors include fat oxidation due to a lack of glucose, especially in the fasting state[5]. As a result, ketone body formation has been observed in multiple clinical studies[6, 7]. Such an adaptation is partially explained by glucagon[8] and fibroblast growth factor-21[9-11].

Hepatic steatosis, which is characterized by the accumulation of intrahepatic triglyceride (IHTG) content, is commonly found in patients with type 2 diabetes [12]. Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver steatosis, and is a risk factor for liver cirrhosis and subsequent hepatic carcinoma in later life. Recently, studies of SGLT2 inhibitors have shown that they have a beneficial effect on NAFLD [13], but limited information is available for quantitative evaluation of IHTG content.

A reduction in skeletal muscle mass by SGLT2 inhibitors might have a negative effect in older or lean patients with type 2 diabetes. The effects of SGLT2 inhibitor treatment on muscle mass vary. Some reports have shown a decrease[14-16] and others have shown no change[17] in skeletal muscle mass with SGLT2 inhibitor treatment. We previously reported that segmental muscle mass in the arms was significantly decreased by ipragliflozin in patients with type 2 diabetes under treatment with insulin [18]. The mechanisms underlying the diverse effects of SGLT2 inhibitor on skeletal mass are unclear. Caloric restriction acutely increases plasma amino acid concentrations[19] and stimulates amino acid oxidation[20]. A possible mechanism for this finding is that protein catabolism is similar to

fat oxidation by SGLT2 inhibitors [21]. However, little is known regarding how amino acid metabolism changes with SGLT2 inhibitor administration.

Therefore, in this study, we determined the effects of dapagliflozin on BM, body composition, and IHTG content in Japanese patients with type 2 diabetes who were treated with oral anti-diabetic agents. We also investigated how amino acid metabolism is affected by administration of dapagliflozin and its relation to changes in body composition.

## 2. Methods

We enrolled patients in a randomized, 24-week, open-label, parallel-group, comparative, clinical trial at Shiga University of Medical Science Hospital, Shiga, Japan, between February 2016 and June 2017. The final date of follow-up was 20 January 2018. This trial was registered with the UMIN Clinical Trials Registry (000020239) and the International Clinical Trials Registry Platform (jRCTs#051180018).

### *2.1 Participants*

The participants were outpatients with type 2 diabetes aged 20–75 years who had glycated hemoglobin (HbA1c) values of 7.0%–10.0% (53–85 mmol/mol) and a BM index ≥ 23 kg/m$^2$. At the time of enrollment, they were all being treated with oral anti-diabetic agents other than SGLT2 inhibitors and had estimated glomerular filtration rates of > 45 mL/min/1.73 m$^2$. The exclusion criteria were as follows: treatment with an SGLT2 inhibitor or a loop diuretic; any contraindication for the use of dapagliflozin; severe ketosis; diabetic coma or precoma; a history of hospitalization within the preceding 6 months for trauma, surgery, or infectious disease; and a history of cerebral infarction, transient ischemic attack, or orthostatic hypotension. The inclusion and exclusion criteria were amended on 15 July 2016 to increase the maximum age from 70 to 75 years. Additionally, patients with a history

of unstable angina or myocardial infarction were not excluded after 15 July 2016, as long as they had been stable for the preceding 6 months. The nature and potential risks of the study were explained to all of the participants, and their written informed consent was obtained. The study was performed in accordance with the principles of the Declaration of Helsinki. The original protocol was approved by the Ethics Committee of Shiga University of Medical Science on 29 September 2015, and the amended protocol was approved on 15 July 2016 (R2015-086).

*2.2 Study design*

After confirming that each patient satisfied the inclusion criteria and did not have any of the exclusion criteria, the principal investigator registered the patients in the research registry. Eligible patients were randomized to either the dapagliflozin (Dapa) or control groups at a 1:1 ratio, and the patients' sex was used as a stratification factor. A web-based, password-protected, randomization system based on a computer-generated random sequence was used. Patient randomization was performed by the clinical research development office, which was independent of the investigators.

The participants in the Dapa group took 5 mg of dapagliflozin orally once daily after breakfast for 24 weeks, and no dose adjustments were made during the trial. To prevent hypoglycemia at the initiation of dapagliflozin administration, the patients' sulfonylurea dose was reduced in accordance with the treatment guidelines as follows: for patients taking > 2 mg/day of glimepiride, the dose was reduced to ≤ 2 mg/day; for patients taking > 1.25 mg/day of glibenclamide, the dose was reduced to ≤ 1.25 mg/day; and for patients taking > 40 mg/day of gliclazide, the dose was reduced to ≤ 40 mg/day. Patients in the control group continued their previous treatments and were allowed to add anti-diabetic agents other than SGLT2 inhibitors during the 24-week study period. In both groups, the treatment target was

an HbA1c value < 7.0% without hypoglycemia. Detailed protocols for the treatment of patients with hypoglycemia or hyperglycemia are provided in Appendix S1.

The primary outcome was the change in total BM (TBM) between baseline and 24 weeks. The secondary outcomes were as follows: changes in body fat mass and fat-free mass changes in bone mineral density as measured using dual energy X-ray absorptiometry (DEXA); changes in subcutaneous fat mass at the umbilicus, visceral fat mass at the umbilicus, liver volume, and iliopsoas surface area as measured by magnetic resonance imaging (MRI); changes in IHTG content as measured by $^{1}$H-magnetic resonance spectroscopy (MRS); and changes in fat mass and lean body mass as measured by bioelectrical impedance analysis (BIA). We also evaluated the changes in serum aspartate aminotransferase, alanine aminotransferase, HbA1c, fasting plasma glucose, insulin, ferritin, ketone body, triglyceride, high-density lipoprotein, low-density lipoprotein, and remnant-like particle-cholesterol concentrations in the two groups. We measured 36 amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, tryptophan, lysine, asparagine, aspartic acid, serine, glutamic acid, glutamine, proline, glycine, alanine, tyrosine, arginine, cystine, taurine, citrulline, ornithine, α-amino butyric acid, sarcosine, α-amino adipic acid, cystathionine, γ-amino β-hydroxybutyric acid, γ-amino butyric acid, homocysteine, hydroxylysine, hydroxyproline, β-alanine, β-aminoisobutyric acid, 3-methylhistidine, and 1-methylhistidine) and changes in blood concentrations of total, essential, non-essential, and branched-chain amino acids (BCAAs) in both groups. Safety-related variables included hypoglycemic episodes, standard laboratory analysis findings, physical examinations, and vital signs. Each variable was measured after 0, 2, 8, 16, and 24 weeks of treatment, except for remnant-like particle-cholesterol, DEXA and MRI variables, and amino acids, which were measured after 0 and 24 weeks of treatment.

*2.3 Body mass and composition*

The BM and composition of each participant were measured using BIA (MC-780A; Tanita, Japan), and the participants wore hospital gowns. Measurements were performed after 0, 2, 8, 16, and 24 weeks of treatment after an 8- to 16-h fast. Segmental body composition values were calculated by multiple electrodes with different current frequencies. Fat and muscle mass in the arms, lower limbs, and trunk were determined. DEXA (GE Healthcare, Madison, WI, USA) was also performed after 0 and 24 weeks of treatment to determine whole-body and segmental body composition.

*2.4 MRI and MRS*

Subcutaneous and visceral fat areas at the level of the umbilicus, and the iliopsoas surface area were measured after 0 and 24 weeks of treatment using an MR scanner (Signa Excite HDxt 3.0T; GE Healthcare Japan, Tokyo, Japan). This examination was only performed in subjects who provided additional consent. Participants with an extreme BM, claustrophobia, pacemaker, or any metal implant with the potential to cause damage in a 3-T MR scanner were excluded from this additional assessment for safety reasons. A three-dimensional workstation (Aquarius iNtuition; TeraRecon Inc., Foster City, CA, USA) was used with a semi-automatic segmentation tool. IHTG content was quantified in a 3-$cm^3$ voxel in the posterior segment of the right lobe of the liver using point resolved spectroscopy, as previously described [22]. Briefly, nine lipid signals (Lip9, 13, 16, 21, 23, 28, 41, 43, and 52&53) were identified in the human liver $^1$H-MRS spectra using the LC-model (Version 6.3-1B, Stephen Provencher, Oakville, ON, Canada). Peak assignments were made on the basis of published data [23]. The lipid mass was calculated using Eq. (1):

$$\text{Lipid mass} = 1/2 \times (\text{Lip13} + 16 + 21 + 23 + 28 + 41 + 43) \times 14 + (\text{Lip52\&53}) \times 13$$

159 + 1/3 × (Lip09) × 15

160 + 1/3 × (Lip09) × 12

161 + 2/3 × (Lip09) × 16 (1)

162 IHTG (%) was calculated relative to water mass using Eq. (2):

163 IHTG = lipid mass / (lipid mass + 1/2 × *I* water × 18) × 100 (2)

164 All of the tests were performed after fasting on separate dates within 2 weeks of the 0-and 24-
165 week time points. Unless an emergency occurred, the results were not revealed to
166 investigators until the database had been locked.

167

168 *2.5 Amino acid analysis*

169 The amino acid profile was evaluated in overnight fasting samples using liquid
170 chromatography-mass spectrometry at 0 and 24 weeks. A 5-mL blood sample was collected
171 in a blood collection tube containing EDTA-2Na. The collected samples were promptly
172 centrifuged at 4°C, and the plasma was frozen at −80°C. Amino acid measurements were
173 performed by SRL Inc. (Tokyo, Japan).

174

175 *2.6 Statistical analysis*

176 We calculated the required sample size on the basis of the results of a study
177 performed in Europe, which showed that the difference in BM change between the Dapa and
178 placebo groups was 2.08 kg [4]. The standard deviation (SD) of the BM change was 2.51 kg
179 in the Dapa group and 2.53 kg in the placebo group. Therefore, we calculated that 24 patients
180 in each group were required for detecting a difference between the groups (power: 80%, two-
181 sided $P = 0.05$). We assumed an attrition rate of 5%; therefore, 26 patients were required in
182 each group.

183 Statistical analyses were performed using SAS (ver. 9.4; SAS Institute Inc., Cary, NC,

USA) on an intention-to-treat basis. Data are expressed as mean ± SD for continuous variables and n (%) for categorical variables. Differences in the baseline characteristics between the groups were evaluated using Student's *t*-test for continuous variables and Pearson's chi-squared test for categorical variables. The primary outcome, a change in TBM between baseline and 24 weeks, was compared between the groups using sex-adjusted analysis of covariance. The secondary outcomes were analyzed with sex-adjusted analysis of covariance or Wilcoxon's rank sum test, depending on the distribution of the variables. Changes in body weight, body composition, and ketone bodies were analyzed with a mixed model to consider multiple time points. Pearson's correlation was used to correlate changes in amino acids with changes in body composition. Subgroup analyses were performed for the change in BM according to baseline age, sex, BMI, HbA1c, fat mass by DEXA, lean mass by DEXA; with or without metformin, and with or without DDP4 inhibitor.

To maintain data independence, the data analysis was performed after the database was locked by two statistical analysts and an outsourcing company. $P < 0.05$ was considered to represent statistical significance.

## 3. Results

### *3.1 Baseline clinical characteristics of the study participants*

Eighty-one eligible patients with type 2 diabetes mellitus were screened at Shiga University of Medical Science Hospital between February 2016 and June 2017, and 52 were enrolled in the present study. Figure 1 shows the study flow chart and CONSORT diagram. The participants were randomly assigned to the Dapa group (n = 26) or the control group (n = 26). Fifty patients completed the study (Dapa group, n = 26; control group, n = 24). Two patients in the control group withdrew their consent before commencing the study protocol because of a personal reason and because of the diagnosis of an incidental pancreatic tumor.

Another patient in the Dapa group discontinued the intervention because of exanthema and hypoglycemia. One patient in the control group discontinued standard treatment because she was required to use a steroid and insulin for pemphigoid, but she was followed up and underwent BM and body composition measurements. Therefore, data from 50 patients (Dapa, n = 26; control, n = 24) were included in the intention-to-treat analysis. IHTG content was measured using $^{1}$H-MRS in 43 patients (Dapa, n = 23; control, n = 20) as a secondary outcome. Table 1 shows the demographic and baseline characteristics of the study participants. The baseline characteristics were not significantly different between the groups, except for thiazolidinedione use and the prevalence of hypertension.

*3.2 Primary outcome*

Patients in the Dapa group showed a greater reduction in TBM than those in the control group. The mean change in TBM from baseline to 24 weeks was −2.4 ± 2.13 kg in the Dapa group and −0.68 ± 1.75 kg in the control group. Therefore, there was a difference in the reduction in TBM −1.72 kg ($P$ = 0.004, 95% confidence interval [CI] −2.85 to −0.59) between the groups (Table 2). The difference in TBM became significant from 2 weeks ($P$ = 0.002) after initiating Dapa treatment (Supplemental Figure S1).

**Table 1. Demographic and baseline characteristics of the participants**

| | **Total** | **Control (OAD)** | **Dapa group (OAD+Dapagliflozin 5 mg)** | ***P*-value** |
|---|---|---|---|---|
| Number of patients | 50 | 24 | 26 | |
| Age (years) | 60.9 ± 9.7 | 62.3 ± 6.5 | 59.7 ± 12.0 | 0.984 |
| Gender (male/female) | 32/18 | 16/8 | 16/10 | 0.706 |
| Height (cm) | 163.1 ± 8.3 | 163.2 ± 8.5 | 163.0 ± 8.3 | 0.861 |
| Body weight (kg) | 74.1 ± 13.2 | 73.6 ± 12.5 | 74.6 ± 14.1 | 0.778 |
| Body Mass Index (kg/m$^2$) | 27.8 ± 3.9 | 27.6 ± 3.8 | 28.0 ± 4.0 | 0.708 |
| Waist circumstance (cm) | 97.2 ± 10.6 | 97.3 ± 12.5 | 97.0 ± 8.8 | 0.683 |
| Systolic BP (mmHg) | 140.8 ± 14.8 | 140.0 ± 13.6 | 141.6 ± 15.9 | 0.888 |
| Diastolic BP (mmHg) | 82.2 ± 10.4 | 80.5 ± 11.6 | 83.7 ± 9.2 | 0.262 |
| HbA1c (mmol/mol, %) | 60.70 ± 5.59<br>7.70 ± 0.51 | 60.98 ± 5.46<br>7.73 ± 0.50 | 60.45 ± 5.82<br>7.68 ± 0.53 | 0.592 |
| eGFR (mL/min/1.73 m$^2$) | 80.3 ± 21.0 | 79.2 ± 17.0 | 81.3 ± 24.5 | 0.946 |
| Duration of type 2 diabetes mellitus (years) | 12.5 ± 8.1 | 13.3 ± 8.1 | 11.8 ± 8.1 | 0.502 |

| | | | | |
|---|---|---|---|---|
| Diabetes-related disease [n(%)] | | | | |
| Retinopathy | 10(18.0) | 3(12.5) | 7(26.9) | 0.638 |
| Nephropathy | 21(32.5) | 10(41.7) | 11(42.3) | 0.374 |
| Neuropathy | 9(18.0) | 2(8.3) | 7(26.9) | 0.091 |
| Medications, [n(%)] | | | | |
| Metformin | 39 (78.0) | 18 (75.0) | 21 (80.8) | 0.623 |
| DPP-4 inhibitor | 37 (74.0) | 18 (75.0) | 19 (73.1) | 0.877 |
| Sulfonylurea | 28 (56.0) | 13 (54.2) | 15 (57.7) | 0.802 |
| Thiazolidinedione | 18 (36.0) | 12 (50.0) | 6 (23.1) | 0.048 |
| α-GI | 10 (20.0) | 5 (20.8) | 5 (19.2) | 0.887 |
| Glinide | 2 (4.0) | 1 (4.2) | 1 (3.8) | 0.954 |
| Anti-hypertensive medication | 29(58.0) | 17(70.8) | 12(46.2) | 0.077 |
| Anti-hyperlipidemic medication | 30(62.5) | 14(60.9) | 16(64.0) | 0.823 |
| Hypertension [n(%)] | 28(56.0) | 17(70.8) | 11(42.3) | 0.042 |
| Dyslipidemia [n(%)] | 36(72.0) | 18(75.0) | 18(69.2) | 0.650 |
| Smoking [n(%)] | | | | |
| Never | 22(44.0) | 12(50.0) | 10(38.5) | 0.714 |
| Former | 16(32.0) | 7(29.2) | 9(34.6) | |
| Current | 12(24.0) | 5(20.8) | 7(26.9) | |
| Current drinker [n(%)] | 30(60.0) | 16(66.7) | 14(53.8) | 0.355 |

Values are mean ± SD for continuous variables. The *P* values indicate the difference between the Dapa group and the control group using the unpaired *t*-test or the chi-square test. Except for HbA1c, all of the data were obtained at enrollment. HbA1c values were measured at baseline. OAD: oral anti-diabetic agent, BP: blood pressure, eGFR: estimated glomerular filtration rate, DPP-4: dipeptidyl peptidase-4, α-GI: alpha glucosidase inhibitor.

227 *3.3 Secondary outcomes*

228 Body composition was evaluated using DEXA. The change in fat mass was significantly
229 different between the groups (control group, −0.70 *vs*. Dapa group, −2.32 kg, $P < 0.01$), but
230 the change in fat-free mass was not (control group, −0.17 *vs*. Dapa group, −0.38 kg, $P =$
231 0.516). Approximately 88% of the change in TBM was due to a reduction in fat mass
232 identified using DEXA (Supplemental Figure S2). Bone mineral density was similar in both
233 groups after 6 months of the intervention (Table 2). The change in body composition
234 evaluated with BIA was significantly different between the groups (−1.18 kg, 95% CI −2.22
235 to −0.14, $P < 0.05$), similar to that in fat mass. The fat-free mass value obtained using DEXA
236 included muscle, organs, and body water, whereas that obtained using BIA approximated
237 skeletal muscle mass. Nevertheless, the change in whole-body skeletal mass measured using
238 BIA also did not differ between the groups (−0.54 kg, 95% CI −1.41 to 0.33, $P = 0.220$).
239 Approximately 90% of the change in TBM could be attributed to the reduction in fat mass
240 identified using BIA. Segmental skeletal mass in the legs was significantly reduced after 2
241 weeks of dapagliflozin treatment ($P = 0.002$), but only tended to be reduced after 24 weeks in
242 the Dapa group with a mixed model (Supplemental Figures S3, S4). The change in HbA1c
243 values was similar in the Dapa and control groups (control group, −4.04 ± 9.29 *vs*. Dapa
244 group, −4.01 ± 7.54 mmol/mol, respectively; $P = 0.984$). Liver enzyme activity tended to
245 decrease more in the Dapa group than in the control group, but this difference was not
246 significant. Ketone bodies were significantly higher in the Dapa group than in the control
247 group at 2 weeks ($P = 0.024$) with the mixed model (Supplemental Figure S5). Other plasma
248 parameters were comparable between the groups (Table 2). The adverse events identified
249 during the study are shown in Supplemental Table S1. Hypoglycemia, dehydration, urinary
250 tract infection, and exanthema occurred in both groups.
251

252 *3.3 Magnetic resonance imaging measurements and intrahepatic triglyceride content*

253 To evaluate the areas of visceral fat, subcutaneous fat, and iliopsoas muscle, MRI
254 was performed in a subset of the participants (43/50) (Table 2). The change in the
255 subcutaneous fat area was significantly different between the groups (−23.0 $cm^2$, 95% CI
256 −57.4 to −11.5, $P = 0.046$), although there was no significant difference in the visceral fat and
257 iliopsoas muscle areas. The change in IHTG content was significantly different between the
258 groups after 24 weeks of treatment (−6.8%, 95% CI −14.2 to 0.7, $P = 0.033$) (Table 2).

259

260 *3.4 Subgroup analysis results*

261 Subgroup analysis of the effect of dapagliflozin on BM loss showed no significant
262 effects of baseline values including sex ($P = 0.39$), body mass ($P = 0.45$), HbA1c ($P = 0.90$),
263 BMI ($P = 0.56$), fat mass by DEXA ($P = 0.81$), lean mass ($P = 0.071$), or metformin use ($P =$
264 0. 91) (Figure 2). In patients who were taking a DPP-4 inhibitor, the difference in the BM
265 change between the DAPA and control groups was −2.52 kg (95% CI −3.77 to −1.26 kg;
266 n=19 and 18, respectively; $P < 0.001$), with a significant decrease in the DAPA group. In
267 patients who were not taking a DPP-4 inhibitor, the difference in BM change between the
268 DAPA and control groups was 0.05 kg (95% CI −2.63 to 2.73 kg; n = 7 and 6, respectively; $P$
269 = 0.966). The use of dapagliflozin, with or without a DPP-4 inhibitor, resulted in a significant
270 difference in BM change ($P = 0.021$) (Figure 2).

**Table 2. Changes in parameters between baseline and week 24 in each treatment group**

| | Control group (OAD) | | | Dapa group (OAD+Dapagliflozin 5 mg) | | | Difference in changes (95% CI) | *P* value |
|---|---|---|---|---|---|---|---|---|
| | Baseline | Week 24 | Change | Baseline | Week 24 | Change | | |
| Body Weight (kg) | 72.90 ± 12.71 | 72.22 ± 12.80 | -0.68 ± 1.75 | 73.92 ± 13.91 | 71.52 ± 14.16 | -2.40 ± 2.13 | -1.72 (-2.85, -0.59) | 0.004 |
| DEXA | | | | | | | | |
| Fat mass (kg) | 24.72 ± 8.14 | 24.03 ± 7.90 | -0.70 ± 2.02 | 25.43 ± 7.70 | 23.12 ± 8.28 | -2.32 ± 1.95 | -1.60 (-2.80, -0.41) | 0.009 |
| Fat-free mass (kg) | 47.71 ± 6.93 | 47.33 ± 7.29 | -0.38 ± 1.59 | 45.86 ± 9.16 | 45.69 ± 9.12 | -0.17 ± 1.17 | 0.27 (-0.55, 1.08) | 0.516 |
| Bone mineral density ($g/cm^2$) | 0.9961 ± 0.1437 | 0.9946 ± 0.1456 | -0.0015 ± 0.0454 | 1.0249 ± 0.1965 | 1.0236 ± 0.1948 | -0.0013 ± 0.0142 | -0.0017 (-0.021, 0.0177) | 0.864 |
| BIA | | | | | | | | |
| Fat mass (kg) | 21.33 ± 7.97 | 20.80 ± 7.55 | -0.53 ± 2.09 | 23.27 ± 7.96 | 21.54 ± 8.55 | -1.73 ± 1.53 | -1.18 (-2.22, -0.14) | 0.027 |
| Muscle mass (kg) | 48.78 ± 9.10 | 48.66 ± 9.35 | -0.13 ± 1.67 | 47.92 ± 9.45 | 47.28 ± 8.96 | -0.64 ± 1.38 | -0.54 (-1.41, 0.33) | 0.220 |
| MRI | | | | | | | | |
| Navel subcutaneous fat area ($cm^2$) | 311.0 (220.3, 411.5) | 286.0 (230.23, 408.8) | -3.5 (-27.0, 12.0) | 341.0 (240.0, 417.0) | 293.0 (234.0, 351.0) | -35.0 (-68.0, 1.0) | -23.0 (-57.4, 11.5) | 0.046 * |
| Navel visceral fat area ($cm^2$) | 133.0 (99.3, 170.8) | 142.5 (95.5, 167.5) | -4.5 (-19.3, 12.8) | 135.0 (10.40, 157.0) | 112.0 (91.0, 155.0) | -19.0 (-34.0, -2.0) | -12.3 (-31.1, 6.5) | 0.113 * |
| Iliopsoas muscle surface area ($cm^2$) | 23.0 (18.5, 27.5) | 23.0 (17.25, 28.75) | -1.0 (-2.0, 0.0) | 24.0 (20.0, 26.0) | 22.0 (18.0, 26.0) | -1.0 (-2.0, -0.0) | 0.5 (-1.0, 2.0) | 0.602 * |
| HbA1c (mmol/mol, %) | 60.98 ± 5.46<br>7.73 ± 0.50 | 56.97 ± 7.74<br>7.36 ± 0.71 | -4.01 ± 7.54<br>-0.37 ± 0.69 | 60.45 ± 5.82<br>7.68 ± 0.53 | 56.42 ± 6.94<br>7.31 ± 0.64 | -4.04 ± 9.29<br>-0.37 ± 0.85 | -0.05 (-4.95, 4.85)<br>-0.00 (-0.45, 0.44) | 0.984 |
| FPG (mmol/L) | 8.06 ± 1.51 | 7.94 ± 2.03 | -0.12 ± 1.56 | 7.98 ± 1.55 | 7.54 ± 1.30 | -0.45 ± 1.77 | -0.32 (-1.28, 0.65) | 0.511 |
| Total cholesterol (mmol/L) | 4.59 ± 0.91 | 4.44 ± 0.72 | -0.15 ± 0.55 | 4.78 ± 0.78 | 4.88 ± 0.81 | 0.10 ± 0.46 | 0.24 (-0.05, 0.53) | 0.097 |
| LDL cholesterol (mmol/L) | 2.62 ± 0.78 | 2.41 ± 0.74 | -0.21 ± 0.46 | 2.69 ± 0.74 | 2.70 ± 0.72 | 0.01 ± 0.34 | 0.22 (-0.01, 0.45) | 0.063 |
| HDL cholesterol (mmol/L) | 1.32 ± 0.29 | 1.37 ± 0.31 | 0.06 ± 0.22 | 1.33 ± 0.31 | 1.42 ± 0.37 | 0.09 ± 0.15 | 0.03 (-0.07, 0.13) | 0.558 |
| Triacylglycerol (mmol/L) | 1.18 ± 0.45 | 1.23 ± 0.48 | 0.05 ± 0.46 | 1.53 ± 0.82 | 1.43 ± 1.00 | -0.10 ± 0.59 | -0.15 (-0.46, 0.15) | 0.325 |
| RLP cholesterol (mmol/L) | 0.11 ± 0.05 | 0.11 ± 0.07 | 0.002 ± 0.060 | 0.15 ± 0.11 | 0.15 ± 0.15 | -0.001 ± 0.089 | -0.004 (-0.048, 0.040) | 0.857 |
| AST (units/L) | 27.6 ± 18.4 | 27.3 ± 14.7 | -0.3 ± 18.9 | 24.7 ± 10.4 | 22.7 ± 9.2 | -2.0 ± 7.8 | -1.9 (-10.0, 6.2) | 0.638 |
| ALT (units/L) | 31.9 ± 23.5 | 32.0 ± 25.3 | 0.1 ± 16.6 | 33.8 ± 22.7 | 27.7 ± 18.3 | -6.1 ± 14.9 | -6.5 (-15.4, 2.4) | 0.147 |
| G-GTP (units/L) | 42.0 ± 34.3 | 49.2 ± 65.7 | 7.1 ± 65.6 | 52.2 ± 65.1 | 42.9 ± 48.5 | -9.3 ± 23.0 | -17.7 (-44.7, 9.3) | 0.193 |
| Ferritin (ng/mL) | 319 ± 419 | 224 ± 226 | -75 ± 273 | 287 ± 242 | 205 ± 185 | -81 ± 107 | -7 (-125, 110) | 0.900 |
| Liver volume (mL) | 1497 (1335, 1723) | 1452 (1239, 1670) | -29 (-118, 5) | 1578 (1416, 1771) | 1528 (1395, 1670) | -72 (-113, 67) | 60 (-52, 173) | 0.961 * |
| IHTG (%) | 19.9 (11.3, 31.0) | 18.4 (10.7, 33.1) | -1.55 (-3.80, 9.78) | 24.3 (15.9, 37.3) | 17.7 (7.6, 31.4) | -6.3 (-12.5, -1.0) | -6.8 (-14.2, 0.7) | 0.033 * |

Values are mean ± SD for continuous variables at baseline, 24 weeks, and for the change during this period. The least-square mean (95% confidence interval) is shown for the difference in this change between the groups. Except for the MRI and IHTG data, n = 24 for the control group and n = 26 for the Dapa group. For MRI and IHTG data, n = 20 for the control group and n = 22 for the Dapa group. The *P* values indicate the results of comparisons of the changes between the Dapa and control groups using analysis of covariance adjusted for sex. *For MRI and IHTG, the *P* values indicate the results of comparisons of changes between the Dapa and control groups using Wilcoxon's rank sum test. IHTG: intrahepatic triglyceride, DEXA: dual-energy X-ray absorptiometry, BIA: bioelectric impedance analysis.

*3.5 Amino acid metabolism*

Fasting plasma amino acid concentrations were measured at baseline and 24 weeks in this study (Supplemental Table S2). Twelve amino acids (sarcosine, α-aminoadipic acid, cystathionine, γ-amino β-hydroxybutyric acid, γ-aminobutyric acid, homocysteine, hydroxylysine, hydroxyproline, β-alanine, β-aminoisobutyric acid, 3-methylhistidine, 1-methylhistidine) were below the measurement sensitivity for most of the samples. There were no significant differences in amino acids between the two groups at baseline. At 24 weeks of treatment, changes in most plasma amino acids, except for serine, showed no significant differences between the control and Dapa groups (Supplemental Table S2).

*3.6 Correlations between changes in amino acids and body composition*

We further analyzed the correlations between changes in amino acid concentrations and body composition as a post-hoc explorative analysis, but we did not find a significant inter-group difference. Pearson's correlation coefficients were calculated for each group and are shown in a heat map (Figure 3). The change in body weight and changes in valine, isoleucine, leucine, and total BCAAs were positively correlated only in the Dapa group. The change in fat mass measured with DEXA and changes in valine, isoleucine, and total BCAAs were also positively correlated in the Dapa group (Figure 4). The change in fat mass measured with BIA and changes in valine and total BCAAs were positively correlated in the Dapa group. Additionally, the change in skeletal muscle mass measured with BIA and changes in valine and total BCAAs were positively correlated. However, no correlations were observed in the control group (Figure 4).

## 4. Discussion

There were three major findings in the present study. First, we showed that the addition of dapagliflozin significantly reduced BM and fat mass in patients with type 2 diabetes receiving oral anti-diabetic agents. Second, skeletal muscle mass showed no significant reduction after treatment with dapagliflozin for 24 weeks, except for an acute reduction in the legs at 2 weeks. Third, changes in the plasma amino acid profile were subtle for most of the amino acids after treatment with dapagliflozin for 24 weeks.

Dapagliflozin add-on treatment significantly reduced BM in patients with type 2 diabetes taking oral anti-diabetic drugs. The difference in TBM loss between the Dapa and control groups was −1.72 kg (95% CI −2.85 to −0.59) (Table 2), which is consistent with the results of previous studies that showed a reduction in BM in patients with type 2 diabetes who were treated with SGLT2 inhibitors[1-3]. Dapagliflozin treatment preferentially reduced body fat, with no significant reduction in whole-body muscle mass after 24 weeks of treatment (Table 2). Previous studies that used DEXA also showed that a reduction in BM resulting from SGLT2 inhibitor treatment was mostly caused by a loss of fat mass, which accounted for approximately 70% of the weight loss achieved in Caucasian patients [4, 24]. However, in the present study, 88% of the reduction in BM was attributed to a reduction in fat mass (Supplemental Figure S2). Because the participants in the present study were relatively old, marginally overweight (BM index: 27.8 ± 3.9 kg/m$^2$), and had a typical Asian body composition, we used several methods to analyze their body composition. Our recent study evaluated the efficacy of ipragliflozin in patients with diabetes on insulin therapy[25]. This previous study showed an 80% reduction in fat mass, but no significant change in lean body mass (DEXA) or muscle mass (BIA), with a significant reduction in muscle mass (BIA) in the arms. In the current study, we found no significant changes in fat-free mass as measured by both DEXA and BIA after 24 weeks of dapagliflozin treatment (Table 2). This

finding is consistent with the area of the iliopsoas muscle measured using MRI (Table 2). Segmental analysis showed that muscle mass (BIA) in the legs was marginally reduced by dapagliflozin treatment at 2 weeks (Supplemental Figure S4). Although the mechanism underling the discrepancy between these two studies is unclear, the background of the subjects may have affected muscle metabolism because subjects in the current study were slightly younger than those in the previous study [25]. Further studies are necessary to confirm long-term effects of SGLT2 inhibitors on skeletal muscle in older or lean subjects.

SGLT2 inhibitors have recently been intensively studied regarding their therapeutic potential for NAFLD and non-alcoholic steatohepatitis[16, 26]. $^{1}$H-MRS has been the gold standard method for the quantification of ectopic fat in the liver and skeletal muscle for longer than 20 years[27-29]. In this study, the Dapa group showed a significantly larger reduction in IHTG content as measured using $^{1}$H-MRS than that in the control group (Table 2). This finding is consistent with that in recent studies that were conducted using the MRI liver proton density fat fraction, which is a newly developed quantitative method for the quantification of IHTG using MRI[30].

In the present study, whole-body fat content, assessed using BIA and DEXA, and intrahepatic TG, assessed using $^{1}$H-MRS, were lower in the Dapa group, but the reduction in visceral fat area at the level of the navel was not statistically significant (Table 2). We believe that this was the result of a technical problem. We measured the area of the subcutaneous and abdominal fat at the level of the navel using MRI instead of the total visceral fat mass using multiple slices. One recent study of the use of a CB-1R inverse agonist showed a correlation between total visceral fat mass and visceral fat area in various cross-sections of the abdomen, as measured using whole-body MRI in 123 obese individuals who lost approximately 7.7 kg [31]. Although a good correlation was obtained between visceral fat area at the level of the navel and total visceral fat mass ($r = 0.850$), there was a poor correlation between the change

in visceral fat area at the level of the navel and the change in total visceral fat mass (r = 0.488). Moreover, in the present study, we only found an approximate 1.72 kg difference between the two groups. Therefore, we believe that we failed to detect a significant change in visceral fat area because of a technical issue.

In the present study, 5 mg of dapagliflozin was chosen instead of 10 mg because this is a common starting dose in Japan. To the best of our knowledge, little is known regarding the dose-response for the effect of SGLT2 inhibitors on body composition. A previous study of the changes in body composition during 26 weeks of treatment with 100 mg or 300 mg of canagliflozin showed that BM, fat mass, and lean mass were significantly lower in both treatment groups than in the placebo group [32]. Although a direct comparison between the 100 mg and 300 mg canagliflozin groups was not made, the data suggest that the 300 mg canagliflozin group tended to experience larger reductions in BM, fat mass, and lean mass than the 100 mg group. On average, in both groups, twice as much fat mass was thought to have been lost than lean mass. Thus, in the present study, we may have underestimated the effect of dapagliflozin on body composition. Further studies are needed to determine the effects of various doses of dapagliflozin on body composition.

We were particularly interested in the changes in amino acid metabolism during intervention because plasma amino acids are changed in subjects under caloric restriction [19] or starvation [34]. In our study, most amino acids, except for serine, were unchanged by the treatment of dapagliflozin at 24 weeks (Supplemental Table S2). We did not see changes in amino acids possibly because BM loss was relatively mild (Table 2). We found a significant increase in plasma ketone bodies, which are a marker for fat oxidation, 2 weeks after dapagliflozin treatment, but this not significant at 24 weeks (Supplemental Figure S5). Follow-up amino acid measurement was only performed at 24 weeks after intervention in this study. Therefore, treatment of dapagliflozin might have had some effect on amino acids at the

acute phase, but had a limited effect on protein catabolism after 24 weeks of intervention. Serine serves as a substrate for gluconeogenesis [34]. SGLT-2 inhibitors increase gluconeogenesis[35]. Therefore, decreased serine concentrations (Supplemental Table S2) may have been due to an increased consumption of serine as a substrate for gluconeogenesis in the Dapa group, although there was no decrease in other glycogenic amino acids such as alanine.

We further examine the correlations between changes in blood amino acid concentrations and BM/body composition in each group to overcome the individual variance of changes in body composition as a post-hoc analysis (Figure 3). We found that the trends were different between the Dapa and control groups. BCAAs were correlated with changes in body weight and fat mass in the Dapa group, but not in the control group. Previous studies have shown that elevated levels of blood BCAAs are associated with obesity and diabetes because of insulin resistance in skeletal muscle [40, 41] and in adipose tissue [42, 43]. Therefore, the positive association between BCAAs and BM in the Dapa group may have been due to improved insulin resistance by dapagliflozin treatment. A recent study in patients with diabetes and cardiovascular disease who were administered 10 mg of empagliflozin for 1 month showed no significant change in plasma BCAAs, but there was an increase in their metabolites[21]. This finding suggests that empagliflozin activates BCAA catabolism. Another recent human study reported increased BCAA secretion into the urine by dapagliflozin treatment, similar to glucosuria [44]. Although we did not have the opportunity to measure amino acid concentrations in urine samples, this phenomenon may explain the positive association of changes in BCAAs and the reduction in body weight in the Dapa group.

This study has three major strengths. First, a comprehensive analysis was made of body composition using DEXA, BIA, and MRI. Second, we provided evidence that SGLT2

inhibitor treatment ameliorated liver steatosis, which was established using $^{1}$H-MRS, in patients with type 2 diabetes. Third, amino acid metabolism was evaluated after SGLT-2 inhibitor treatment.

This study also had some important limitations. First, the number of participants was relatively small, although we calculated the required number of participants to prove the effect of dapagliflozin on BM. The wide heterogeneity of the sample with respect to age, HbA1c, intrahepatic TG content, and the concomitant use of other medications might have caused type 2 errors in the interpretation of the effects of dapagliflozin on secondary outcomes. Second, the study had an open-label design, which may have introduced bias. Third, amino acid measurements were only made at the end of the intervention, which might have meant that acute changes were missed. Because of these reasons, any generalization of the findings to other populations must be made with caution.

## 5. Conclusion

Twenty-four weeks of dapagliflozin add-on treatment effectively reduces BM, mainly by reducing fat mass, in inadequately controlled patients with type 2 diabetes who are treated with oral anti-diabetic agents. Whole-body muscle mass is maintained, but IHTG content is reduced by dapagliflozin treatment. Although there was a small difference in the change of plasma serine concentrations between the two groups in this study, most amino acids showed subtle changes after treatment of dapagliflozin for 24 weeks. This finding suggests limited effects on skeletal muscle catabolism. Further investigations are necessary to confirm the long-term effects of SGLT2 inhibitors on body composition.

## Acknowledgments

The authors thank the participants and the Shiga University of Medical Science Anti-

Diabetic Drugs Intervention Trial-2 (SUMS-ADDIT-2) investigators for their involvement in this study. A complete list of members of SUMS-ADDIT-2 is provided in the Supplementary Materials. We also thank Keiko Kosaka, Keiko Asano, Megumi Matsuo, Naoko Yamanaka, and Ryoko Matsubae for their expert technical assistance.

We thank Edanz Group (www.edanzediting.com/ac) for editing the English text of drafts of this manuscript.

## Funding

This work was supported by AstraZeneca and Ono Pharmaceutics. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Declarations of interest

HM received research support unrelated to this study from Astellas Pharma Inc., AstraZeneca K.K., Kowa Pharmaceutical, Nippon Boehringer Ingelheim, Ono Pharmaceutical, Taisho Toyama Pharmaceutical, Tanabe Mitsubishi, Sunstar, and Kyowa Kirin, and lecture fees from Astellas Pharma, AstraZeneca, Ono Pharmaceutical, Novo Nordisk Pharma, Eli Lilly Japan, MSD, Daiichi Sankyo, Dainippon Sumitomo, Nippon Boehringer Ingelheim, Mitsubishi Tanabe Pharma, Sanofi, and Takeda Pharmaceutical. K Morino received grants from Astellas and Sanwa Kagaku Kenkyusho that were unrelated to this study, and a consultancy fee from Kyocera. HA received lecture fees from Bayer, Daiichi Sankyo, Fukuda Denshi, and Takeda Pharmaceutical, and consultancy fees from Kyowa Hakko Kirin.

No other potential conflicts of interest relevant to this study are declared.

## Author contributions

Kayo Horibe: Data curation, Writing-original draft preparation. Katsutaro Morino: Conceptualization, Methodology, Funding acquisition, Project administration, Investigation, Writing-original draft preparation, Reviewing and Editing. Itsuko Miyazawa: Investigation, Writing, Reviewing and Editing. Sachiko Tanaka: Data curation and Statistical analysis. Keiko kondo: Data curation and Statistical analysis. Daisuke Sato: Investigation. Natsuko Ohashi: Investigation. Shogo Ida: Investigation. Tsuyoshi Yanagimachi: Review and Editing. Masahiro Yoshimura: MRI/MRS analysis. Ryuta Itho: MRI/MRS analysis. Kiyoshi Murata: MRI/MRS analysis, Methodology. Katsuyuki Miura: Methodology, Statistical analysis. Hisatomi Arima: Methodology, Statistical analysis, Review and Editing. Yukihiro Fujita: Review and Editing. Satoshi Ugi: Conceptualization, Investigation. Hiroshi Maegawa: Conceptualization, Methodology, Supervision, Review and Editing.

**Figure legends**

**Figure 1 | Study flow chart and CONSORT diagram**

ITT: intention to treat

**Figure 2 | Sub-group analysis**

The graph shows the change in body mass during 24 weeks of dapagliflozin treatment (red) and in the control group (blue) for each of the various subgroups. Values are mean ± SD. BL: baseline, M: male, F: female, Lo: Low, Hi: high

**Figure 3 | Heat map showing Pearson's correlation coefficient between changes in amino acids and clinical parameters**

A heat map shows the correlations of the changes in amino acids versus changes in weight,

fat mass (DEXA), lean body mass (DEXA), fat mass (BIA), and muscle mass (BIA). Shades of red and blue represent positive and negative correlations, respectively.

**Figure 4 | Correlations between BCAAs and body compositional change**

Dispersion graphs, Pearson correlations (r) and *P* values (*P*) for changes in amino acids and body compositional variables. The confidence level for ellipses is 95%. Val: valine, Leu: leucine, Iso: isoleucine, BCAAs: total branched chain amino acids, LBM: lean body mass, MUS: muscle

**SUPPLEMENTARY MATERIAL**

Additional supplementary material may be found in the online version of this article:

**Figure S1** | Changes in body mass during the study period

**Figure S2** | Change in body composition as measured using DEXA

**Figure S3** | Segmental changes in body composition as measured using DEXA and BIA

**Figure S4** | Changes in segmental muscle mass over the study period

**Figure S5** | Change in serum ketone bodies over the study period

**Table S1** | Major adverse events during the study

**Table S2 |** Changes in plasma amino acid concentrations between baseline and week 24 in each treatment group

**Appendix S1** | Detailed protocol for patients with hypoglycemia or hyperglycemia

**Appendix S2** | Complete list of members of SUMS-ADDIT-2

**Doc S1** | CONSORT checklist

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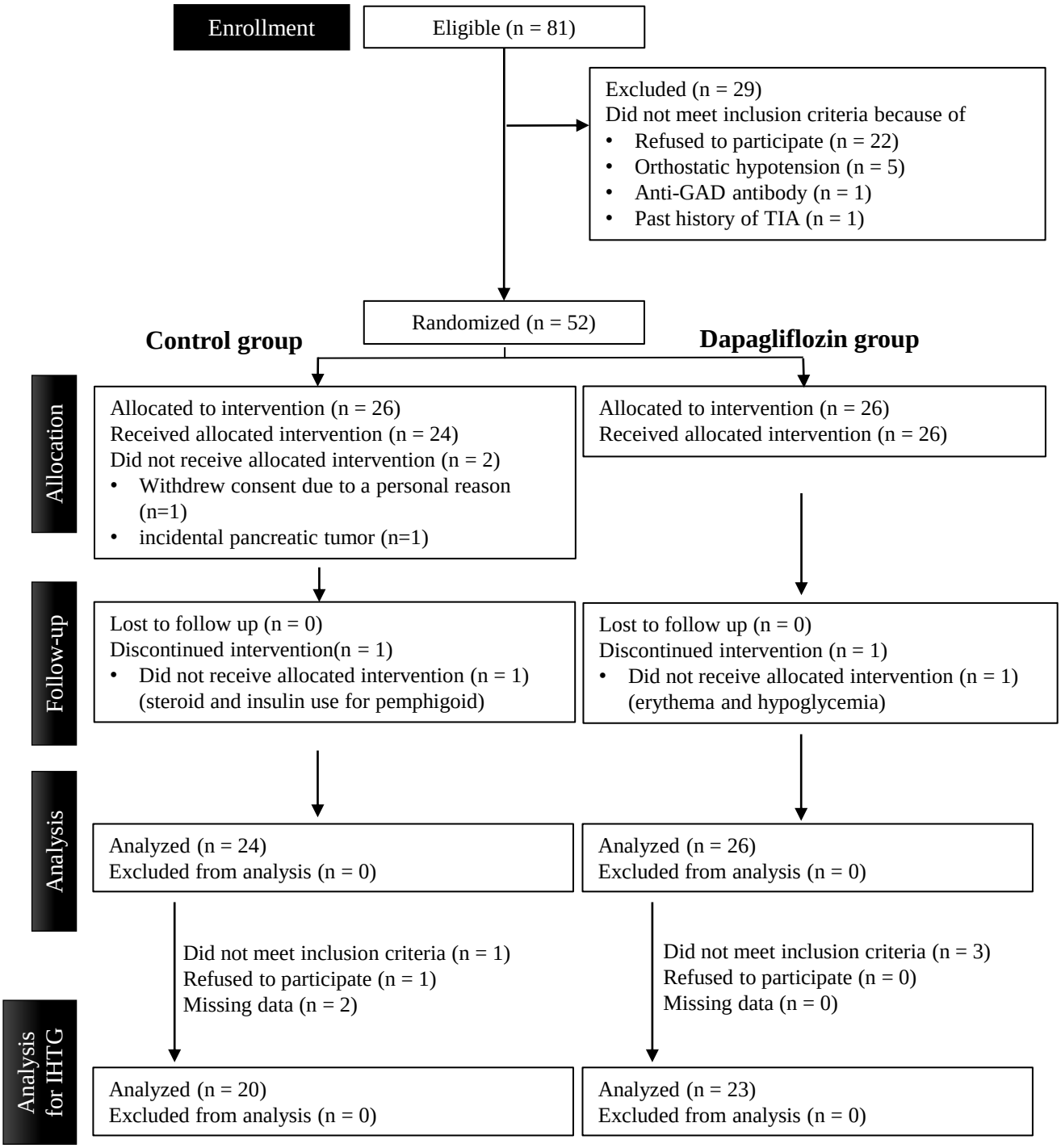
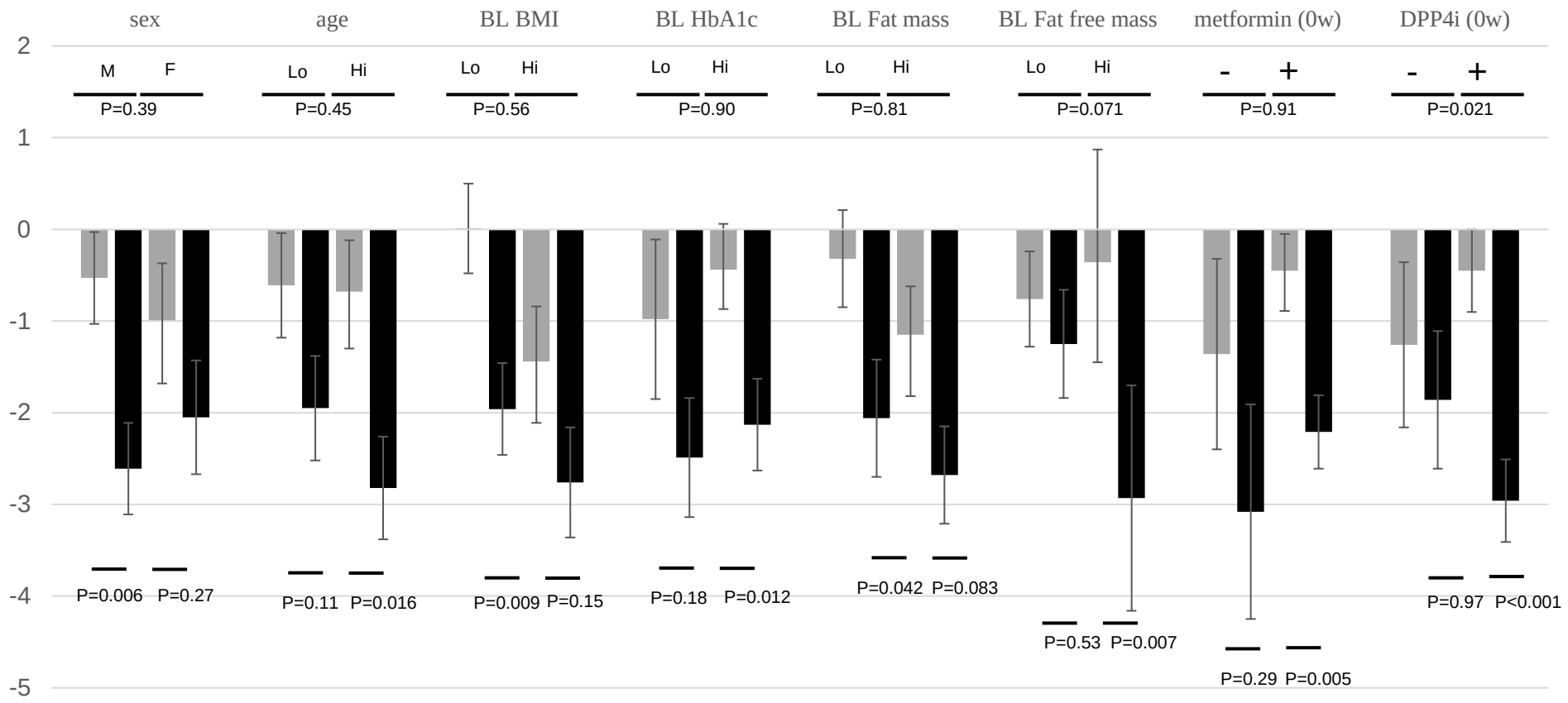
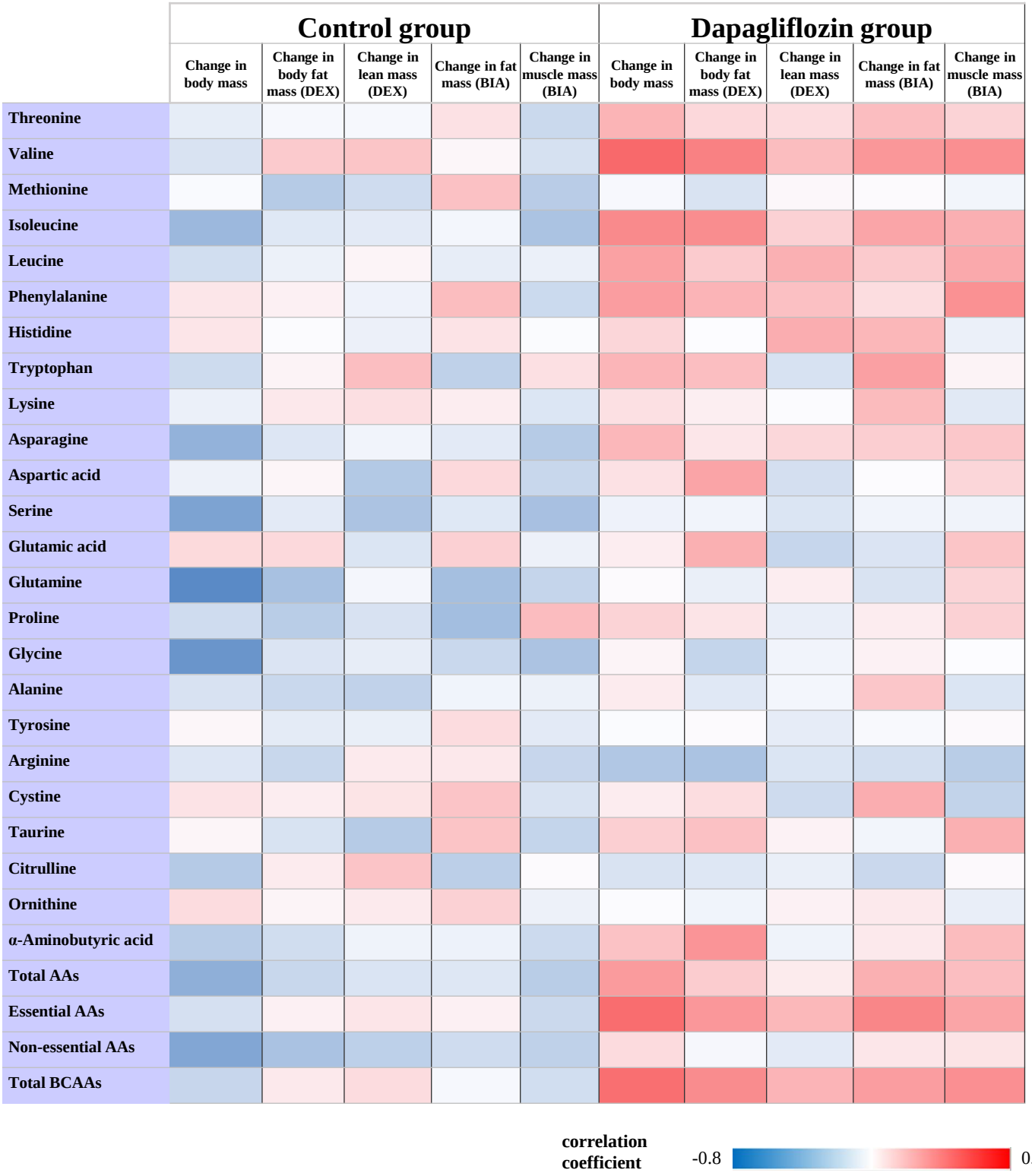
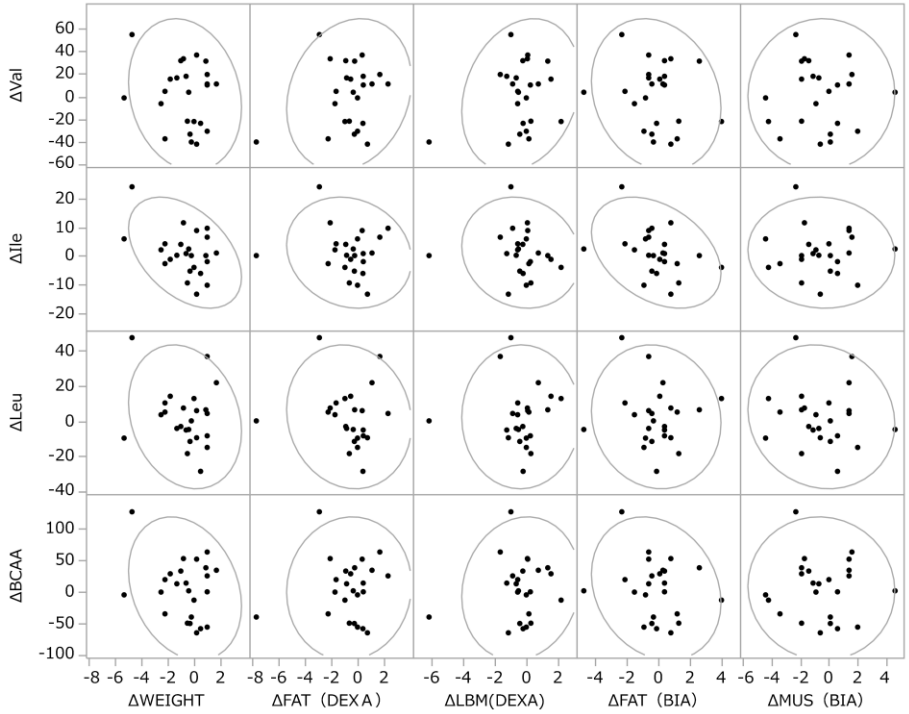

Enrollment
Eligible (n = 81)
Excluded (n = 29)
Did not meet inclusion criteria because of
• Refused to participate (n = 22)
• Orthostatic hypotension (n = 5)
• Anti-GAD antibody (n = 1)
• Past history of TIA (n = 1)
Randomized (n = 52)
Control group
Dapagliflozin group
Allocation
Allocated to intervention (n = 26)
Received allocated intervention (n = 24)
Did not receive allocated intervention (n = 2)
• Withdrew consent due to a personal reason (n=1)
• incidental pancreatic tumor (n=1)
Allocated to intervention (n = 26)
Received allocated intervention (n = 26)
Follow-up
Lost to follow up (n = 0)
Discontinued intervention(n = 1)
• Did not receive allocated intervention (n = 1) (steroid and insulin use for pemphigoid)
Lost to follow up (n = 0)
Discontinued intervention (n = 1)
• Did not receive allocated intervention (n = 1) (erythema and hypoglycemia)
Analysis
Analyzed (n = 24)
Excluded from analysis (n = 0)
Analyzed (n = 26)
Excluded from analysis (n = 0)
Did not meet inclusion criteria (n = 1)
Refused to participate (n = 1)
Missing data (n = 2)
Did not meet inclusion criteria (n = 3)
Refused to participate (n = 0)
Missing data (n = 0)
Analysis for IHTG
Analyzed (n = 20)
Excluded from analysis (n = 0)
Analyzed (n = 23)
Excluded from analysis (n = 0)

# Body mass change (kg)

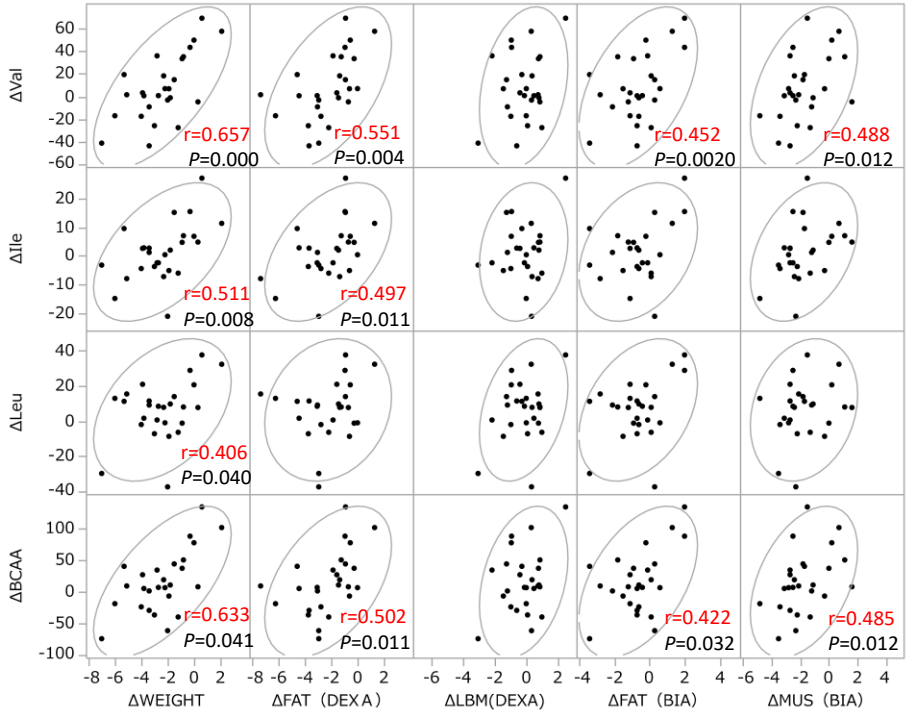



Control group
Dapagliflozin group
Change in body mass
Change in body fat mass (DEX)
Change in lean mass (DEX)
Change in fat mass (BIA)
Change in muscle mass (BIA)
Change in body mass
Change in body fat mass (DEX)
Change in lean mass (DEX)
Change in fat mass (BIA)
Change in muscle mass (BIA)
Threonine
Valine
Methionine
Isoleucine
Leucine
Phenylalanine
Histidine
Tryptophan
Lysine
Asparagine
Aspartic acid
Serine
Glutamic acid
Glutamine
Proline
Glycine
Alanine
Tyrosine
Arginine
Cystine
Taurine
Citrulline
Ornithine
α-Aminobutyric acid
Total AAs
Essential AAs
Non-essential AAs
Total BCAAs
correlation coefficient
-0.8
0.8

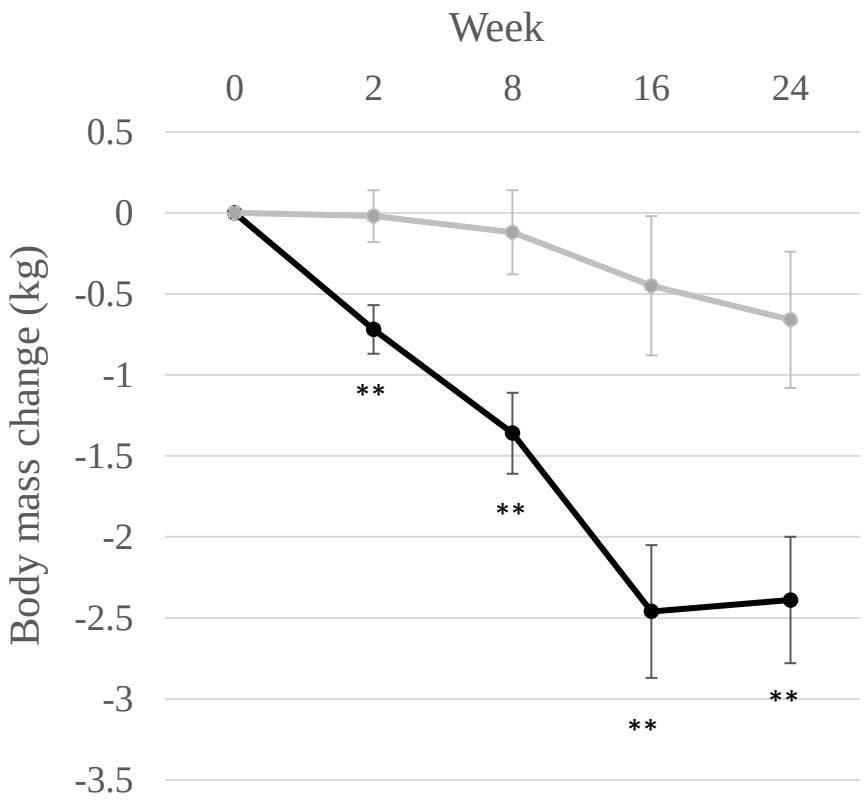
## Control group




## Dapagliflozin group

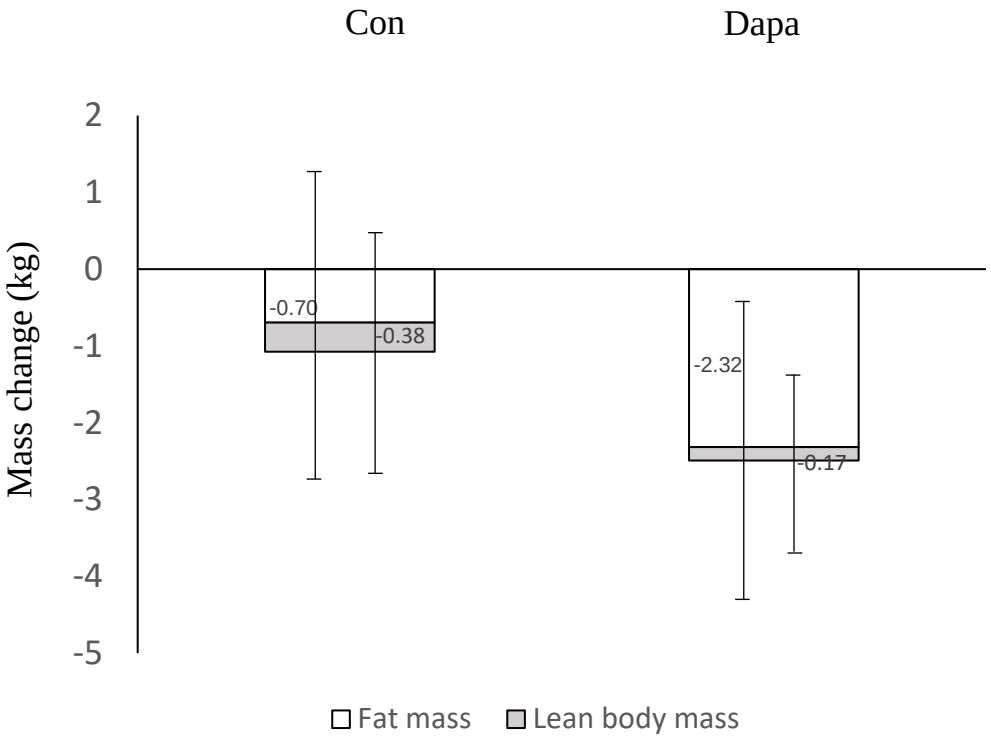


# Supplemental Fig. S1



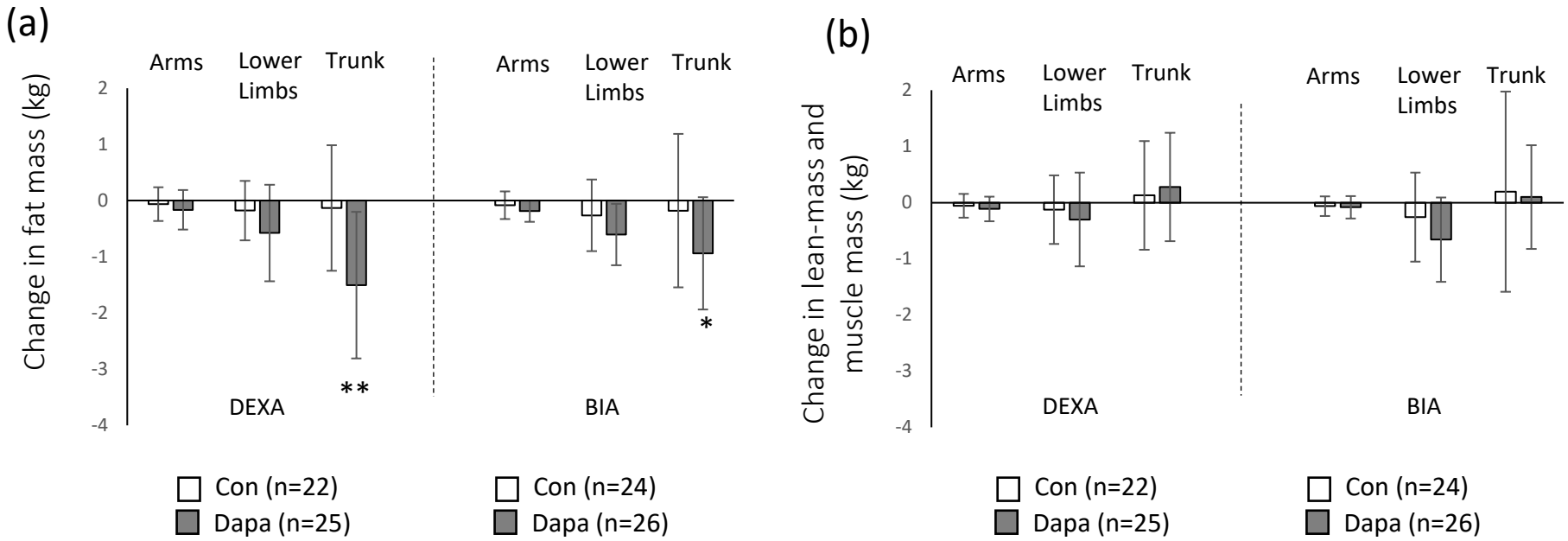

**Supplemental Fig. S1. Changes in body mass over the study period**
Values are least square mean ± SE. The *P*-values are for comparisons of the change in the Dapa group (black) and the Con group (gray), according to mixed model analysis. * $P < 0.05$, ** $P < 0.01$.

# Supplemental Fig. S2



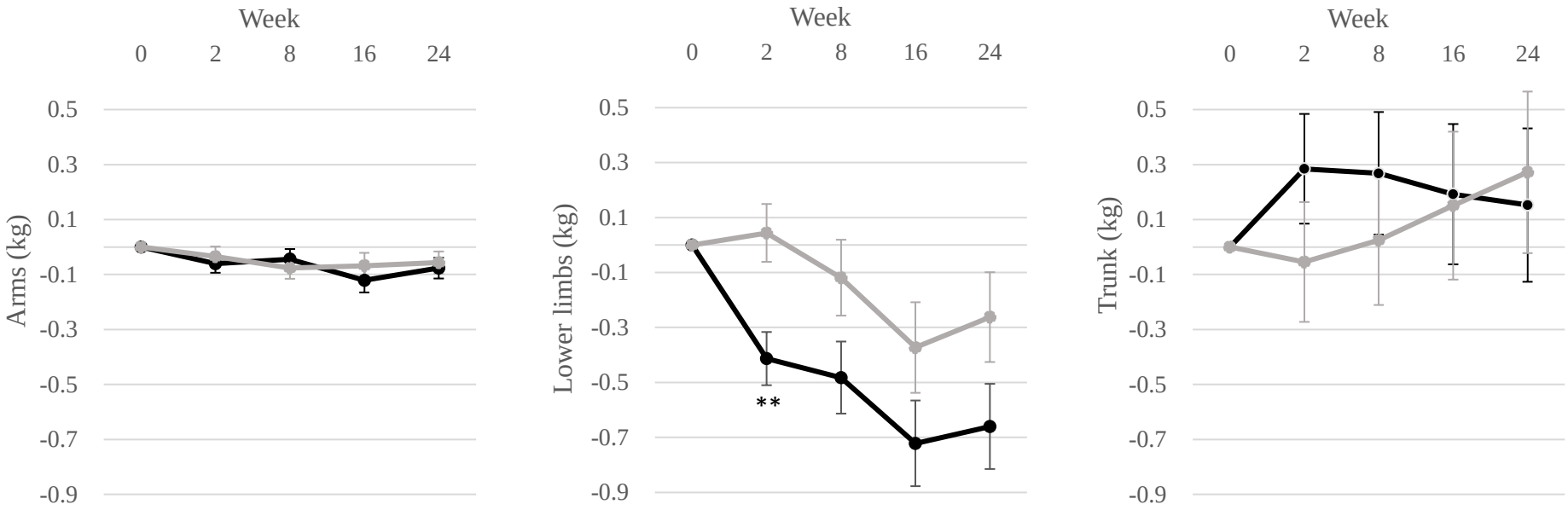

**Supplemental Fig. S2. Changes in body composition, measured using DEXA**
Values are mean ± SD.

# Supplemental Fig. S3



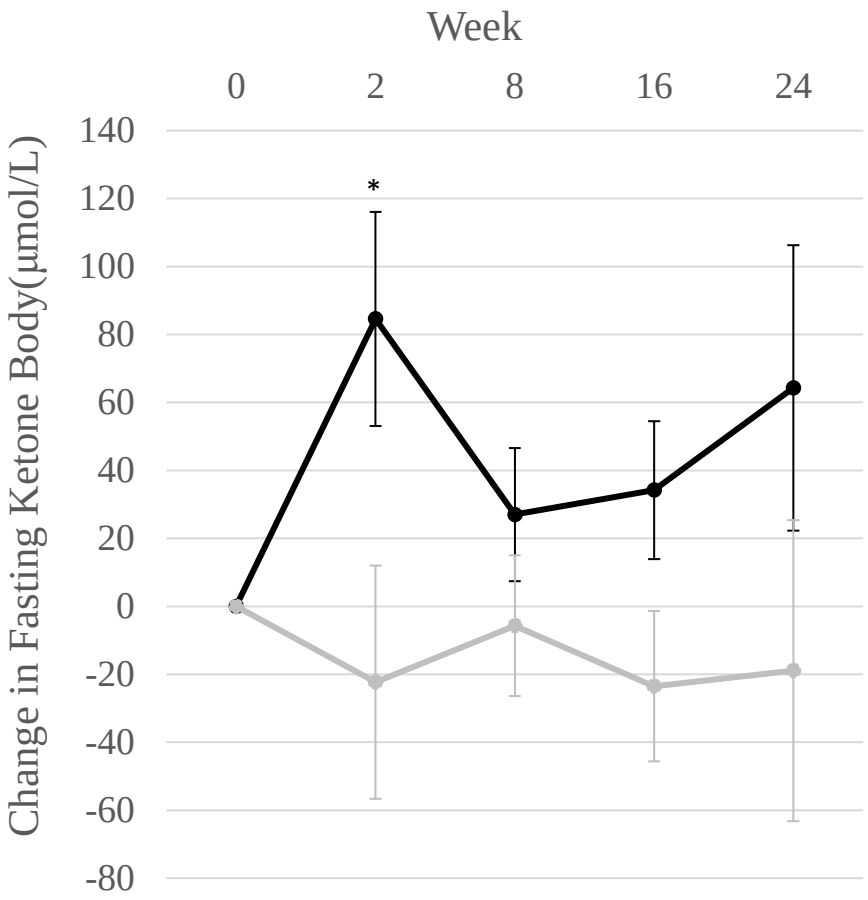

**Supplemental Fig. S3. Segmental changes in body composition, measured using DEXA and BIA**
(a) Change in fat mass measured with DEXA and BIA after 24 weeks of intervention.. (b) Change in lean-mass measured with DEXA and muscle mass measured with BIA after 24 weeks intervention.Values are mean ± SD, The P-values show comparisons of changes between the group receiving add-on Dapagliflozin(Dapa) and the group receiving no additional treatment(Con) using ANCOVA adjusted for sex. *p<0.05, **p<0.01.

# Supplemental Fig. S4




**Supplemental Fig.S4. Changes in muscle mass over the study period**
Values are least square mean ± SE. The *P*-values are for comparisons of the change in the Dapa group (black) and the Con group (gray), according to mixed model analysis. * $P < 0.05$, ** $P < 0.01$.

# Supplemental Fig. S5




**Supplemental Fig. S5. Changes in fasting ketone body over the study period**
Values are least square mean ± SE. The *P*-values are for comparisons of the change in the Dapa group (black) and the Con group (gray), according to mixed model analysis. * $P < 0.05$.

# Supplemental Table S1

Major adverse events during the study

| Events | | | Number of patients, n (%) | |
|---|---|---|---|---|
| | Con group (n=24) | Dapa group (n=26) | Con group (n=24) | Dapa group (n=26) |
| Any adverse event | 41 | 51 | 16 (67) | 22 (85) |
| Hypoglycemia | 6 | 7 | 4 (17) | 6 (23) |
| Exanthema/ Skin itch | 7 | 4 | 6 (25) | 4 (15) |
| Muscle / joint pain | 6 | 3 | 5 (21) | 3 (12) |
| Dehydration | 4 | 6 | 4 (17) | 5 (19) |
| Upper respiratory infection | 3 | 6 | 3 (13) | 5 (19) |
| Urinary tract infection | 1 | 3 | 1 (4) | 2 (8) |
| Orthostatic hypotension | 0 | 3 | 0 | 3 (12) |
| Genital itch Candidal vaginitis | 0 | 0 | 0 | 0 |

Hypoglycemia, dehydration, urinary tract infection, exanthema, and orthostatic hypotension were the pre-defined major adverse events.

**Supplement Table S2. Changes in plasma amino acid concentrations between baseline and week 24 in each treatment group**

| | Control group (OAD) | | | | Dapa group ( OAD + Dapagliflozin 5 mg) | | | | Difference in change (95% CI) | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline (mean ± SD) | Week 24 (mean ± SD) | Change (mean ± SD) | P value | Baseline (mean ± SD) | Week 24 (mean ± SD) | Change (mean ± SD) | P value | | |
| Threonine (nmol/mL) | 126.28 ± 22.18 | 130.80 ± 26.28 | 4.53 ± 20.53 | 0.291 | 116.61 ± 27.30 | 110.90 ± 18.56 | -5.71 ± 22.19 | 0.201 | 10.57 (-1.62, 22.77) | 0.088 |
| Valine (nmol/mL) | 238.70 ± 27.27 | 240.24 ± 25.53 | 1.53 ± 27.50 | 0.787 | 246.83 ± 42.93 | 254.70 ± 43.39 | 7.88 ± 29.18 | 0.181 | -6.89 (-22.97, 9.19) | 0.393 |
| Methionine (nmol/mL) | 24.50 ± 34.0 | 25.40 ± 4.88 | 0.91 ± 3.61 | 0.228 | 23.42 ± 3.61 | 23.59 ± 3.25 | 0.18 ± 2.40 | 0.710 | 0.74 (-1.01, 2.49) | 0.401 |
| Isoleucine (nmol/mL) | 68.30 ± 12.27 | 69.43 ± 11.77 | 1.13 ± 7.89 | 0.492 | 70.98 ± 15.98 | 72.33 ± 16.73 | 1.35 ± 9.85 | 0.490 | -0.24 (-5.40, 4.93) | 0.927 |
| Leucine (nmol/mL) | 128.55 ± 19.41 | 130.95 ± 19.88 | 2.40 ± 16.73 | 0.491 | 136.15 ± 27.80 | 142.47 ± 24.86 | 6.32 ± 16.53 | 0.063 | -3.98 (-13.56, 5.61) | 0.409 |
| Phenylalanine (nmol/mL) | 55.62 ± 6.74 | 54.823 ± 5.99 | -0.79 ± 5.35 | 0.476 | 56.81 ± 11.00 | 57.53 ± 10.42 | 0.72 ± 4.62 | 0.435 | -1.45 (-4.30, 1.41) | 0.313 |
| Histidine (nmol/mL) | 76.69 ± 8.34 | 76.81 ± 10.15 | 0.13 ± 7.57 | 0.936 | 72.74 ± 10.78 | 73.46 ± 8.89 | 0.72 ± 6.48 | 0.575 | -0.53 (-4.56, 3.50) | 0.794 |
| Tryptophan | 48.11 ± 7.55 | 48.95 ± 6.65 | 0.84 ± 5.25 | 0.440 | 50.02 ± 9.40 | 49.33 ± 9.66 | -0.70 ± 4.77 | 0.464 | 1.55 (-1.34, 4.43) | 0.286 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (nmol/mL) | | | | | | | | | | |
| Lysine (nmol/mL) | 193.14 ± 31.33 | 200.55 ± 30.73 | 7.41 ± 23.68 | 0.139 | 186.80 ± 19.16 | 188.47 ± 19.68 | 1.67 ± 18.63 | 0.652 | 5.38 (-6.68, 17.43) | 0.374 |
| Asparagine (nmol/mL) | 43.73 ± 6.39 | 45.62 ± 7.77 | 1.89 ± 6.65 | 0.178 | 42.2 ± 6.74 | 43.25 ± 6.47 | 1.04 ± 4.17 | 0.216 | 0.88 (-2.28, 4.05) | 0.577 |
| Aspartic acid (nmol/mL) | 3.95 ± 1.76 | 3.40 ± 1.50 | -0.55 ± 2.07 | 0.206 | 3.62 ± 1.17 | 3.31 ± 1.29 | -0.31 ± 1.42 | 0.273 | -0.24 (-1.25, 0.78) | 0.640 |
| Serine (nmol/mL) | 107.98 ± 18.81 | 112.13 ± 20.90 | 4.15 ± 9.70 | 0.047 | 104.66 ± 16.74 | 99.38 ± 15.62 | -5.27 ± 11.22 | 0.024 | 9.61 (3.63, 15.59) | 0.002 |
| Glutamic acid (nmol/mL) | 64.98 ± 20.81 | 59.85 ± 22.61 | -5.12 ± 24.55 | 0.317 | 62.01 ± 23.05 | 58.28 ± 19.50 | -3.80 ± 15.09 | 0.211 | -1.07 (-12.61, 10.48) | 0.854 |
| Glutamine (nmol/mL) | 566.80 ± 79.95 | 578.80 ± 95.73 | 12.00 ± 64.00 | 0.368 | 537.33 ± 74.99 | 518.50 ± 69.77 | -18.84 ± 45.55 | 0.045 | 30.96 (-0.83, 62.74) | 0.056 |
| Proline (nmol/mL) | 176.74 ± 42.98 | 173.75 ± 45.3 | -2.99 ± 28.39 | 0.611 | 167.23 ± 42.32 | 169.95 ± 46.58 | 2.72 ± 20.58 | 0.506 | -5.72 (-19.92, 8.47) | 0.421 |
| Glycine (nmol/mL) | 198.93 ± 54.18 | 206.29 ± 51.77 | 7.36 ± 26.84 | 0.192 | 195.98 ± 69.45 | 199.74 ± 49.01 | 3.76 ± 21.89 | 0.390 | 3.41 (-10.61, 17.43) | 0.627 |
| Alanine (nmol/mL) | 427.85 ± 105.23 | 422.61 ± 144.05 | -5.24 ± 68.18 | 0.710 | 400.39 ± 73.84 | 400.33 ± 85.94 | -0.05 ± 59.66 | 0.996 | -6.17 (-42.58, 30.25) | 0.735 |
| Tyrosine (nmol/mL) | 65.20 ± 12.97 | 63.26 ± 11.56 | -1.95 ± 7.29 | 0.204 | 58.73 ± 16.00 | 58.52 ± 15.28 | -0.21 ± 6.98 | 0.878 | -1.64 (-5.71, 2.44) | 0.423 |
| Arginine | 82.72 ± 15.18 | 85.06 ± 16.08 | 2.33 ± 10.45 | 0.285 | 75.24 ± 12.11 | 75.85 ± 13.81 | 0.61 ± 8.53 | 0.720 | 1.63 (-3.82, 7.07) | 0.551 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (nmol/mL) | | | | | | | | | | |
| Cystine (nmol/mL) | 32.35 ± 14.61 | 32.68 ± 14.89 | 0.33 ± 8.90 | 0.858 | 30.33 ± 14.33 | 29.83 ± 16.34 | -0.51 ± 12.36 | 0.836 | 0.50 (-5.46, 6.46) | 0.868 |
| Taurine (nmol/mL) | 70.72 ± 19.4 | 61.91 ± 17.55 | -8.81 ± 25.14 | 0.099 | 68.74 ± 18.47 | 69.05 ± 19.55 | 0.31 ± 24.80 | 0.949 | -9.10 (-23.48, 5.29) | 0.210 |
| Citrulline (nmol/mL) | 27.85 ± 9.68 | 28.98 ± 9.61 | 1.13 ± 5.33 | 0.310 | 24.22 ± 6.43 | 25.77 ± 7.81 | 1.55 ± 5.31 | 0.149 | -0.43 (-3.49, 2.64) | 0.781 |
| Ornithine (nmol/mL) | 54.24 ± 13.14 | 53.18 ± 9.05 | -1.06 ± 8.64 | 0.553 | 46.70 ± 10.94 | 47.08 ± 8.94 | 0.39 ± 5.54 | 0.723 | -1.45 (-5.549 2.70) | 0.485 |
| α-Aminobutyric acid (nmol/mL) | 20.86 ± 4.28 | 21.34 ± 7.80 | 0.48 ± 7.54 | 0.758 | 20.69 ± 6.95 | 21.76 ± 6.83 | 1.07 ± 4.46 | 0.232 | -0.62 (-4.15, 2.70) | 0.725 |
| Total AA (nmol/mL) | 2890.0 ± 211.6 | 2912.2 ± 261.0 | 22.2 ± 208.11 | 0.606 | 2783.2 ± 242.5 | 2775.7 ± 232.6 | -7.49 ± 135.44 | 0.780 | 28.15 (-71.82, 128.13) | 0.574 |
| Essential AA (nmol/mL) | 959.9 ± 85.0 | 978.0 ± 85.7 | 18.1 ± 84.5 | 0.306 | 960.3 ± 121.6 | 972.8 ± 105.8 | 12.5 ± 69.5 | 0.371 | 5.16 (-39.17, 49.49) | 0.816 |
| Non-essential AA (nmol/mL) | 1930.2 ± 165.6 | 1934.3 ± 217.0 | 4.13 ± 158.79 | 0.900 | 1822.9 ± 152.9 | 1803.0 ± 153.4 | -19.9 ± 98.4 | 0.312 | 22.99 (-52.20, 98.19) | 0.541 |
| Total BCAA (nmol/mL) | 435.6 ± 56.3 | 440.6 ± 52.7 | 5.05 ± 46.22 | 0.598 | 454.0 ± 81.7 | 469.5 ± 82.4 | 15.55 ± 48.83 | 0.117 | -11.10 (-38.33, 16.13) | 0.416 |

Values are mean ± SD for continuous variables at baseline, 24 weeks, and for the change during this period. The least-square mean (95%

confidence interval) is shown for the difference in this change between the groups. n = 24 for the control group and n = 26 for the Dapa group. The *P* values indicate the results of comparisons of the changes between the Dapa and control groups, using analysis of covariance adjusted for sex.

AA: amino acids, BCAA: branched chain amino acids.

# Appendix S1

**Treatment guidelines**

In both the Dapa and Con groups, the target of treatment is to achieve an HbA1c below 7.0%, without inducing hypoglycemia. During the treatment period, diet and exercise management is to be continued as before.

Reduce the dose of or withdraw SUs for 2 weeks after the initiation of dapagliflozin administration, in order to reduce the risk of hypoglycemia associated with concomitant SU use.

**Dapagliflozin group**

1) Administer dapagliflozin 5 mg once daily orally after breakfast. In order to prevent hypoglycemia, reduce the dosage of SUs at the initiation of dapagliflozin administration.

For those patients taking >2 mg/day of glimepiride, reduce the dose to ≤2 mg/day.

For those patients taking >1.25 mg/day of glibenclamide, reduce the dose to ≤1.25 mg/day.

For those patients taking >40 mg/day of gliclazide, reduce the dose to ≤40 mg/day.

The discontinuation of SUs is at the physician's discretion.

2) For hypoglycemia: when blood glucose <70 mg/dl or when hypoglycemic symptoms are strongly suspected, instruct the patient to consume carbohydrates promptly. If the physician frequently diagnoses hypoglycemia, reduce the dose of or discontinue oral agents in the following order: SUs, glinides, thiazolidines, alpha-GIs, metformin, then DPP-4 inhibitors.

3) If a physician identifies a rapid or marked reduction in HbA1c, the dosage of oral agents should be reduced according to the treatment guidelines above.

4) If a physician identifies worsening blood glucose control, add oral agents to the therapeutic regimen according to the following treatment guidelines.

Determine if the patient has an acute metabolic disorder by interview, physical examination, and blood gas analysis, etc. The physician should decide whether participation should be discontinued and/or insulin therapy started. If there were reductions in the doses of medication or discontinuation at the initiation of the study or during dapagliflozin administration, it is permissible to resume administration of these drugs. The maximum dosage is the dose at the time of patient registration. The order of administration resumption should be determined by the physician so that no hypoglycemia occurs and an HbA1c <7.0% can be achieved. Even if the conditions above are met and hyperglycemia or a high HbA1c is present, the dapagliflozin dosage should not be increased during the intervention period.

**Control group**

1) Oral antidiabetic agents may be changed to achieve an HbA1c of <7.0%, without causing hypoglycemia, according to the following treatment guidelines.

2) For hypoglycemia: when blood glucose <70 mg/dl or when hypoglycemic symptoms are strongly suspected, instruct the patient to consume carbohydrates promptly. If the physician frequently diagnoses hypoglycemia, reduce the dose of or discontinue oral agents in the following order: SUs, glinides, thiazolidines, alpha-GIs, metformin, then DPP-4 inhibitors.

3) If a deterioration in blood glucose control is identified (an increase in blood glucose or HbA1c), add an oral agent according to the following treatment guidelines.

Determine if the patient has an acute metabolic disorder by interview, physical examination, and blood gas analysis, etc. Determine whether participation should be started with insulin therapy. If a DPP-4 inhibitor has not been administered, add one. If a DPP-4 inhibitor has already been administered, after evaluating the contraindications and any history of adverse reactions to metformin, start this drug and increase the dose in 500 mg or 750 mg steps, up to 1,500 mg. The dose can be increased to 2,250 mg at the physician's discretion. When a DPP-4 inhibitor and a metformin dose ≥1,500 mg have already been administered, or these drugs cannot be administered due to contraindications or adverse drug reactions, add an alpha-glucosidase inhibitor. If an alpha-glucosidase inhibitor has already been administered, increase the dosage of the alpha-glucosidase inhibitor to three tablets, administered in three divided doses per day.

When a DPP-4 inhibitor, metformin ≥1,500 mg, and an alpha-glucosidase inhibitor (three tablets administered in three divided doses per day) have already been administered or these drugs cannot be administered due to contraindications or adverse drug reactions, consider the addition of a thiazolidinedione at 15 mg/day if there is no history of adverse drug reaction or bladder cancer. Do not increase the thiazolidinedione dosage except if more than 30 mg has already been administered. When a DPP-4 inhibitor, metformin ≥1,500 mg, an alpha-glucosidase inhibitor (three tablets administered in three divided doses per day) and a thiazolidinedione have already been administered, or these drugs cannot be administered due to contraindications or adverse drug reactions, consider the administration of a glinide. If any glinide has already been administered at a dose of two or fewer tablets/day, increase the dosage to three tablets/day. Do not use sulfonylureas unless they were already being used at the time of enrollment.

The treatment described above is to be continued for 24 weeks. If the discontinuation criteria are met, participation should be discontinued at that time and any necessary follow-up procedures should be implemented.

# Appendix S2

## Shiga University of Medical Science Anti-Diabetic Drugs Intervention Trial-2 (SUMS-ADDIT-2) Research group

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Safety committee: Motozumi Okamoto (Otsu Red Cross Hospital) and Takashi Nomiyama (Fukuoka University).
CRO: Center for Clinical Research and Advanced Medicine in the SUMS. Chief: Hiromu Kutsumi; Data managers: Teruko Ueda and Shoji Momokawa; Study manager: Mitsuru Kawanishi.
Statistical assistance: DOT World Co., Ltd.
Clinical data measurement: SRL Inc. and CMIC Pharma Science.
DEXA measurement: The staff of the Department of Radiology in the SUMS Hospital.