Diabetes Care
American Diabetes Association

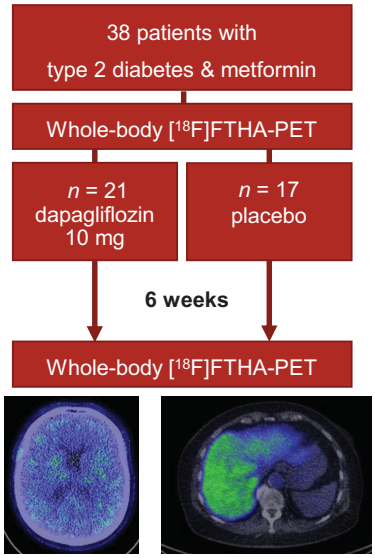
# SGLT2 Inhibitor Dapagliflozin Increases Skeletal Muscle and Brain Fatty Acid Uptake in Individuals With Type 2 Diabetes: A Randomized Double-Blind Placebo-Controlled Positron Emission Tomography Study

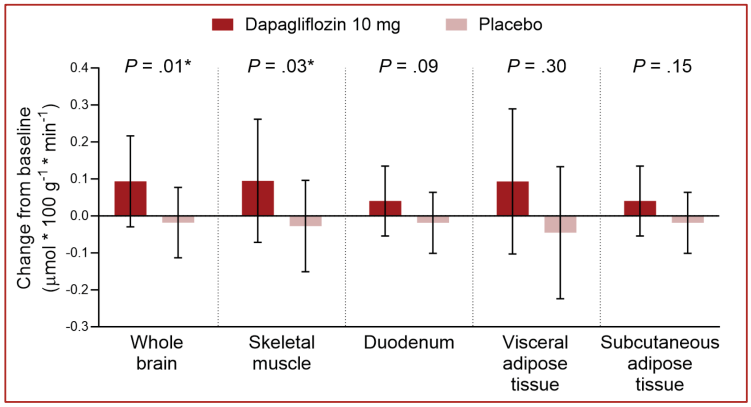
Aino Latva-Rasku, Eleni Rebelos, Jouni Tuisku, Richard Aarnio, Achol Bhowmik, Helmi Keskinen, Sanna Laurila, Minna Lahesmaa-Hatting, Laura Pekkarinen, Henrik Isackson, Anna K. Kirjavainen, Jukka Koffert, Kerstin Heurling, Lauri Nummenmaa, Ele Ferrannini, Jonas Oldgren, Jan Oscarsson, and Pirjo Nuutila

*Diabetes Care* 2024;47(9):1–8 | https://doi.org/10.2337/dc24-0470

## Dapagliflozin Increases Skeletal Muscle and Brain Fatty Acid Uptake in Individuals With Type 2 Diabetes

### A Randomized Double-Blind Placebo-Controlled Positron Emission Tomography (PET) Study




**Conclusions**

Increase in fatty acid uptake in response to sodium–glucose cotransporter 2 inhibitor treatment extends from the liver to skeletal muscle and the brain

## ARTICLE HIGHLIGHTS

- **Why did we undertake this study?**
  We aimed to characterize the extent of the effects of dapagliflozin on tissue-specific fatty acid metabolism in humans.
- **What is the specific question(s) we wanted to answer?**
  We were interested in exploring which tissues contribute to the increased whole-body fatty acid consumption during sodium–glucose cotransporter 2 (SGLT2) inhibitor treatment.
- **What did we find?**
  We discovered that fatty acid uptake was increased in skeletal muscle and the brain.
- **What are the implications of our findings?**
  Because SGLT2 inhibitor treatment also reduces body fat, our study suggests that fatty acid uptake and oxidation are enhanced by dapagliflozin in several different tissues. In the brain, this change might be a sign of improved myelin and membrane synthesis.

# SGLT2 Inhibitor Dapagliflozin Increases Skeletal Muscle and Brain Fatty Acid Uptake in Individuals With Type 2 Diabetes: A Randomized Double-Blind Placebo-Controlled Positron Emission Tomography Study

https://doi.org/10.2337/dc24-0470

Aino Latva-Rasku,[1,2] Eleni Rebelos,[1] Jouni Tuisku,[1] Richard Aarnio,[1] Achol Bhowmik,[1] Helmi Keskinen,[1] Sanna Laurila,[1,3] Minna Lahesmaa-Hatting,[1] Laura Pekkarinen,[1,2] Henrik Isackson,[4,5] Anna K. Kirjavainen,[6] Jukka Koffert,[1] Kerstin Heurling,[7] Lauri Nummenmaa,[1,8] Ele Ferrannini,[9] Jonas Oldgren,[4,10] Jan Oscarsson,[11] and Pirjo Nuutila[1,2]


## OBJECTIVE

**The aim of this study was to investigate the impact of the sodium–glucose cotransporter 2 (SGLT2) inhibitor dapagliflozin on tissue fatty acid (FA) uptake in the skeletal muscle, brain, small intestine, and subcutaneous and visceral adipose tissue of individuals with type 2 diabetes by using positron emission tomography (PET).**

## RESEARCH DESIGN AND METHODS

**In a 6-week randomized double-blind placebo-controlled trial, 53 patients with type 2 diabetes treated with metformin received either 10 mg dapagliflozin or placebo daily. Tissue FA uptake was quantified at baseline and end of treatment with PET and the long-chain FA analog radiotracer 14(*R*,*S*)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid. Treatment effects were assessed using ANCOVA, and the results are reported as least square means and 95% CIs for the difference between groups.**

## RESULTS

**A total of 38 patients (dapagliflozin *n* = 21; placebo *n* = 17) completed the study. After 6 weeks, skeletal muscle FA uptake was increased by dapagliflozin compared with placebo (1.0 [0.07, 2.0] μmol · 100 g$^{-1}$ · min$^{-1}$; *P* = 0.032), whereas uptake was not significantly changed in the small intestine or visceral or subcutaneous adipose tissue. Dapagliflozin treatment significantly increased whole-brain FA uptake (0.10 [0.02, 0.17] μmol · 100 g$^{-1}$ · min$^{-1}$; *P* = 0.01), an effect observed in both gray and white matter regions.**

## CONCLUSIONS

**Six weeks of treatment with dapagliflozin increases skeletal muscle and brain FA uptake, partly driven by a rise in free FA availability. This finding is in accordance with previous indirect measurements showing enhanced FA metabolism in response to SGLT2 inhibition and extends the notion of a shift toward increased FA use to muscle and brain.**


Sodium–glucose cotransporter 2 (SGLT2) inhibitors suppress glucose reabsorption in renal proximal tubules, which results in glycosuria and reduced circulating glucose levels (1). Since the widespread adoption of SGLT2 inhibitors, it has become

[1]Turku PET Centre, University of Turku, Turku, Finland
[2]Department of Endocrinology, Turku University Hospital, Turku, Finland
[3]Heart Center, Turku University Hospital, Turku, Finland
[4]Clinical Physiology and Cardiology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[5]Integrative Physiology, Medical Cell Biology, Uppsala University Hospital, Uppsala, Sweden
[6]Radiopharmaceutical Chemistry Laboratory, Turku PET Centre, University of Turku, Turku, Finland
[7]Antaros Medical AB, Gothenburg, Sweden
[8]Department of Psychology, University of Turku, Turku, Finland
[9]National Research Council Institute of Clinical Physiology, Pisa, Italy
[10]Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden
[11]Late-Stage Development, Cardiovascular, Renal, and Metabolism, BioPharmaceuticals Research and Development, AstraZeneca, Gothenburg, Sweden

Corresponding author: Aino Latva-Rasku, aino.e.latva-rasku@utu.fi

Received 5 March 2024 and accepted 10 June 2024

Clinical trial reg. no. NCT03387683, clinicaltrials.gov

This article contains supplementary material online at https://doi.org/10.2337/figshare.26042449.

evident that they also modulate whole-body energy metabolism (1). Most notably, this class of drugs has been suggested to enhance the use of fatty acids (FAs) and ketone bodies as fuels at the expense of glucose use. Studies using indirect calorimetry have shown that SGLT2 inhibitors reduce the respiratory exchange ratio, enhance lipid oxidation, and increase circulating levels of free FAs (FFAs) and ketones (2–4). At the tissue level, FA uptake has been studied by using positron emission tomography (PET). We previously reported that 6 weeks of treatment with dapagliflozin increases hepatic, but not myocardial, FA uptake measured with PET using the long-chain FA analog 14(*R*,*S*)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid ([$^{18}$F]FTHA) (5,6). [$^{18}$F]FTHA undergoes the initial steps of β-oxidation, but the sulfur substitution at the sixth carbon inhibits its full degradation (6). Consequently, only 30–36% of the $^{18}$F-fluorine from [$^{18}$F]FTHA enters the mitochondria in the liver and skeletal muscle, whereas the majority is incorporated into triacylglycerols (TAGs) and phospholipids in these tissues and the brain (7–9). Although most of the $^{18}$F-label at the C-14 is trapped intracellularly, $^{18}$F-containing compounds, such as TAGs, become present in the circulation as early as 10–20 min after injection, with the portion of intact [$^{18}$F]FTHA being low (5–20%) after 30 min (7,10).

Two prior studies have used [$^{11}$C]palmitate, a radiotracer used to characterize FA oxidation in addition to uptake rate (11), to explore the effects of SGLT2 inhibitors in humans. Similarly to our findings, empagliflozin did not affect myocardial FA uptake or oxidation when accounting for elevated serum FFA concentrations, whereas both myocardial FA uptake and oxidation decreased if only radiotracer transfer rates were considered (12). An extension of this publication reported increased FA uptake in the visceral, but not subcutaneous, adipose tissue (13). However, because radiotracer uptake rates were not reported, it is challenging to evaluate the effect of increased serum FA levels based on the reported values.

Here, we investigated the effects of dapagliflozin on skeletal muscle, adipose tissue, intestinal, and brain FA uptake by using [$^{18}$F]FTHA. We hypothesized that the treatment would be associated with enhanced skeletal muscle FA uptake.

## RESEARCH DESIGN AND METHODS

### Study Design

This was a double-blind randomized parallel-group study with the primary objective of investigating the effects of dapagliflozin treatment on function and metabolism in the myocardium (clinical trial reg. no. NCT03387683) (5), and FA uptake in other tissues was studied as an exploratory objective. The study was conducted at the Turku PET Centre, Turku University Hospital, Turku, Finland, and Uppsala University Hospital, Uppsala, Sweden, from February 2018 to March 2019.

The study comprised five visits: a screening visit 1 to 21 days before first PET study visit, an interim telephone call, a baseline PET study visit, an end-of treatment visit 6 weeks after the first PET study visit, and a safety follow-up telephone call. On the second visit, patients were randomly assigned at a 1:1 ratio to receive either 10 mg dapagliflozin or placebo daily. Randomization was performed in blocks of two without stratification, with the scheme generated by Parexel (Parexel International, Durham, NC). Randomization was performed by a central telephone service. Compliance was evaluated based on the amount of returned study medicine.

All study procedures, PET data analyses, and laboratory analyses were performed by investigators blinded to the treatment.

The study protocol was approved by the Finnish Medicines Agency Fimea, the Independent Ethics Committee of the Southwest Finland Hospital District, and the Regional Ethics Committee of Uppsala, Sweden. The study was conducted according to the principles of the Declaration of Helsinki. All patients provided written informed consent before any study procedures. A negative pregnancy test result performed on site before every PET imaging visit was required from all female participants of childbearing age.

### Study Patients

Patients with type 2 diabetes diagnosed at least 6 months before the study based on the American Diabetes Association 2017 criteria, age 40–75 years and with a BMI of $\geq$25 kg · m$^{-2}$, were eligible if they had been treated with a stable dose of metformin for at least 6 weeks before screening and had an $HbA_{1c}$ of 42–75 mmol · mol$^{-1}$ (6.0–9.0%), normal left ventricular ejection fraction ($\geq$50%), and no significant signs or symptoms of coronary artery disease. Exclusion criteria included diagnosis of any other type of diabetes, uncontrolled hypertension ($\geq$160/100 mmHg measured at screening), history of stroke, atrial fibrillation, valvular disease, estimated glomerular filtration rate (eGFR) of $<$45 mL · min$^{-1}$ · 1.73 m$^{-2}$, unstable or rapidly progressing renal disease or severe hepatic impairment (Child-Pugh class C), and use of loop diuretics or antidiabetic drugs other than metformin.

Of the 87 patients participating in the screening visit, 53 were considered eligible, and 38 of those eligible (dapagliflozin $n$ = 21; placebo treatment $n$ = 17) also completed the [$^{18}$F]FTHA studies; 28 of these patients were studied in Turku, Finland, and 10 in Uppsala, Sweden (5).

### [$^{18}$F]FTHA-PET Protocol

The PET studies were conducted after a minimum of 6–8 h of fasting and, at the end-of-treatment visit, 4–6 h after the last dose of study drug and a minimum of 12 h after taking any other medications. The [$^{18}$F]FTHA radiotracer was manufactured in the local radiochemistry laboratories as described earlier (14).

Before the PET scan, two venous catheters were inserted into opposite forearms. One was used for blood sampling and the other for radiotracer administration. Image acquisition was performed on three GE Healthcare Discovery MI PET/computed tomography (CT) scanners and one GE Healthcare Discovery 690 PET/CT (GE Healthcare, Milwaukee, WI). Patients were examined with the same scanner at baseline and end of study. Before radiotracer administration, a low-dose CT or magnetic resonance scan was performed. The dose of [$^{18}$F]FTHA was based on body weight, with the target of 2 MBq · kg$^{-1}$. Right after radiotracer bolus injection, a 32-min dynamic scan was started from the thoracic area (12 × 15, 4 × 30, 2 × 120, 1 × 180, and 4 × 300 s), and a 10-min static scan was performed on the brain 52–62 min postinjection. Image data were reconstructed using iterative reconstruction (three iterations; 16 subsets; 5 mm postfilter).

### PET Data Analysis

All PET data were corrected for dead time, decay, and photon attenuation based on low-dose CT. Radiotracer uptake into peripheral tissues was assessed from dynamic

frames 12–32 min after injection and, in the brain, starting from a single static image from 52 to 62 min after injection. In peripheral tissues, volumes of interest (VOIs) were drawn freehand with Carimas software (version 2.10) (15). Paraspinal muscles were used for skeletal muscle analyses, the left ventricular wall was used for the myocardium, the duodenal wall was chosen to represent the small intestine, and a mean of several VOIs drawn in subcutaneous and intraperitoneal visceral adipose tissue were used for adipose tissue analysis. For the brain, an automatized pipeline was used for analysis (16). First, the PET data were spatially normalized to MNI152 standard space using an in-house [$^{18}$F]FTHA template. Then, three predefined VOIs (i.e., cortical gray matter, white matter, and whole brain) were used to extract the time activity curves.

To obtain the rate of tissue radiotracer uptake with respect to radiotracer availability in the circulation, the tissue fractional [$^{18}$F]FTHA uptake rate (FUR; mL · [mL · min]$^{-1}$) was calculated by dividing tissue accumulated activity measured from the images by the integral of radiometabolite-corrected plasma radioactivity from injection to the midpoint of the selected PET frame (17,18). Because [$^{18}$F]FTHA FUR is considered to express a fraction of total FA uptake, tissue FA uptake was calculated by multiplying FUR by serum FFA concentration during the scan and divided by tissue density (in units of μmol · 100 g$^{-1}$ · min$^{-1}$). Whole-body [$^{18}$F]FTHA clearance was calculated by dividing the injected dose by the plasma radioactivity area under the curve (AUC) from injection to extrapolated infinity (7).

Because [$^{18}$F]FTHA is metabolized rapidly, plasma input was corrected by measuring the remaining intact fraction of the radiotracer at 5, 10, 20, and 30 min after injection by using thin-layer chromatography with autoradiography (8,9), and the input curve was forced to zero after 30 min based on previous studies showing only minuscule amounts of unmetabolized [$^{18}$F]FTHA after this time point (19). Serum FFA were measured 5 min before injection and 32 min into the scan.

### Laboratory Measurements

Fasting plasma glucose was analyzed by the hexokinase enzymatic method (Roche Diagnostics, Indianapolis, IN) and $HbA_{1c}$ by ion-exchange high-performance liquid chromatography (Bio-Rad, Hercules, CA), and International Federation of Clinical Chemistry (IFCC) $HbA_{1c}$ was calculated using the following formula: IFCC = (NGSP − 2.15) · 0.09148$^{-1}$. Enzymatic colorimetric assays were used for serum FFA (Roche Modular and Cobas Analyzer; Wako Chemicals, Richmond, VA), plasma β-hydroxybutyrate (LiquiColor; Stanbio Laboratory, Boerne, TX), and plasma lactate (Roche Diagnostics).

### Statistical Analysis

Distribution of the data was evaluated with the Shapiro-Wilk test, and logarithmic transformation was performed on nonnormally distributed parameters ($HbA_{1c}$, daily dose of metformin). Baseline differences between groups were evaluated with the independent samples *t* test or Mann-Whitney *U* test for nonnormally distributed data (β-hydroxybutyrate). Treatment effects in anthropometric measurements, laboratory tests, and tissue [$^{18}$F]FTHA and FFA uptake rates were analyzed using ANCOVAs.

The treatment effect on the brain was investigated at voxel level using a statistical parametric mapping toolbox (SPM12; Wellcome Trust Centre for Neuroimaging, London, U.K.) running on Matlab (Mathworks, Natick, MA). The effects were tested using the *t* test with the FUR difference images (post minus pre), which were smoothed with an 8-mm Gaussian filter before analysis. The resulting statistical maps were corrected for multiple comparisons at cluster level using a false discovery rate at $P < 0.05$ (16).

Associations between changes in laboratory results and fractional radiotracer uptake were studied using Pearson correlation. Baseline results are reported as means and SDs, and treatment effects as least square means with 95% CIs for the difference between groups. For β-hydroxybutyrate, the value 0.09 μmol · L$^{-1}$ was used for patients with results below the measurement range (i.e., <0.1 μmol · L$^{-1}$). A per-protocol analysis using the complete [$^{18}$F]FTHA uptake data set ($N = 38$) was conducted. Statistical analyses other than the brain statistical parametric mapping analyses were performed using IBM SPSS Statistics for Windows (version 29.0; IBM Corp., Armonk, NY).

### Data and Resource Availability

The data sets analyzed in the current study are available from the corresponding author on reasonable request, in accordance with the AstraZeneca data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

## RESULTS

### Patient Characteristics

Baseline characteristics of both treatment arms are reported in Table 1. There were no significant differences between treatment groups concerning sex distribution, age, BMI, glycemia, or time since diagnosis, and all patients were Caucasian. The higher baseline mean β-hydroxybutyrate level resulted from a single elevated value (0.57 μmol · L$^{-1}$) in the placebo group. The daily doses of metformin ranged from 500 to 3000 mg similarly in both groups. Compliance with study medication was ≥95% in both treatment arms, and no adverse effects were reported.

### Effects of Dapagliflozin on Body Weight and Circulating Metabolites

There were significant reductions in body weight, BMI, fasting plasma glucose, and $HbA_{1c}$ after 6 weeks of treatment in the dapagliflozin group compared with the placebo group. Serum fasting β-hydroxybutyrate levels increased, whereas there was no difference in fasting lactate or serum FFA concentrations during the scan between the placebo and dapagliflozin groups (Table 1).

### Changes in Peripheral FFA Uptake

There were no significant differences in tissue [$^{18}$F]FTHA FUR or FA uptake between treatment arms at baseline (Table 2). As previously shown in a larger data set from the same study, dapagliflozin treatment was associated with a significant increase in liver [$^{18}$F]FTHA and FA uptake (5).

In skeletal muscle, the FA uptake rate was higher in the dapagliflozin group compared with the placebo group when accounting for the levels of circulating FFA. In the duodenum, the increases in [$^{18}$F]FTHA and FA uptake did not reach statistical significance, despite the percentage change being similar to that in skeletal muscle (Table 2, Fig. 1, and Supplementary Table 1).

In the dapagliflozin group, except for a modest negative correlation between a decrease in plasma glucose and an

**Table 1—Baseline and end-of-treatment characteristics**

| | Baseline | | | End of treatment | | |
|---|---|---|---|---|---|---|
| | Dapagliflozin ($n$ = 21) | Placebo ($n$ = 17) | $P$ | LSM difference | 95% CI for difference | $P$ |
| Age, years | 64 ± 8 | 66 ± 6 | 0.32 | | | |
| Sex | | | 0.19 | | | |
| Male | 15 | 8 | | | | |
| Female | 6 | 9 | | | | |
| T2D duration, years | 6.3 ± 4.4 | 8.6 ± 6.8 | 0.28 | | | |
| Metformin dose, mg | 1,345 ± 768 | 1,294 ± 830 | 0.69 | | | |
| BMI, kg · $m^{-2}$ | 30.4 ± 3.6 | 28.9 ± 3.0 | 0.17 | −0.5 | −0.7, −0.2 | <0.001 |
| Glucose, mmol · $L^{-1}$ | 7.7 ± 1.3 | 7.5 ± 1.35 | 0.70 | −0.63 | −1.0, −0.2 | 0.003 |
| $HbA_{1c}$, % | 6.7 ± 0.49 | 6.6 ± 0.71 | 0.64 | −0.2 | −0.3, −0.1 | 0.009 |
| $HbA_{1c}$, mmol · $mol^{-1}$ | 49.7 ± 5.2 | 48.5 ± 7.8 | 0.60 | −2.2 | −3.8, −0.7 | 0.006 |
| β-OH-but, μmol · $L^{-1}$ | 0.13 ± 0.06 | 0.21 ± 0.14 | 0.02 | 0.27 | 0.03, 0.51 | 0.03 |
| FFA mean, mmol · $L^{-1}$ | 0.79 ± 0.21 | 0.78 ± 0.25 | 0.83 | 0.06 | −0.05, 0.18 | 0.27 |
| Lactate, mmol · $L^{-1}$ | 1.50 ± 0.57 | 1.34 ± 0.45 | 0.34 | −0.17 | −0.37, 0.04 | 0.11 |
| Insulin, pmol · $L^{-1}$ | 55.4 ± 24.9 | 55.7 ± 31.1 | 0.18 | −8.1 | −21.7, 5.5 | 0.24 |
| Glucagon, pmol · $L^{-1}$ | 13.4 ± 8.4 | 13.7 ± 7.8 | 0.90 | 0.4 | −2.1, 2.9 | 0.75 |
| Insulin/glucagon | 5.1 ± 3.0 | 4.1 ± 3.0 | 0.28 | −0.4 | −1.4, 0.6 | 0.45 |

Baseline data are expressed as means ± SDs and treatment effects as least square means (LSMs) and 95% CIs. $P$ values are for difference between dapagliflozin and placebo at baseline and for treatment effects at end of treatment.

increase in hepatic [$^{18}$F]FTHA and FA uptake ($n$ = 17; $r$ = −0.49; $P$ = 0.045 and $r$ = −0.48; $P$ = 0.045, respectively), the changes in serum FFA levels, plasma β-hydroxybutyrate, lactate, or glucagon/insulin ratio were not correlated with the changes in tissue FA or [$^{18}$F]FTHA uptake. Accordingly, no significant association between tissue FA or [$^{18}$F]FTHA uptake and circulating substrate levels or glucagon/insulin ratio were observed at baseline in the whole study group ($N$ = 38).

**Table 2—Tissue fractional [$^{18}$F]FTHA uptake and FA uptake rates at baseline and effect of treatment**

| | Baseline | | | End of treatment | | |
|---|---|---|---|---|---|---|
| | Dapagliflozin ($n$ = 21) | Placebo ($n$ = 17) | $P$ | LSM difference | 95% CI for difference | $P$ |
| Fractional [$^{18}$F]FTHA uptake rate, mL · (mL · min)$^{-1}$ · 1,000 | | | | | | |
| Skeletal muscle | 6.5 ± 1.5 | 7.5 ± 1.9 | 0.07 | 0.58 | −0.26, 1.43 | 0.17 |
| Duodenum | 31 ± 9.8 | 33 ± 9.7 | 0.50 | 5.4 | −0.37, 11.06 | 0.07 |
| Liver | 207 ± 46 | 216 ± 43 | 0.55 | 35 | 13, 57 | 0.003 |
| Visceral AT | 4.5 ± 1.2 | 5.7 ± 2.4 | 0.08 | 0.54 | −0.43, 1.5 | 0.27 |
| Subcutaneous AT | 3.2 ± 0.71 | 3.5 ± 0.76 | 0.13 | 0.25 | −0.22, 0.72 | 0.29 |
| Myocardium | 47 ± 10 | 49 ± 13 | 0.62 | 4.4 | −0.17, 10 | 0.15 |
| White matter | 8.6 ± 2.1 | 9.2 ± 1.0 | 0.27 | 1.3 | 0.48, 2.1 | 0.003 |
| Gray matter | 7.6 ± 1.9 | 8.2 ± 1.4 | 0.34 | 1.3 | 0.35, 2.2 | 0.008 |
| Whole brain | 7.3 ± 1.7 | 7.7 ± 1.0 | 0.34 | 1.1 | 0.41, 1.8 | 0.003 |
| FA uptake rate, μmol · 100 $g^{-1}$ · $min^{-1}$ | | | | | | |
| Skeletal muscle | 0.49 ± 0.16 | 0.56 ± 0.22 | 0.24 | 0.11 | 0.01, 0.20 | 0.03 |
| Duodenum | 2.4 ± 1.2 | 2.5 ± 1.1 | 0.79 | 0.59 | −0.10, 1.3 | 0.09 |
| Liver | 15 ± 5.4 | 16 ± 6.7 | 0.67 | 4.5 | 0.80, 8.2 | 0.02 |
| Visceral AT | 0.39 ± 0.16 | 0.46 ± 0.24 | 0.29 | 0.09 | −0.04, 0.22 | 0.30 |
| Subcutaneous AT | 0.27 ± 0.09 | 0.29 ± 0.10 | 0.43 | 0.04 | −0.04, 0.11 | 0.15 |
| Myocardium | 3.6 ± 1.0 | 3.6 ± 1.2 | 0.99 | 0.11 | −0.51, 0.74 | 0.71 |
| White matter | 0.41 ± 0.13 | 0.46 ± 0.14 | 0.26 | 0.11 | 0.03, 0.20 | 0.01 |
| Gray matter | 0.37 ± 0.13 | 0.41 ± 0.13 | 0.30 | 0.10 | 0.02, 0.18 | 0.01 |
| Whole brain | 0.35 ± 0.11 | 0.39 ± 0.11 | 0.34 | 0.10 | 0.02, 0.17 | 0.01 |

Baseline data are expressed as means ± SDs and treatment effects as least square means (LSM) and 95% CIs. $P$ values are for difference between dapagliflozin and placebo at baseline and for treatment effects at end of treatment. AT adipose tissue.

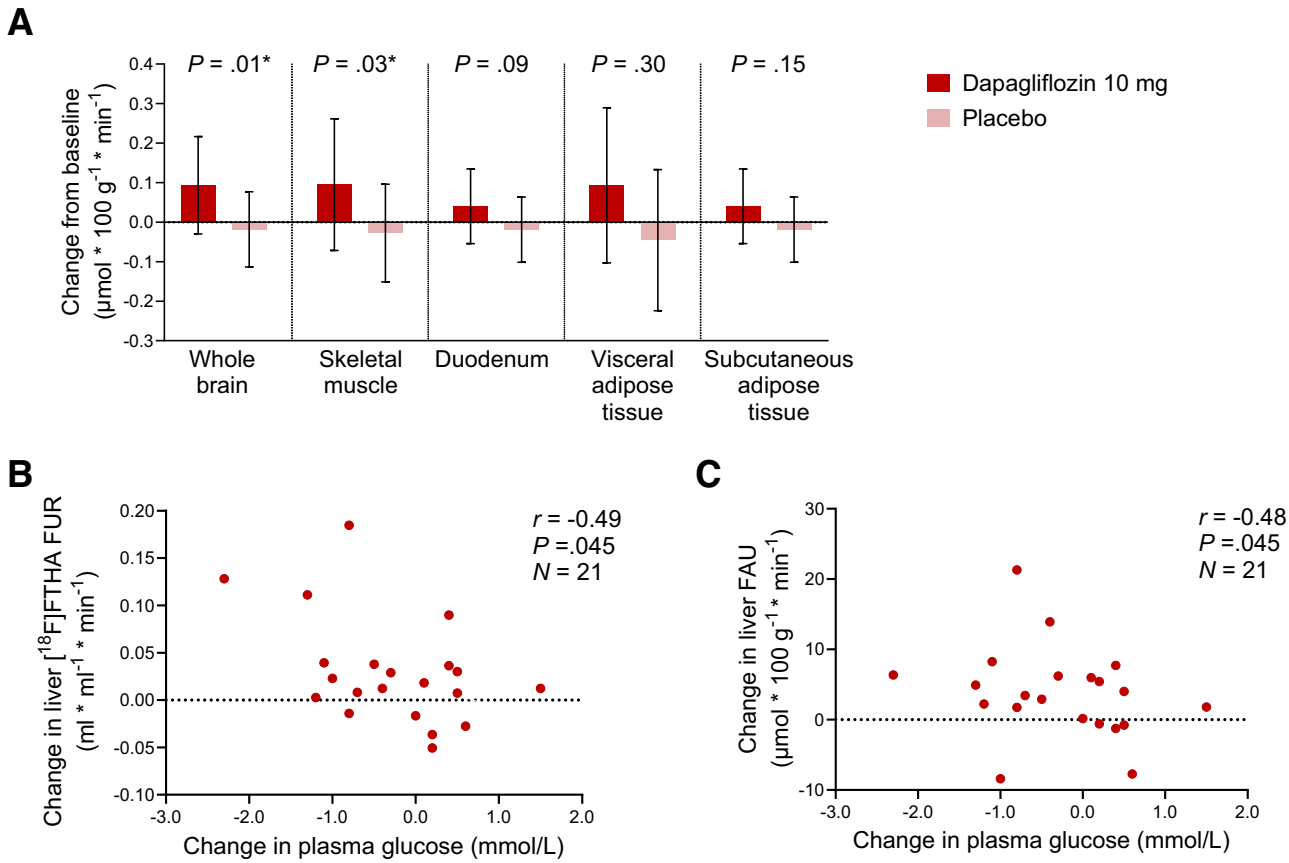



**Figure 1**—*A*: Dapagliflozin treatment induced a significant increase in the brain and skeletal muscle FA uptake (FAU), whereas the treatment effect was not statistically significant in the intestine or adipose tissue (dapagliflozin group $n = 21$; placebo group $n = 17$). *B* and *C*: In the dapagliflozin treatment arm, the increases in hepatic [$^{18}$F]FTHA and FAU from baseline to end of treatment were greater in patients who presented a more significant decrease in plasma glucose. Changes are reported as means and 95% CIs. *$P < 0.05$. AT, adipose tissue.

The change in plasma glucose level did not correlate with changes in the [$^{18}$F]FTHA FUR or FA uptake rate in any tissue other than the liver.

### Increased Brain FA Uptake

In the dapagliflozin group, brain [$^{18}$F]FTHA FUR and FA uptake were increased globally, with [$^{18}$F]FTHA FUR increasing by 22.1–28.2% and FA uptake by 28.0–34.4% in all brain regions (Table 2 and Fig. 2).

The brain fractional [$^{18}$F]FTHA uptake rate was significantly correlated with that in the liver and duodenum at baseline in the whole group ($N = 38$; $r = 0.51$; $P = 0.001$ and $r = 0.59$; $P < 0.001$, respectively). In the dapagliflozin treatment arm, the increase in brain [$^{18}$F]FTHA FUR was also correlated with the [$^{18}$F]FTHA FUR changes in the liver and duodenum ($n = 20$; $r = 0.64$; $P = 0.003$ and $r = 0.74$; $P < 0.001$, respectively). In comparison, no similar association could be found between the brain and skeletal muscle or adipose tissue (all $P > 0.6$) (Supplementary Fig. 1).

### No Significant Effects on Plasma [$^{18}$F]FTHA Radiometabolites

Fractions of unmetabolized [$^{18}$F]FTHA of total plasma radioactivity were similar between groups at baseline, averaging 88.6% (SD 6.3) at 5 min, 69.3% (SD 11.3) at 10 min, 42.6% (SD 10.1) at 20 min, and 27.2% (SD 6.8%) at 30 min after radiotracer injection. The parent fractions were not affected by dapagliflozin ($P > 0.2$ at each time point). In both groups and at both visits, the decline in radioactivity in plasma evened after 20 min, signaling an increase in circulating radiometabolites of [$^{18}$F]FTHA. During both baseline and end-of-treatment visits, the differences in the AUC of metabolite-corrected plasma radioactivity versus total plasma activity averaged 3% (SD 9) during the 32-min thoracal scan and 7% (SD 13) when measured until the end of the brain scan and were not affected by treatment ($P = 0.19$).

## CONCLUSIONS

This is the first study to report the effects of an SGLT2 inhibitor on tissue-specific FA uptake in the skeletal muscle, small intestine, and brain in humans in vivo. The findings are in line with previous results in type 2 diabetes showing a decrease in the respiratory exchange ratio, indicating a systemic substrate shift from glucose to FA oxidation in response to SGLT2 inhibitor treatment (2,4). The results in the liver and myocardium were discussed in detail in the first publication on the study (5).

In skeletal muscle, the increase in FFA uptake is at least in part accounted for by the increase in serum FFA levels, because the increase in the [$^{18}$F]FTHA uptake rate was less pronounced than the calculated FA uptake rate. The mean 22% increase in skeletal muscle FA uptake might reflect enhanced (3) β-oxidation (20), but also lipid buildup in muscle cells (21), as reported earlier during SGLT2 inhibitor treatment, because in the muscle, [$^{18}$F]FTHA is mostly bound to complex lipids (6). Of note, an increase in intramyocellular as opposed to intercellular lipid content, as is seen in athletes, has been linked to improved

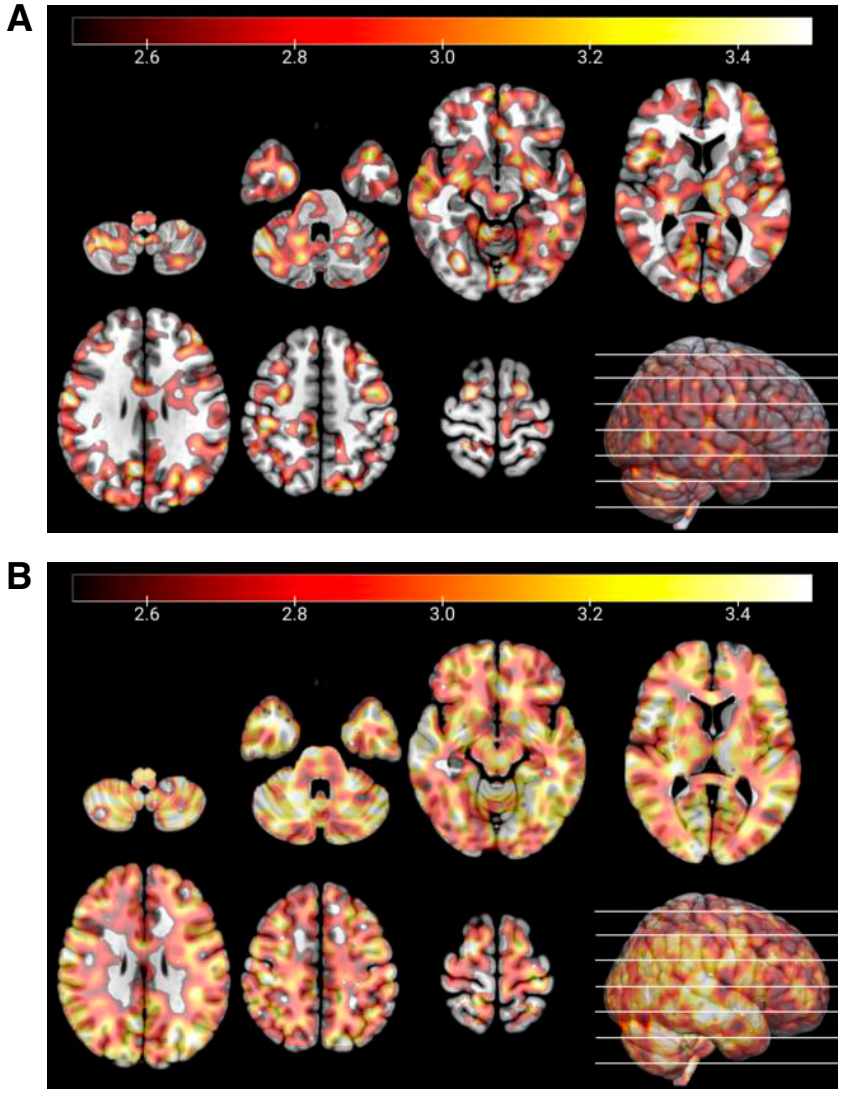



**Figure 2**—Brain [$^{18}$F]FTHA FUR (*A*) and FA uptake (*B*) were increased globally. The figures were made by statistical parametric mapping from the whole study population to compare treatment effects. Brighter color indicates higher T score (i.e., more significant difference between dapagliflozin and placebo).

mitochondrial function (22) and has also been observed after dapagliflozin treatment in individuals with prediabetes (23). Whether our finding might indicate a positive shift in FA processing within the tissue cannot be confirmed from the data collected.

Despite the small intestine being a significant producer of TAGs from dietary fats, plasma FFAs are mainly used as sources of energy or for the buildup of phospholipids in enterocytes (24). In the current study, the rate of intestinal FA uptake in patients with type 2 diabetes was similar to that reported previously in individuals with severe obesity, with the uptake rates being elevated in comparison with those in healthy controls (25).

We did not observe changes in visceral or subcutaneous adipose tissue FA uptake. A previous report showed a significant decrease in adipose tissue volume in both depots after treatment with SGLT2 inhibitors (26). In contrast to our findings, Lauritsen et al. (13) reported increased FA uptake in visceral, but not in subcutaneous, adipose tissue after 4 weeks of treatment with empagliflozin. Because GLUT4 expression was also reported to decrease, possibly hindering glycerol production and lipid storage in white adipocytes, the authors attributed this finding to increased lipid turnover in visceral adipose tissue and increased FA availability. Importantly, [$^{11}$C]-palmitate was used in that study, so the use of different radiotracers could explain the apparently discrepant results. The most surprising finding in our study was the 30% increase in brain [$^{18}$F]FTHA and FA uptake by dapagliflozin. In the whole study group, baseline FA uptake rates in white and gray matter were similar to those found in individuals with insulin resistance (27). Others have reported a two- to threefold higher brain [$^{18}$F]FTHA uptake rate in individuals with severe obesity compared with healthy lean controls (8), but because of different timeframes for PET scanning, direct comparison with the current results is not feasible. Although the final fate of FAs taken up by the brain remains uncertain, a majority of FAs are likely stored as lipids rather than consumed as fuels. This is supported by a previous study reporting that $>$69% of total [$^{18}$F]FTHA radioactivity was recovered in the brain lipid pool, mostly as TAGs (53%) and phospholipids (7%) (8).

Although it has been suggested that the brain might be able to oxidize up to 20% of the FAs it stores (28), this capacity is likely very limited to restrain oxidative stress (29). Also, the total amount of oxygen that would be needed for the complete oxidation of the measured FA uptake in the current study would exceed the actual brain oxygen consumption (30). Because recent studies have indicated improved cognitive function and reduced risk of Alzheimer disease after longer-term treatment with SGLT2 inhibitors (31,32), it seems unlikely that these drugs would upregulate brain FA oxidation to such a degree that would increase reactive oxygen species production. Consequently, it can be hypothesized that the increased cerebral FA uptake associated with insulin resistance (27) and obesity (8) is different from the mechanism explaining the increased uptake after treatment with dapagliflozin. A possible explanation for the increased FA uptake seen after dapagliflozin treatment is the enhanced myelination in the white matter and the improved rate of membrane turnover in the gray matter, both of which have been shown to be impeded in type 2 diabetes (33,34), but this must be considered speculative, because it cannot be established based on the results of the current or previous studies. Nevertheless, increased FA oxidation enabled by enhanced mitochondrial oxidative capacity likely has a less significant effect.

TAG trafficking is also a potential link between brain, liver, and small intestine FA uptake in the current study, but because this association has not been

described before, there is no direct evidence supporting the proposition. Still, because the liver and small intestine are the main sources of TAGs in the body, the brain has been shown to readily take up TAGs (35), and the brain was scanned late enough to allow accumulation of [$^{18}$F]FTHA-carrying TAGs.

Unfortunately, brain FA and [$^{18}$F]FTHA uptake are still inadequately understood to fully elucidate the current findings (36), limiting the interpretation of the results. Based on current knowledge, [$^{18}$F]FTHA should not significantly degrade to $^{18}$F-carrying ketone bodies, so ketonemia associated with SGLT2 inhibitor treatment cannot explain the results. Unfortunately, the radiometabolites of [$^{18}$F]FTHA are largely uncharacterized, so we cannot determine the contribution of metabolites when calculating the total uptake. A previous study using an earlier timepoint showed twofold higher uptake rates compared with the uptake rates found in this study (8), so it is therefore important to avoid comparing uptake rates when the time used for detection of uptake is different.

We did not measure $^{18}$F-containing compounds in TAGs or the change in cholesterol ester levels during treatment. At any rate, the results mostly represent FA uptake in the brain, free or released from TAGs. Because we did not quantify plasma total radioactivity after 30 min, increased rerelease of [$^{18}$F]FTHA into the circulation after dapagliflozin could lead to an overestimation of the fractional uptake rate in the brain. However, because total plasma activity at 30 min postinjection was low, ~6% of the peak activity, the majority of the radiotracer was likely trapped in tissues during the early minutes of the scan. Furthermore, the difference between the AUCs of radiometabolite corrected versus total plasma radioactivity was not affected by the treatment, indicating changes in total metabolites did not influence the results. We also note that [$^{18}$F]FTHA is a false long-chain saturated FA analog, so the results should not be interpreted to describe FA uptake or metabolism in general. Finally, the duration of the treatment was 6 weeks, so it might be that the observed changes would not persist with longer treatment.

In conclusion, 6 weeks of dapagliflozin treatment induced a significant increase in skeletal muscle and brain FA uptake in individuals with type 2 diabetes, observed via direct in vivo metabolic imaging. We hypothesize that these changes mirror different tissue-specific changes in response to SGLT2 inhibitor treatment: an increased uptake in response to abundant circulating FFAs in skeletal muscle, an enhanced capacity to use FAs as fuels via β-oxidation in the skeletal muscle and liver, and enhanced myelination and membrane repair in the brain. However, additional studies of the effects of SGLT2 inhibitors on tissue metabolism are warranted, especially concerning the changes in the central nervous system, to further characterize the changes in FA use in the brain.

**Acknowledgements.** The authors thank study nurses Sanna Himanen, Mia Koutu (Turku), and Helena Wall (Uppsala) and the volunteers who participated in this study.

**Funding.** The study was funded by AstraZeneca AB (Gothenburg, Sweden). The work was also supported by a personal grant from the Finnish Medical Foundation (A.L.-R.).

**Duality of Interest.** K.H. is an employee of Antaros Medical. E.F. consults with Boehringer-Ingelheim, Eli Lilly, and Oramed Pharmaceuticals and has received research grant support from Janssen and Sanofi. J.Os. is employed by AstraZeneca Gothenburg. No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** A.L.-R., E.R., S.L., M.L.-H., L.P., and H.I. performed the study visits. A.L.-R., E.R., S.L., E.F., J.Ol., J.Os., and P.N. participated in study planning. A.L.-R. and J.T. performed the statistical analyses. A.L.-R., J.T., A.B., H.K., J.K., K.H., and L.N. performed PET data analyses. R.A. performed the radiotracer metabolite analysis. A.K.K. performed [$^{18}$F]FTHA production. All authors commented on the manuscript. P.N. was the principal investigator and is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Parts of this study were presented at the 59th Annual Meeting of the European Association for the Study of Diabetes, Hamburg, Germany, 4 October 2023.

**Handling Editors.** The journal editors responsible for overseeing the review of the manuscript were Liz Selvin and Justin Basile Echouffo-Tcheugui.

## References

1. Scheen AJ. Sodium-glucose cotransporter type 2 inhibitors for the treatment of type 2 diabetes mellitus. Nat Rev Endocrinol 2020;16:556–577
2. Ferrannini E, Baldi S, Frascerra S, et al. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. Diabetes 2016;65:1190–1195
3. Op den Kamp YJM, de Ligt M, Dautzenberg B, et al. Effects of the SGLT2 inhibitor dapagliflozin on energy metabolism in patients with type 2 diabetes: a randomized, double-blind crossover trial. Diabetes Care 2021;44:1334–1343
4. Daniele G, Xiong J, Solis-Herrera C, et al. Dapagliflozin enhances fat oxidation and ketone production in patients with type 2 diabetes. Diabetes Care 2016;39:2036–2041
5. Oldgren J, Laurila S, Åkerblom A, et al. Effects of 6 weeks of treatment with dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on myocardial function and metabolism in patients with type 2 diabetes: a randomized, placebo-controlled, exploratory study. Diabetes Obes Metab 2021;23:1505–1517
6. DeGrado TR, Coenen HH, Stocklin G. 14(R,S)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid (FTHA): evaluation in mouse of a new probe of myocardial utilization of long chain fatty acids. J Nucl Med 1991;32:1888–1896
7. Guiducci L, Grönroos T, Järvisalo MJ, et al. Biodistribution of the fatty acid analogue $^{18}$F-FTHA: plasma and tissue partitioning between lipid pools during fasting and hyperinsulinemia. J Nucl Med 2007;48:455–462
8. Karmi A, Iozzo P, Viljanen A, et al. Increased brain fatty acid uptake in metabolic syndrome. Diabetes 2010;59:2171–2177
9. Takala TO, Nuutila P, Pulkki K, et al. 14(R,S)-[$^{18}$F]Fluoro-6-thia-heptadecanoic acid as a tracer of free fatty acid uptake and oxidation in myocardium and skeletal muscle. Eur J Nucl Med Mol Imaging 2002;29:1617–1622
10. Labbé SM, Croteau E, Grenier-Larouche T, et al. Normal postprandial nonesterified fatty acid uptake in muscles despite increased circulating fatty acids in type 2 diabetes. Diabetes 2011;60:408–415
11. Bergmann SR, Weinheimer CJ, Markham J, Herrero P. Quantitation of myocardial fatty acid metabolism using PET. J Nucl Med 1996;37:1723–1730
12. Lauritsen KM, Nielsen BRR, Tolbod LP, et al. SGLT2 inhibition does not affect myocardial fatty acid oxidation or uptake, but reduces myocardial glucose uptake and blood flow in individuals with type 2 diabetes: a randomized double-blind, placebo-controlled crossover trial. Diabetes 2021;70:800–808
13. Lauritsen KM, Voigt JH, Pedersen SB, et al. Effects of SGLT2 inhibition on lipid transport in adipose tissue in type 2 diabetes. Endocr Connect 2022;11:e210558
14. Savisto N, Viljanen T, Kokkomäki E, Bergman J, Solin O. Automated production of [18 F]FTHA according to GMP. J Labelled Comp Radiopharm 2018;61:84–93
15. Rainio O, Han C, Teuho J, et al. Carimas: an extensive medical imaging data processing tool for research. J Digit Imaging 2023;36:1885–1893
16. Karjalainen T, Tuisku J, Santavirta S, et al. Magia: robust automated image processing and kinetic modeling toolbox for PET neuroinformatics. Front Neuroinform 2020;14:3
17. Thie JA. Clarification of a fractional uptake concept. J Nucl Med 1995;36:711–712
18. Ishizu K, Nishizawa S, Yonekura Y, et al. Effects of hyperglycemia on FDG uptake in human brain and glioma. J Nucl Med 1994;35:1104–1109
19. Mäki MT, Haaparanta M, Nuutila P, et al. Free fatty acid uptake in the myocardium and skeletal muscle using fluorine-18-fluoro-6-thia-

heptadecanoic acid. J Nucl Med 1998;39:1320–1327
20. Wallenius K, Kroon T, Hagstedt T, et al. The SGLT2 inhibitor dapagliflozin promotes systemic FFA mobilization, enhances hepatic β-oxidation, and induces ketosis. J Lipid Res 2022;63:100176
21. Op den Kamp YJM, Gemmink A, de Ligt M, et al. Effects of SGLT2 inhibitor dapagliflozin in patients with type 2 diabetes on skeletal muscle cellular metabolism. Mol Metab 2022;66:101620
22. Meex RCR, Schrauwen-Hinderling VB, Moonen-Kornips E, et al. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes 2010;59:572–579
23. Veelen A, Andriessen C, Op den Kamp Y, et al. Effects of the sodium-glucose cotransporter 2 inhibitor dapagliflozin on substrate metabolism in prediabetic insulin resistant individuals: a randomized, double-blind crossover trial. Metabolism 2023;140:155396
24. Gangl A, Ockner RK. Intestinal metabolism of plasma free fatty acids. Intracellular compartmentation and mechanisms of control. J Clin Invest 1975;55:803–813
25. Koffert J, Ståhle M, Karlsson H, et al. Morbid obesity and type 2 diabetes alter intestinal fatty acid uptake and blood flow. Diabetes Obes Metab 2018;20:1384–1390
26. Latva-Rasku A, Honka M-J, Kullberg J, et al. The SGLT2 inhibitor dapagliflozin reduces liver fat but does not affect tissue insulin sensitivity: a randomized, double-blind, placebo-controlled study with 8-week treatment in type 2 diabetes patients. Diabetes Care 2019;42:931–937
27. Honkala SM, Johansson J, Motiani KK, et al. Short-term interval training alters brain glucose metabolism in subjects with insulin resistance. J Cereb Blood Flow Metab 2018;38:1828–1838
28. Ebert D, Haller RG, Walton ME. Energy contribution of octanoate to intact rat brain metabolism measured by 13C nuclear magnetic resonance spectroscopy. J Neurosci 2003;23:5928–5935
29. Schönfeld P, Reiser G. Why does brain metabolism not favor burning of fatty acids to provide energy? Reflections on disadvantages of the use of free fatty acids as fuel for brain. J Cereb Blood Flow Metab 2013;33:1493–1499
30. Goyal MS, Vlassenko AG, Blazey TM, et al. Loss of brain aerobic glycolysis in normal human aging. Cell Metab 2017;26:353–360.e3
31. Low S, Goh KS, Ng TP, et al. Association between use of sodium-glucose co-transporter-2 (SGLT2) inhibitors and cognitive function in a longitudinal study of patients with type 2 diabetes. J Alzheimers Dis 2022;87:635–642
32. Wu C-Y, Iskander C, Wang C, et al. Association of sodium-glucose cotransporter 2 inhibitors with time to dementia: a population-based cohort study. Diabetes Care 2023;46:297–304
33. Wang D-Q, Wang L, Wei M-M, et al. Relationship between type 2 diabetes and white matter hyperintensity: a systematic review. Front Endocrinol (Lausanne) 2020;11:595962
34. Roy B, Ehlert L, Mullur R, et al. Regional brain gray matter changes in patients with type 2 diabetes mellitus. Sci Rep 2020;10:9925
35. Banks WA, Owen JB, Erickson MA. Insulin in the brain: there and back again. Pharmacol Ther 2012;136:82–93
36. Tracey TJ, Steyn FJ, Wolvetang EJ, Ngo ST. Neuronal lipid metabolism: multiple pathways driving functional outcomes in health and disease. Front Mol Neurosci 2018;11:10