***SUPPLEMENTARY MATERIAL***

***Mixed Meal Tolerance Test Protocol***

The mixed meal tolerance test (MTT) consisted of a standard meal (18% protein, 31% fat, and 51% carbohydrate, total caloric content 585 kcal) that was consumed within 10 min. Tracers ([2H5]glycerol and 6,6-[2H2]glucose) were infused as a primed-constant infusion starting 120 minutes before the meal and continued until the end of the study (360 min after meal ingetsion) for the measurement of lipolysis and glucose fluxes, i.e. glucose production and clearance (1). The MTT contained 1.5g of U13C- glucose to measure glucose rate of absorption (1).

Arterialized blood samples were taken before the meal and then at regular intervals after the meal for 6 hours (i.e., 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min). Measurements of plasma apolipoprotein A and B concentration and lipidomic analyses were conducted on samples taken at 0, 180min and 360min considering that after mixed meals plasma TAG concentrations rise within 2-3 hours, reaching a plateau at 3-4 hours, and return to baseline by 6 hours (2).

***Measurement of the lipidomic profile***

Fasting plasma lipidomic profile was evaluated by liquid chromatography/quadrupole time-of-flight mass spectrometry (LC 1290 Infinity/MS Q-TOF -6545 Agilent Technology, Santa Clara, CA) equipped with an electrospray ionization source. Briefly, 10 µL of human plasma was deproteinized with 150 µL of cold methanol (Merck-Sigma-Aldrich, Darmstadt, Germany) and 10 µL of internal standard and centrifuged at 14000 rpm for 20 min. Subsequently, the supernatant was transferred into 0.2-mL glass inserts in screw-top vials with Teflon-lined caps (Agilent, Santa Clara, CA) and injected into the LC-MS QTOF. For liquid chromatography analysis, we used an Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm 1.8-Micron column at 50°C. Mobile phase A was water with 0.1% formic acid and mobile phase B was isopropanol/acetonitrile (1:1, v:v) with 0.1% formic acid. The injection volume was 1 µl and the untargeted acquisition was performed in positive mode.

The quantitative targeted analysis of the spectra (n=94) was performed with the Agilent MassHunter Profinder B.06.00, a mass spectrometry-based batch-targeted feature extraction software (Agilent, Santa Clara, CA). Lipid concentrations were calculated by relating the peak area of each lipid species to the peak area of the corresponding internal standard added to each sample before deproteinization within each lipid class; the internal standards were DAG(C34:0), TAG(C45:0), PC(C34:0), PE(C34:0), LPC(C17:0), SM(d18:1/17:0), CER(18:1/17:0) (Avanti Polar Lipids, Alabaster, AL and Larodan, Solna, SE). The proportion of unsaturated and saturated fat was evaluated using the number of double bonds for each lipid species and was considered as follows: 0-1 double bounds as saturated and ≥2 double bonds as unsaturated for DAG, PC and PE; 0-2 double bounds as saturated and ≥3 double bonds as unsaturated for TAG; 0 double bounds as saturated and ≥1 double bond as unsaturated for CER and LPC.

Plasma FFA composition was measured by gas chromatography-mass spectrometry (GC7890-MS5975, Agilent Technology, Santa Clara, CA) with electron ionization (EI). Briefly, 20 μL of plasma sample was mixed with heptadecanoic acid (C17:0) (Merck-Sigma-Aldrich, Darmstadt, Germany) as internal standard, 200 μL of methanol:chloroform 2:1, 100 μL of chloroform (Merck-Sigma-Aldrich, Darmstadt, Germany), and 100 μL of MilliQ water, vortexed and centrifuged at 14000 rpm for 20 min). The organic phase was dried under nitrogen flux, reconstituted with 80 μL of acetonitrile (Sigma-Aldrich), derivatized with 20 μL of N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Merck-Sigma-Aldrich, Darmstadt, Germany) for 40 min at 75ºC and measured by GC-MS equipped with a capillary column (DB-5MS J&W, l 30 m; i.d. 0.25 mm; film thickness 0.25 µm, J&W, Agilent). FFA composition included myristic (C14:0), palmitoleic (C16:1), palmitic (C16:0), linoleic (C18:2), oleic (C18:1) and stearic (C18:0) acid. Retention times for each FFA were identified by a single injection of known standards (Merck-Sigma-Aldrich, Darmstadt, Germany). The percentage of each FFA was calculated as the area under the peak divided by the total area. Saturated FFA were calculated as the sum of myristic acid, palmitic acid, and stearic acid, and unsaturated FFA were calculated as the sum of palmitoleic acid, oleic acid, and linoleic acid.

***Hormone measurements***

Plasma insulin was measured by electro-chemiluminescence (COBASe411 instrument, Roche, Indianapolis, USA), and plasma glucagon by radioimmunoassay (Millipore Corporation, Billerica, MA, USA). Plasma GLP-1 and GIP concentrtaions were measured using a Milliplex® kit (Merck KGaA, Darmstadt, Germany) on Luminex® (Millipore Corporation, Billerica, MA, USA).

***Fasting and postprandial insulin resistance measurements***

Insulin resistance during fasting state was assessed as HOMA-IR, Hepatic-IR and Adipo-IR indexes, while in postprandial state OGIS180 was used as index of peripheral insulin sensitivity, as previously reported (1; 3).

***Tracer measurements and Calculations of lipid fluxes***

Tracer enrichments were measured in plasma samples at all times by gas chromatography/mass spectrometry (GCMS *5975* Agilent Technologies, Fullerton, CAUSA) as described previously (1).

Tracer data during fasting and postprandial state were analyzed with mathematical modeling for the quantification of rate of appearance of glycerol and glucose fluxes (i.e., glucose production, oral glucose rate of appearance during postprandial state and glucose clearance) as previously described (1; 3).

FFA uptake by peripheral tissues (Rd\_FFA) was calculated as

Rd\_FFA(t) (umol/min) = Lipolysis(t) (umol/min) – dFFA(t)/dt x Vol (ml/kg) x BW (kg)

* Lipolysis(t) was estimated as 3 times Ra\_glycerol(t), considering that during TAG hydrolysis 1 glycerol and 3 FFAs are released.
* dFFA(t)/dt = [FFA(t)-FFA(t1)]/(t-t1); FFA(t) and FFA(t1) are the concentrations (umol/ml) measured at time t and at (t1), i.e., the time point just before time (t);
* Vol is the volume of distribution that for FFA considered equal to plasma volume of distribution (4) and was estimated according to the formula proposed by Lemmens for subjects with severe obesity (5), i.e. Vol (ml/kg)=

References

1. Gastaldelli A: Measuring and estimating insulin resistance in clinical and research settings. Obesity. 2022;30:1549-1563

2. Lairon D, Lopez-Miranda J, Williams C: Methodology for studying postprandial lipid metabolism. Eur J Clin Nutr. 2007;61:1145-1161

3. Camastra S, Astiarraga B, Tura A, Frascerra S, Ciociaro D, Mari A, Gastaldelli A, Ferrannini E: Effect of exenatide on postprandial glucose fluxes, lipolysis, and ss-cell function in non-diabetic, morbidly obese patients. Diabetes Obes Metab. 2017;19:412-420

4. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, Wolfe RR: Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. The American journal of physiology. 1993;265:E380-391

5. Lemmens HJ, Bernstein DP, Brodsky JB: Estimating blood volume in obese and morbidly obese patients. Obes Surg. 2006;16:773-776