Online Supplemental Materials

for

Subcutaneous adipose tissue metabolic function and insulin sensitivity in people with obesity

Han-Chow E. Koh, Stephan van Vliet, Terri A. Pietka, Gretchen A. Meyer, Babak Razani, Richard Laforest, Robert J Gropler, and Bettina Mittendorfer

Study participants

The data reported here were obtained from fifteen healthy lean people and thirty-seven people with obesity who participated in two different studies (ClinicalTrials.gov numbers NCT02994459 and NCT03408613) that used the same experimental protocol. The studies were approved by the Human Research Protection Office at Washington University School of Medicine in St. Louis, MO, USA. Participants were recruited by using the Washington University Volunteer for Health registry, through advertising in the local (Washington University and general St. Louis Metropolitan area) community, and through word-by-mouth. Written informed consent was obtained from all participants before they enrolled in the study. All participants completed a comprehensive medical examination, including a history and physical examination, a resting electrocardiogram, standard blood tests, and an oral glucose tolerance test before being enrolled in the study. They were eligible to participate in this study if: i) they were sedentary (<1.5 hour of exercise/week) and weight stable (no more than ±2 kg weight change for at least 3 months); ii) they were between 18 years and 70 years old; iii) their body mass index was ≥18.5 and <25.0 kg/m^2 (lean) or ≥30.0 and <50.0 kg/m^2 (obese); and iii) their maximum body circumference was <60 cm (to fit in the PET scanner). Potential participants were excluded if they exercised or engaged in vigorous physical activities for ≥1.5 h per week, had evidence of chronic illness or significant organ dysfunction, took medications or dietary supplements that interfere with insulin action or glucose or lipid metabolism (including, anti-

diabetes medications, lipid lowering medications, antihypertensive mediations, steroids, weight loss medications and weight loss supplements, anticoagulants, antiplatelet therapies, and antibiotics), or consumed tobacco products or excessive amounts of alcohol (>115 g alcohol per week). Pregnant and lactating women were also excluded.

Anthropometric assessments

Participants' height and body mass were measured to the nearest millimeter and dekagram. Body surface area was calculated as the product of (body mass in kg × 0.425) and (height in cm × 0.725), multiplied by 0.007184 (DuBois & DuBois 1916). Fat-free mass, total body fat mass, and the android/gynoid fat distribution (i.e., adipose tissue in the trunk vs the thigh) were assessed by using dual energy X-ray absorptiometry (Lunar iDXA, GE Healthcare). Intra-abdominal and abdominal SAT volumes were measured by using magnetic resonance imaging (Prisma 3T, Siemens).

Metabolic testing procedures

Each participant completed: i) a basal metabolic study to determine basal glucose and palmitate kinetics by using intravenous [6,6-$^{2}H_2$]glucose and [U-^{13}C]palmitate infusions, and ii) a two-stage hyperinsulinemic-euglycemic clamp procedure in conjunction with [6,6-$^{2}H_2$]glucose and [U-^{13}C]palmitate infusions and PET scanning after injections of [^{15}O]water and [^{18}F]fluorodeoxyglucose to determine whole-body, SAT, and muscle insulin-stimulated glucose uptake, SAT perfusion, and insulin action on adipose tissue triglyceride lipolysis. Fourteen participants (ten obese, four lean) also completed the PET scanning protocol after [^{18}F]fluorodeoxyglucose injection during basal conditions to determine basal SAT and muscle glucose uptake rates.

Basal metabolic study. Participants were instructed to abstain from alcohol and exercise and vigorous physical activities for three days before being admitted to the Clinical and Translational

Research Unit (CTRU) on the day before testing. Between 1900 h and 2000 h on the day of admission, they consumed a standard meal (800 kcal, 50% from carbohydrates, 30% from fat, and 20% from protein) and then fasted (except for water) overnight. At ~0600 h the following morning, a catheter was inserted into an antecubital vein to infuse [6,6-$^{2}H_{2}$]glucose (infusion rate: 0.22 μmol per kg body wt·min^{-1}, priming dose: 20 μmol/kg body wt) and [U-^{13}C]palmitate (6.0 nmol per kg fat-free mass·min^{-1}) bound to human albumin for 240 min. Another catheter was inserted into a hand vein, and the hand was warmed to 55°C for blood sampling. Participants who completed PET scanning during basal conditions, were transferred to the PET/CT scanner (Siemens Biograph True Point/True View) after ~150 min where ~1.2 GBq [^{15}O]water was administered as a bolus and 2 min of dynamic PET scanning of the torso was performed. Subjects were then quickly repositioned and a second dose of [^{15}O]water was administered followed by 2 min of PET scanning of the thigh. At ~200 min, ~185 MBq [^{18}F]FDG was administered intravenously and 40 min dynamic PET scanning of the torso, followed by 30 min dynamic PET scanning of the thigh was performed. Low dose CT scans (120 kVp, 50 mAs effective) were performed for attenuation correction and to delineate the muscle regions of interest. Blood samples to determine plasma glucose and palmitate enrichments, and substrate and hormone concentrations were collected immediately before the start of the tracer infusions and then every 10 min from 150 min to 240 min. A SAT biopsy from the periumbilical region was obtained approximately 2 hours after the start of the study to evaluate adipocyte size and gene expression.

Two-stage hyperinsulinemic-euglycemic pancreatic clamp. Participants were instructed to abstain from alcohol and exercise and vigorous physical activities for three days before being admitted to the Clinical and Translational Research Unit (CTRU) on the day before testing. Between 1900 h and 2000 h, they consumed a standardized meal (800 kcal, 50% from carbohydrates, 30% from fat, and 20% from protein) and then fasted, except for water, until the completion of the study (Supplemental Figure 4) the next day. At ~0600 h, a catheter was

inserted into an antecubital vein to infuse the metabolic tracers, hormones, and dextrose; another catheter was inserted into a radial artery for blood sampling. Another catheter was inserted in the arterial artery of one arm for blood sampling. Participants were then transferred to the Center for Clinical Imaging Research, where a two-stage hyperinsulinemic-euglycemic pancreatic clamp procedure was performed. Constant infusions of octreotide (45 ng/kg FFM/min), glucagon (1.5 ng/kg FFM/min), growth hormone (6 ng/kg FFM/min) were started at time 0 and maintained for 390 min. Insulin was infused at 10 mU/m^2 body surface area/min for the first 120 min, then at 50 mU/m^2 body surface area/min, both initiated with a two-step priming dose; [6,6-$^2H_2$]glucose was infused at 0.22 µmol/kg/min for the first 120 min, and then at 0.11 µmol/kg/min (Conte et al 2012; Smith et al. 2016). Dextrose, enriched with [6,6-$^2H_2$]glucose (2.5%) was infused at a variable rate to maintain plasma glucose concentration (monitored every 10 min) at ~6.1 mmol/l during the hyperinsulinemic clamp procedure. We chose a pancreatic clamp procedure to minimize differences in plasma insulin concentration during the clamp procedure in healthy lean people and people with obesity (Conte et al 2012; Ter Horst et al. 2020). Approximately 270 min after starting the pancreatic clamp procedure, participants were transferred to the PET/CT scanner (Siemens Biograph True Point/True View) and the [^{15}O]water, [^{18}F]FDG injection and PET/CT scanning protocol described above was performed while the clamp procedure was continued. Blood samples to determine plasma glucose and palmitate enrichments, and substrate and hormone concentrations were collected immediately before the start of the hormone infusions and then every 10 min during PET scanning. Due to technical issues we were unable to obtain thigh glucose uptake measurements in nine of the fifty-two participants. Abdominal subcutaneous adipose tissue oxygen tension was assessed by using an oxygen-sensitive fiber-optic probe (Oxylite^{TM}, Oxford Optronix, Ltd) (Cifarelli et al. 2020). Biopsies from abdominal SAT were obtained under local anesthesia immediately after the PET scanning protocol to evaluate the mRNA expression of genes involved in glucose uptake and disposal.

Sample processing and analysis

Blood samples were collected in chilled tubes containing heparin or EDTA and stored at -80 °C until final analyses. Plasma glucose concentration was determined by using the glucose oxidase method (YSI 2300 STAT, YSI Inc, Yellow Springs, OH). Plasma insulin concentration was determined by using an automated immunoassay (Elecsys®, Roche Diagnostics). Plasma glucose and palmitate tracer-to-tracee ratios and plasma fatty acid concentrations were determined by using gas-chromatography coupled with mass spectrometry (Smith et al 2016; Mittendorfer et al 2003). SAT samples used to determine adipocyte size were collected in osmium tetroxide solution and analyzed by using an automated Coulter counter (Laforest et al 2017). SAT samples used for histological examination were fixed in 10% neutral buffered formalin and embedded in paraffin; sections were then stained with hematoxylin and eosin (H&E) and Masson's trichrome for digital image analysis at 20x (H&E) and 10x (trichrome) magnification. SAT samples used to determine mRNA expression were rinsed in ice-cold saline, snap-frozen in liquid nitrogen, and then stored at 80 °C before RNA was isolated and sequenced at the Washington University Genome Technology Access Center.

Calculations

Palmitate rate of appearance (Ra) in plasma was calculated by dividing the [U-^{13}C]palmitate infusion rate by the plasma palmitate tracer-to-tracee ratio (Mittendorfer 2003). Unlabeled glucose Ra in plasma was calculated by dividing the [$^{2}H_{2}$]glucose infusion rate by the plasma glucose tracer-to-tracee ratio (Smith 2016). Whole-body glucose uptake rate was calculated as the sum of unlabeled glucose Ra in plasma and the glucose tracer infusion rate. The hepatic insulin sensitivity index (which assesses the inhibitory effect of insulin on endogenous glucose production) was calculated as the inverse of the product of basal glucose Ra and plasma insulin concentration (DeFronzo 1988, Smith 2020). The insulin sensitivity index of adipose tissue lipolysis (which assesses the inhibitory effect of insulin on adipose tissue lipolytic rate) was

calculated as the inverse of the product of basal palmitate Ra in relation to fat mass and basal plasma insulin concentration (Fabbrini 2012). Tissue perfusion was determined by using a 1-compartment model to fit the arterial blood and tissue-specific activity time courses after [^{15}O]water injection (Muzik 2013). Glucose uptake rates (µmol/kg tissue/min) in abdominal and thigh SAT and major muscle groups was calculated by using Patlak graphical analysis of the tissue and arterial blood specific activity time curves (Kelley 1999, Patlak 1985) and tissue-specific lumped constants (Kelley 1999, Virtanen 2001). Total SAT glucose uptake (µmol/min) was calculated as the product of depot-specific glucose uptake and SAT mass; total muscle glucose uptake was calculated as the product of the average glucose uptake rate in the individual muscle groups and muscle mass (Buckinx, 2018).

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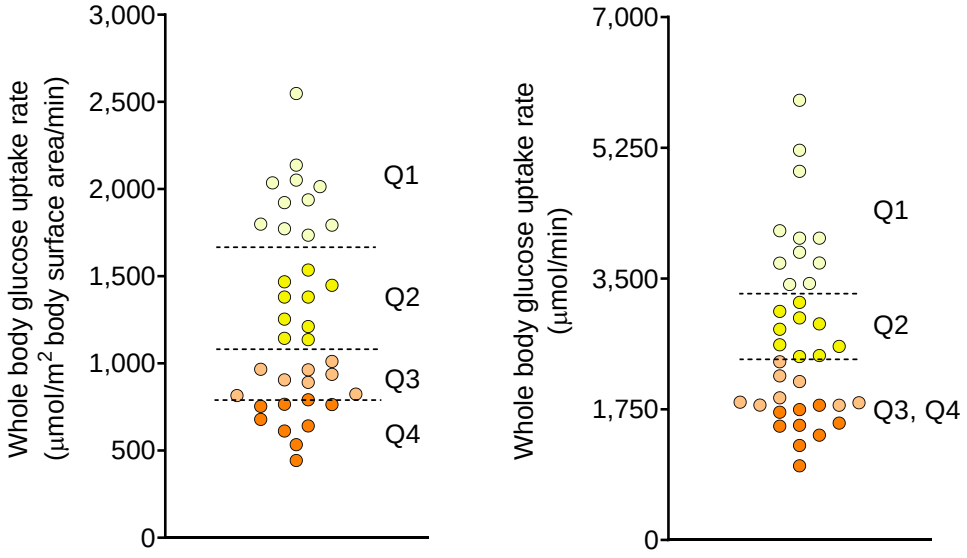
Supplemental Table 1. Plasma glucose, insulin and C-peptide concentrations and endogenous glucose production rate during basal conditions and during the two-stage hyperinsulinemic-euglycemic pancreatic clamp procedure

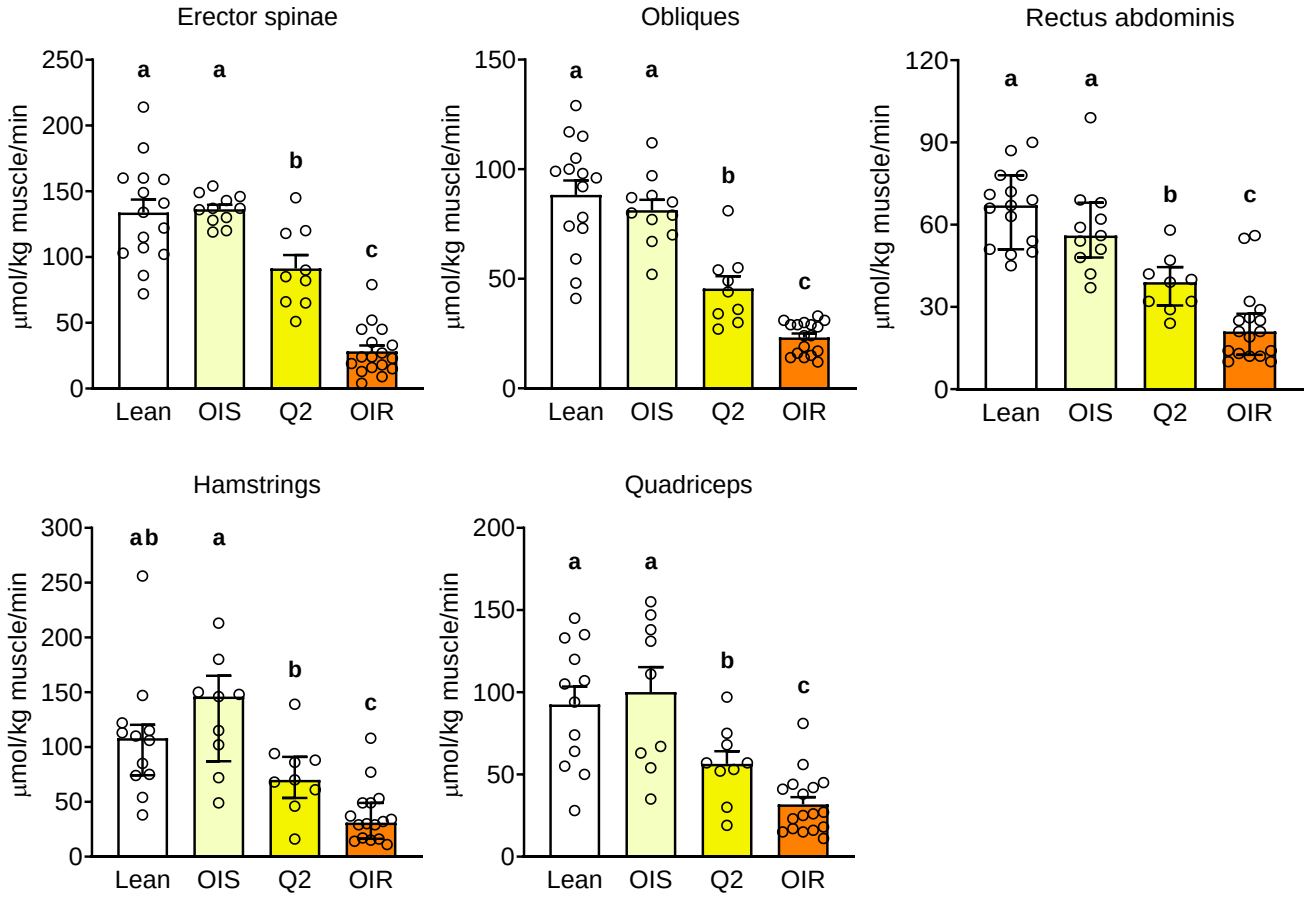
	Lean	Obese		
		Insulin-sensitive	Quartile 2	Insulin-resistant
Basal				
Glucose (mmol/l)	5.0 (4.5, 5.2)	4.8 (4.6, 5.3)	5.4 (4.8, 5.6)	5.0 (4.9, 5.3)
Arterial insulin (pmol/l)	40 (29, 48)[a]	52 (50, 63)[b]	79 (61, 94)[b]	114 (98, 164)[c]
C-peptide (nmol/l)	0.55 (0.36, 0.66)[a]	0.74 (0.66, 0.85)[b]	0.94 (0.79, 1.01)[b]	1.21 (1.01, 1.35)[c]
Endogenous glucose Ra (µmol/kg FFM/min)	14.4 ± 2.2	13.2 ± 3.5	14.7 ± 2.0	13.8 ± 2.5
Stage 1				
Glucose (mmol/l)	6.1 (5.8, 6.4)	6.3 (6.2, 6.4)	6.4 (6.1, 7.1)	6.6 (6.2, 7.1)
Arterial insulin (pmol/l)	80 (74, 103)[a]	113 (88, 147)[ab]	109 (90, 126)[ab]	128 (112, 160)[b]
C-peptide (nmo/l)	0.14 (0.11, 0.23)[a]	0.25 (0.20, 0.29)[b]	0.24 (0.20, 0.30)[b]	0.46 (0.35, 0.64)[c]
Endogenous glucose Ra (µmol/kg FFM/min)	11.7 ± 3.0	11.7 ± 2.8	12.4 ± 2.9	13.7 ± 2.6
Stage 2				
Glucose (mmol/l)	6.2 (6.0, 6.5)	6.3 (5.9, 6.6)	6.2 (5.7, 6.4)	5.8 (5.8, 6.0)
Arterial insulin (pmol/l)	623 (583, 699)	697 (669, 741)	647 (545, 786)	704 (620, 752)
C-peptide (nmol/l)	0.07 (0.05, 0.12)[a]	0.11 (0.09, 0.16)[a]	0.13 (0.08, 0.18)[a]	0.21 (0.15, 0.30)[b]
Endogenous glucose Ra (µmol/kg FFM/min)	0.3 (0.0, 4.0)	0.7 (0.3, 1.6)	1.3 (0.3, 3.3)	3.2 (1.6, 4.4)

Values are mean ± SD or median (IQR). Values in each row not sharing letters are significantly different from each other, $p<0.05$.

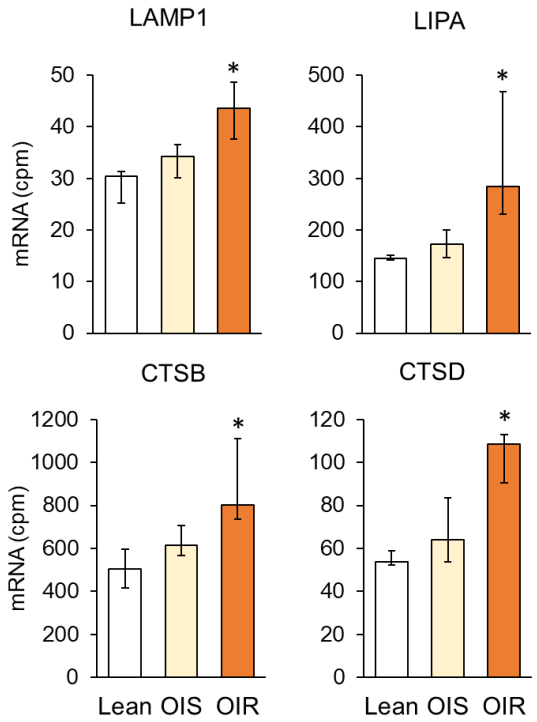
Abbreviations: FFM, fat-free mass; Ra, rate of appearance.



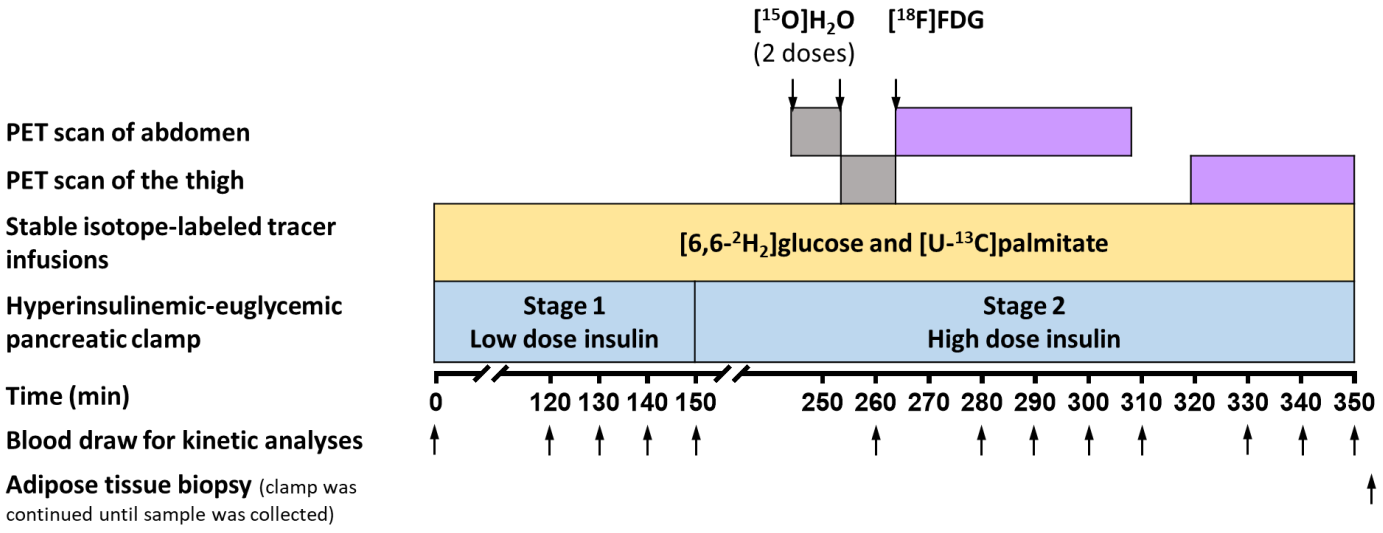
Supplemental Figure 1. Insulin-stimulated whole-body glucose uptake rate in participants with obesity according to quartiles (indicated by dashed horizontal lines). Whole-body glucose uptake rate was expressed in µmol/min/m^2 body surface area (left) and µmol/min (right). *Abbreviations*: Q, quartile.



Supplemental Figure 2. Insulin-stimulated glucose uptake rate in different skeletal muscle groups in lean participants and participants with obesity who were grouped, by quartiles, according to insulin-simulated whole-body glucose uptake rate into those who are most insulin-sensitive and those who are progressively more insulin-resistant. Values are mean ± SEM (upward error bar only) or median (IQR). Bars not sharing letters are significantly different from each other, $p<0.05$. *Abbreviations*. OIR, obese insulin-resistant (Q3/4); OIS, obese insulin-sensitive (Q1); Q, quartile.



Supplemental Figure 3. mRNA expression of lysosome markers in abdominal subcutaneous adipose tissue. Values are median (IQR). * effect of insulin resistance, $p<0.05$. *Abbreviations.* CTSB, cathepsin B; CTSD, cathepsin D; LAMP1, lysosomal-associated membrane protein 1; LIPA, lysosomal acid lipase; OIR, obese insulin-resistant (Q3/4); OIS, obese insulin-sensitive (Q1); Q, quartile.



Supplemental Figure 4. Outline of the hyperinsulinemic-euglycemic pancreatic clamp procedure in conjunction with stable isotope-labeled glucose and palmitate tracer infusions and positron-emission tomography imaging of subcutaneous adipose tissue and skeletal muscles in the abdomen and thigh after ^{15}O-labeled water and ^{18}F-fluorodeoxyglucose injections. Blood samples for substrate kinetic analyses and a subcutaneous abdominal adipose tissue biopsy were collected at specific time points as indicated in the figure. *Abbreviations*: FDG, fluorodeoxyglucose; H_2O, water; PET, positron emission tomography.