SUPPLEMENTAL INFORMATION

Obesity is associated with increased basal and postprandial β-cell insulin secretion even in the absence of insulin resistance

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Body composition analysis

Participants' body fat mass and fat-free mass were determined by using dual energy X-ray absorptiometry (Lunar iDXA, GE Healthcare Lunar, Madison, WI). Intra-abdominal adipose tissue volume and intrahepatic triglyceride content were assessed by using magnetic resonance imaging (Commean et al. 2011, Hong et al. 2011).

Metabolic testing

Study 1 (lean and insulin sensitive obese)

Basal metabolic study. Participants were admitted to the Clinical and Translational Research Unit (CTRU) in the late afternoon. At 1900 h, they consumed a standard meal 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 210 min. Another catheter was inserted into a hand vein, and arterialized blood samples (heated hand technique) were obtained between 180 min and 210

min to determine plasma glucose and palmitate enrichments, and substrate and hormone concentrations.

Two-stage hyperinsulinemic-euglycemic pancreatic clamp. Participants were admitted to the CTRU in the late afternoon. At 1900 h, they consumed a standard meal and then fasted (except for water) overnight. At ~0600 h the following morning, a catheter was inserted into an antecubital vein to infuse metabolic tracers, hormones, and dextrose; another catheter was inserted into a radial artery for blood sampling. Participants were then transferred to the Clinical Translational Imaging Unit, where constant infusions of [6,6-$^{2}H_{2}$]glucose (infusion rate: 0·165 µmol per kg body wt·min^{-1}, priming dose: 14.9 µmol/kg body wt) and [U-^{13}C]palmitate (4.5 nmol per kg fat-free mass·min^{-1}) bound to human albumin, octreotide (45 ng per kg fat-free mass·min^{-1}), glucagon (1.5 ng per kg fat-free mass·min^{-1}), and growth hormone (6.0 ng per kg fat-free mass·min^{-1}) were started and maintained for 360 min. For the initial 120 min, insulin was infused at 10 mU/m^{2} body surface area [BSA]·min^{-1} (initiated with a two-step priming dose of 40 mU/m^{2} BSA·min^{-1} for 5 min followed by 20 mU/m^{2} BSA·min^{-1} for 5 min); for the remaining 240 min, insulin was infused at 50 mU/m^{2} BSA·min^{-1} (initiated with a two-step priming dose of 200 mU/m^{2} BSA·min^{-1} for 5 min followed by 100 mU/m^{2} BSA·min^{-1} for 5 min). The infusion rates of [6,6-$^{2}H_{2}$]glucose and [U-^{13}C]palmitate were reduced to 0.11 µmol per kg body wt·min^{-1} and 3.0 nmol per kg fat-free mass·min^{-1}, respectively, at the start of the high dose insulin infusion to account for the decreases in endogenous glucose production and palmitate rate of appearance in plasma. Dextrose, enriched to 2.5% with [6,6-$^{2}H_{2}$]glucose, was infused at a variable rate to maintain plasma glucose concentration (monitored every 10 min) at ~6.0 mM during insulin infusion. Plasma glucose, insulin, and C-peptide concentrations during the clamp are shown in Supplemental Table 2. Approximately 200 min after starting the hyperinsulinemic clamp procedure, participants were transferred to the PET/CT scanner where ~185 MBq [^{18}F]FDG was administered intravenously and a 40 min PET scan of the abdomen was performed. 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 from 80 min to 120 min (stage 1, low dose insulin infusion) and from 320 min to 360 min (stage 2, high dose insulin infusion).

Oral glucose tolerance test. Participants were admitted to the CTRU in the morning after they fasted for ~12 h overnight at home. At ~0700 h, a catheter was inserted into a hand vein for blood sampling. Participants ingested 75 g of glucose within 5 min. Blood samples were obtained immediately before and then every 30 min for 120 min after glucose ingestion to determine plasma substrate and hormone concentrations.

Study 2 (people with obesity before and after weight loss)

Each participant completed a hyperinsulinemic-euglycemic clamp procedure and a glucose ingestion protocol before and after 15%-20% diet induced weight loss. For the clamp procedure, participants were admitted to the CTRU in the late afternoon. At 1900 h, they consumed a standard meal and then fasted (except for water) overnight. At ~0600 h the following morning, a catheter was inserted into an antecubital vein to infuse dextrose and insulin (50 mU/m^2 BSA·min^{-1}, initiated with a two-step priming dose of 200 mU/m^2 BSA·min^{-1} for 5 min followed by 100 mU/m^2 BSA·min^{-1} for 5 min) for 240 min (Smith et al. 2016). Another catheter was inserted into a radial artery or a vein in the hand that was warmed to 55 °C for blood sampling. Dextrose was infused at a variable rate to maintain plasma glucose concentration (monitored every 10 min) at ~5.5 mM during insulin infusion. Blood samples to determine plasma glucose, fatty acid, and insulin concentrations were collected before the start of the insulin and dextrose infusions and then every 10 min from 210 min to 240 min. On a separate occasion, approximately one week apart, participants were admitted to the CTRU to complete a glucose ingestion protocol, after an overnight fast. At ~0700 h, a catheter was inserted into a hand vein for blood sampling. Participants then ingested 50 g (n=3) or 75 g (n=3) of glucose within 5 min; the amount of

glucose ingested was the same before and after weight loss. Blood samples were obtained immediately before and then every 30 min for 120 min after glucose ingestion to determine plasma glucose, insulin, and C-peptide concentrations. After completion of these tests, participants completed a weight loss therapy program that included a low-calorie diet of self-prepared foods and weekly individual dietary counseling sessions. Initial dietary recommendations were based on an estimate of each subject's total daily energy expenditure (1.5 × measured resting energy expenditure, assessed by using an automated metabolic measurement system [TrueOne 2400, ParvoMedics, Salt Lake City, UT). A structured meal plan was emphasized, and the recommended meal pattern consisted of three meals and two snacks daily. Dietary intake was adjusted as needed based on each subject's rate of weight loss to ensure weight loss targets were achieved. After subjects achieved 15% - 20% weight loss, a weight maintenance diet was prescribed to maintain a stable body weight (<2% change) for at least 3 weeks before all testing procedures were repeated. The average time needed to achieve the targeted weight loss was 37 ± 8 weeks.

Sample processing and analysis

Blood samples were collected in chilled tubes containing heparin or EDTA and placed on ice; plasma was separated by centrifugation within 30 min of collection and then 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); insulin and C-peptide concentrations were determined by using automated immunoassays (Elecsys®, Roche Diagnostics). Plasma incretin (glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1) and glucagon concentrations were determined by immunoassay (Luminex Corp., Austin, TX). 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).

References

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Supplemental Table 1. Basal glucose, palmitate and insulin kinetics

	Lean (n=8)	Obese (n=8)
Palmitate rate of appearance in plasma		
μmol/min	69 (16)	119 (20)*
μmol/kg fat-free mass/min	1.5 (0.4)	2.1 (0.3)*
μmol/m^2 body surface area/min	40 (10)	56 (6)*
μmol/kg fat mass/min	3.5 (2.6, 5.1)	2.7 (2.2, 3.7)
Glucose rate of appearance in plasma		
μmol/min	610 (66)	701 (136)
μmol/kg fat-free mass/min	13.8 (2.1)	12.8 (3.7)
μmol/m^2 body surface area/min	351 (37)	332 (63)
Insulin secretion rate		
pmol/min	125 (43)	214 (56)*
pmol/kg fat-free mass/min	2.7 (0.9)	4.1 (1.3)*
pmol/m^2 body surface area/min	69 (20)	107 (24)*

Values are mean (SD) or medians (IQR). * Value significantly different from Lean value, $p<0.05$.

Supplemental Table 2. Plasma glucose, insulin, and C-peptide concentrations and insulin secretion and clearance rates during basal conditions and during the two-stage hyperinsulinemic-euglycemic pancreatic clamp procedure

	Lean (n=8)	Obese (n=8)
Basal		
Glucose (mmol/l)	5.0 (0.3)	5.0 (0.2)
Insulin (pmol/l)	38 (13)	57 (15)*
C-peptide (ng/ml)	1.7 (0.6)	2.4 (0.6)*
Insulin secretion rate (pmol/min)	125 (43)	214 (56)*
Insulin clearance rate (l/min)	3.4 (0.9)	3.8 (0.8)
Stage 1 – low dose insulin infusion		
Glucose (mmol/l)	6.4 (0.8)	6.4 (0.5)
Insulin (pmol/l)	87 (22)	122 (31)*
C-peptide (ng/ml)	0.46 (0.21)	0.72 (0.17)*
Total (endogenous and exogenous) insulin appearance (pmol/min)	139 (13)	191 (27)*
Insulin clearance rate (l/min)	1.7 (0.5)	1.6 (0.3)
Stage 2 – high dose insulin infusion		
Glucose (mmol/l)	6.1 (0.5)	6.2 (0.5)
Insulin (pmol/l)	667 (108)	715 (70)
C-peptide (ng/ml)	0.21 (0.09)	0.34 (0.12)*
Total (endogenous and exogenous) insulin appearance (pmol/min)	537 (26)	663 (55)*
Insulin clearance rate (l/min)	0.82 (0.12)	0.93 (0.10)

Values are mean (SD). * Value significantly different from Lean value, $p<0.05$.

Supplemental Table 3. Effect of obesity-associated insulin hypersecretion in the absence of insulin resistance on indices that are commonly used to assess insulin resistance

	Lean	Obese
HOMA-insulin resistance index[a]	1.2 (0.3)	1.9 (0.8)*
Matsuda insulin sensitivity index[b]	5.9 (2.1)	4.0 (1.7)**
OGIS index[c]	421 (36)	377 (27)*

Values are mean (SD). HOMA, homeostasis model assessment; OGIS, oral glucose insulin sensitivity.

* Value significantly different from Lean value, $p<0.05$; ** $p=0.069$.

[a] The HOMA insulin resistance index was calculated by dividing the product of the fasting plasma glucose and insulin concentrations (in mmol/l and mU/l, respectively) by 22.5 (Matthews et al. 1985).

[b] The Matsuda index was calculated as 10,000/square root of [fasting plasma glucose x fasting plasma insulin concentrations] x [mean glucose x mean insulin concentration during the oral glucose tolerance test] (Matsuda et al. 1999) with glucose expressed as mg/dl and insulin as mU/l.

[c] The OGIS was derived by using the plasma insulin and glucose concentrations during the oral glucose tolerance in a model described by Mari and colleagues (Mari et al. 2001) with glucose expressed as mg/dl and insulin as mU/l.

References

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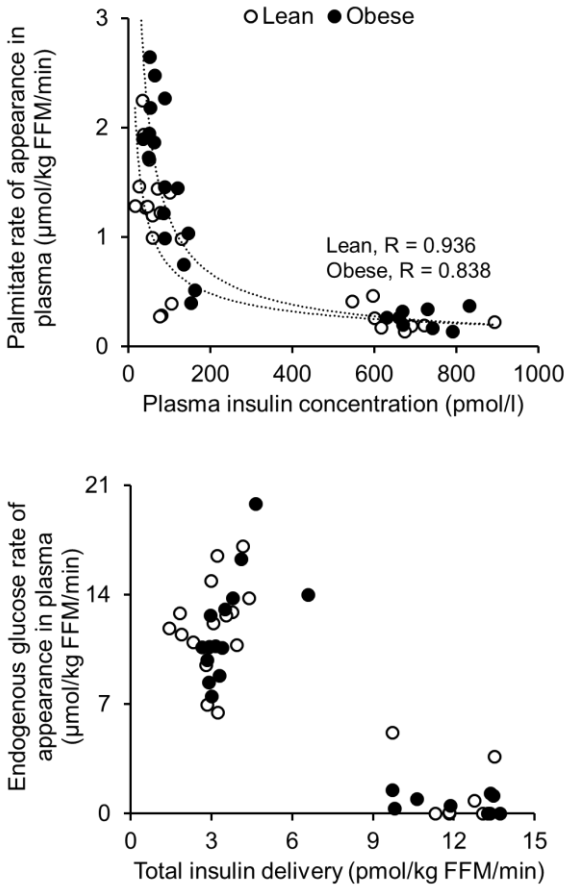
Supplemental Table 4. Body mass and composition and whole body insulin sensitivity before and after weight loss

	Before	After
Body mass index (kg/m^2)	35 (2)	28 (2)*
Body mass (kg)	91 (6)	74 (6)*
Fat-free mass (kg)	46 (6)	43 (5)*
Body fat (% of total body mass)	49 (4)	42 (4)*
Fat mass (kg)	45 (6)	31 (5)*
Glucose infusion rate ([µmol/min]/[pmol insulin/l])[a]	4.9 (1.0)	4.0 (1.0)*
Glucose infusion rate ([µmol/kg FFM/min]/[pmol insulin/l])[a]	0.11 (0.02)	0.09 (0.02)

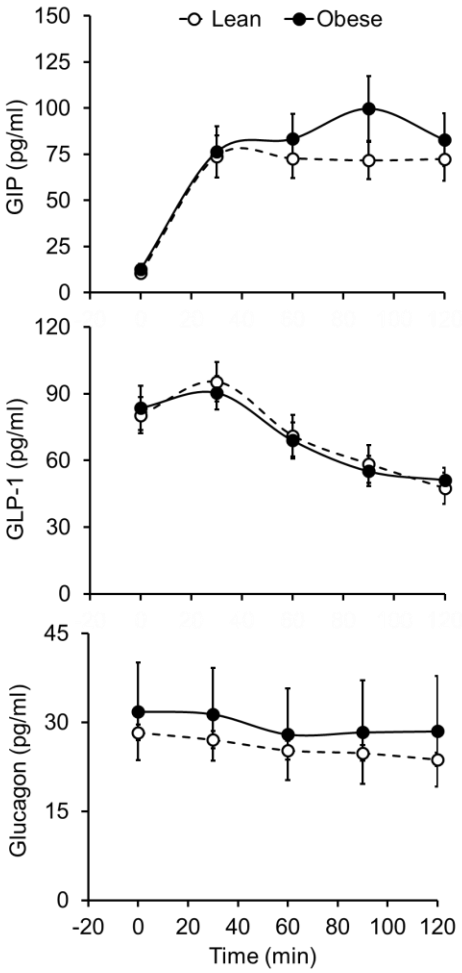
Values are mean (SD). FFM: fat-free mass.

[a] Glucose infusion rate needed to maintain euglycemia during the hyperinsulinemic-euglycemic clamp procedure.

* Value significantly different from Before value, $p<0.05$.



Supplemental Figure 1. Relationships between plasma insulin concentration and palmitate rate of appearance in plasma relative to fat-free mass (top) and total insulin delivery (insulin secretion rate during basal conditions and sum of insulin secretion rate and intravenous insulin infusion during the clamp procedure) and endogenous glucose appearance in plasma during basal conditions and during the hyperinsulinemic-euglycemic clamp (bottom) the Lean (n=8) and Obese (n=8) groups. FFM, fat-free mass.



Supplemental Figure 2. Plasma glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 (GLP-1), and glucagon concentrations before and after glucose ingestion in the Lean (n=8) and Obese (n=8) groups. Data are mean ± SEM.