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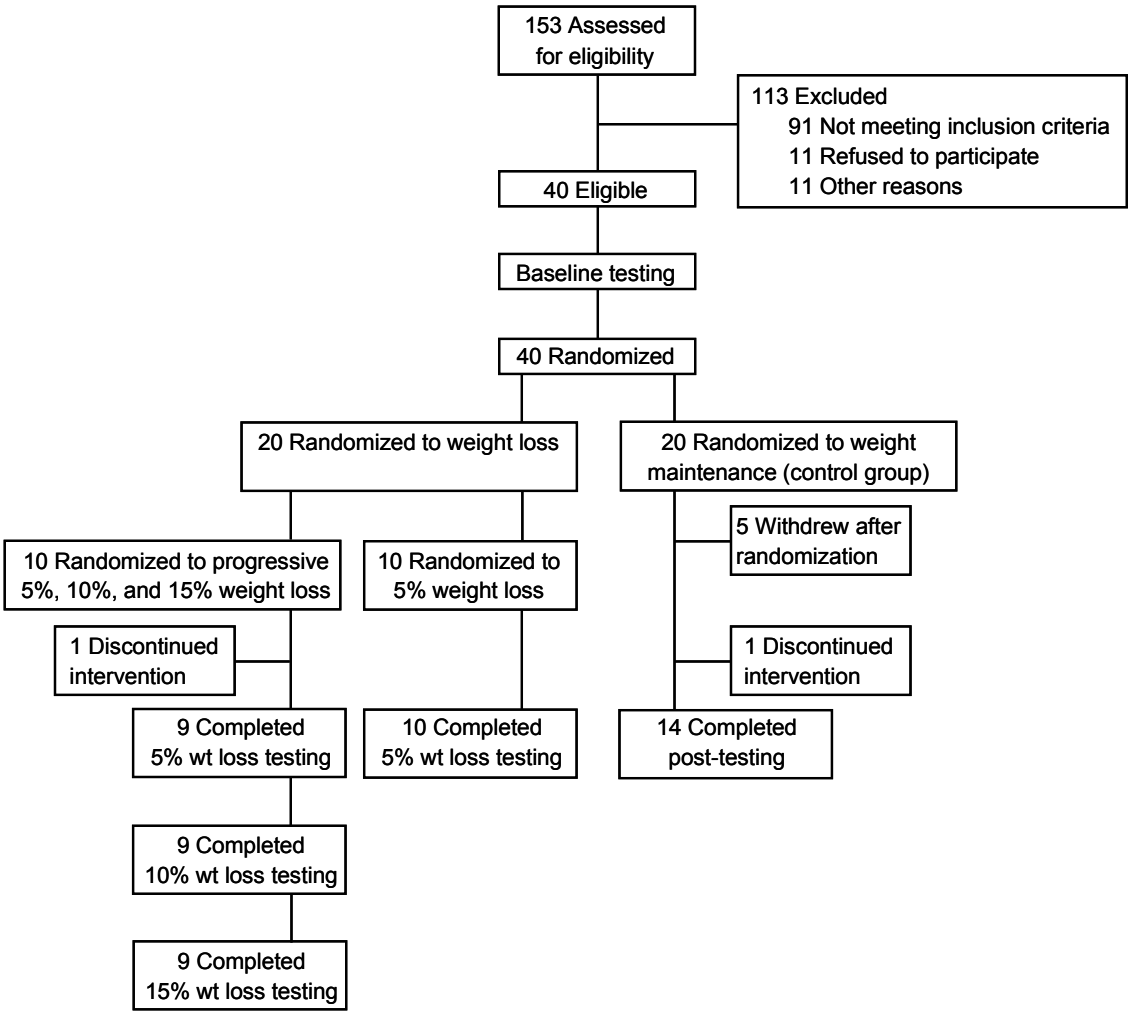
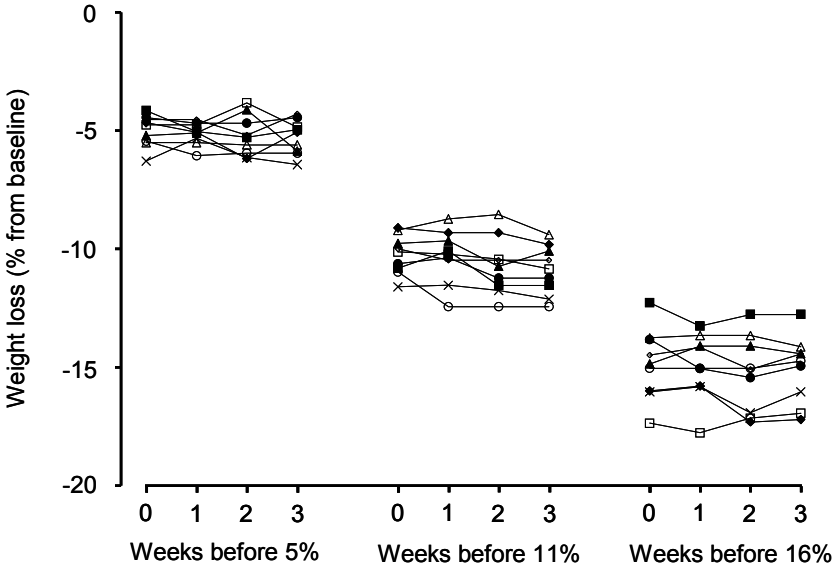
Supplemental Figure S1, related to Experimental Procedures: CONSORT Flow Diagram



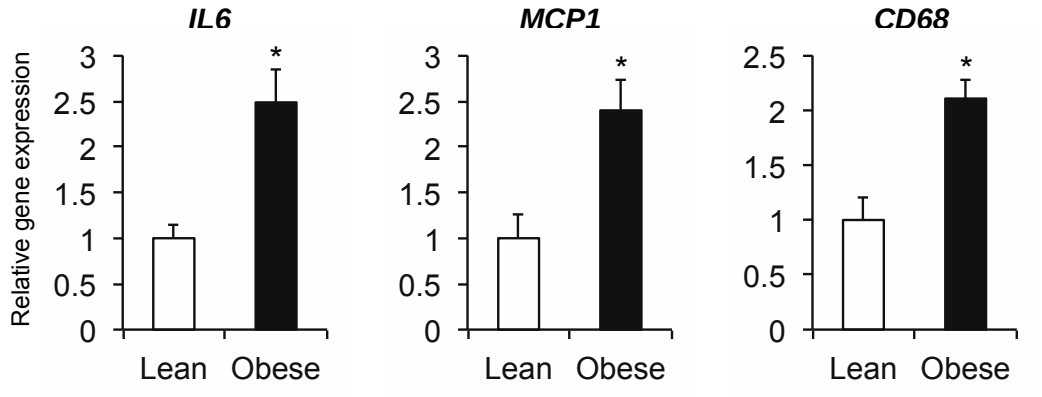
Flow of participants through screening procedures, baseline testing, randomization into weight loss and weight maintenance, and post-intervention testing.

Supplemental Figure S2, related to Experimental Procedures: Weight loss during the three weeks preceding metabolic testing



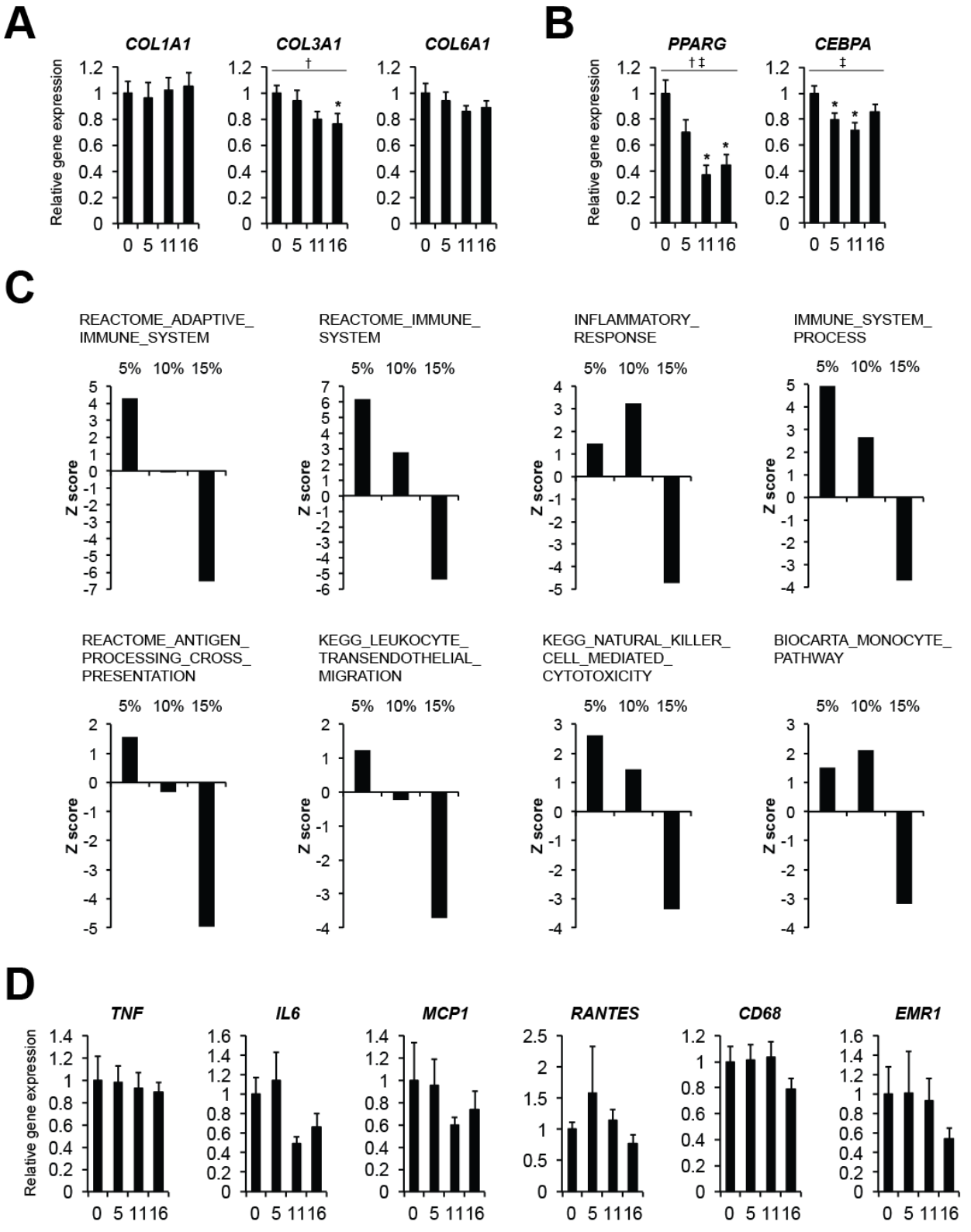
Weight loss data for individual subjects are shown (n = 9) for the three weeks preceding metabolic testing at each weight loss interval. Individual changes ranged from 0.1% to 1.7% at 5% weight loss, 0.5% to 1.5% at 11% weight loss, and 0.3% to 1.6% at 16% weight loss; the average change was 0.9% in all three instances.

Supplemental Figure S3, related to Table 1: Obesity is associated with adipose tissue inflammation



Subcutaneous adipose tissue expression of genes involved in inflammation was determined by real-time PCR in people who were lean (n=12, white bars) and obese (n=31, black bars). Data are means ± SEM. The differences between groups (lean vs. obese) were evaluated with the Student's t-test for normally distributed variables or the Mann-Whitney U test for not normally distributed variables. *P<0.05 vs. lean.
Abbreviations: *IL6*, interleukin-6; *MCP1*, monocyte chemotactic protein 1; *CD68*, cluster of differentiation 68.

Supplemental Figure S4, related to Figure 2: Effect of progressive weight loss on selected markers of extracellular matrix (ECM) structure, adipogenesis, and inflammation in subcutaneous adipose tissue



Subcutaneous adipose tissue gene expression of selected markers of ECM structure (A) and adipogenesis (B), representative biological pathways involved in immune function and inflammation (PAGE) (C), and gene expression of inflammatory markers (D) in subcutaneous adipose tissue, before (0) and after progressive 5% (5), 11% (11), and 16% (16) weight loss (n = 9). Non-normally distributed variables were log transformed for analysis and back transformed for presentation. Data are means ± SEM. **P*<0.05 vs. baseline; †*P*<0.05 for the linear component and ‡*P*<0.05 for the quadratic component of the main effect of time.

Abbreviations: *COL1A1*, collagen, type I, alpha 1; *COL3A1*, collagen, type III, alpha 1; *COL6A1*, collagen, type VI, alpha 1; *PPARG*, peroxisome proliferator-activated receptor gamma; *CEBPA*, CCAAT/enhancer-binding protein alpha; *TNF*, tumor necrosis factor; *IL6*, interleukin-6; *MCP1*, monocyte chemotactic protein 1; *RANTES*, regulated on activation, normal T cell expressed and secreted; *CD68*, cluster of differentiation 68; *EMR1*, EGF-like module-containing mucin-like hormone receptor-like 1.

Supplemental Table S1, related to Table 1: Metabolic characteristics and markers of inflammation in lean and obese subjects

	Lean (n = 12)	Obese (n = 33)	*P*-value
Weight (kg)	62.3 ± 4.4	106.4 ± 15.8	<0.001
Body mass index (kg/m^2)	22.9 ± 1.3	37.9 ± 4.3	<0.001
Body fat (%)	34.3 ± 5.5	46.9 ± 5.6	<0.001
Fat free mass (kg)	40.6 ± 3.3	56.1 ± 9.5	<0.001
Visceral adipose tissue (cm^3)	715 ± 403	1428 ± 534	<0.001
Intrahepatic triglyceride (%)	1.6 (1.2, 3.3)	7.0 (4.1, 12.7)	<0.001
Glucose (mg/dL)	94 ± 5	97 ± 8	0.304
Insulin (mU/L)	5.2 (2.1, 6.7)	19.2 (13.8, 23.0)	<0.001
HOMA-IR score	1.2 (0.5, 1.5)	4.4 (3.0, 5.7)	<0.001
Triglyceride (mg/dL)	85 (59, 111)	131 (89, 131)	0.010
HDL-cholesterol (mg/dL)	58 (51, 76)	42 (33, 48)	0.010
LDL-cholesterol (mg/dL)	114 (101, 141)	102 (90, 129)	0.472
Leptin (ng/mL)	16.7 ± 9.5	43.1 ± 18.2	<0.001
Adiponectin (µg/mL)	14.6 (9.8, 19.6)	5.2 (4.0, 6.9)	<0.001
C-reactive protein (mg/L)	0.75 (0.26, 1.59)	3.70 (2.32, 4.89)	<0.001
Interleukin-6 (ng/mL)	1.1 ± 0.4	2.2 ± 0.8	<0.001
WBC count (10^3/mL)	5.3 ± 1.1	6.6 ± 1.8	0.026
Palmitate Ra suppression (%)	69.5 ± 10.2	52.3 ± 12.4	<0.001
Glucose Ra suppression (%)	75.3 (67.5, 85.0)	70.1 (63.1, 75.5)	<0.001
Glucose Rd stimulation (%)	389 (331, 498)	183 (109, 221)	<0.001

Data are means ± SD for normally distributed variables or medians (quartile 1, quartile 3) for not normally distributed variables. The differences between groups (lean vs. obese) were evaluated with the Student t-test for normally distributed variables or the Mann-Whitney U test for not normally distributed variables.
Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WBC, white blood cell.

Supplemental Table S2, related to Figure 2: Adipose tissue biological pathways significantly affected by progressive weight loss

Please refer to the separate Excel spreadsheet.

Supplemental Table S3, related to Experimental Procedures: Sequence of primers for real-time PCR

Gene	Accession Number	Forward primer (5'-3')	Reverse primer (5'-3')
36B4	NM_001002	GTGATGTGCAGCTGATCAAGACT	GATGACCAGCCCAAAGGAGA
TNF	NM_000594	GAGGCCAAGCCCTGGTATG	CGGGCCGATTGATCTCAGC
IL6	NM_000600	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
MCP1	NM_002982	CAGCCAGATGCAATCAATGCC	TGGAATCCTGAACCCACTTCT
RANTES	NM_002985	CCAGCAGTCGTCTTTGTCAC	CTCTGGGTTGGCACACACTT
CD68	NM_001251	CTTCTCTCATTCCCCTATGGACA	GAAGGACACATTGTACTCCACC
EMR1	NM_001256255	GCTGTGATACTGTTCTTGATGGT	CAGCATCGGCAGCCCATAA
ABCG1	NM_016818	ATTCAGGGACCTTTCCTATTCGG	CTCACCACTATTGAACTTCCCG
ABCA1	NM_005502	ACCCACCCTATGAACAACATGA	GAGTCGGGTAACGGAAACAGG
APOE	NM_000041	GTTGCTGGTCACATTCCTGG	GCAGGTAATCCCAAAAGCGAC
CETP	NM_000078	GGCCAAGTCAAGTATGGGTTG	ACAGACACGTTCTGAATGGAGA
SCD	NM_005063	ACACTTGGGAGCCCTGTATG	GCAGCCGAGCTTTGTAAGA
FADS1	NM_013402	CTACCCCGCGCTACTTCAC	CGGTCGATCACTAGCCACC
FADS2	NM_004265	TGACCGCAAGGTTTACAACAT	AGGCATCCGTTGCATCTTCTC
ELOVL6	NM_024090	AACGAGCAAAGTTTGAACTGAGG	TCGAAGAGCACCGAATATACTGA
SPARC	NM_003118	TGAGGTATCTGTGGGAGCTAATC	CCTTGCCGTGTTTGCAGTG
MFAP5	NM_003480	GGGTCAATAGTCAACGAGGAGA	CTGTAGCGGGATCATTCACCA
LOX	NM_001178102	CGGCGGAGGAAAACTGTC	TCGGCTGGGTAAGAAATCTGA
LOXL2	NM_002318	GGGTGGAGGTGTACTATGATGG	CTTGCCGTAGGAGGAGCTG
ANGPT1	NM_001146	AGCGCCGAAGTCCAGAAAAC	TACTCTCACGACAGTTGCCAT
ADAM12	NM_021641	CGAGGGGTGAGCTTATGGAAC	GCTTTCCCGTTGTAGTCGAATA
NQO1	NM_001025433	GAAGAGCACTGATCGTACTGGC	GGATACTGAAAGTTCGCAGGG
DHCR24	NM_014762	GCCGCTCTCGCTTATCTTCG	GTCTTGCTACCCTGCTCCTT
UCHL1	NM_004181	CCTGTGGCACAATCGGACTTA	CATCTACCCGACATTGGCCTT
COL1A1	NM_000088	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
COL3A1	NM_000090	GGAGCTGGCTACTTCTCGC	GGGAACATCCTCCTTCAACAG
COL6A1	NM_001848	ACAGTGACGAGGTGGAGATCA	GATAGCGCAGTCGGTGTAGG
PPARG	NM_015869	ATGGGTGAAACTCTGG	CGACATTCAATTGCCA
CEBPA	NM_001287435	AGGGTCTCTAGTTCCACGCC	CAAGGGGAAGCCCAGCCTATA

Supplemental Experimental Procedures, related to Experimental Procedures

Lifestyle intervention program

The dietary intervention included a low-calorie diet of self-prepared foods to achieve 5% weight loss, followed by the use of solid and liquid meal replacements, as needed, to achieve the 10% and 15% weight loss targets. The macronutrient content of the diet throughout the study was comprised of ~50-55% of energy as carbohydrate, 30% of energy as fat, and 15-20% of energy as protein. Diet and behavioral education were provided in individual weekly 1-hour sessions, led by an experienced weight management dietitian or behavioral psychologist, throughout the study. A structured meal plan was emphasized, and the recommended meal pattern consisted of 3 meals and 2 snacks daily. The behavioral program used cognitive-behavioral techniques to foster dietary adherence, and handouts that summarized the educational content were provided. The initial recommended dietary energy intake was individualized based on the participant's estimated daily energy expenditure (determined by each subject's measured resting energy expenditure multiplied by 1.5). Dietary intake was adjusted as needed based on each subject's rate of weight loss to help ensure weight loss and weight maintenance targets were achieved. Participants who were not achieving the desired rate of weight loss received additional support from the study dietitian or behavioral psychologist, including more intense self-monitoring, re-evaluating previous goals and setting new short-term goals, and more aggressive use of meal replacements.

Sample processing and analyses

Metabolite concentrations. Plasma glucose concentration was determined by the glucose oxidase method on an automated glucose analyzer (Yellow Spring Instruments Co, Yellow Springs, OH). Plasma insulin and C-peptide concentrations were measured by using electrochemiluminescence technology (Elecsys 2010; Cobas / Roche Diagnostics, Indianapolis, IN). Total plasma triglyceride, LDL and HDL cholesterol, and ALT concentrations were determined by following standard procedures in the Barnes Jewish Hospital Clinical Core laboratory. Plasma concentrations of leptin and adiponectin (EMD Millipore, St Charles, MO), CRP, IL-6, and MCP-1 (R&D Systems, Minneapolis, MN) were measured by using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Plasma FFA concentrations were quantified by using gas chromatography with flame ionization detection (Hewlett-Packard 5890-II, Palo Alto, CA).

Tracer-to-tracee ratios. Plasma glucose and palmitate tracer-to-tracee ratios (TTR) were determined by using electron impact ionization gas chromatography-mass spectroscopy (GC-MS; MSD 5973 system with capillary column; Hewlett-Packard; Palo Alto, CA).

Calculations used to evaluate metabolic function

Substrate kinetics. The Ra of glucose in plasma was calculated by dividing the glucose tracer infusion rate by the average plasma glucose TTR during the last 30 min of the basal period, stage 1, and stage 2 of the hyperinsulinemic euglycemic-clamp procedure. Glucose Ra during basal conditions represents endogenous glucose Ra, an index of hepatic glucose production rate, which equals basal glucose Rd from plasma. During the clamp procedure, hepatic glucose production rate was calculated by subtracting the glucose infusion rate (i.e., dextrose solution plus tracer added to it) from total glucose Ra (endogenous plus exogenous); glucose Rd was calculated as total glucose Ra (i.e., the sum of endogenous glucose Ra and the rate of infused glucose). Palmitate Ra, an index of FFA flux and adipose tissue lipolysis, was calculated by dividing the palmitate tracer infusion rate by the average plasma palmitate TTR obtained during the final 30 min of the basal period and stage 1 of the clamp procedure.

Insulin sensitivity. Hepatic and adipose tissue insulin sensitivity were assessed as the relative decrease in glucose and palmitate Ra, respectively, from basal to low-dose insulin infusion (stage 1) of the clamp procedure. Skeletal muscle insulin sensitivity was determined as the relative increase in glucose Rd from basal to high-dose insulin infusion (stage 2) of the clamp procedure.

Glucose tolerance, insulin clearance, and β-cell function. Glucose, insulin and C-peptide areas-under-the-curve (AUCs) were calculated by using the trapezoidal rule to assess glucose tolerance and insulin clearance (insulin AUC relative to C-peptide AUC). The β-cell insulin secretion rate (ISR) response sensitivity to plasma glucose was assessed by using oral minimal model analysis of plasma C-peptide concentrations (Breda et al., 2002) (SAAM II version 2, University of Washington, Seattle, WA), to obtain Φ-static (ISR response sensitivity to glucose concentration), Φ-dynamic (ISR response sensitivity to the rate of increase in glucose concentration), and Φ-total (overall ISR response sensitivity to glucose). Overall β-cell function, which provides an assessment of insulin secretory response in relationship to insulin sensitivity (Bergman et al., 2002), was calculated as the product of total β-cell responsivity (Φ-total) and the insulin-stimulated increase in glucose Rd during high-dose insulin infusion.

Supplemental references

Bergman, R.N., Ader, M., Huecking, K., and Van Citters, G. (2002). Accurate assessment of beta-cell function: the hyperbolic correction. Diabetes *51 Suppl 1*, S212-220.

Breda, E., Toffolo, G., Polonsky, K.S., and Cobelli, C. (2002). Insulin release in impaired glucose tolerance: oral minimal model predicts normal sensitivity to glucose but defective response times. Diabetes *51 Suppl 1*, S227-233.