SUPPLEMENTARY DATA

Title: Increased Lipolysis, Diminished Adipose Tissue Insulin Sensitivity and Impaired β-cell Function Relative to Adipose Tissue Insulin Sensitivity in Obese Youth with Impaired Glucose Tolerance (IGT)

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Materials

Metabolic Studies

Participants were recruited through newspaper advertisements, flyers posted in the medical campus, city bus routes and the outpatient clinics in the Weight Management and Wellness Center and the Division of Pediatric Endocrinology. All participants were admitted twice within a 1-4 week period to the Pediatric Clinical and Translational Research Center for a hyperinsulinemic-euglycemic clamp, to assess *in vivo* (peripheral) insulin sensitivity, and a hyperglycemic clamp, to assess insulin secretion, performed in random order. Each clamp evaluation was performed after a 10- to 12-hr overnight fast. Fasting hepatic glucose production was measured before the start of the hyperinsulinemic-euglycemic clamp, with a primed (2.2 µmol/kg) constant infusion of [6,6-$^2H_2$]glucose (Isotech, Miamisburg, OH, USA) at 0.22 µmol/kg/min for a total of 2 hours. Whole-body lipolysis was measured at baseline and during the hyperinsulinemic-euglycemic clamp by the use of a prime (1.2 µmol/kg) constant rate (0.08 µmol/kg/min) infusion of [$^2H_5$]glycerol (MSD Isotopes, St. Louis, MO, USA), which was started 2 hours before the clamp. The pyrogen-free isotope was dissolved in 0.9 % sodium chloride and sterilized by passing through a 0.22-µm Millipore filter (Bedford, MA, USA). After the baseline isotope infusion period, *in vivo* (peripheral) insulin sensitivity was evaluated during a 3-hr hyperinsulinemic (80 mu/m^2/min)- euglycemic clamp. Plasma glucose was clamped at approximately 100 mg/dL with a variable rate infusion of 20% dextrose in water. The glucose infusion was adjusted based on arterialized plasma glucose measurements every 5 minutes and blood was sampled every 10 to 15 minutes for determination of insulin and FFA concentrations and plasma isotopic enrichment. Continuous indirect calorimetry (Deltatrac Metabolic Monitor; Sensormedics, Anaheim, CA, USA) was used to measure CO_2 production, O_2 consumption, and respiratory quotient for 30 min at baseline and at the end of the 3-hr hyperinsulinemic-euglycemic clamp. First- and second-phase insulin secretion were assessed during a 2-hr hyperglycemic (225 mg/dL) clamp. Plasma glucose was increased rapidly to 225 mg/dL by a bolus infusion of dextrose and maintained at that level by a variable rate infusion of 20% dextrose for 2 hours, with frequent measurement of glucose and insulin concentrations. Glucose tolerance status was determined with HbA1c and/or a 2-hr oral glucose tolerance test (OGTT; 1.75 g/kg, maximum 75 g). Fasting blood samples were obtained for determination of lipid profile.

Biochemical Measurements

Plasma glucose was determined by the glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH, USA), and insulin by a commercially available human specific insulin radioimmunoassay kit (catalog no. HI-14K; Linco/Millipore, St. Charles, MO, USA). FFA concentration was determined using enzymatic colorimetric methods with a Wako (non-esterified fatty acids) NEFA-HR test kit (Wako, Osaka, Japan). Plasma lipid concentrations were determined using the standards of the Centers for Disease Control and Prevention. HbA1c was measured by high-performance liquid chromatography (Tosoh Medics, Inc., San Francisco, CA, USA).

Deuterium enrichment of glycerol in the plasma was determined according to previously described methods (1,2). Plasma samples were deproteinized with barium hydroxide and zinc sulfate. The supernatant was purified through a column of mixed-bed anion and cation ion-exchange chromatography. Acetate derivatives of glycerol were prepared by adding pyridine and acetic anhydride to the dried eluted samples. Derivatized samples were analyzed for ^2H enrichment on a Hewlett Packard gas chromatograph-mass spectrometer (GC-MS) system (5985A; Hewlett Packard, Palo Alto, CA, USA) as reported (1,2). Selected ion monitoring software was used to monitor charge-to-mass ratio (*m/z*) (145) *m* and *m/z* 148 (*m* + 3), representing unlabeled and ^2H-labeled glycerol, respectively. Standard curves of known enrichments of glycerol were performed with each assay.

Deuterium enrichment of glucose in the plasma was determined on Hewlett-Packard GC-MS system (5985A; Hewlett Packard, Palo Alto, CA, USA) according to a modification of a previously reported method (3,4). Plasma samples were deproteinized with 2-propanol and subjected to sequential

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cation and anion exchange chromatography. The pentaacetate derivative of glucose was analyzed for ^{2}H enrichment on the GC-MS in the electron impact mode. Selected ion monitoring of the *m*-190 ion (mass-to-charge ratio 200) and the corresponding *m* + 2 ion (mass-to-charge ratio 202), reflecting unlabeled and labeled glucose, was performed. Standard curves of known enrichments of glucose were performed with each assay.

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Supplementary Table 1. Whole-body lipolysis (GlyRa) at fasting and during hyperinsulinemic-euglycemic calmp in obese youth with IGT vs. NGT.

Variable	NGT (n=97)	IGT (n=41)	P
Fasting			
GlyRa per total body (µmol/min)	301.6 ± 15.3	405.9 ± 26.9	<0.01
GlyRa per Kg fat free mass (µmol/kgFFM/min)	5.9 ± 0.3	8.0 ± 0.6	<0.01
GlyRa per Kg fat mass (µmol/kgFM/min)	9.1 ± 0.6	9.6 ± 0.7	<0.01
Hyperinsulinemic-euglycemic clamp steady-state			
GlyRa per total body (µmol/min)	158.6 ± 9.8	200.5 ± 17.7	0.05
GlyRa per Kg fat free mass (µmol/kgFFM/min)	3.2 ± 0.2	3.9 ± 0.3	0.07
GlyRa per Kg fat mass (µmol/kgFM/min)	4.4 ± 0.4	4.7 ± 0.4	0.05

Values are reported as the mean ± SEM.

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