ONLINE SUPPLEMENT

Healthy volunteers were recruited from the general population of the Delaware Valley. The protocol was approved by the Institutional Review Board of the University of Pennsylvania (Penn) and subjects gave written informed consent. Exclusion criteria included any known chronic or recurrent medical disorders; any screening laboratory abnormality; tobacco use; use of any prescription medication (except oral contraception) or supplemental vitamins; and positive urine pregnancy test. Twenty subjects were recruited, equally divided by gender, and came to Penn's Clinical Translational Research Center (CTRC) for three visits: Visit 1 for screening; Visit 2, two weeks later, for frequently sampled intravenous glucose tolerance (FSIGT) testing, dual-energy x-ray absorbitometry estimation of body adiposity and dietary counseling; and Visit 3, two weeks later, consisting of an overnight acclimatization phase, a 24-hour saline control phase, and a 24-hour post-LPS study phase (60-hours total). Lipopolysaccharide (US standard reference endotoxin, lot # CC-RE-LOT-1+2; Clinical Center, Pharmacy Department at the National Institutes of Health) was given intravenously as a 3 ng/kg bolus at 6AM on day 2. A total of 18 blood samples were collected, 9 before and 9 after LPS for plasma and Qiagen PAX tubes (Qiagen Inc, Valencia, CA) facilitated collection of whole blood RNA [11]. Subcutaneous adipose samples were collected by core needle aspiration biopsy from the gluteal region 30 minutes before and on three occasions (4, 12, and 24 hrs) after LPS. Samples were divided in two and either snap frozen or treated with RNA Later®, and stored at -70C until analyzed.

Metabolic and Inflammatory Markers: Plasma levels of insulin (radio-immunoassays (RIA), Linco Research, St Charles, MO), tumor necrosis factor alpha (TNF), interleukin-6 (IL-6), and monocyte chemoattractant protwin-1 (MCP-1) (all Linco Multiplex ELISAs on Luminex IS100; Austin TX) were measured according to the manufacturers' guidelines. All samples were assayed in duplicate and pooled human plasma samples were included to assess variability. The intra- and inter-assay c.v.'s of pooled human plasma were 4.1% and 11.6% for insulin; 8.66% and 20.4% for TNF; 8.7% and 10.9% for IL-6; and 3.6% and 7.2% for MCP-1**.** Plasma lipids and glucose were measured enzymatically (Wako Diagnostics, Richmond, VA) on a Hitachi 912 automated chemistry system in a Center for Disease Control-certified lipid laboratory as described previously [1, 2].

Real time PCR and quantification**:** RNA extraction was performed using the RNAeasy total RNA kit (Qiagen, Valencia, CA). Whole blood (n=20) and adipose (n=11) mRNA was subjected to RT-PCR and subsequent quantitative PCR (qPCR) on an Applied Biosystems 7300 Real-Time PCR System (ABI, Foster City, CA) for measurement of interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF), tumor necrosis factor induced protein 3 (A20), resistin, suppressor of cytokine signaling (SOCS)-1, SOCS-2, SOCS-3, SOCS-6, insulin receptor, insulin receptor substrate (IRS)-1, IRS-2, IRS-3, IRS-4, GLUT-4, MCP-1 and chemokine CXCL10 mRNA (supplement Table 1). Macrophage marker human epidermal growth factor module-containing mucin-like receptor 1 (EMR1-F4/80) [3] mRNA was assayed in adipose.

Quantitative PCR was performed with TaqMan Universal PCR Master Mix (ABI) under standard conditions. Variability in total cDNA concentrations between samples was normalized by subtracting the beta-actin C_t value from the target C_t value for each sample. The comparative C_t method was used to analyze changes in gene expression [4]. The ΔC_t for each post-LPS

sample was compared to the mean ΔC_t for all pre-LPS samples in a single individual using the relative quantitation $2^{-(\Delta\Delta Ct)}$ method to determine fold-change from baseline.

Protein isolation and Western blotting: Human adipose samples were homogenized (50mg/mL RIPA buffer) using a Qiagen TissueLyser. Lysates were delipidated by incubation with isopropanol at 4°C for 15 min prior to centrifugation at 8000rpm for 10 min at 4°C. The protein protein pellet was resuspended in RIPA buffer. Protein concentration was quantified using the Pierce bicinchoninic acid (BCA) kit (Thermo Fisher Scientific, Rockford, IL). Equal concentrations (10μg) of lysate were reduced, separated by SDS-PAGE electrophoresis, transferred to nitrocellulose membranes and blocked in 5% bovine serum albumin (BSA) in TBS-tween prior to overnight incubation (at 4°C) with primary antibody. Blots were probed with serine (473) phosphorylated AKT (Cell Signaling Technology, MA), whole cell IRS1 (Upstate, Millipore, MA), Glut4 (Chemicon, Millipore, MA), MCP1 (Abcam, MA), A20 (eBioscience, CA) and β-actin (Cell Signaling Technology, MA). Blots were washed in TBST, incubated with appropriate secondary antibody (BioRad, CA) and visualized by chemiluminescence.

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2. Anderson, P.D., et al., *Innate Immunity Modulates Adipokines in Humans.* J Clin Endocrinol Metab, 2007.
3. Khazen, W., et al., *Expression of macrophage-selective markers in human and rodent adipocytes.* FEBS Lett, 2005. **579**(25): p. 5631-4.
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Supplement Table 1. Real-time Quantitative PCR Assays

Gene	Forward primer	Reverse primer	Fluorescent probe
IL-6	ABI Assay on Demand Assay ID# Hs00174131-m1		
TNF	ABI Assay on Demand Assay ID# Hs00174128_m1		
Resistin	agccatcaatgagaggatcca	tccaggccaatgctgcttat	5'[fam]-aggtcgccggctccctaatatttaggg-[tamra]3'
MCP-1	Hs00234140_m1		
SOCS-1	5’-ctaagcaccggcagacaagag-3’	5’-ctcagcgatagtaaagtgcatggt-3’	5’[FAM]-aggcgtgttcctgggacttggctt-[tamra]3’
SOCS-6	5’-ctcttacaagcccatcatgctatg-3’	5’-tccttgagccagtctggtagtaca-3’	5’[FAM]-aaggtcggtggcgagtgatccctc-[tamra]3’
SOCS-2	Hs00374416_m1		
SOCS-3	Hs00269575_s1		
IR	Hs00169631_m1		
IRS-1	Hs00178563_m1		
IRS-2	Hs00275843_s1		
GLUT-4	Hs01559106_g1		
EMR-1	Hs00173562_m1		
β-actin	5’-ctcctcctgagcgcaagtactc-3’	5’-tcgtcatactcctgcttgctgat-3’	5’[FAM]-ccatcctggcctcgctgtcca-[tamra]3’

Supplement Table 2. Baseline Abundance of Insulin Receptor Substrate (IRS) and Suppressors of Cytokine Signaling (SOCS) mRNAs in Adipose and Whole Blood Relative to beta-Actin

	Delta CT Adipose **Mean (SD)**	**Delta CT Whole Blood** **Mean (SD)**
SOCS-1	10.4 (0.78)	8.22 (0.11)
SOCS-2	7.2 (0.65)	12.7 (0.17)
SOCS-3	6.9 (0.35)	6.94 (0.11)
SOCS-6	6.92 (0.3)	11.76 (0.5)
IRS-1	5.6 (0.65)	11.4 (0.23)
IRS-2	4.9 (0.63)	5.09 (0.13)
IRS-3	Not detectable	11.98 (0.55)
IRS-4	Not detectable	Not detectable
Insulin receptor	4.13 (0.58)	16.4 (0.55)

The delta CT for each mRNA relative to the CT for beta-Actin is presented. Lower delta CTs indicates more abundant mRNAs.