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Pre-gravid obesity associates with increased maternal endotoxemia and metabolic inflammation

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Abstract

Obese pregnant women develop severe insulin resistance and enhanced systemic and placental inflammation, suggesting associated modifications of endocrine and immune functions. Activation of innate immunity by endotoxins/lipopolysaccharides (LPS) has been proposed as a mechanism for enhancing metabolic alterations in disorders with insulin resistance. The aim of this study was to characterize the immune responses developed by the adipose tissue AT and their potential links to maternal endotoxemia in pregnancy with obesity. Blood and subcutaneous abdominal AT were obtained from 120 lean and obese women (term pregnancy) recruited at delivery. Gene expression was assessed in AT and stromal vascular cells isolated from a subset of 24 subjects from the same cohort. Doubling of plasma endotoxin concentrations indicated subclinical endotoxemia in obese compared with lean women. This was associated with significant increase in systemic CRP and IL-6 but not TNF-alpha concentrations. AT inflammation was characterized by accumulation of CD68+ macrophages with a 3-fold increased gene expression of the macrophage markers CD68, EMR1 and CD14. Gene expression for cytokines IL-6, TNF-α, IL-8, and MCP1 and for LPS - sensing CD14, TLR4, TRAM2 was 2.5-5 fold higher in stromal cells of obese compared to lean. LPS-treated cultured stromal cells of obese women expressed a 5-16 fold stimulation of the same cytokines up-regulated in vivo. Our data demonstrate that subclinical endotoxemia is associated with systemic and AT inflammation in obese pregnant women. Recognition of bacterial pathogens may contribute to the combined dysfunction of innate immunity and the metabolic systems in AT.

INTRODUCTION

Obesity is a major concern in pregnancy, because it has severe adverse outcomes including an increased risk of spontaneous miscarriage, congenital anomalies, as well as metabolic dys-regulation manifested as preeclampsia or gestational diabetes (1). These maternal conditions increase the risk for in utero metabolic programming with the development of excess adiposity and lowered insulin sensitivity in the fetus (2, 3). The combination of the physiological insulin resistance of pregnancy with that in obesity makes pregnancy complicated with obesity a condition of severe insulin resistance (4). Obese pregnant women

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also develop enhanced systemic and placental inflammation, suggesting associated modifications of endocrine and immune functions (5, 6).

Modifications of the AT (AT) secretome are features central to the propagation of inflammation in obesity. The accumulation of macrophages, with an increased synthesis of TNF-alpha and other adipo-cytokines represents the core of the inflammatory responses initiated within the AT (7). Concomitantly, the alteration of adipose endocrine functions with abnormal production of adipocyte hormones including but not exclusively leptin, adiponectin, and resistin contributes to increased metabolic dysfunction (8, 9). In pregnancy, the placenta makes an additional contribution to the pre-gravid systemic changes because of its capacity to deliver cytokines in the maternal circulation and the strong similarity between the placenta and AT secretome (10). The disruption of the normal links between the secretory and circulatory systems eventually evolves into the triangular loop associating obesity, inflammation and insulin resistance (11). However, neither the nature of these links nor the pathways towards the progression of the inflammation to other organs such as liver, skeletal muscle or the vascular system are yet characterized (12). Metabolic inflammation represents a newer concept combining chronic metabolic disturbances with low grade inflammatory responses which engage the release of pro-inflammatory cytokines by several organs (13). Rather than translating into the classic inflammatory response to injury, metabolic inflammation is a milder persistent condition triggered by a plethoric nutrient environment and/or energy imbalance. Mechanisms leading to the activation of the innate immune system have recently generated significant interest in the quest to the basis for metabolic diseases (14). Endotoxin, the lipopolysaccharide complex present in the outer membrane of gram negative bacteria is a potent external stimulus of the innate immune system (15). An evolving model is proposing that metabolic endotoxemia originating from the diet or the environment disrupts the balance between the immune and the metabolic systems, hence favoring excess lipid storage in AT (16, 17).

Our hypothesis is that compared to lean women, obesity pre-gravid triggers endotoxemia and AT inflammation which extend into pregnancy. The aim of this study was to characterize the factors which bring together inflammatory and metabolic changes in the adipose tissue of obese compared to lean pregnant women. We report that obese pregnant women have subclinical endotoxemia associated with insulin resistance and increased cytokines in maternal circulation. The systemic changes are associated with increased AT stromal inflammation, macrophage accumulation and the recruitment of genes in LPS sensing pathways. We propose that the low grade endotoxemia of the obese women may represent an environmental stimulus to activate pro-inflammatory responses within the AT.

METHODS AND PROCEDURES

Study subjects

120 women with a singleton pregnancy were recruited at the time of admission for elective cesarean delivery at term (38-40 weeks). Obesity was defined as pre-gravid body mass index (BMI) >30. The protocol was approved by the MetroHealth Medical Center Institutional Review Board and Clinical Research unit (CRU) Scientific Review Committee. Volunteers gave their written informed consent in accordance with the MetroHealth Medical Center guidelines for the protection of human subjects. Subcutaneous abdominal AT (5-8 g) was obtained at the incision site before opening of the fascia. Maternal blood was drawn on admission to labor and delivery, prior to placement of an intravenous line for hydration.

Isolation of stromal vascular cells

AT samples were snap-frozen in liquid nitrogen within 5 min of biopsy or immediately processed for cell isolation. Adipocytes were isolated by digestion of fresh AT with 1mg/ml collagenase (Worthington Biochemical, Lakewood, NJ) in Hanks buffered solution for 45 min at 37°C. Cells from the stromal vascular fraction (SVF) were pelleted by centrifugation 20 min at 1500 g. The SVF pellet was re-suspended in erythrocyte lysis buffer, centrifuged, resuspended in RPMI medium and counted. One aliquot of SVF cells was immediately frozen and the remaining cells were plated at a density of approximate 1.5 - 1.8 × 10^6 cells/well in 12 well culture plates (precoated with 1% gelatin) containing RPMI medium containing 10 % FCS and 1% penicillin/streptomycin. For in vitro experiments, the cells were changed to fresh medium after overnight plating followed by incubation for 24 hrs in the presence or absence of 100 ng/ml Lipopolysaccharide (LPS) (L4391, Sigma Aldrich, St. Louis, MO). All the reagents used for SVF isolation were endotoxin-free as evaluated by the Limulus Amoebocyte lysate assay (LONZA, Walskerville, MD).

Analysis of RNA expression

Total RNA was isolated from intact AT fragments of lean and obese subjects and from cultured SVF cells using Trizol reagent (Invitrogen, Carlsbad, CA). Gene expression was monitored by real-time PCR using a Roche thermal cycler (Roche Applied Science, Indianapolis, IN) with Lightcycler Fast-start DNA Sybr Green 1 master mix and primers from Integrated DNA Technologies (Coralville, IA). Specific primers were designed within the 3′ coding region of the genes. CD14 (BC-010507) forward: 5′-tctctgtccccacaagttcc-3′ reverse: 5-cccgtccagtgtcaggttatc-3′; CD68 (NM_001040059) forward: 5′-gaaccccaacaaaaccaag-3′ reverse: 5′-gatgagaggcagcaagatg-3′; toll-like receptor 4 (TLR4) (NG-011475) forward: 5′-cccaccactcaccagctaat-3′ reverse: 5′-gccctgtggttcagagaaag-3′; translocating chain-associated membrane protein 2 (TRAM2) (NM-012288) forward: 5′-ctgtgatgctccgtctcaaa-3′ reverse: 5′-gcagccataaggcaagagac-3′; EGF-like module containing, mucin-like, hormone receptor-like 1 EMR-1 (NM_001974) forward: 5′-ccaaggggataagatgaag-3′ reverse: 5′-caccaaggagatgattaatgcc-3″; interleukin-6 (IL-6) (NM_000600) forward: 5′-tacccccaggagaagattcc-3′ reverse: 5′-ttttctgccagtgcctcttt-3′; tumor necrosis factor alpha (TNF-α) (NM-000594) forward: 5′-tccttcagacaccctcaacc-3′ reverse: 5′-aggccccagtttgaattctt-3′, monocyte chemotactic protein-1 (MCP1) (NM_002982) forward: 5′-gacaagcaaacccaaactcc-3′ reverse: 5′-gcaatttccccaagtctctg-3′; interleukin-8 (IL-8) (NM_000584) forward: 5′-gtgcagttttgccaaggagt-3′ reverse: 5′-ctctgcacccagttttcctt-3′; leptin (NM_000230) forward: 5′-gaagaccacatccacacacg-3′ reverse: 5′-agctcagccagacccatcta-3′; peroxisome proliferator-activated receptor gamma (PPARγ) (NM_138711) forward: 5′-agaagcctgcatttctgcat-3′ reverse: 5′-tcaaaggagtgggagtggtc-3′; adiponectin (NM_004797) forward: 5′-cctaagggagacatcggtga-3′ reverse: 5′-gtaaagcgaatgggcatgtt-3′; β-actin (NM_001101) forward: 5′-ggacttcgagcaagagatgg-3′ reverse: 5′-agcactgtgttggcgtacag-3′. Quantitation of relative gene expression was performed by comparative C_T method and expressed as fold difference between groups. All data were expressed relative to β-actin.

Immunohistochemistry and size measurement of adipocytes

AT sections were fixed in 10% formalin and included in paraffin. Endogenous peroxidase of deparaffinized sections was neutralized by hydrogen peroxide 3% for 10 min. Antigen unmasking was performed by incubating the slides in a 100 mM Tris-HCL pH 10 buffer at 98°C for 45 min. The non specific reactions were blocked by incubation for 30 min with normal swine serum and sections were incubated with anti-CD68 antibodies for 30 min. The biotinylated secondary antibody was applied for 30 min followed by the streptavidin-horseradish peroxidase conjugate for 15 min. Peroxidase was developed using the DAB working solution (LSAB+ system, DAKO) and washed in deionised water. The DAB-

chromogen was added to develop the enzyme activity. Sections were counterstained using hematoxylin Gill II (Sigma Aldrich). Digitized images were obtained using a Nikon E600 microscope equipped with DXM200 camera. Image analysis and adipocyte measures were performed using the Image J software from NIH 1.35q (Bethesda, MD). The numbers of adipocytes and macrophages were counted in ten randomly chosen areas with a 40x magnification by two independent researchers. The number of macrophages was normalized to 100 adipocytes. The monoclonal antibody against human CD68 (clone PGM-1) was from Dako Cytomaton (Glostrup, Danemark). The secondary antibodies anti-Rb Biotin and Streptavidin-HRP were from Vector (Burlingame, CA).

Plasma assays

Plasma was separated by centrifugation at 4°C and kept frozen at −20°C for glucose, insulin and adipo-cytokine assays. Adiponectin was measured using an ELISA kit and leptin using a radiometric assay (Linco Research, ST. Charles, MO) with intra-assay CVs of 7.4 and 2.6-4-6 % respectively. TNF-α and interleukin-6 were assayed by ELISA (Quantiglo kit, R&D System, Minneapolis, MN) with the following CVs: 5.3-7.8 and 2.6-3.4 %. CRP was measured by ELISA (Alpha Diagnostics International, San Antonio, TX) with CV 3.0-4.7%. Glucose was measured by YSI 2300 STAT Plus Glucose & Lactate Analyzer (YSI Incorporated, Yellow Springs, Ohio). Insulin was measured by radioimmunoassay commercial kits (Linco Research) with intra-assays coefficient of variations (CV) 2.9-6.0%. The insulin resistance indices were calculated according to the homeostasis model assessment (HOMA-IR): fasting plasma insulin (μU/ml) x fasting glucose (mmol/liter)/ 22.5. Plasma endotoxin determination was performed with the chromogenic endpoint Limulus Amoebocyte Lysate assay for the detection of Gram negative bacterial endotoxin (QCL-1000® Lonza, Walkersville, MD). Standard endotoxin dilutions were prepared in borosilicate glass tubes to minimize LPS adhesion to walls and each point was prepared in triplicate. Under these conditions the lower limit of detection was 0.1 EU/ml. Plasma samples were heat inactivated for 5 min at 70° C to minimize protease interference. All samples were diluted 1:2 with 50 mM Tris Buffer which contain less than 0.005 EU/ ml(Lonza) and assayed in duplicate. sCD14 was assayed by ELISA (Cell Sciences, Canton, MA). All plasma samples were run in duplicate.

Measurement of cytokine release

Concentrations of TNF-α, IL-8 and MCP1 were measured by Quantikine ELISA kits according to the manufacturer instructions (R&D Systems, Minneapolis, MN) with the following CVs: 0.1-8.1%, 0.4-4.7% and 0.1-3.2%, respectively. IL-6 concentration was determined by using ELISA (RayBioTech**,** Norcross GA) with CV 0.1-7.2%. The range of quantification for IL-6 is 1.37 to 1000 pg/ml, for TNF-α is 0.5 to 32 pg/ml and for IL-8 and MCP1 is 31.2 to 2000 pg/ml.

Statistical analysis

All values are presented as mean + SEM. Differences between dependent variables were examined with or one-way or two-way repeated measures analysis of variance (ANOVA). Statistical significant mean differences were identified with a Fisher's PLSD post-hoc test. The data were analyzed using the Statview II statistical package (Abacus Concepts, Berkeley, CA). Statistical significance was set at $p < 0.05$.

RESULTS

Subjects characteristics

Obese pregnant women displayed all major metabolic alterations usually associated with the metabolic syndrome (Table 1). Per the definition of obesity the average pre-gravid BMI of the lean group was 22.0+2.0 and that of the obese group was 38.4+6.0 and it was associated with maternal hyperleptinemia (73.2+15.6 for obese vs. 32.6+20.4 ng/ml for lean women). The obese women exhibited marked insulin resistance with doubling of HOMA-IR indices (5.3+1.7 vs. 2.2+1.1, $p<0.0001$) and increased plasma insulin concentrations (25.4+12.7 vs. 11.8+5.6 μU/ml, $p<0.0001$) in term pregnancy.

Low grade endotoxemia associates with systemic inflammation in obese pregnant women

Endotoxemia measured by the Limulus Amoebocyte assay was 2-fold higher (1.0+0.5 vs. 0.5+0.2 EU/ml, $p<0.006$) in obese compared to lean women (Table 2). The concentration of the circulating soluble CD14, ligand of endotoxin (LPS) was not different between groups. The mild endotoxemia was associated with an increased concentration in the inflammatory markers CRP (12472+8042 vs. 8074+6463 ng/ml), $p<0.005$) and IL-6 (4.5+3.4 vs. 2.4+1.4 pg/ml, $p<0.002$). By contrast plasma TNF-α concentrations were similar in obese and lean women.

Metabolic and immune alterations of AT of obese women

The cellular and molecular characteristics of AT were analyzed in a subset of 24 (11 obese and 13 lean) subjects from the same cohort. The mean values for pre-gravid BMI (38.3+1.8 vs. 22.0+0.4), plasma insulin (25.7+12.4 vs. 9.9+1.7 μU/ml, $p<0.0001$) and leptin 51.9.2+22.5 vs. 29.9+20.4 ng/ml, $p<0.0001$) were higher in obese than lean subjects and did not differ significantly from the main cohort. The adipocyte size was significantly larger in obese vs. lean (96.1+5.2 vs. 87.2+1.4 μm). Macrophages were identified based on their immuno-reactivity for CD68, the human homolog of mouse macrosialin, a transmembrane glycoprotein highly expressed in monocytes and macrophages. The percentage of CD68 positive cells in stromal vascular compartment was 10-fold higher in AT of obese women (Figure 1). The expression of adipo-cytokines and inflammatory markers was assessed in intact AT from obese pregnant women (Figure 2). The leptin gene showed a 12% increased expression whereas the expression of adiponectin and its master transcriptional regulator peroxisome proliferators-activated receptor γ were decreased by 32 and 43 % respectively. The expression of EMR1 (human homolog of mouse F480), CD68, and CD14, cell surface markers of tissue macrophages was enhanced 2-3 fold (Figure 2), suggesting that the AT of obese women combines metabolic and immune alterations.

Enhanced stromal inflammation in AT of women with pre-gravid obesity

To further characterize the inflammatory condition, the expression of chemokines and pro-inflammatory cytokines was compared between cells of the stromal fraction (SVF) and intact AT of obese women. Compared to AT, there was a 22-to 324 fold increase in IL-6, TNF-α, and IL-8 expression in freshly isolated SVF cells suggesting that cells of the stromal compartment make a functional contribution to AT inflammation (Table 3). Stromal inflammation was further characterized in cells isolated from lean and obese pregnant women. TNF-α, IL-6, IL-8 and MCP1 exhibited a 2 to 5 fold higher expression in cells of obese compared to lean women (Figure 3 A). The increase in cytokine gene expression was accompanied by an activation (2 to 4.5 fold) of the membrane CD14 antigen, the toll like receptor 4 (TLR4) and the TRIF-related adaptor molecule (TRAM2), a set of genes implicated in sensing and transducing the effects of LPS into the cells (Figure 3 B).

Next, the effect of LPS to stimulate an inflammatory response in adipose cells was investigated in vitro, using plated SVF cells. Incubation with LPS (100 ng/ml) for 24h stimulated the activation of cytokine expression by 4 to 16 fold in SVF isolated from obese pregnant women compared to a 2 to 7 fold in SVF from lean pregnant women (Figure 4 A, B). The release of cytokines from the stimulated SVF cells into the culture medium exhibited a pattern similar to that of gene expression with an accumulation of MCP1, IL-8, IL-6 and TNF-α (Figure 4 A, B).

DISCUSSION

Low grade endotoxemia in obese pregnant women

We report that low grade maternal endotoxemia (circulating endotoxin) associates with insulin resistance and systemic inflammation in pregnant women with pre-gravid obesity. The difference between the endotoxin circulating concentrations in obese and lean pregnant suggest that the increased endotoxemia relates to obesity rather than to the pregnancy condition. Although pregnancy per se is a chronic low grade inflammatory condition even in lean subjects (18) there is no information to date relating to endotoxin levels in normal or pathologic gravidas. The doubling in circulating endotoxin in pregnant obese women is in line with the mild endotoxemia associated with type 2 diabetes in human and in LPS treated mice (16, 17, 19). The absence of significant change in the concentration of soluble CD14, a systemic ligand of LPS suggests that the increased endotoxemia in obese pregnant women is reflected by unbound circulating LPS or other endotoxins (20). As point of fact, the LAL assay we have used to assess plasma endotoxin levels does not provide a specific measure of LPS but also recognizes other types of endotoxins (21). LPS, is a complex lipopolysaccharide released from the cell wall of most gram negative bacteria which is ubiquitous in the environment e.g. air, water, food. LPS is also continuously released upon the turnover of bacteria which colonize the digestive tract so that there is a chronic variable exposure in vivo (22). Hence, modifications of gut microbiota have been proposed as a potential nutritional-related stimulus to modify metabolic homeostasis through LPS mediated pathways (23). In this context, circulating endotoxins appear as potential linkers between the activation of innate immunity and the development of metabolic disorders such as obesity and diabetes (24).

Recruitment of inflammatory and LPS sensing pathways in stromal vascular cells

A number of recent studies have documented that experimental endotoxemia mimicked by exposure to LPS in humans induces AT inflammation and insulin resistance through the activation of the adipose tissue secretome (25-27). The activation of toll like receptor 4 (TLR4), by the CD14-LPS complex is a proximal signaling step engaging the innate immune system into the fight against bacterial pathogens which is functional in adipocytes (28, 29). TLR4 signaling progresses towards the generation of an intracellular cytokine cascade through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-*k*B) activation (30, 31). Accumulation of macrophages in AT of obese pregnant women was accompanied by the enhanced expression of TLR4 associated genes as well as genes for chemotactic and inflammatory cytokines. A similar activation pattern was replicated in vitro by incubating isolated adipose stromal cells with LPS. Taken together, our data suggest that increased systemic LPS in the plasma of obese pregnant women may represent an exogenous stimulus to activate cellular signals leading to adipo-cytokines production. The recent observation that diet induced modification of the gut flora with probiotics ameliorates the insulin resistance and glucose homeostasis of pregnant women brings support to our current hypothesis (32).

Combination of adipose inflammation and altered expression of metabolic genes

The decreased gene expression of adiponectin, a marker of insulin sensitivity, and of proliferator-activated receptor γ, a master transcriptional regulator of fat storage suggests an active contribution of AT metabolism to the increased insulin resistance of obese compared to lean pregnant women (33). Changes in adiponectin mRNA levels were not associated with a significant decrease of circulating plasma adiponectin hence, rather than a systemic regulation, these data suggest an autocrine or paracrine effect of adiponectin in the adipose cells. TNF-α has long been identified as a link between inflammation and insulin resistance (34). We did not observe an increase in systemic TNF-α in obese women despite their pronounced insulin resistance. This may represent a human signature in the setting of metabolic inflammation which reflects different mechanisms of cytokine action as previously reported in humans and mice (35). The leptin gene expression in AT was not reflecting the marked hyperleptinemia of obese women. To our knowledge this is the first report that hyperleptinemia in pregnancy is not reflected by an increase in leptin mRNA level in AT. The regulation of the AT leptin gene may be specific to pregnancy because of the additional placental expression of leptin (36). Variability has been previously documented in humans between leptin gene expression in AT and increased adiposity (37, 38) with either increased (39, 40) or unchanged (41) mRNA levels. This suggests that complex patterns of regulation of the leptin gene and secretion rate from fat are still not fully understood.

These data support the concept that the circulating concentration of leptin and TNF-α may be poor surrogate of their mechanism of action at the cellular level (36). Taken together our data indicate that an intricate combination of metabolic and immune alterations in the adipose tissue associates with mild inflammatory systemic changes. Hence, obesity in pregnancy is a situation associated with meta-inflammation as proposed by Hotamilsligil for chronic inflammation associated with other metabolic syndromes (13).

Evolving model for the role of metabolic inflammation in pregnancy

Our data suggest that functional crosstalks evolve between the environment of the obese pregnant woman and her AT. Our proposed model is that the recognition and sensing of bacterial derived pathogens initiates the transduction of immuno-modulatory signals within the adipose cells. The increased circulating endotoxemia is an indication of maternal environmental changes in pregnancy with obesity, the origin of which remains to be established.

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REFERENCES

1. Catalano PM, Ehrenberg HM. The short- and long-term implications of maternal obesity on the mother and her offspring. BJOG. 2006; 113:1126–1133. [PubMed: 16827826]
2. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. Diabetes Care. 2009; 32:1076–1080. [PubMed: 19460915]
3. Freeman DJ. Effects of maternal obesity on fetal growth and body composition: implications for programming and future health. Semin Fetal Neonatal Med. 2010; 15:113–118. [PubMed: 19853544]
4. Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. Am J Obstet Gynecol. 1999; 180:903–916. [PubMed: 10203659]

5. Stewart FM, Freeman DJ, Ramsay JE, Greer IA, Caslake M, Ferrell WR. Longitudinal assessment of maternal endothelial function and markers of inflammation and placental function throughout pregnancy in lean and obese mothers. J Clin Endocrinol Metab. 2007; 92:967–975.
6. Challier JC, Basu S, Bintein T, et al. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. Placenta. 2008; 29:274–281. [PubMed: 18262644]
7. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112:1796–1808. [PubMed: 14679176]
8. Curat CA, Wegner V, Sengenès C, et al. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. Diabetologia. 2006; 49:744–747. [PubMed: 16496121]
9. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese human. Diabetes. 2007; 56:1010–1013. [PubMed: 17287468]
10. Hauguel-de Mouzon S, Guerre-Millo M. The placenta cytokine network and inflammatory signals. Placenta. 2006; 27:794–798. [PubMed: 16242770]
11. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007; 132:2169–2180. [PubMed: 17498510]
12. Lee DE, Kehlenbrink S, Lee H, Hawkins M, Yudkin JS. Getting the message across: mechanisms of physiological cross talk by adipose tissue. Am J Physiol Endocrinol Metab. 2009; 296:E1210–E1229. [PubMed: 19258492]
13. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006; 444:860–867. [PubMed: 17167474]
14. Philpott DJ, Girardin SE. Gut microbes extend reach to systemic innate immunity. Nat Med. 2010; 16:160–161. [PubMed: 20134466]
15. Ulevitch RJ, Tobias PS. Recognition of gram-negative bacteria and endotoxin by the innate immune system. Curr Opin Immunol. 1999; 11:19–22. [PubMed: 10047547]
16. Creely SJ, McTernan PG, Kusminski CM, et al. Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab. 2007; 292:E740–E747. [PubMed: 17090751]
17. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007; 56:1761–1772. [PubMed: 17456850]
18. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynecol. 1998; 179:80–86. [PubMed: 9704769]
19. Dasu MR, Devaraj S, Park S, Jialal I. Increased toll-like receptor activation and TLR ligands in recently diagnosed type 2 diabetes subjects. Diabetes Care. 2010; 33:861–868. [PubMed: 20067962]
20. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science. 1990; 249:1431–1433. [PubMed: 1698311]
21. Bryant CE, Spring DR, Gangloff M, Gay NJ. The molecular basis of the host response to lipopolysaccharide. Nature Reviews Microbiology. 2010; 8:8–14.
22. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006; 124:837–848. [PubMed: 16497592]
23. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444:1022–1023. [PubMed: 17183309]
24. DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. Mayo Clin Proc. 2008; 83:460–469. [PubMed: 18380992]
25. Ghanim H, Abuaysheh S, Sia CL, et al. Increase in plasma endotoxin concentrations and the expression of toll-like receptors and suppressor of cytokine signaling -3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. Diabetes Care. 2009; 32:2281–2287. [PubMed: 19755625]

26. Mehta NN, McGillicuddy FC, Anderson PD, et al. Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. Diabetes. 2010; 59:171–181.
27. Shah R, Lu Y, Hinkle CC, et al. Gene profiling of human adipose tissue during evoked inflammation in vivo. Diabetes. 2009; 58:2211–2219. [PubMed: 19581417]
28. Bès-Houtmann S, Roche R, Hoareau L, et al. Presence of functional TLR2 and TLR4 on human adipocytes Histochem. Cell Biol. 2007; 127:131–137.
29. Kopp A, Buechler C, Neumeier M, et al. Innate immunity and adipocyte function: ligand-specific activation of multiple toll-like receptors modulates cytokine, adipokine, and chemokine secretion in adipocytes. Obesity. 2009; 17:648–656. [PubMed: 19148127]
30. Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. Cytokine. 2008; 42:145–151. [PubMed: 18304834]
31. Pasparakis M. Regulation of tissue homeostasis by NF-l[kappa]lB signalling: implications for inflammatory diseases. Nature Reviews Immunology. 2009; 9:778–788.
32. Laitinen K, Poussa T, Isolauri E, the Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota Group. Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. Br J Nutr. 2009; 101:1679–1687. [PubMed: 19017418]
33. Iwaki M, Matsuda M, Maeda N, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes. 2003; 52:1655–1663. [PubMed: 12829629]
34. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993; 259:87–91. [PubMed: 7678183]
35. Arner P. Resistin: yet another adipokine tells us that men are not mice. Diabetologia. 2005; 48:2203–2205. [PubMed: 16193286]
36. Lepercq J, Cauzac M, Lahlou N, Timsit J, Girard J, Auwerx, Hauguel-de Mouzon S. Overexpression of placental leptin in diabetic pregnancy: a critical role for insulin. Diabetes. 1998; 47:847–50. [PubMed: 9588462]
37. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1995; 1:1155–1161. [PubMed: 7584987]
38. Ranganathan S, Maffei M, Kern PA. Adipose tissue OB mRNA expression in humans: discordance with plasma leptin and relationship with adipose TNF-alpha expression. J Lipid Res. 1998; 39:724–730. [PubMed: 9555938]
39. Lönnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese(ob) gene in adipose tissue of human obese subjects. Nat. Med. 1995; 1:950–953. [PubMed: 7585223]
40. Halleux CM, Servais I, Reul BA, Detry R, Brichard SM. Multihormonal Control of *ob* Gene Expression and Leptin Secretion from Cultured Human Visceral Adipose Tissue: Increased Responsiveness to Glucocorticoids in Obesity. The Journal of Clinical Endocrinology & Metabolism. 1998; 83:902–910. [PubMed: 9506746]
41. Clapham JC, Smith SA, Moore GB, Hughes MG, Azam H, Scott A, Jung RT. Plasma leptin concentrations and OB gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinaemia in lean and obese humans. Int J Obes Relat Metab Disord. 1997; 21:178–183.

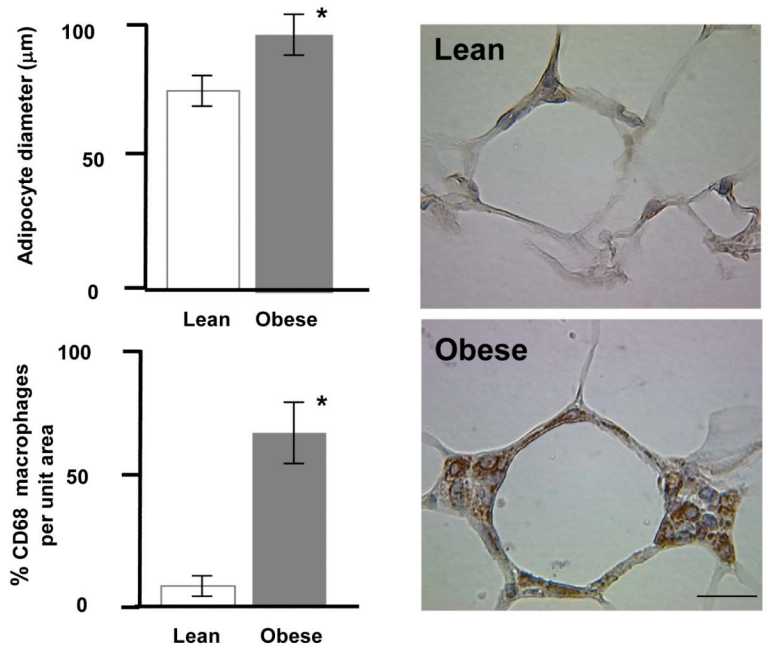

Figure 1. Accumulation of CD68+ macrophages in the adipose tissue vascular stroma

Right panel : histochemical analysis of subcutaneous adipose tissue shows rare CD68+ staining (dark brown) in a section from a representative lean woman (pre-gravid BMI: 22.5). In contrast, the section from an obese woman (pre-gravid BMI: 31.7) shows an increased number of dark brown stained cells accumulating in the stroma vascular space in between the adipocytes. Scale bar: 20 μm. Magnification X 100.

Left panel: quantification of adipocytes size (top) and CD68+ macrophage number (bottom) in adipose tissue sections. Mean + SEM of n = 14 adipose tissue sections from obese (solid bars) and n = 7 from lean (open bars) women. Statistical significance: $*p < 0.001$.

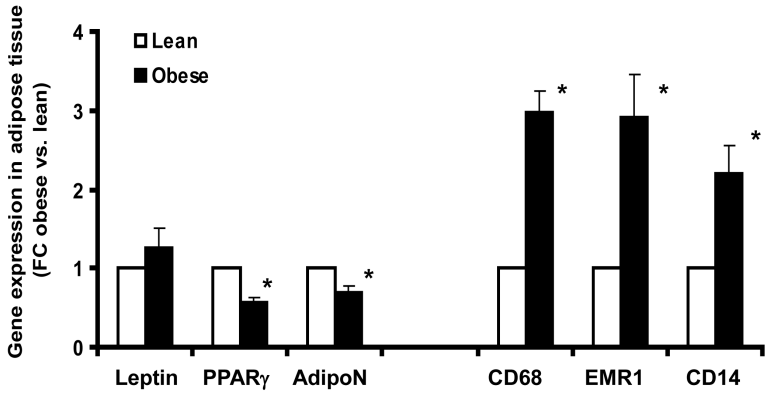

Figure 2. Endocrine and immune related genes in adipose tissue of obese pregnant women

Quantitative RT-PCR analysis of total RNA isolated from abdominal subcutaneous adipose tissue of 14 obese and 11 lean women undergoing term elective C-section. Data (mean ± SEM) were expressed as fold changes in obese vs. lean after normalization to β-actin. Statistical significance: * $p < 0.05$. Lean: open bars, obese: solid bars.

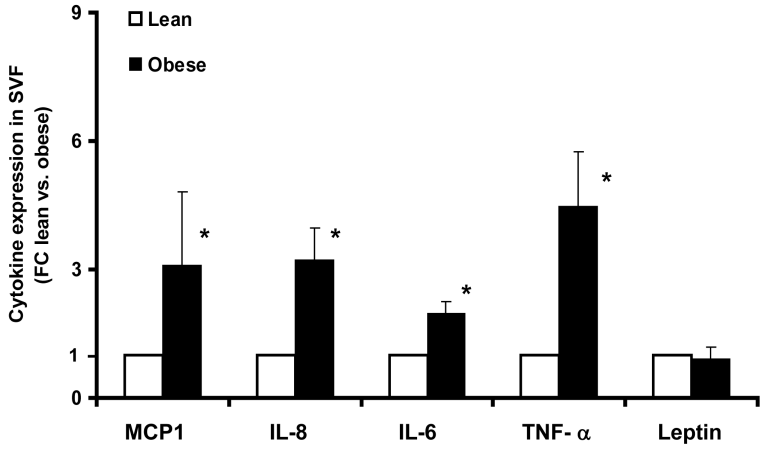

Figure 3. A. Increased cytokine expression in isolated stromal vascular cells of obese pregnant women

Total RNA was isolated from cultured stromal vascular cells from 11 obese and 4 lean women. MCP1, IL-8, IL-6, TNF-α and leptin mRNA levels were measured by quantitative RT-PCR analysis. B. Expression of endotoxin sensitive genes is increased in isolated stromal vascular cells of obese pregnant women. Total RNA was isolated from cultured stromal vascular cells from 8 obese and 4 lean women. CD14, TLR4, and TRAM2 mRNA levels were measured by quantitative RT-PCR analysis. Data (mean ± SEM) were expressed as fold changes in obese vs. lean after normalization to β-actin. Statistical significance: * $p < 0.05$. Lean: open bars, obese: solid bars

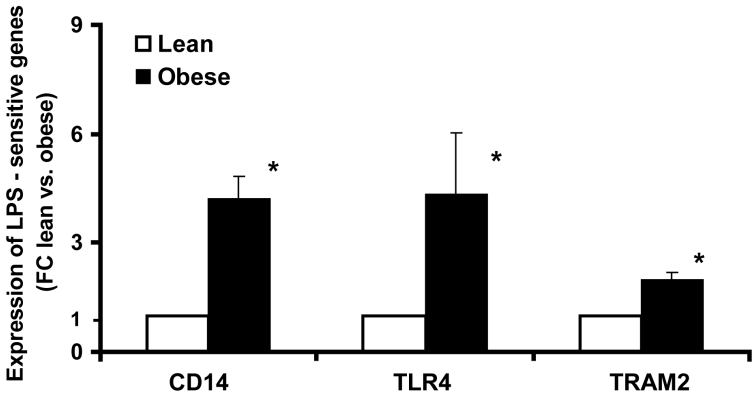

Figure 4. LPS stimulates the expression and the release of cytokines and chemokines in stromal vascular cells in pregnant women

Quantitative RT-PCR analysis of total RNA isolated from LPS-stimulated cultured stromal vascular cells from 10 obese and 5 lean women. Cytokine secretion was measured by ELISA as described in Methods. Data (mean ± SEM) were expressed as fold changes in LPS - treated vs. untreated cells in each group: obese (A) and lean (B). Real-time Ct values were normalized to β-actin. Statistical significance: * $p < 0.05$; no treatment: open bars, LPS treatment: solid bars

TABLE 1

Anthropometrics and metabolic characteristics of studied groups

	Lean women n=55	Obese women n=65	*p*-value
Maternal age (years)	27.9 ± 6.0	27.6 ± 5.7	0.82
Gestational age at delivery (weeks)	38.8 ± 0.6	38.8 ± 1.6	0.78
Pre-pregnancy BMI (kg/m^2)	22.0 ± 2.0	38.4 ± 6.0	0.0001
Term pregnancy BMI (kg/m^2)	28.3 ± 3.3	42.9 ± 6.2	0.0002
HOMA-IR index	2.2 ± 1.2	5.0 ± 3.4	0.0001
Insulin (μU/ml)	11.7 ± 5.6	25.4 ± 12.7	0.0001
Adiponectin (μg/ml)	12.9 ± 0.5	10.5 ± 3.3	0.09
Leptin (ng/ml)	32.6 ± 20.4	73.2 ± 35.6	0.0001

Maternal blood was obtained at the time of admission for elective C-section after overnight fasting. Data are means ± SD.

TABLE 2

Endotoxemia parameters of systemic inflammation in lean and obese women

	Lean women n=55	Obese women n=65	*p*-value
LPS (EU/ml)	0.5 ± 0.2	1.0 ± 0.5	0.006
sCD14 (mg/ml)	1.6 ± 0.6	1.4 ± 0.4	0.56
CRP (ng/ml)	8074 ± 6463	12283 ± 7929	0.005
IL-6 (pg/ml)	2.4 ± 1.4	4.6 ± 3.3	0.002
TNF-α (pg/ml)	1.4 ± 0.9	1.3 ± 0.5	0.67

Blood was obtained at the time of admission for term elective C-section after overnight fasting. Data are means ± SD.

TABLE 3

Enrichment of expression of pro-inflammatory genes in adipose cells of obese women

	Adipose tissue (AT)	Un-plated SVF cells
MCP1	1	22.0 ± 2.6
IL-8	1	324.3 ± 48.1
IL-6	1	141.0 ± 26.9
TNF-α	1	180.0 ± 32.4

SVF: stromal vascular cells isolated from subcutaneous abdominal adipose tissue biopsies.MCP1, IL-8, IL-6 and TNF-α gene expression was measured by real-time PCR as described in Methods.