Published in final edited form as:

Cytokine. 2015 February ; 71(2): 405–408. doi:10.1016/j.cyto.2014.09.009.

Abdominal visceral adiposity influences CD4⁺ T cell cytokine production in pregnancy

Marlies K. Ozias^{a,1,*}, Shengqi Li^{a,2}, Holly R. Hull^a, William M. Brooks^{b,c}, Margaret G. Petroff^e, and Susan E. Carlson^a

^aDepartment of Dietetics and Nutrition, University of Kansas Medical Center, Kansas City, Kansas, USA

^bDepartment of Neurology, University of Kansas Medical Center, Kansas City, Kansas, USA

^cHoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, Kansas, USA

^eDepartment of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, USA

1. Introduction

Women with pregravid obesity are at a greater risk for pregnancy complications [1]. The biological mechanisms responsible for these clinical outcomes are not well characterized, although there is a strong link to the increased inflammation that occurs in obesity [2]. Obese pregnant women categorized by their pre-pregnancy body mass index (BMI) have greater circulating C-reactive protein (CRP) and IL-6 accompanied by both adipose and placental innate inflammation at delivery compared to healthy weight pregnant women [3, 4]. Another investigation found differences in circulating CRP, IL-6, and CCL2 associated to excess adiposity in the first and second trimesters which disappeared by the third trimester [5]. While that study did measure adiposity by skinfold thickness, the majority of research on the impact of obesity in pregnancy uses pre-pregnancy BMI to categorize obesity. Abdominal adiposity is more physiologically relevant to obesity-induced health risks. Abdominal subcutaneous and visceral adiposity are associated with the systemic inflammation seen in obesity-induced health risks and have the potential to play a greater role than whole-body adiposity in obesity-related pregnancy complications. For example, greater abdominal subcutaneous fat thickness measured at 18-22 gestational weeks by ultrasound increases the risk of gestational diabetes mellitus, cesarean delivery, and fetal macrosomia [6].

© 2014 Elsevier Ltd. All rights reserved.

*Corresponding author. Department of Endocrinology, Phoenix Veterans Affairs Healthcare System, 650 E Indian School Road, Phoenix, AZ, 85012, USA. Phone: 1-660-864-9068, Fax: 1-602-200-6004, address: mkozias@gmail.com.

¹currently at Department of Endocrinology, Phoenix Veterans Affairs Healthcare System, Phoenix, AZ, USA.

²currently at Brooklyn Hospital Center, Brooklyn, NY, USA.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Chronic, low-grade, inflammation occurs in obesity. In addition to higher circulating IL-6, TNF- α [7], and CRP [8], PBMC in obese individuals have elevated production of TNF- α and IFN- γ and decreased IL-10 compared to lean individuals [9]. Additionally, peripheral CD4⁺ [10, 11] and adipose CD3⁺ [12] T cell populations increase in individuals with an obese BMI. However, the CD4⁺ T cell subpopulations change depending on the severity of the obesity-induced complications. In diet-induced obese mice, CD4⁺ T helper (Th) 1 cells initiate obesity-induced insulin resistance [13] and obese individuals with diabetes mellitus have a larger adipose CD4⁺ Th1 cell population than normoglycemic lean and obese subjects [14]. In contrast, the increase in CD4⁺ T cell population in metabolically healthy obese individuals comes from circulating Th2 and regulatory T (Treg) cell populations while the Th1 and Th17 cell counts remain similar to those in lean subjects [11]. The characterization of CD4⁺ T cells in pregnant obese women has not been performed.

T cells are routinely characterized by their secretion of cytokines, chemokines, and growth factors (collectively referred to as cytokines herein). The majority of research on the impact of obesity in pregnancy focuses on macrophage-related inflammation. In addition, obesity categorized by BMI is characterized by increased circulating inflammation and increased CD4⁺ T cell population [10]. It is not known how the CD4⁺ T cell cytokine profile changes in pregnant women based on their weight status (BMI) or adipose tissue mass. The intent of this study was to compare weight status and several measures of adipose tissue mass, including abdominal visceral and subcutaneous fat mass with the stimulated CD4⁺ T cell cytokine profile in women during the 3rd trimester of pregnancy.

2. Methods

2.1 Participants

Women from the Kansas City metropolitan area were enrolled between February 2012 and March 2013 for this study. Women were eligible if they were English-speaking, 18-44 years old with a singleton pregnancy, planning to delivery at the University of Kansas Hospital, and had a pre-pregnancy BMI between 18.5-40 kg/m² based on self-reported height and weight. Exclusion criteria included diabetes, hypertension, smoking or drug use during pregnancy, chronic medical conditions known to influence inflammation status, or fear of enclosed spaces. The research protocol and informed consent process adhered to the Declaration of Helsinki and were approved by the Institutional Review Boards/Human Subjects Committees at University of Kansas Medical Center.

2.2 Isolation of CD4⁺ T cells and cytokine analysis

Blood samples were obtained from subjects in the third trimester (n=35; between 35 to 39 weeks gestation). PBMC were isolated from blood collected in heparin-coated tubes (BD Vacutainer, Franklin Lakes, NJ). Blood was layered onto Histopaque 1077 (Sigma-Aldrich, St. Louis, MO) and centrifuged for 30 minutes at 400 $\times$ g. Mononuclear cell fraction was collected and washed with RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL Penicillin/ 100 μ g/mL Streptomycin, 2mM L-glutamine, 100 μ M β -mercaptoethanol before cell viability and count were assessed. PMBCs were frozen in 90% FBS/10% DMSO in liquid nitrogen until CD4⁺ T cell isolation and stimulation could be

performed on all samples. Frozen samples were resuscitated by rapidly raising the temperature to 37°C and gradual aliquots of complete warmed RPMI added to 5 times the original volume to prevent osmotic shock. CD4⁺ T cells were isolated from PBMC by positive selection (Miltenyi Biotec Inc., Auburn, CA). CD4⁺ purity was assessed by flow cytometry (LSR II, BD Biosciences, San Jose, CA) using FITC anti-human CD4 antibody (Biolegend, San Diego, CA) and estimated to be 98 ± 1% and 96 ± 1% in fresh and frozen samples, respectively. In duplicate, cells were stimulated with PMA (50 ng/mL) plus A23187 (250 ng/mL) for 72 hours. Samples were harvested on ice and supernatants were collected by centrifugation at 18,400 × g for 10 minutes at 4° C and stored at -20° C until analysis. CD4⁺ T cell cytokine expression was determined by Milliplex® xMAP technology (Invitrogen Corp, Camarillo, CA) according to manufacturer's instructions. Cytokine supernatants were measured on a Luminex 200 (Austin, TX). Samples were analyzed on one plate to eliminate intervariability.

2.3 Body fat measurement

To determine total body fat and abdominal subcutaneous and visceral fat mass, dual x-ray absorptiometry and magnetic resonance imaging (MRI; Siemens Skyra 3T) were performed as previously described [15].

2.4 Statistical analyses

Subjects' descriptive statistics are presented as mean values ± SD (Table 1). Women were recruited for the study in three categories based on their pre-pregnancy weight and height: normal-weight (18.5-24.9), overweight (25-29.9), and obese 30.0-40.0). Differences between the three BMI categories were analyzed with one-way ANOVA. In order to normalize the absolute value of abdominal fat mass to each subject's size, each abdominal compartment of adipose was divided by the amount of total body fat mass. Linear relationships were calculated between body composition and CD4⁺ T cell cytokines with Pearson correlation. Actual cytokine medians and ranges are found in Supplemental Table 1. Shapiro-Wilks test was used to test normality, with outcomes variables not normally distributed log-transformed prior to all analyses. All data were analyzed with IBM SPSS Statistics 17.0 software (SPSS, Chicago, IL). *P* values presented are two-tailed and significance was set at 0.05.

3. Results and discussion

There were no associations between the BMI categories (healthy weight, overweight, obese) and CD4⁺ T cell-produced cytokines (data not shown). Out of the thirty-five detectable cytokines measured only FGF-2 was related (inversely) to total body fat mass ($r=-0.467$, $P=0.009$). We found no relationships between abdominal subcutaneous fat mass and CD4⁺ T cell cytokines.

In contrast, abdominal visceral fat mass correlated with CD4⁺ T cell cytokine production. Ten cytokines were inversely related to visceral fat mass and two cytokines (FGF-2 and GM-CSF) trended toward a significant inverse relationship (Table 2). Notably, IL-17A was negatively correlated with increased visceral fat mass ($r=-0.525$) as well as IL-10 ($r=-0.549$)

and soluble CD40 ligand ($r=-0.491$). Additionally, IFN- γ ($r=-0.501$), TNF- β ($r=-0.513$), and IL-12p70 ($r=-0.507$) had significant negative associations with visceral fat mass. Inverse relationships were also observed between visceral fat mass and CD4⁺ T cell expressed chemokine concentrations: CCL3 ($r=-0.599$) and IL-8 ($r=-0.721$). Lastly, two growth factors were inversely related to visceral fat mass: G-CSF ($r=-0.580$) and FLT-3L ($r=-0.506$).

To our knowledge, no one has assessed the nature of peripheral CD4⁺ T cells in relationship to obesity in pregnancy even though obese individuals have increased CD4⁺ T cell populations [10, 11]. Total body fat mass (with one exception) and abdominal subcutaneous fat mass were not related to any cytokine response. Only abdominal visceral fat mass was associated with peripheral blood CD4⁺ T cell cytokine production with lower production of cytokines, growth factors and chemokines. Perhaps as important as finding a relationship between abdominal visceral fat mass and CD4⁺ T cell responses, we did not find a relationship between a commonly used BMI and cytokine production. Our results reinforce the ideas that a) visceral fat mass is an important measurement of fat mass, as opposed to body mass, and b) it is a health risk.

Our original hypothesis, based on previous studies of obesity in pregnancy, was that visceral and subcutaneous abdominal adiposity would correlate to an inflammatory-skewed CD4⁺ T cell cytokine production. Specifically, Challier *et al.* [16] found women with a pre-pregnancy obese BMI have increased PBMC mRNA expression of CD14, CD68, TNF- α , and IL-6, indicating increased monocyte activation compared to women with a pre-pregnancy normal weight BMI. To our surprise, we found no relationships between cytokines to total body fat or BMI but did see a pattern of cytokines suggesting a decreased inflammatory response in relation to visceral adiposity. While CD4⁺ T cell populations increase in obesity, the increase is from the Th2 and Treg cell populations rather than Th1 or Th17 when insulin resistance is not present [11, 12]. Women in this study experienced pregnancies without gestational or other types of diabetes so their CD4⁺ T cell responses should have favored a less inflammatory lymphocyte profile than had they been diabetic [17]. Overall, our data suggest that CD4⁺ T cells in late pregnancy may be less responsive in pregnant women with greater abdominal visceral fat mass. Future studies measuring both adaptive and innate immune response in relationship to abdominal adiposity would provide insight into the impact of visceral fat mass and subsequent inflammatory or anti-inflammatory status on pregnancy.

A limitation of our study is that we did not measure the peripheral CD4⁺ T cell populations responsible for the cytokine expression. However, cytokine expression is typically used in exploratory studies to categorize the response of immune cells. Additionally, *ex vivo* PMA stimulation induces a broad spectrum of cytokine production and may not represent the true nature of the subjects' CD4⁺ T cell profile. A final limitation was limited sample volume preventing us from determining unstimulated basal cytokine levels. Strengths include quantification of total body fat mass and abdominal visceral and subcutaneous fat masses in addition to using BMI as an indicator of fat mass. By quantifying CD4⁺ T cell cytokine production, we obtained information about the CD4⁺ T cell populations and gained insight into the relationship of the adaptive immune response to fat mass in pregnancy. The 41-cytokine multiplex assay allowed us to identify cytokines with associations to increased

visceral adiposity that have not been identified until now. Additionally, the robust response of CD4⁺ T cell IL-2 expression indicates overall cell viability.

Visceral fat is an ectopic fat store and hypothesized to play a greater role in the metabolic dysfunction in obesity due to increased secretion of inflammatory cytokines into circulation [18]. Interestingly, we found a dampened CD4⁺ T cell cytokine response to increased abdominal visceral fat mass. We conclude that abdominal visceral fat plays an important role in immune response in pregnancy and is a better indicator of impaired peripheral CD4⁺ T cell immune function than BMI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to acknowledge the nursing staffs at our participating clinics and hospital whose support was essential to recruitment and availability of blood samples; Hoglund Brain Imaging Center for the creation of the MRI adipose protocol and financial support; the University of Kansas Medical Center Flow Cytometry Core Laboratory, which is sponsored, in part, by the NIH/NIGMS COBRE grant P30 GM103326, Dr. Eric Gumprecht for manuscript preparation; and our subjects for their time.

Funding: This study is supported by the Sam E. and Mary F. Roberts Foundation, and the National Institutes of Health through grants P30 HD02528, UL1 TR000001, and S10 RR029577. The Hoglund Brain Imaging Center is supported by a generous gift from Forrest and Sally Hoglund.

References

1. Sebire NJ, Jolly M, Harris JP, et al. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. *Int J Obes Relat Metab Disord*. 2001; 25(8):1175–82. [PubMed: 11477502]
2. Wolf M, Sandler L, Hsu K, et al. First-trimester C-reactive protein and subsequent gestational diabetes. *Diabetes Care*. 2003; 26(3):819–24. [PubMed: 12610043]
3. Basu S, Haghiac M, Surace P, et al. Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. *Obesity (Silver Spring)*. 2011; 19(3):476–82. [PubMed: 20930711]
4. Roberts KA, Riley SC, Reynolds RM, et al. Placental structure and inflammation in pregnancies associated with obesity. *Placenta*. 2011; 32(3):247–54. [PubMed: 21232790]
5. Friis CM, Paasche Roland MC, Godang K, et al. Adiposity-related inflammation: effects of pregnancy. *Obesity (Silver Spring)*. 2013; 21(1):E124–30. [PubMed: 23505192]
6. Suresh A, Liu A, Poulton A, et al. Comparison of maternal abdominal subcutaneous fat thickness and body mass index as markers for pregnancy outcomes: A stratified cohort study. *Aust N Z J Obstet Gynaecol*. 2012; 52(5):420–6. [PubMed: 23045985]
7. Kern PA, Ranganathan S, Li C, et al. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab*. 2001; 280(5):E745–51. [PubMed: 11287357]
8. Visser M, Bouter LM, McQuillan GM, et al. Elevated C-reactive protein levels in overweight and obese adults. *JAMA*. 1999; 282(22):2131–5. [PubMed: 10591334]
9. Dicker D, Salook MA, Marcoviciu D, et al. Role of peripheral blood mononuclear cells in the predisposition of obese individuals to inflammation and infection. *Obes Facts*. 2013; 6(2):146–51. [PubMed: 23595216]
10. Womack J, Tien PC, Feldman J, et al. Obesity and immune cell counts in women. *Metabolism*. 2007; 56(7):998–1004. [PubMed: 17570264]

11. van der Weerd K, Dik WA, Schrijver B, et al. Morbidly obese human subjects have increased peripheral blood CD4+ T cells with skewing toward a Treg- and Th2-dominated phenotype. *Diabetes*. 2012; 61(2):401–8. [PubMed: 22228716]
12. Zeyda M, Huber J, Prager G, et al. Inflammation correlates with markers of T-cell subsets including regulatory T cells in adipose tissue from obese patients. *Obesity (Silver Spring)*. 2011; 19(4):743–8. [PubMed: 20508627]
13. Winer S, Chan Y, Paltser G, et al. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med*. 2009; 15(8):921–9. [PubMed: 19633657]
14. Fabbrini E, Cella M, McCartney SA, et al. Association Between Specific Adipose Tissue CD4 + T-Cell Populations and Insulin Resistance in Obese People. *Gastroenterology*. 2013
15. Ozias MK, Li S, Hull HR, et al. Relationship of circulating adipokines to body composition in pregnant women. *Adipocyte*. 2014 accepted for publication.
16. Challier JC, Basu S, Bintein T, et al. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta*. 2008; 29(3):274–81. [PubMed: 18262644]
17. Mahmoud F, Abul H, Omu A, et al. Lymphocyte sub-populations in gestational diabetes. *Am J Reprod Immunol*. 2005; 53(1):21–9. [PubMed: 15667522]
18. Fontana L, Eagon JC, Trujillo ME, et al. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes*. 2007; 56(4):1010–3. [PubMed: 17287468]

Highlights

- Visceral fat correlates to decreased CD4⁺ T cell cytokines in pregnancy.
- Pre-pregnancy body mass index has no relationship to CD4⁺ T cell cytokine profile.
- Whole body adiposity had minimal correlation to CD4⁺ T cell cytokines.

Table 1
Descriptive statistics of subjects by BMI category

	Healthy weight n = 16 Mean ± SD (Range)	Overweight n = 7 Mean ± SD (Range)	Obese n = 12 Mean ± SD (Range)
Age (y)	28 ± 6 ^a (19-42)	29 ± 4 (23-33)	30 ± 5 (21-37)
Nulliparity (n)	8	5	3
Gestational weight gain (kg)	15.1 ± 4.3 (6.8-22.3)	17.5 ± 5.6 (10.9-24.1)	14.3 ± 6.0 (1.8-24.5)
Pre-pregnancy BMI (kg/m ²)	21.6 ± 1.4 (18.9-23.6)	27.4 ± 1.7 (25.1-29.4)	34.2 ± 2.3 (31.3-38.6)
Total body fat mass (kg)	22.7 ± 4.8 (14-33)	34.3 ± 4.0 (29-40)	41.6 ± 9.8 [*] (27-53)
Abdominal subcutaneous fat mass (g)	4125.0 ± 1138.9 ^b (2449-5576)	5767.0 ± 360.0 (5315-6034)	8950.4 ± 1912.2 [*] (5848-10891)
Abdominal visceral fat mass (g)	577.5 ± 242.2 (324-997)	760.0 ± 441.5 (347-1171)	1248.0 ± 986.4 (506-3302)

^a n = 35;

^b n = 18 for abdominal fat mass measurements.

^{*} P = 0.005 compared to healthy weight and overweight BMI categories, measured by one-way ANOVA. Body mass index, BMI.

Table 2
Pearson correlation of CD4⁺ T cell cytokines to visceral fat mass (g) / total body fat mass (kg)

Nonsignificant ($P > 0.05$)			Significant ($P \leq 0.05$)
IL-1 α	IL-5	IL-7	TNF- β [*]
IL-1RA	IL-6	IFN- α 2	IFN- γ [*]
IL-12p40	IL-4	IL-15	IL-12p70 [*]
TNF- α	IL-13	IL-9	IL-17A [*]
IL-1 β	IL-2	PDGF-AA	sCD40L [*]
CCL2	CCL7	CX3CL1	FLT-3L [*]
CCL4	CXCL10	CXCL1	G-CSF [*]
CCL22	CCL11	VEGF	IL-10 [*]
GM-CSF [#]	FGF-2 [#]		CCL3 [†]
			IL-8 [‡]

^{*} $P \leq 0.05$;

[†] $P \leq 0.01$;

[‡] $P \leq 0.001$;

[#] $P = 0.055$. Cytokines without normal distribution were log transformed prior to bivariate Pearson correlation.