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American Diabetes Association

Sam, Susan, Steven Haffner, Michael H. Davidson, Ralph B. D'Agostino, Steven Feinstein, George Kondos, Alfonso Perez, Theodore Mazzone. "Relation of Abdominal Fat Depots to Systemic Markers of Inflammation in Type 2 Diabetes" Diabetes Care 32(5): 932-937. (2009)
https://hdl.handle.net/2144/2633

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Relation of Abdominal Fat Depots to Systemic Markers of Inflammation in Type 2 Diabetes

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OBJECTIVE — Both visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) have been linked to systemic inflammation in nondiabetic cohorts. We examined the relationships between VAT and SAT and systemic inflammatory markers in a large well-characterized cohort of subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Three hundred eighty-two subjects with type 2 diabetes in the CHICAGO (Carotid Intima-Media Thickness in Atherosclerosis Using Pioglitazone) study cohort underwent abdominal computed tomography to determine SAT and VAT distribution. Fasting blood was obtained for measurement of inflammatory markers. The relationships between inflammatory markers and BMI, SAT, and VAT were examined using regression models adjusted for age, sex, diabetes treatment, duration of diabetes, smoking, statin use, and HbA1C.

RESULTS — VAT was positively related to CRP, monocyte chemoattractant protein (MCP), intracellular adhesion molecule (ICAM)-1, and plasminogen activator inhibitor type 1 (PAI-1) antigen before adjustment for BMI. After adjustment for BMI, the relationship to CRP was lost but positive associations with MCP ($P < 0.01$), PAI-1 ($P < 0.0001$), ICAM-1 ($P < 0.01$), and vascular cell adhesion molecule ($P = 0.01$) were evident. BMI was positively related to CRP ($P < 0.0001$) and IL-6 ($P < 0.01$) even after adjustment for VAT and SAT. SAT was not related to any inflammatory marker after adjustment for BMI.

CONCLUSIONS — In this large group of subjects with type 2 diabetes, BMI was most strongly associated with CRP and IL-6 levels. SAT was not associated with markers of systemic inflammation. The size of the VAT depot provided information additional to that provided by BMI regarding inflammatory markers that are strongly related to vascular wall remodeling and coagulation. Our findings suggest that adipose tissue distribution remains an important determinant of systemic inflammation in type 2 diabetes.

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Received 13 October 2008 and accepted 5 February 2009.

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pioglitazone compared with glimepiride on carotid intima-media thickness in subjects with type 2 diabetes (10). The details of the study have been previously reported (10). Data included in this report were obtained before randomization to treatment groups. The study was approved by central and local institutional review board committees, and all participants provided written informed consent. All subjects underwent measurements of height, weight, and waist and hip circumference by a trained nurse at the baseline visit. Waist circumference was measured at the smallest circumference between the ribs and iliac crest, and hip circumference was measured at maximum circumference between the iliac crest and crotch to the nearest 0.1 cm.

Subjects underwent an abdominal CT scan for determination of VAT, SAT, and total abdominal adipose tissue (TAT). Abdominal adipose tissue content and distribution were quantified by CT scan at the level of L4–L5 vertebrae while the subjects were in supine position with both arms stretched above the head. A single 6-mm slice was taken during suspended respiration after a normal expiration. TAT was measured by delineating the body surface with a receiver operator instrument and then by computing the adipose tissue volume using an attenuation range of −190 to −30 HU. VAT area was quantified by delineating the abdominal cavity at the internal aspect of the abdominal wall and the posterior aspect of the vertebral body with the receiver operator instrument. SAT area was calculated by subtracting VAT from TAT volume. To obtain VAT, SAT, and TAT volumes, the area for each fat component was multiplied by the slice thickness. Fasting blood samples were obtained at the baseline visit for measurement of inflammatory markers and A1C. Inflammatory markers were measured using kits according to the manufacturer’s instructions: plasma CRP (Roche Diagnostics, Indianapolis, IN), intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (R&D Systems, Minneapolis, MN), matrix metalloproteinase (MMP)9 (R&D Systems), and PAI-1 (Trinity Biotech USA, St. Louis, MO). Fibrinogen was measured by direct coagulation analysis (Dade Behring Marburg, Marburg, Germany), human insulin by ELISA (Linco, St. Charles, MO), and A1C by high-performance liquid chromatography (Bio-Rad, Hercules, CA). IL-6 was measured by ELISA (Quantikine HS; R&D Systems).

**Statistical methods**

Log transformation of the data was performed when it was necessary to achieve homogeneity of variance. Sex differences in inflammatory biomarkers, VAT, SAT, TAT, and BMI were compared by ANCOVA adjusted for age, BMI, baseline diabetes treatment, duration of diabetes, years of smoking, statin use, and A1C.

Age- and sex-adjusted Pearson correlation coefficients were used to test relationships between each inflammatory marker and SAT, VAT, and BMI. SAT, VAT, and BMI were first standardized to mean 0 and SD 1. We calculated regression coefficients quantifying the estimated change in log-transformed biomarker per SD increase in SAT, VAT, or BMI separately and then transformed back to estimate the percent change in each biomarker. The multivariable analyses were repeated with addition of BMI to the models when assessing the relationship between VAT or SAT and inflammatory markers or with the addition of both VAT and SAT to the model when assessing the relationship between BMI and inflammatory markers. The associations between VAT and inflammatory markers were further examined by multivariable models after addition of SAT, or of hip circumference, to models that included BMI. Similar analyses were performed to evaluate the associations between SAT and inflammatory markers before and after adjustment for BMI or for BMI and VAT. To further examine the role of smoking, we repeated all of the above analyses by placing smoking as a categorical variable instead of years of smoking as follows: current smokers (n = 58), ex-smokers (n = 186), and nonsmokers (n = 127). To evaluate whether the associations between abdominal fat depots and inflammatory markers were related to the degree of obesity, we repeated the above analyses in two groups based on a median split of BMI. Analyses were performed using the 11.0 PC package of SPSS statistical software (SPSS, Chicago, IL). P ≤ 0.01 was considered significant in order to adjust for evaluation of multiple inflammatory markers for their relationship to VAT (which was our primary analysis).

**RESULTS** — The baseline characteristics of study subjects are presented in Table 1. The mean age was 61 years. Thirty-eight percent of subjects were women. Fifty-five percent were on statin therapy, and 65% were current or former smokers. Subjects were on the following diabetes therapy at the time of assessment in the study: 15% were not taking any medications for diabetes, 15% were taking sulfonylureas, 29% were taking metformin. 31% were taking a combination of metformin and sulfonylureas, and 10% were on insulin therapy. The average ± SD BMI was 32.5 ± 5.1 kg/m², the mean duration of type 2 diabetes was 92 ± 86 months, and the mean A1C was 7.4 ± 0.9%. The median (interquartile range) CRP level was 2.7 mg/l (1.4–5.5), ICAM-1 was 241 ng/ml (196–294), MCP was 61 pg/ml (43–61), VCAM-1 was 670 mg/dl (537–816), fibrinogen was 322 mg/dl (249–391), MMP9 was 439 ng/ml (305–613), PAI-1 was 29 ng/ml (20–43), and IL-6 was 2.49 pg/ml (1.8–3.8) (data not shown).

Men had a higher amount of VAT than women (P < 0.0001), whereas women had a higher amount of SAT than men (P < 0.0001) (Table 2). BMI and

| Table 1—Baseline characteristics of study participants |
| Age (years) | 61 ± 8 |
| BMI (kg/m²) | 32.5 ± 5.1 |
| Waist circumference (cm) | 108 ± 13 |
| Hip circumference (cm) | 113 ± 12 |
| Duration of type 2 diabetes (months) | 92 ± 86 |
| A1C (%) | 7.4 ± 0.9 |
| Smoking | |
| Current | 16 |
| Former | 49 |
| Never | 35 |
| Diabetes therapy | |
| None | 15 |
| Sulfonylurea | 15 |
| Metformin | 29 |
| Sulfonylurea and metformin | 31 |
| Insulin | 10 |
| Statin use | |
| Statin | 55 |
| No statin | 45 |
| Sex | |
| Men | 62 |
| Women | 38 |

Data are means ± SD or %. Study participants are subjects with type 2 diabetes (n = 382).
Table 3—Age- and sex-adjusted Pearson correlation coefficients between log-transformed inflammatory markers and BMI, waist circumference, TAT, VAT, SAT, and A1C

|            | BMI  | Waist | TAT   | VAT  | SAT  | A1C  |
|------------|------|-------|-------|------|------|------|
| CRP        | 0.34*| 0.26* | 0.28* | 0.18*| 0.17*| 0.03*|
| ICAM-1     | 0.12 | 0.16* | 0.17† | 0.21*| 0.08 | -0.02|
| MCP        | 0.05 | -0.03 | 0.06  | 0.16*| -0.01| 0.003|
| VCAM-1     | -0.04| 0.05  | 0.02  | 0.10 | -0.04| 0.02 |
| Fibrinogen | 0.16*| 0.13  | 0.12  | 0.07 | 0.08 | -0.04|
| MMP9       | 0.13 | 0.18* | 0.13  | 0.17*| -0.04| -0.03|
| PAI-1      | 0.14†| 0.18* | 0.18† | 0.29*| 0.05 | -0.04|
| IL-6       | 0.21 | 0.19* | 0.12  | 0.07 | 0.04 | 0.07 |

*p ≤ 0.001, †p ≤ 0.01.
associated with any inflammatory markers. Interestingly, adjusting for SAT (index of central SAT) or hip circumference (index of peripheral SAT) did not reduce the importance of VAT for predicting systematic inflammatory markers. Our findings suggest that both BMI and VAT are correlates of systemic inflammation in obese subjects with type 2 diabetes. Furthermore, VAT provides information additional to BMI for a number of systemic inflammatory markers that are strongly associated with vascular remodeling and coagulation (13–18).

An increase in inflammatory markers has been associated with increased risk for metabolic abnormalities and cardiovascular disease (25,19). Expansion of adipose tissue explains these associations, as it promotes a systemic inflammatory response. Inflammatory molecules such as TNF-α, IL-6, serum amyloid A, and MCP-1 are produced in significant quantity by ATMs and adipocytes (2,5,19). Recently, it has been shown that obesity is associated with increased ATM infiltration (up to 40%) and a change in ATM polarization to a more proinflammatory state (3,4). Both SAT and VAT are known to secrete inflammatory cytokines in vitro and have been implicated in metabolic disorders (7–9). Subcutaneous abdominal fat is divided into superficial and deep layers by a fascial plane, and recent evidence suggests that there maybe metabolic differences between the two components (20). For example, deep but not superficial subcutaneous abdominal tissue has been associated with peripheral insulin resistance and features of metabolic syndrome (20). We were not able to separate these compartments in the current study.

A recent study from the Framingham cohort has shown that both VAT and SAT are associated with CRP and a number of other inflammatory markers, independent of BMI; however, the associations were stronger for VAT (11). Subjects in the CHICAGO cohort were more obese compared with the Framingham cohort, and all had type 2 diabetes, whereas the prevalence of type 2 diabetes in the Framingham cohort was 10% (11). There-...
ter the onset of diabetes, they also suggest that relationships between specific adipose tissue depots and inflammatory markers may be modified by the onset of diabetes. Similar to previous studies, we observed sex differences in CRP and fibrinogen levels with higher levels in women (21). We also found a stronger association between CRP and BMI, independent of VAT and SAT, in women.

In multivariable fully adjusted models, an increase in VAT was strongly associated with an increase in PAI-1 levels independent of BMI. A higher PAI-1 plasma level has been linked to a higher risk of coronary heart disease in subjects with type 2 diabetes (15). Both animal and human studies suggest that PAI-1 expression is higher in VAT than SAT (9,22). Our data support the strong association between VAT and PAI-1 levels, independent of BMI, in type 2 diabetes. MCP-1 is a potent chemotactic factor for monocytes (23) and has been associated with cardiovascular disease and diabetes (16). In the Framingham cohort, MCP-1 was more strongly associated with VAT than with SAT (11). Our findings are similar to the Framingham cohort, as we also observed a strong correlation between VAT and MCP-1 that was independent of BMI. ICAM-1 and VCAM-1 are members of the cellular adhesion molecule family that have been implicated in inflammatory and atherosclerotic processes (13). Elevated levels of both have been reported in obesity (24). In the Framingham cohort, both SAT and VAT were associated with ICAM-1 but neither of these relations persisted after adjustment for BMI (11). In contrast, in our study, VAT was associated with ICAM-1 before and after adjustment for BMI. In contrast to findings in predominantly nondiabetic populations, we found no independent relationships among BMI, VAT or SAT, and fibrinogen or MMP9 in fully adjusted models (11,25).

Although prospective studies will be necessary to determine the causal nature of these associations, our results suggest that in obese subjects with type 2 diabetes, both BMI and VAT are important drivers of systemic inflammation. While BMI is strongly associated with CRP and IL-6 levels, VAT is the primary determinant of ICAM, VCAM, MCP-1, and PAI-1. ICAM and VCAM are found in the vessel wall where their level of expression is related to atherosclerotic plaque remodeling (13,17,18). MCP-1 and PAI-1 are significant markers of cardiovascular disease risk (14–16). Our findings indicate that adipose tissue distribution remains an important determinant of systemic inflammation in type 2 diabetes. They underscore the importance of managing excess adiposity, including that in the visceral fat depot, for optimally managing cardiovascular risk in subjects with type 2 diabetes.

Acknowledgments — This analysis was supported by National Institutes of Health Grant DK-71711 (to T.M.) and by an institutional award from the University of Illinois at Chicago.

The CHICAGO study was sponsored and funded by Takeda Global Research & Development. No other potential conflicts of interest relevant to this article were reported.

S.S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. 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.
2. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793–1801.
3. 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.
4. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007;117:175–184.
5. Ridker PM. Inflammatory biomarkers and risks of myocardial infarction, stroke, diabetes, and total mortality: implications for longevity. Nutr Rev 2007;65:S253–S259.
6. Cancello R, Henegar C, Viguere N, Taleb S, Poitou C, Rouault C, Coupage M, Peloux V, Hugol D, Bouilhot JL, Bouloum A, Barbatelli G, Cinti S, Svensson PA, Barsh GS, Zucker JD, Basdevant A, Langin D, Clement K. Reduction of macrophage infiltration and chemokina expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 2005;54:2277–2286.
7. Fried SK, Bunka DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation with glucocorticoid. J Clin Endocrinol Metab 1998;83:847–850.
8. Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J Clin Endocrinol Metab 2005;90:2282–2289.
9. Alessi MC, Petretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. Diabetes 1997;46:860–867.
10. Mazzone T, Meyer PM, Feinstein SB, Davidson MH, Kondos GT, D’Agostino RB Sr, Perez A, Provost JC, Halfner SM. Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes: a randomized trial. JAMA 2006;296:2572–2581.
11. Pou KM, Massaro JM, Hofmann U, Van’s, RS, Maurovich-Horvat P, Larson MG, Keaney JF Jr, Meigs JB, Lipinska I, Kathiresan S, Murabito JM, O’Donnell C, Benjamin EJ, Fox CS. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 2007;116:1234–1241.
12. Abe M, Matsuda M, Kobayashi H, Miyata Y, Nakayama Y, Komuro R, Fukuthara A, Shimomura I. Effects of statins on adipose tissue inflammation: their inhibitory effect on myD88-independent IRF3/IFN-beta pathway in macrophages. Arterioscler Thromb Vasc Biol 2008;28:871–877.
13. Abe Y, El-Masri B, Kimball KT, Pownall H, Reilly CF, Osmundsen K, Smith CW, Ballantyne CM. Soluble cell adhesion molecules in hyperterglycemia and potential significance on monocyte adhesion. Arterioscler Thromb Vasc Biol 1998;18:723–731.
14. Juhan-Vague I, Alessi MC, Morange PE. Hypofibrinolysis and increased PAI-1 are linked to atherothrombosis via insulin resistance and obesity. Ann Med 2000;32 (Suppl. 1):78–84.
15. Brazionis L, Rowley K, Jenkins A, Itsiopoulos C, O’Dea K. Plasminogen activator inhibitor-1 activity in type 2 diabetes: a different relationship with coronary heart disease and diabetic retinopathy. Arterioscler Thromb Vasc Biol 2008;28:786–791.
16. De Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, McCabe CH, Cannon CP, Braunwald E. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107:690–695.
17. O’Brien KD, Allen MD, McDonald TO, Chait A, Harlan JM, Fishbein D, McCarty J, Ferguson M, Hudkins K, Benjamin CD,
Lobb R, Alpers CE. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques. Implications for the mode of progression of advanced coronary atherosclerosis. J Clin Invest 1993;92:945–951

18. Pradhan AD, Rifai N, Ridker PM. Soluble intercellular adhesion molecule-1, soluble vascular adhesion molecule-1, and the development of symptomatic peripheral arterial disease in men. Circulation 2002;106:820–825

19. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. Lancet 2008;371:1800–1809

20. Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. Am J Physiol Endocrinol Metab 2000;278:E941–E948

21. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131–2135

22. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 1996;2:800–803

23. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 2006;116:1494–1505

24. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, D’Andrea F, Molinari AM, Giugliano D. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation 2002;105:804–809

25. Festa A, D’Agostino R Jr, Williams K, Karter AJ, Mayer-Davis EJ, Tracy RP, Haffner SM. The relation of body fat mass and distribution to markers of chronic inflammation. Int J Obes Relat Metab Disord 2001;25:1407–1415