Ethnicity, Insulin Resistance, and Inflammatory Adipokines in Women at High and Low Risk for Vascular Disease

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OBJECTIVE — We sought to compare the relationship between body composition, insulin resistance, and inflammatory adipokines in Aboriginal Canadian women, who are at high risk of vascular disease, with white women.

RESEARCH DESIGN AND METHODS — A subgroup of the First Nations Bone Health Study population, consisting of 131 Aboriginal women and 132 matched white women, was utilized. Body composition was determined by whole-body dual X-ray absorptiometry, and blood analytes were measured after an overnight fast.

RESULTS — After excluding individuals with diabetes, A1C, BMI, percent trunk fat, and homeostasis model assessment of insulin resistance (HOMA-IR) were greater in First Nation women compared with white women, whereas adiponectin, retinol binding protein (RBP)4, and insulin-like growth factor binding protein-1 (IGFBP-1) were lower. First Nation women had more trunk fat for any given level of total fat than white women. There were no differences in resistin, leptin, tumor necrosis factor (TNF)-α, or C-reactive protein (CRP) levels between First Nation and white women. Insulin resistance correlated with leptin and inversely with adiponectin levels in both First Nation and white women. There were weak correlations between insulin resistance and TNF-α, interleukin-6, and CRP, but these were not significant after correction for body fat. No correlation was found between RBP4 and insulin resistance. ANCOVA revealed a higher HOMA-IR adjusted for total body fat in First Nation women than in white women ($P = 0.015$) but not HOMA-IR adjusted for trunk fat ($P > 0.2$).

CONCLUSIONS — First Nation women are more insulin resistant than white women, and this is explained by trunk fat but not total fat. Despite the increased insulin resistance, inflammatory adipokines are not significantly increased in First Nation women compared with white women.

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An increased prevalence of vascular disease in insulin-resistant states such as pre-diabetes, type 2 diabetes, and the metabolic syndrome has been long recognized (1). There is considerable debate whether insulin resistance is the primary event in atherosclerosis, with consequent activation of proinflammatory signaling pathways, or, alternatively, whether low-grade inflammation and subsequent insulin resistance accounts for the association of diabetes and vascular disease (2).

Aboriginal Canadian populations, which include First Nation, Metis, and Inuit individuals (3), have an increased prevalence of atherosclerosis and cardiovascular and peripheral vascular disease (4,5). First Nations are Aboriginal individuals signatory to treaties and/or recognized by the Canadian Federal Government as a fiduciary responsibility and represent the large majority of Aboriginal individuals living in Canada (3). While type 2 diabetes is more prevalent among Canadian men than women in the general population, the reverse is true for the First Nation population (6,7). In the First Nation population, obesity is more prevalent among men than women, but the prevalence of metabolic syndrome and type 2 diabetes appears to be greater for women than men, suggesting that First Nation women may be more insulin resistant than their male counterparts (6,8). Studies in Canadian Aboriginal populations have found elevated adipocytokines such as tumor necrosis factor (TNF)-α, C-reactive protein (CRP), and leptin (9,10), whereas adiponectin levels were found to be reduced (11). In some of these studies, percent body fat determined by electric impedance was reported to be elevated. However, there have been no reports of a systematic comparison of body composition and insulin resistance in women from ethnic groups at high risk of vascular disease with the general female population.

In this study, we have compared body composition, insulin resistance, and adipokines in a large cohort of First Nation women with an age-matched cohort of white women from the general Canadian population.

RESEARCH DESIGN AND METHODS

— The study population was based on the urban participants from a population-based, cross-sectional survey of osteoporosis: the First Nations Bone Health Study. The design and recruitment of this study is described in detail elsewhere (12). All subjects completed an entrance questionnaire that included information about health status and medications. We excluded individuals with either a history of diabetes or a fasting plasma glucose level >6.9 mmol/l. In addition, we excluded individuals with an elevated A1C (>6.0%) to minimize the number of subjects with normal fasting plasma glucose (but impaired glucose tol-

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Abbreviations: CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; IGFBP-1, insulin-like growth factor binding protein-1; IL, interleukin; RBP, retinol binding protein; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Comparison of demographic, anthropomorphic, and biochemical parameters*

	White women	First Nation women	*P* value
n	132	131	
Age (years)	45.6 ± 14.1	41.1 ± 10.2	
Pre-/transitional/postmenopausal	67/36/29	77/44/10	0.005
Weight (kg)	73.9 ± 16.3	77.8 ± 16.4	0.040
BMI (kg/m^2)	27.52 ± 6.29	29.20 ± 5.87	0.011
Total fat (kg)	26.78 ± 10.63	29.37 ± 9.74	0.022
Total lean mass (kg)	42.59 ± 6.33	43.72 ± 7.05	
Trunk fat (kg)	12.90 ± 5.99	15.63 ± 5.96	<0.001
Trunk fat (%)	16.69 ± 4.68	19.53 ± 4.13	<0.001
Plasma glucose (mmol/l)	5.03 ± 0.41	5.16 ± 0.53	
A1C (%)	5.50 ± 0.29	5.58 ± 0.29	0.013
Insulin (pmol/l)	49.65 (31.66)	59.0 (48.2)	0.002
HOMA-IR	1.54 (1.07)	1.93 (1.66)	0.002
Resistin (ng/ml)	16.74 ± 6.36	16.81 ± 6.03	
Adiponectin (μg/ml)	17.67 ± 9.26	14.95 ± 8.37	0.012
Leptin (ng/ml)	33.93 ± 25.90	33.77 ± 24.21	
TNF-α (pg/ml)	5.45 ± 1.25	5.60 ± 1.33	
IL-6 (ng/ml)	1.18 (1.08)	1.51 (1.43)	<0.001
CRP (μg/ml)	2.05 (3.09)	2.37 (3.49)	
RBP4 (ng/ml)	47.59 (20.88)	44.24 (15.31)	0.008
IGFBP-1 (ng/ml)	12.45 (19.55)	11.92 (10.33)	0.002

Data are means ± SD or median (interquartile range).

erance) or undiagnosed type 2 diabetes. After these exclusions, data from a total of 132 white and 131 First Nation women were available for analysis. All participants provided written informed consent. The study was approved by the University of Manitoba's Research Ethics Board.

Assays and measurements

Dual-energy X-ray absorptiometry measurements. Body composition (lean, fat, and bone mass) was derived from whole-body dual-energy X-ray absorptiometry (Hologic QDR-4500; Hologic, Waltham, MA). A single trained operator was used to perform all dual-energy X-ray absorptiometry scans. Fat mass and lean tissue mass parameters were analyzed using the manufacturer's software. Trunk fat, defined as the absolute amount of fat in the trunk region, including thorax, abdomen, and pelvis, was calculated.

Glucose and insulin assays and homeostasis model assessment of insulin resistance calculations. Blood samples were obtained after an overnight fast, separated, and stored in aliquots at −70°C until analysis. Blood glucose was measured with a glucose oxidase method (Yellow Springs). Insulin was measured using a two-site chemiluminescent immunometric assay (Immulite insulin; Diagnostic Products Corporation), which has 8.5% cross-reactivity with proinsulin. All samples were assayed in a single run with reagent pooled from several kits. Insulin resistance was calculated using homeostasis model assessment of insulin resistance (HOMA-IR) (13). Insulin-like growth factor binding protein-1 (IGFBP-1) was measured with reagents from Diagnostic Systems Laboratories (Webster, TX).

Adipokines and inflammatory markers. Total adiponectin was measured by radioimmunoassay using reagents from Linco Research (St. Charles, MO), whereas resistin and leptin were measured by enzyme-linked immunoassay kits obtained from Biovendor Laboratory Medicine (Brno, Czech Republic). The specificity, sensitivity, and coefficient of variation of these assays in our laboratory have been previously reported (14). TNF-α and interleukin (IL)-6 were measured by a Quantikine HS assay from R&D Systems (Minneapolis, MN). The sensitivity of the assay was 0.12 and 0.039 pg/ml, and the within-assay coefficients of variation were 5 and 7%, respectively. Serum retinol binding protein (RBP)4 was measured with an enzyme-linked immunosorbent assay kit from Alpco Diagnostics (Windham, NH). The sensitivity and interassay coefficients of variation were 100 pg/ml and 5%, respectively. C-reactive protein (CRP) was measured with a high-sensitivity assay kit from BioQuatt (San Diego CA). The sensitivity and coefficients of variation of the assay were 100 ng/ml and 4%, respectively.

Statistical analysis

Normally distributed data are expressed as means ± SD. Measurements that were non–normally distributed (such as HOMA-IR) are reported as median (quartile range). Group differences in continuous measurements were identified with the Wilcoxon's rank-sum test. χ^2 analysis was used to compare the frequency of various conditions in the two populations. The relationship between two variables was assessed with Spearman's rank correlation coefficient. Partial correlations were computed on ranks after controlling for ethnicity, trunk fat, and total fat (15). ANCOVA was used to compare covariate relationships between the two populations (regression line intercepts and parallelism). Non–normally distributed variables were log transformed to obtain normal distributions before ANCOVA. Statistical analysis was performed using SPSS 11.0 for Windows software.

RESULTS

Differences in demographic, anthropomorphic, and biochemical parameters

Weight, BMI, total fat, trunk fat, A1C, fasting insulin, IL-6, and HOMA-IR were significantly greater in First Nation women compared with white women, while adiponectin, RBP4, and IGFBP-1 were significantly lower in First Nation women (Table 1). However, there were no significant differences in resistin, leptin, TNF-α, or CRP levels. There were significantly fewer postmenopausal First Nation women, and, as a group, the First Nation women were slightly younger than the white population, suggesting that age itself was not a major cause of the increased insulin resistance apparent in the First Nation women.

First Nation women had a larger percentage of their total fat as trunk fat than nondiabetic white women (52.5 ± 5.9 vs. 46.9 ± 5.7%, $P < 0.001$). In both groups, there was a significant relationship between trunk fat and total fat. First Nation women had a significantly greater increment in trunk fat for each additional kilogram of total fat (ANCOVA $P = 0.028$ for comparison of regression slopes) and sig-

Table 2—*Spearman correlation coefficients between HOMA-IR and other parameters in nondiabetic populations*

	White women	First Nation women	All women
BMI	0.569*	0.660*	0.634*
IGFBP-1	−0.585*†	−0.554*†	−0.592*†
Total fat	0.576*	0.623*†	0.607*†
Trunk fat	0.603*	0.697*	0.668*
Resistin	0.008	0.180‡	0.097
Adiponectin	−0.415*†	−0.451*†	−0.451*†
Leptin	0.525*	0.672*†	0.595*†
TNF-α	0.042	0.186‡	0.107
IL-6	0.272§	0.373*	0.347*
CRP	0.250§	0.390*	0.324*
RBP4	0.088	0.005	0.008

*$P < 0.005$, ‡$P < 0.05$, §$P < 0.01$. †Significant after controlling for trunk fat.

nificantly greater trunk fat computed at the covariate means ($P < 0.001$ for comparison of regression least-squares means).

Determinants of insulin resistance

HOMA-IR was strongly correlated with BMI, total body fat, and trunk fat in both groups and when the population was considered as a whole (Table 2). The strongest correlation was observed with trunk fat. ANCOVA regression analysis for HOMA-IR and trunk fat for the two populations showed no significant ethnicity effect. While regression analysis for HOMA-IR and total fat indicated that there was no difference in the regression slopes, the intercept was significantly higher in First Nation compared with white women ($P > 0.2$ for comparison of regression slopes; $P = 0.015$ for comparison of intercepts). That is, for any given amount of total fat, First Nations had a higher HOMA-IR than white women (Fig. 1). When total fat was replaced by trunk fat in the ANCOVA model, no ethnicity difference was seen ($P = 0.19$ for comparison of regression slopes; $P > 0.2$ for comparison of intercepts).

A significant inverse correlation was observed between IGFBP-1 and HOMA-IR and between adiponectin and HOMA-IR, while leptin showed a strong positive correlation in all groups. Interestingly, even after correction for trunk fat, the relationships between HOMA-IR and both adiponectin and leptin remained significant (in all comparisons except for HOMA-IR versus leptin in the white women). Resistin, TNF-α, and CRP showed a weak, but significant, positive correlation with HOMA-IR, but none of these correlations remained significant after correction for trunk fat. RBP4 did not correlate with HOMA-IR in either group or the entire study population.

The contribution of adipose tissue to adipokine levels

The correlations between fat mass and adipokines are shown in Table 3. RBP4 levels did not correlate with either total or trunk fat in either group, and there were only weak correlations for TNF-α in First Nation women. Leptin strongly correlated with total body and trunk fat, and in each group, the correlation was slightly greater with total than trunk fat. Similarly, adiponectin levels were inversely correlated with fat mass in all groups, and, in contrast with leptin, the correlation was stronger for trunk than total fat. Resistin correlated with total and trunk fat only in the First Nation women, while IL-6 levels correlated with total and trunk fat in both groups. CRP correlated with both total and trunk fat in both control and First Nation women. All of these correlations remained significant after controlling for HOMA-IR.

CONCLUSIONS — Several epidemiological studies (4–8,16) have reported a higher prevalence of diabetes, insulin resistance, and cardiovascular disease in various ethnic groups including Native Americans and Canadians. Some studies (9,10) have reported increased levels of inflammatory markers and their association with insulin resistance or percent body fat in Aboriginal versus white populations. However, there are no reports that specifically address markers of insulin resistance and inflammatory markers in Aboriginal women compared with white women.

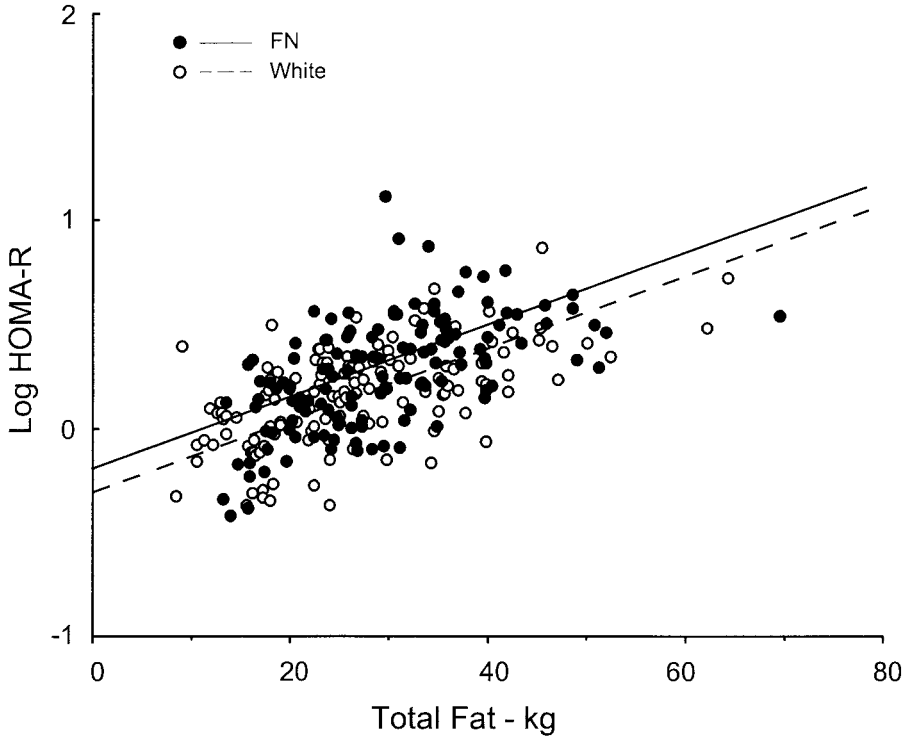

Figure 1— *The relationship between insulin resistance and total fat in First Nation (FN) and white women. ANCOVA was used to examine differences in the slope and intercept of the lines of best fit. The slopes did not differ significantly, whereas the intercepts were significantly different. The predicted regression lines based on the ANCOVA model are shown.*

Table 3—*Spearman correlation coefficients between adipose tissue and adipokine levels in nondiabetic populations with and without correction for insulin resistance*

	White women		First Nation women		All women	
	Total fat	Trunk fat	Total fat	Trunk fat	Total fat	Trunk fat
Resistin	0.162	0.146	0.203*†	0.192†‡	0.203*†	0.192†‡
Adiponectin	−0.366*†	−0.406*†	−0.423*†	−0.483*†	−0.423*†	−0.483*†
Leptin	0.781*†	0.743*†	0.782*†	0.748*†	0.782*†	0.748*†
TNF-α	0.128	0.124	0.139§	0.137§	0.139§	0.137§
IL-6	0.528*†	0.574*†	0.513*†	0.566*†	0.513*†	0.566*†
CRP	0.514*†	0.533*†	0.543*†	0.548*†	0.543*†	0.548*†
RBP4	0.088	0.112	0.022	0.013	0.022	0.013

*$P < 0.005$, ‡$P < 0.01$, §$P < 0.05$. †Significant after controlling for HOMA-IR.

First Nation women had significantly greater total fat mass than white women and a greater proportion of their adipose mass as trunk fat. Differences in trunk fat explained the difference in insulin resistance observed between First Nation and white women. While there was no significant difference in the slope of the relationship between log HOMA-IR and total body fat in the two groups of women, First Nation women had a significantly higher intercept, indicating that at any given level of total body fat, First Nation women were more significantly insulin resistant than white women.

Consistent with increased insulin resistance, First Nation women had lower adiponectinand IGFBP-1 but greater IL-6 levels than white women. However, the majority of inflammatory markers such as resistin, TNF-α, or CRP were not significantly increased in First Nation women and would not explain the difference in insulin sensitivity between the two study groups.

Although we recognize that dysglycemia and type 2 diabetes represent a continuum and that excluding subjects with type 2 diabetes might have biased our results against finding differences between the First Nation and control women, we chose to concentrate our analysis of insulin resistance on subjects who did not have self-reported diabetes or biochemical evidence of type 2 diabetes because the validity of the HOMA-IR determinations in type 2 diabetes patients is questionable (17,18).

In both First Nation and white women, as in other reports (19,20), leptin levels directly correlated with insulin resistance and body fat. However, despite significantly greater amounts of body fat in First Nation compared with white women, leptin levels were not significantly increased. This may reflect the relatively lower expression of leptin in visceral fat compared with subcutaneous fat (21) and the tendency for First Nation women to have a significantly larger proportion of their fat as trunk fat.

In this study, as previously observed (14), the adipokine that correlated best with insulin resistance was leptin. The magnitude of the correlation coefficient for HOMA-IR and leptin was comparable with HOMA-IR and IGFBP-1, a sensitive marker of insulin resistance (22), and was significantly higher than that observed for the relationship between HOMA-IR and adiponectin. While there was no difference between resistin levels in First Nation and white women, resistin levels did significantly correlate with both total fat and trunk fat in First Nation women but not in white women consistent with previous reports (14,23). In First Nation women alone, there was a weak positive correlation between HOMA-IR and resistin levels. This correlation was not seen in the white women and was lost when data were controlled for trunk fat, suggesting that this association was due to the correlation of both HOMA-IR and resistin levels with trunk fat in First Nation women. In contrast to the rodent, where resistin appears to be important in modulating hepatic insulin sensitivity (24,25), the contribution of resistin to insulin resistance in human subjects appears to be relatively minor. While a few studies have reported a weak positive association between insulin resistance and resistin levels (14,26,27), the majority of studies have not (23,28–33). Resistin levels in human subjects are thought to correlate more closely with inflammation than with insulin resistance (28,30,34).

RBP4 has recently been proposed to be an adipocytokine (35,36) and was elevated in insulin-resistant mice (36). These authors also found that plasma RBP4 levels determined by Western blot were elevated in a small sample of obese diabetic and nondiabetic subjects compared with lean subjects, but there was no difference between obese nondiabetic and obese diabetic subjects despite the latter being more insulin resistant (36). Other studies found RBP4 levels to correlate with poor metabolic control in both type 2 (37) and type 1 diabetic patients (38). These observations suggest that elevated RBP4 concentrations result from hyperglycemia, rather than insulin resistance, which is consistent with the lack of correlation between RBP4 levels and either fat mass or HOMA-IR in the present study.

TNF-α levels have been reported to be significantly elevated in insulin-resistant type 2 diabetic subjects (39,40). Although First Nation and control women had similar levels of TNF-α, a weak correlation was found between TNF-α and HOMA-IR but only in the First Nation individuals.

First Nation women had elevated IL-6 levels, but they did not have significantly higher CRP or TNF-α levels. This contrasts with previous reports where CRP levels have been found to be elevated in diabetic and insulin-resistant individuals (9,10,39,41). However, in the Diabetes Heart Study, there was no difference in CRP levels between type 2 diabetic subjects and their unaffected siblings (42), although a significant relationship was found between BMI and CRP in the same study as in the data reported here. Although First Nation women had elevated IL-6 levels, the correlation of IL-6 with HOMA-IR became insignificant after correction for trunk fat. This is consistent with the concept that truncal obesity precedes inflammation in the development of insulin resistance (43).

Significant limitations to this study include the cross-sectional design and

lack of prospectively defined clinical end points such as the development of diabetes. Although the sample sizes were adequate for the analyses undertaken, a much larger population would be required to assess cardiovascular events. Our finding may not be applicable to men since they were not the target of the First Nations Bone Health Study. We used an indirect measure of insulin resistance, HOMA-IR, since it is well suited to epidemiological studies and shows a satisfactory correlation with other quantitative measures such as the euglycemic-hyperinsulinemic glucose clamp (17). Correlations were adjusted for trunk fat and total fat, but no adjustment was made for other potentially important covariates such as physical activity. Although we excluded women with known or biochemical evidence of type 2 diabetes, this is unlikely to have excluded all women who were destined to develop diabetes in the future, particularly since women with impaired fasting glucose were included in the study.

In summary, our observations in First Nation women, a population that is at high risk for vascular disease, suggest that these women are more insulin resistant due in part to their tendency to accumulate adipose tissue in the truncal region. However, despite the increase in insulin resistance, we observed no significant increase in inflammatory cytokines, with the exception of IL-6. Although limited by cross-sectional nature, these data suggest that inflammation is unlikely to be the primary cause of the insulin resistance, as has been suggested by some investigators (44,45).

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