Plasma Adiponectin and Leptin Levels, Body Composition, and Glucose Utilization in Adult Women With Wide Ranges of Age and Obesity

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OBJECTIVE — The purpose of this study was to determine the relationships between plasma adiponectin and leptin levels, total and central obesity, and glucose utilization across the adult age span.

RESEARCH DESIGN AND METHODS — We studied 148 women aged 18–81 years with a BMI range of 17.2–45.3 kg/m². Total percent body fat was determined by dual-energy X-ray absorptiometry and abdominal fat by computed tomography. Glucose tolerance in non-type 2 diabetic volunteers was determined with an oral glucose tolerance test. Glucose utilization X-ray absorptiometry and abdominal fat by computed tomography. Glucose tolerance in non–expect that adiponectin would be lower in older individuals because of an increase in adipose tissue (3–6). Human adiponectin has 244 amino acids, and the molecular weight of the monomer is 26,413. However, it circulates in polymeric form. Adiponectin appears to be linked to glucose homeostasis since plasma adiponectin levels are lower in diabetic subjects (7,8) and are positively correlated with glucose utilization (M) (9). Adiponectin levels are lower in obese (defined by BMI) than in nonobese subjects (6,10). Furthermore, adiponectin levels increase after weight reduction (11). In addition, plasma adiponectin concentrations are negatively correlated with total body fat and waist-to-thigh ratio (9). It is unknown whether adiponectin is related to direct measures of abdominal obesity (e.g., visceral fat and subcutaneous abdominal fat), which is known to be associated with insulin resistance (12). Moreover, to our knowledge, plasma adiponectin levels as a function of age have only been examined in a Japanese population (6). Although Arita et al. (6) did not find an age effect, one might expect that adiponectin would be lower in older individuals because of an increase in obesity with aging.

We tested the hypothesis that adiponectin levels would be negatively associated with visceral fat, positively

RESULTS — Adiponectin concentrations did not differ by age-groups. There were significant age effects for BMI, percent body fat, visceral fat, subcutaneous abdominal fat, VO2max, and M. Adiponectin levels were lower in the prediabetic women (n = 18) than in the normal glucose-tolerant women (n = 108) and the women with type 2 diabetes (n = 22) (both P < 0.05). Univariate correlations revealed significant negative relationships between plasma adiponectin levels and BMI, percent body fat, visceral fat, subcutaneous abdominal fat, fasting leptin, and fasting insulin and positive relationship with M (all P < 0.05). In a multiple stepwise regression model to predict adiponectin, only M remained in the model at P < 0.001. Multivariate analyses revealed a significant relation for M as a function of adiponectin, insulin, and VO2max.

CONCLUSIONS — The data suggest that plasma adiponectin does not change with age but levels are negatively associated with percent body fat, visceral fat, subcutaneous abdominal fat, insulin, and leptin levels in women. Adiponectin is positively associated with M across the age span in women.
associated with glucose utilization, and decline with age. Additionally, we examined the relationship between plasma adiponectin and leptin levels. Thus, the purpose of this study was to determine the relationships among adiponectin levels, total and central obesity, and glucose utilization across the adult age span.

**RESEARCH DESIGN AND METHODS** — One-hundred forty-eight women (22 African Americans and 126 Caucasians) with a mean age 50.3 ± 1.25 years (range 18–81) and BMI 23.84 volunteered to participate in the study. Subjects were screened by medical history questionnaire, physical examination, and fasting blood profile, and in 137 women a graded exercise treadmill test was performed in an attempt to exclude those with cardiovascular disease. Women were either sedentary (<20 min of aerobic exercise 2 times/week) or athletes (n = 40; swimmers, runners, and triathletes), as previously described (13). Unless the patient had known diabetes and was being treated with oral hypoglycemic agents (n = 9), subjects underwent a 75-g 2-h oral glucose tolerance test (14) with blood samples drawn at baseline and at 120 min for measurement of plasma glucose levels. Volunteers were grouped by glucose tolerance status (normal glucose tolerance [NGT], prediabetic, and diabetic) and by age (young <40 years, middle 40–59 years, and old ≥60 years). None of the women with type 2 diabetes were receiving insulin or thiazolidinediones. 13 were newly diagnosed and not on any medication, and 10 were on medication, including 5 on sulfonlyureas and 5 on biguanides. All subjects were non-smokers and had no evidence of cancer, liver, renal or hematological disease, or other medical disorders with the exception of glucose intolerance. All methods and procedures for the study were approved by the Institutional Review Boards of the University of Maryland, the University of British Columbia, and the Johns Hopkins Bayview Medical Center. Each participant provided written informed consent to participate in the study.

**Body composition**

Height (cm) and weight (kg) were measured to calculate BMI as weight (kg)/height (m²). In 126 women, waist circumference was measured at the narrowest point superior to the hip, divided by the circumference of the hip, and measured at its greatest gluteal protuberance to obtain waist-to-hip ratio (WHR) (15). Fat mass, fat-free mass (FFM) (16) and bone mineral content, was determined by dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) in 145 subjects. Computed tomography scan of the abdomen (n = 126) was performed using a PQ 6000 Scanner (Marconi Medical Systems, Cleveland, OH) to determine visceral adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAT) areas as previously described (16).

**Maximal oxygen uptake**

Maximal oxygen uptake (VO_{2\text{max}}) was measured using a continuous treadmill test protocol as previously described (17). Validation for attainment of VO_{2\text{max}} included meeting two of the following three criteria: 1) a plateau in oxygen uptake with an increased workload as evidenced by a difference in oxygen uptake of <2 ml·kg⁻¹·min⁻¹, 2) a respiratory exchange ratio >1.10; and 3) a maximal heart rate within 10 bpm of the age-predicted maximal value.

**Hyperinsulinemic-euglycemic clamps**

All testing was performed in the morning after a 12-h overnight fast. All subjects were weight stabilized (<1 kg) for at least 2 weeks before metabolic testing. Peripheral tissue sensitivity to exogenous insulin was measured in 140 women using the hyperinsulinemic-euglycemic clamp technique (18) with 10-min priming and continuous infusion of insulin (240 pmol·m⁻²·min⁻¹, Humulin; Eli Lilly, Indianapolis, IN) (1). Insulin concentrations have been previously reported for these studies (13,19) and are ~480 pmol/l. In the diabetic volunteers, plasma glucose was allowed to drop to 5.3 mmol/l and clamped at that level for the duration of the study. The mean plasma glucose level for all other volunteers averaged 5.2 mmol/l (13,19). The coefficient of variance did not exceed 5.0% in any of the studies.

**Analysis of blood samples**

Blood samples were collected in heparinized syringes and placed in prechilled test tubes containing 1.5 mg EDTA/ml of blood and aprotinin (400 KIU/ml) in a total volume that was 4% of the sample volume. The blood samples were centrifuged at 4°C, and a 1-ml aliquot of plasma was rapidly frozen (80°C) for subsequent hormone analysis. All determinations were performed in duplicate. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Immunoassay insulin and leptin were determined as previously described (13,20). Human adiponectin levels were determined by a newly developed specific human adiponectin radioimmunoassay (Linco Research, St. Charles, MO). The inter- and intra-assay coefficient of variations were <10% at ED20, ED50, and ED80 concentrations of the standard range (1–200 ng/ml). Since circulating levels are in μg/ml concentrations, samples were diluted 1/500 in assay buffer before estimation.

**Statistical analyses**

The mean concentration of glucose and insulin was calculated for each sample time point for the clamp. The trapezoidal rule was used to calculate the integrated response over 30-min intervals for each subject. The integrated response was divided by its time interval to compute mean concentrations. M for the last 60-min interval of the clamp, calculated from the amount of glucose infused after correction for glucose-equivalent space (glucose space correction), was used in the analyses. Adiponectin and insulin concentrations were log transformed to achieve a more normal distribution. All data were analyzed using SAS version 8.2 (SAS, Cary, NC). The data were analyzed for the total group, as well as by glucose tolerance status (normal, prediabetic, and diabetic) and age-groups (young <40 years, middle 40–59 years, and old ≥60 years). Standard methods were used to compute means, SE, and Pearson correlation coefficients. The mean values for categorical variables were computed using ANOVA (proc generalized linear model using Bonferroni corrections). Multiple regression models were used to examine the effects of age, body fat, adiponectin, leptin, fitness, basal glucose, and insulin levels on glucose utilization. All standard tests were two tailed. Data are means ± SE, and P < 0.05 was regarded as statistically significant.

**RESULTS** — The mean value and range of the variables for the entire group are presented in Table 1. As shown, there
was a wide range of age, total body obesity, fat distribution (waist circumference 60–130.7 cm and WHR 0.68–1.03), central fat, physical fitness, glucose utilization, and adiponectin concentrations.

Table 1 also shows the physical and metabolic characteristics, as well as adiponectin concentrations, in women across the three age-groups. There were significant effects of age-groups on BMI, percent body fat, VAT, SAT, $V_{O2\text{max}}$, fasting leptin, glucose, insulin concentrations, and $M$. Nevertheless, plasma adiponectin concentrations did not differ between the age-groups (young versus middle, $P = 0.45$; young versus old, $P = 0.85$; middle versus old, $P = 0.55$).

The physical and metabolic characteristics as well as adiponectin concentrations in women grouped by glucose tolerance status are presented in Table 2. There were 108 women with NGT, 18 women were prediabetic, and 22 had diabetes. Glucose tolerance, as expected, was associated with many of the variables. Differences in adiponectin levels were observed as a function of glucose tolerance status. Adiponectin levels were lower in the prediabetic than those with NGT but were not different in women with diabetes. Comparisons of adiponectin (ANOVA) between NGT and prediabetic women and between prediabetic and diabetic women were significant at the 0.05 level. Even if prediabetic and diabetic subjects are matched by BMI and age, the differences between groups remain significant for adiponectin ($P = 0.03$). The relationships between age, body composition, fitness, and glucose metabolism variables and plasma adiponectin concentrations in women are shown in Table 3. Age per se was not correlated with plasma adiponectin concentrations. However, BMI, percent body fat, waist circumference, WHR, VAT (Fig. 1A), SAT (Fig. 1B), and plasma leptin were negatively correlated with adiponectin. In a univariate analyses, $V_{O2\text{max}}$ was also significantly associated with adiponectin levels. We then tested if the relationship between adiponectin and $V_{O2\text{max}}$ was the same across the age-groups. A positive relationship was found in the middle and old age-groups, while an inverse relationship was found in the young group, which was significantly different from the two older groups. The two older groups were not different from each other. Therefore, we could not adjust for $V_{O2\text{max}}$ across the age span. Finally, plasma glucose concentrations were not associated with adiponectin levels, but in-

### Table 1—Physical and metabolic characteristics of the women

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Normal subjects (n)</th>
<th>Prediabetic subjects (n)</th>
<th>Diabetic subjects (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (N = 148)</td>
<td>Young, &lt;40 years</td>
<td>Middle, 40–59 years</td>
<td>Old, ≥60 years</td>
</tr>
<tr>
<td>29</td>
<td>58</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Prediabetic subjects (n)</td>
<td>0</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Nondiabetic subjects (n)</td>
<td>1</td>
<td>9</td>
<td>12</td>
</tr>
</tbody>
</table>

Data are means ± SE (range) or means ± SE (n). *P < 0.05, young vs. middle; †P < 0.05, old vs. middle; ‡P < 0.05, old vs. young.
Adiponectin, age, and insulin resistance

Table 3—The relationships between plasma adiponectin concentrations and body composition and glucose metabolism variables in women

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>–0.02</td>
</tr>
<tr>
<td>Weight</td>
<td>–0.30*</td>
</tr>
<tr>
<td>BMI</td>
<td>–0.32*</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>–0.26*</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>–0.28*</td>
</tr>
<tr>
<td>WHR</td>
<td>–0.30*</td>
</tr>
<tr>
<td>VAT</td>
<td>–0.28*</td>
</tr>
<tr>
<td>SAT</td>
<td>–0.27*</td>
</tr>
<tr>
<td>VO_{2max}</td>
<td>0.25*</td>
</tr>
<tr>
<td>Fasting plasma leptin</td>
<td>–0.28*</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.04</td>
</tr>
<tr>
<td>Fasting plasma insulin</td>
<td>–0.24*</td>
</tr>
<tr>
<td>M</td>
<td>0.28*</td>
</tr>
</tbody>
</table>

*P < 0.005.

sulin and M did correlate with plasma adiponectin (Fig. 1C).

In a multiple stepwise regression model to predict adiponectin, we put in the variables age, M, percent body fat, VAT, SAT, insulin, glucose, leptin, and VO_{2max}. Only M remained in the model at P < 0.001. To examine the contribution of the variables on M, a backward elimination multiple regression analysis was performed with the following dependent variables: age, percent body fat, VAT, SAT, adiponectin, insulin, leptin, and VO_{2max}. In the final model, percent fat, VAT, SAT, adiponectin, insulin, glucose, and VO_{2max} all contributed significantly at P < 0.05. However, examination of the parameter coefficients clearly indicated that there was collinearity between percent fat, VAT, and SAT. Therefore, we ran three separate models when only one of the estimates of fatness (percent fat, VAT, or SAT) was entered into the model. VAT and percent fat did not contribute significantly to M in their final two corresponding models, whereas adiponectin, insulin, and VO_{2max} all contributed significantly at P < 0.05. In the model with SAT, SAT replaced VO_{2max} so that adiponectin, insulin, and SAT contributed significantly to M.

CONCLUSIONS—The results of our study indicate that age does not influence plasma adiponectin levels. Glucose tolerance status was associated with adiponectin levels, with prediabetic volunteers having lower concentrations than normal or diabetic volunteers. Visceral fat, subcutaneous abdominal fat, and fasting leptin were negatively associated with adiponectin, and M was positively associated with adiponectin in women across the age span. However, multivariate analysis revealed that only M is a significant independent predictor of adiponectin.

Although we hypothesized that adiponectin would decline with age because of the increase in obesity with age, our data failed to support this hypothesis. Only one other study (6) has reported that plasma adiponectin concentrations did not correlate with age when adjusted

Figure 1—A: Relationship of VAT area with plasma adiponectin concentrations in women (r = –0.28, P = 0.002). B: Relationship of SAT area with plasma adiponectin concentrations in women (r = –0.27, P = 0.001). C: Relation of M with plasma adiponectin concentrations in women (r = 0.28, P < 0.001).
for BMI. When we divided our women into three age-groups, it appeared that the youngest age-group (<40 years) had the lowest adiponectin levels and women in the 40–59 age-group had the highest, but the differences in adiponectin concentrations across age were not statistically significant. The younger women were significantly leaner, had less abdominal fat, were more insulin sensitive, and had a higher VO2max. Thus, we expected to have to correct for differences in adiposity to determine whether adiponectin declined with age, but this was not necessary. We also cannot explain the differential effect of age in the relation between VO2max and adiponectin among the age-groups, and further studies are required.

Several studies have examined the relationship between plasma adiponectin concentrations and adiposity by BMI (6,7,9) and percent body fat (9). We confirm the established relation between plasma adiponectin levels and BMI and percent body fat with a range of body fat (10–57%) larger than that previously reported. To our knowledge, the relationship between plasma adiponectin concentrations and direct measures of central obesity was not previously known. As we hypothesized, we found that women with lower amounts of VAT and SAT had higher adiponectin concentrations in a cohort of women with a large variability in abdominal fat. Plasma adiponectin levels were also negatively correlated with measures of abdominal girth (waist circumference) and fat distribution (WHR). Thus, adiponectin concentrations are associated with total and central adiposity.

Although plasma adiponectin concentrations have been shown to be lower in diabetic than nondiabetic subjects (7–9), our results are inconsistent with these reports. However, in our study, prediabetic women have adiponectin levels two-fold lower than those in normal or diabetic women. Differences in adiponectin concentrations between individuals with NGT and those patients with diabetes disappear after adjustment for M and/or fasting plasma insulin concentrations, which suggested that plasma adiponectin concentrations are more closely related to insulin sensitivity and fasting insulinemia than glycosylation (9). Plasma adiponectin levels are inversely correlated with fasting glucose (7) and insulin (7,9) and positively correlated with glucose utilization (9). We have previously reported (20) the association between plasma leptin levels, hyperglycemia, hyperinsulinemia, and body composition. In the current study, plasma adiponectin levels are negatively correlated with plasma leptin levels, which is similar to that reported in Japanese women (10). We also found an inverse association between plasma adiponectin concentration and fasting insulin levels and a direct association between adiponectin and glucose utilization. Because the women in our study included trained athletes across the age span who were very insulin sensitive (13) and also included sedentary women with and without diabetes, the highest and lowest values of insulin-stimulated glucose disposal were ~50-fold different. Thus, across a wide range of insulin sensitivity, higher adiponectin levels are associated with greater insulin-stimulated glucose disposal. In our multiple regression analyses, adiponectin was an independent predictor of M.

Adiponectin levels have a reported genetic heritability of 46% (21). Moreover, several quantitative trait loci have been identified that have significant evidence of linkage for obesity-related phenotypes with serum adiponectin levels (21). Genetic polymorphisms in the adiponectin gene have been identified (22,23) and shown to be associated with obesity and insulin resistance (24). Yet, the mechanism by which adiponectin influences insulin sensitivity in humans is unclear. There are several disease states in which a decrease in adiponectin is associated with insulin resistance (e.g., lipodystrophy) (8). Adiponectin treatment reverses this in animal models (25). It would be interesting to see if adiponectin treatment reverses insulin resistance in humans.

We conclude from our data that plasma adiponectin levels do not decline with age, are negatively associated with visceral and subcutaneous abdominal fat and plasma leptin, and are positively associated with glucose utilization across the adult age span. Additional studies are needed to determine whether changes in adiponectin concentrations with treatments or interventions are associated with improvements in insulin sensitivity in humans and the mechanisms whereby this occurs.

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