Novel Associations Between Bioavailable Estradiol and Adipokines in Elderly Women With Different Phenotypes of Obesity

Implications for Atherogenesis

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Background—Peripheral adiposity confers protection against diabetes and atherosclerosis in elderly women. The underlying mechanisms, however, remain to be elucidated.

Methods and Results—On the basis on dual-energy X-ray absorptiometry measurements of central fat mass (CFM) and peripheral fat mass (PFM), we identified 290 elderly women with distinct forms of body fat distribution (lean, peripheral obesity, central obesity, or general obesity). Study parameters were plasma tumor necrosis factor-α, interleukin (IL)-6, adiponectin, estradiol, sex hormone–binding globulin, insulin resistance, and aortic calcification, graded on lateral radiography. In peripherally and generally obese women, plasma estradiol and insulin resistance were significantly lower, whereas sex hormone–binding globulin and adiponectin were significantly higher compared with centrally obese women independent of age, body mass index, total fat mass, and smoking habits (all \( P < 0.05 \)). After adjustment for these confounders, IL-6 in centrally obese women was comparable with that seen in generally obese (similar high CFM%) but significantly higher than in peripherally obese women and lean women (low CFM%). Atherosclerosis was less severe in generally obese (2.5±0.3) compared with centrally obese women (5.0±0.7, \( P = 0.001 \)). In multiple regression analysis, total fat mass, body fat distribution, insulin resistance, estradiol, current smoking, treated hyperlipidemia, and treated hypertension contributed independently to the variation of aortic calcification (\( R = 0.55 \), SEE = 3.60, \( P < 0.001 \)).

Conclusions—Abundant presence of PFM in generally obese women is associated with increased plasma adiponectin and higher insulin sensitivity, which could explain the apparent protection against the atherogenic effects of IL-6 derived from CFM. Low peripheral exposure to estradiol appears to be a sine qua non of maintained adiponectin secretion from PFM. (Circulation. 2004;110:2246-2252.)

Key Words obesity ■ insulin ■ atherosclerosis ■ hormones ■ aging

The insulin resistance (IR) syndrome is an established risk factor for macrovascular disease and type 2 diabetes, two of the most significant health problems among the elderly (>65 years).\(^1,2\) In the past decade, there has been a marked increase in the prevalence of obesity in women, which draws attention to the need for better understanding the implications for cardiovascular health.\(^3\) In elderly women, atherogenic effects of obesity is mainly a function of body fat distribution rather than body mass index (BMI) per se. The abundant presence of peripheral fat mass (PFM) appears to counteract the diabetogenic/atherogenic trends of central fat mass (CFM) through mechanisms involving insulin sensitization.\(^4-6\) Mediators of this protective association, however, remain poorly understood.

Adipose tissue secretes a variety of bioactive substances, which can modulate IR and atherogenesis. Insulin resistance and accelerated atherogenesis in centrally obese women are associated with increased plasma levels of proinflammatory cytokines (interleukin [IL]-6 and tumor necrosis factor [TNF]-α) and decreased plasma levels of the antiinflammatory adipokine adiponectin.\(^7\) However, mechanisms by which PFM counteracts the unfavorable balance of proinflammatory and antiinflammatory adipokines have not been systematically investigated. Given the established insulin-sensitizing, antiinflammatory, and antiatherogenic effects of adiponectin in animals,\(^8\) a constitutive contribution to the circulating adiponectin pool might explain the protective effects of PFM.

Observations on pregnant animals and women have pointed out that increased/long exposure of body fat mass to estradiol may promote IR, involving inhibition of adiponectin.\(^9-11\) Women with low sex hormone–binding globulin (SHBG) are at particularly increased risk for gesta-
tional diabetes,12 which also points to the role of increased peripheral exposure to sex steroids. After menopause, adipocytes are primary sources of endogenous estrogens in women. Women with upper-body obesity have lower plasma SHBG and higher estradiol compared with women with lower-body obesity,13 the latter finding suggesting regional differences in the enzymatic conversion of steroid hormones in visceral versus subcutaneous adipocytes.14–16 Potential influence of body fat distribution on estrogen metabolism and depot-specific secretion of adipokines with direct consequences for IR and atherogenesis has received moderate attention.

Therefore, the aim of the study was to analyze (1) the independent associations between fat distribution and circulating levels of fat-derived hormones/adipokines and (2) whether phenotype-specific patterns of these hormones can explain the association of body fat distribution with IR and aortic calcification (AC) in elderly women.

Methods
Participants were 290 healthy women, 60 to 85 years of age, who were selected from a large cohort to obtain groups with distinct body fat distribution. Methods of selection have been described and illustrated earlier in detail.4 Briefly, CFM (sum of subcutaneous and visceral fat mass of the trunk) and PFM (fat mass of legs and arms) of all 1356 subjects were measured by dual-energy X-ray absorptiometry (DEXA) (Hologic QDR4500). Fat depots were expressed as percentage of total body soft tissue mass and were scored on a scale from 1 to 4 (1, <25th; 2, 25 to 50th; 3, 50 to 75th; and 4, >75th percentile). Scores could be combined in 4×4 ways, of which 1 to 1 indicated lean women; 1 to 4, women with peripheral obesity; 4 to 1, women with central obesity; and 4 to 4, women with general obesity (Figure 1 in Reference 4). Twelve women with ongoing hormone replacement therapy were excluded from further analyses. All participants signed an approved consent form, and the study was conducted in accordance with the Helsinki Declaration II. The local ethics committee approved the study protocol.

Traditional cardiovascular factors collected were BMI, blood pressure, level of education, smoking habits, daily coffee consumption, regular alcohol consumption, weekly fitness activities, presence of treated diabetes mellitus, treated hypertension, and treated hyperlipidemia. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA<sub>IR</sub>) index (fasting insulin [µU/mL] × fasting glucose [mmol/L])/22.5). Calcified deposits in the lumbar aorta were visualized on lateral radiographs and graded from 0 to 24, as described earlier in detail.4 All plasma analyses were performed in samples from fasting subjects. Adiponectin was measured with high-sensitive radioimmunoassay (Linco), whereas IL-6 and TNF-α were measured with ELISA (R&D). Total plasma 17β-estradiol and SHBG were measured by the Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics), using the recently introduced Elecsys-Estradiol II (detection limit, 18.4 pmol/L; intra-assay CV, 3.6%) and Elecsys-SHBG assays (detection limit, 0.35 nmol/L; intra-assay CV, 2.6%). Measured total estradiol divided by SHBG (free estradiol index, FEI) estimated the bioavailable pool.

Statistical Analysis
Results shown are mean±SEM unless otherwise indicated. Statistical analysis was performed with the use of SPSS software, version 11.01. Characteristics of the four phenotypes were compared by means of ANOVA or the Kruskal-Wallis test. General linear models compared the means of fat-derived hormones/adipokines, SHBG, HOMA<sub>IR</sub>, and AC after adjustment for confounders (age, BMI, total fat mass [TFM], and smoking). A forced-entry model of multiple regression analysis, including a dummy variable adjusting for the four groups of body fat distribution, identified independent contributors to the variation of AC. Associations and differences were statistically different at a level of P<0.05.

Results
Cardiovascular Profile
Characteristics of the four groups of women with different phenotypes of obesity are summarized in Table 1. There were statistically significant differences between the four groups in terms of body size (BMI) and overall obesity (TFM), which were inversely associated with the frequency of current smoking and the overall duration of smoking.

Body Fat Distribution
Lean women (low CFM%) and centrally obese women (high CFM%) had comparable low PFM%, whereas peripherally obese women (low CFM%) and generally obese (high CFM%) women had comparable high PFM% (Figure 1).

Independent Impact of Body Fat Distribution
Means of HOMA<sub>IR</sub> and AC in women with different forms of body fat distribution before and after adjustment for potential confounders (age, smoking, BMI, and TFM) are shown in Figure 2. Under both conditions, peripherally and generally obese women showed significantly lower IR compared with centrally obese women. When comparing AC, the significantly lower AC scores of generally obese women compared with lean women and centrally obese women were no longer present after adjustment for overall obesity. Instead, centrally and generally obese women showed significantly higher AC scores compared with women with peripheral obesity and lean women.

Figure 3 indicates the means of the different adipokines/cytokines before and after adjustment for the same confounders. Under both conditions, the comparison of CFM%-matched groups revealed significantly higher plasma adiponectin in women with high compared with those with low PFM%. The highest adiponectin concentrations were apparent in peripherally obese women, whereas the lowest concentrations were observed in centrally obese women. IL-6 in centrally and generally obese women was comparable, especially after adjustment for overall obesity, whereas lean women and peripherally obese women displayed significantly lower plasma IL-6. Plasma TNF-α did not show significant differences between the four groups.

Figure 4 indicates means of estradiol, SHBG, and bioavailable estradiol before and after adjustment for age, smoking, BMI, and TFM. Lean and peripherally obese women were not different in terms of plasma estradiol despite significant differences in PFM%. In contrast, the higher CFM% in centrally obese women compared with lean women was accompanied with significantly increased estradiol. Interestingly, when adjusting for TFM, general obese women had significantly lower estradiol compared with centrally obese women despite comparable CFM%. Plasma SHBG was the lowest in centrally obese women, whereas peripherally and generally obese women had comparable high levels of the transport protein. Accordingly, peripherally and generally obese women had the lowest bioavailable estradiol followed by lean women and centrally obese women.

Independent Contributors to IR and AC
In multiple regression analysis including the HOMA<sub>IR</sub> index as dependent variable and independent variables outlined in
Table 2, only adiponectin emerged as an independent contributor (β = −0.243, *P* = 0.001) to the variation of the HOMA IR index (*R* = 0.48, SEM = 2.47, *P* < 0.001). When changing the dependent variable to AC, the independent direct correlates of AC were body fat distribution, insulin resistance, current smoking, estradiol, treated hyperlipidemia, and treated hypertension, whereas total body fat mass was an independent inverse correlate of AC (Table 2).

**Insulin Resistance and Atherosclerosis Adjusted for the Proposed Mediators**

Means of IR and AC obtained after adjustment for confounders and the hypothesized mediators (bioavailable estradiol, IL-6, and adiponectin) of the described associations are shown in Figure 5. After these adjustments, no significant differences between the four groups were apparent.

**Discussion**

Comparison of women with distinct forms of body fat distribution provided a unique setting for investigating associations of body fat distribution with fat-derived hormones, IR, and AC. The striking findings were the better insulin sensitivity and less severe atherosclerosis of generally obese women compared with centrally obese women despite more pronounced overall and comparable CFM% in the former group. The question to be answered was whether constitutive contribution of subcutaneous adipocytes to circulating adiponectin—which has insulin-sensitizing, anti-inflammatory, and antiatherogenic effects⁹—could underlie the protective associations of PFM.

Centrally obese women had lower adiponectin compared with lean women, illustrating the adverse impact of visceral adiposity on circulating adiponectin. However, generally obese women compared with centrally obese women had higher plasma adiponectin, which appears to be in line with the constitutive contribution of PFM. In further support, subcutaneous adipocytes (both abdominal and gluteal) of nondiabetic obese subjects were shown to have maintained high adiponectin expression/secretion compared with the low expression/secretion of visceral adipocytes.¹⁷,¹⁸ In diabetic subjects, adiponectin expression/secretion of subcutaneous adipocytes is also low,¹⁸ suggesting that hormonal milieu
characteristics for insulin-resistant/centrally obese subjects may inhibit adiponectin in both fat depots.

Hormonal changes characteristic for centrally obese women versus generally and peripherally obese women included markedly decreased SHBG and increased estradiol levels. The comparable high SHBG of generally and peripherally obese women suggests that signals from PFM stimulate hepatic SHBG release, a notion recently raised by Ducluzeau et al.\textsuperscript{19} However, high PFM\% is also prone to lower plasma estradiol, which could be explained, at least in part, by a contrasting impact of obesity on the 17β-hydroxysteroid dehydrogenase (17β-HSD)/aromatase ratio in visceral (increased ratio) and peripheral adipocytes (decreased ratio).\textsuperscript{16} High ratio promotes estradiol formation, whereas low ratio facilitates peripheral aromatization and formation of estrone, a 12-fold weaker form of estrogen.\textsuperscript{13} Thus, even though estradiol formation is expected to be increased in the visceral compartment of both centrally and generally obese women, the higher PFM\% in the latter group appears to decrease the circulating pool either by converting or metabolizing estradiol.

Results of the study suggest that estradiol production and bioavailability have important implications for circulating adiponectin and whole-body IR. In support of this, (1) high plasma levels of bioavailable estradiol predict IR independent of the degree of overall and central adiposity,\textsuperscript{20} (2) adiponectin is independently and inversely associated with plasma estradiol (but not with bioavailable testosterone),\textsuperscript{21} (3) increasing plasma estradiol in the course of pregnancy is associated with decreasing adiponectin and increasing IR, particularly in women with low SHBG, and these alterations ameliorate after delivery,\textsuperscript{9}–\textsuperscript{12,22} and (4) in mice, ovariectomy increases plasma adiponectin, which can be reversed by estradiol replacement.\textsuperscript{9} These observations provide strong
support for the role of endogenous estradiol in the modulation of adiponectin secretion and IR. Insulin, especially when administered with insulin sensiti-
zers, is a potent stimulator of the adiponectin gene. Similar to the reported differences in adiponectin expression and estradiol formation, insulin responsiveness also appears to be different between subcutaneous and visceral adipocytes of nondiabetic obese subjects; the latter showing decreased responsiveness attributable to reduced insulin receptor auto-

phosphorylation and reduced expression and tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1). Prolonged exposure of adipocytes to increased plasma estradiol, for example, during pregnancy or in women with central obesity, appears to be prone to decreased IRS-1

TABLE 2. Independent Contributors to Variation of Aortic Calcification

<table>
<thead>
<tr>
<th>Factor</th>
<th>Standardized β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat mass, kg</td>
<td>-0.529</td>
<td>0.006</td>
</tr>
<tr>
<td>Body fat distribution*</td>
<td>0.310</td>
<td>0.042</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>0.221</td>
<td>0.001</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.191</td>
<td>0.005</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.135</td>
<td>0.039</td>
</tr>
<tr>
<td>Treated hypertension</td>
<td>0.122</td>
<td>0.055</td>
</tr>
<tr>
<td>Treated hyperlipidemia</td>
<td>0.122</td>
<td>0.055</td>
</tr>
</tbody>
</table>

R² = 0.55, SEE = 3.60, P < 0.001. Other variables in the model were age, years since menopause, BMI, level of education, regular alcohol and coffee consumption, former smoking, weekly fitness activity, treated diabetes mellitus, systolic and diastolic pressure, SBH6, and adipokines/cytokines (adiponectin, IL-6, TNF-α).

*Groups of body fat distribution are represented in the model as a dummy variable.

Figure 4. Association of body fat distribution with estradiol, SHBG, and bioavailable estradiol. Black and white bars indicate mean ± SEM obtained before and after adjustment for age, BMI, total fat mass, and smoking, respectively. *P < 0.05; a, versus lean women; b, versus peripherally obese women; c, versus centrally obese women; d, versus generally obese women.

Figure 5. Insulin resistance and AC in women with different forms of body fat distribution after adjustment for age, smoking, BMI (confounders), bioavailable estradiol, IL-6, and adiponectin (mediators).
expression. Indeed, experimental studies confirm that the impact of estradiol on IRS-1 expression in adipocytes is dose and time dependent. 

Low IRS-1 expression of subcutaneous adipocytes has been recently proposed as a marker of IR, low adiponectin expression/secretion, and accelerated atherogenesis. 

Because centrally obese women in the present study had similarly high IR, low adiponectin, and signs of accelerated atherogenesis, it is tempting to postulate that the decreased expression of IRS-1 in subcutaneous adipocytes could reflect increased cellular exposure to estradiol. The apparent independent contribution of estradiol to the variation of AC found by the present study also points in this direction.

Generally and centrally obese women revealed comparably high levels of IL-6, indicating that contribution of fat mass to circulating IL-6 is mainly from the central compartment, with minor additional contribution from the peripheral compartment. 

The contribution of adipocytes to circulating TNF-α is minimal, yet this adipokine is known to be a potent autocrine regulator of IL-6 secretion in adipocytes. In centrally obese subjects with IR, expression of both proinflammatory adipokines is increased, which might also reflect the increased exposure of adipocytes to estradiol. The high interstitial concentration of IL-6 and its ability to decrease the expression of IRS-1 and the adiponectin gene suggest that this adipokine might be a mediator of the apparent adverse effects of estradiol on adipocytes. 

Ultimate answers, however, demand in situ studies measuring the reactive changes in the cellular expression and interstitial concentration of adipokines in visceral fat in response to pharmacological inhibitors of estradiol formation.

Although plasma IL-6 was comparable in generally and centrally obese women, the severity of AC was markedly different, pointing to the importance of other factors. The significantly higher bioavailable estradiol and lower adiponectin of centrally compared with generally obese women nurtures the hypothesis that prolonged and increased exposure of subcutaneous adipocytes to estradiol may eliminate the protective contribution of PFM to circulating adiponectin, under which conditions the dominant presence of IL-6 may promote accelerated atherogenesis. The atherogenic effects of IL-6 are conferred mainly through stimulation of the release of C-reactive protein from hepatic and vascular cells and induction of IR in the liver and the skeletal muscle. The recent observation that these latter organs are primary sites of adiponectin receptors provides hints to why atherogenesis in postmenopausal women is a critical function of the relative presence of proinflammatory and antiinflammatory adipokines. Elimination of the differences in HOMA IR and AC between the four groups by adjustment for bioavailable estradiol, IL-6, and adiponectin further nurtures the notion that these factors represent key mechanisms of IR and atherogenesis in elderly women.

Some methodological limitations deserve mentioning. DEXA is not able to provide direct measures of visceral fat mass, but comparative studies have demonstrated that it provides a good alternative to computer tomography for predicting visceral fat in elderly women. Nevertheless, the present study cannot give definite answers to the contribution of the subcutaneous and the visceral compartment in the trunk region. Although the few available human studies comparing the expression and secretion of adipokines (IL-6 and adiponectin) in adipocytes from well-defined compartments have provided useful hints, much remains to be elucidated pertaining to the regional differences in the function of adipose tissue in elderly women.

In summary, the novel associations of the present study provide reasons to believe that endogenous estradiol metabolism, which appears to be a function of body fat distribution, has important implications for adipokine secretion from adipocytes with direct implications for whole-body IR, inflammation, and atherogenesis in postmenopausal women. Because this study focused on the putative role of endogenous estradiol, future research should clarify whether central obesity has any implication for increased susceptibility to the adverse cardiovascular effects of hormone replacement therapy in diabetic patients early after initiation of the therapy (<1 year).

Disclosure

Dr Christiansen is CEO of the Center for Clinical and Basic Research, an organization that performs clinical phase II and III and postmarketing trials for the pharmaceutical industry and that has worked with many sponsors that have been involved in the area of osteoporosis and postmenopausal complaints. He is a major shareholder in this company and a member of the board.

References


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