Relationship between Blood Adipocytokines and Resting Energy Expenditure in Young and Elderly Women

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Summary It has been demonstrated in a previous study that resting energy expenditure (REE) is associated with adiponectin levels in the blood. However, body composition was not taken into consideration in that study. The purpose of the present study was to again investigate the relationship between blood adipocytokines and REE, adjusted by body composition, in both young and elderly women. REE and blood adipocytokines were measured in 115 young (age: 22.3±2.1 y, BMI: 21.3±1.9 kg/m²) and 71 elderly (63.4±6.5 y, 22.9±2.3 kg/m²) women. Dual energy X-ray absorptiometry was used to measure percent body fat. Fat mass and fat free mass (FFM) were calculated. REE (kcal/d and kcal/kg BW/d) was lower in elderly women than in young women, but no significant difference was observed in REE, expressed as kcal/kg FFM/d, between the two groups. Although elderly women had a higher percent body fat and higher serum leptin concentrations than young women, plasma adiponectin concentrations did not differ between young and elderly women. In elderly women, REE (kcal/d) was significantly and inversely correlated with plasma adiponectin concentration (r=-0.386, p<0.001), but REE expressed per kilogram of BW or FFM was not significantly correlated. Furthermore, no significant correlation was observed between REE (kcal/d) and concentrations of plasma adiponectin or serum leptin, after adjusting for potential confounders such as body composition and hormones, in either age group. These results suggest that adipocytokines do not influence REE in adult women.

Key Words resting energy expenditure, adiponectin, leptin, age, female adults

Resting energy expenditure (REE) accounts for 60 to 80% of total daily energy expenditure and is the basis for estimating energy requirements. In the field of energy metabolism, early investigators showed intense interest in establishing the factors contributing to REE (1–3).

Recent progress has shown that adipocytokines, which are bioactive substances secreted from adipocytes (4, 5), play an important role in regulation of basal metabolic rate (6). In particular, much attention is paid to adiponectin, which is suggested to play a role in improving insulin resistance and protecting against arteriosclerosis (7–9). In addition, rodent studies suggest that energy expenditure is regulated by adiponectin (6, 10–13). Further, Ruige et al. (14) recently demonstrated that a low resting metabolic rate (RMR: kcal/d) is strongly and inversely associated with high adiponectin levels in overweight or obese humans. Together these studies suggest that there is a relationship between adiponectin and REE.

It is well known that body mass, especially fat free mass (FFM), is a useful parameter for estimating REE (15–17). Fat mass (FM) is also an important predictor of REE in elderly people even though it is low-metabolic-rate tissue (17–21). However, in a previous study conducted to investigate the relationship between adiponectin and REE, body composition (FFM and FM) was not taken into consideration (14). It is therefore necessary to assess the effect of adiponectin on REE after adjusting for the confounding influence of body composition.

Therefore, we re-evaluated the relationship between blood adipocytokines and REE adjusted by body compo-
position in young and elderly women. We did not find any significant relationship between plasma adiponectin concentration and REE adjusted by body composition in either age group.

**MATERIALS AND METHODS**

**Subjects.** One hundred and fifteen young women (22.3±2.1 y) and 71 elderly women (63.4±6.5 y), who were at least 3 y (13.5±7.5 y) post-menopause, were recruited for the study. Women who had BMI (kg/m²) which fell outside the range BMI<18.5, BMI>30 were excluded. There were 4 young and 17 elderly subjects who were overweight (25≤BMI<30). Included participants were not using any medications, including estrogen-replacement drugs. All subjects were informed of the purpose and possible risks of the study and then provided written informed consent, as approved by the Ethical Committee at the National Institute of Health and Nutrition in Japan.

**Study protocol and indirect measurement of REE.** Participants came to the National Institute of Health and Nutrition in the early morning. They were asked to minimize any waking prior to their laboratory visit for the REE measurement. REE was measured directly by open-circuit indirect calorimetry. Measurements were performed between 0700 and 0900 h in a room at constant temperature (23–25 °C). After entering the laboratory, subjects rested in the supine position for at least 30 min, and wore a Hans-Rudolph full face mask (Hans Rudolph Inc., Kansas City, MO, USA). Two samples of expired air were collected in Douglas bags over each of two 10 min periods, and the mean of the two values was used for analysis. For young subjects, all measurements were made during the follicular phase of the menstrual cycle.

An oxygen and carbon dioxide analyzer (Arco-1000A, Arco System, Japan) was used to analyze the rate of oxygen consumption and carbon dioxide production. The volume of expired air was determined using a dry gas volume meter (DC-5, Shinagawa, Japan) and converted to standard temperature, pressure and dry gas (STPD). Gas exchange results were converted to REE (kcal/d) using Weir’s equation (22).

**Body composition analysis.** Body weight (BW) was measured to the nearest 0.1 kg using an electronic scale (Inner Scan BC-600, TANITA Co., Japan), and height (Ht) was measured to the nearest 0.1 cm using a stadiometer (YL-65, YAGAMI Inc., Japan). Body mass index (BMI) was calculated by dividing BW in kilograms by the Ht in meters squared (kg/m²). The percentage of whole body fat (% body fat) was measured using dual energy X-ray absorptiometry (Hologic QDR-4500 DXA Scanner, Hologic Inc., Waltham, MA, USA). Manufacturer’s software version 11.2 for Windows was used to analyze the % body fat. FFM and FM were calculated by BW and % body fat.

**Blood samples.** Venous blood samples (fasting for at least 12 h) were collected for measurements of serum glucose, glycosylated hemoglobin (HbA₁c), total cholesterol, HDL-cholesterol, triglycerides, estradiol (E₂), total triiodothyronine (T₃), leptin, and plasma adiponectin. Serum and plasma samples were stored at −80°C for subsequent analysis. All blood parameters were analyzed by SRL, Inc. (Tokyo, Japan) and Mitsubishi Chemical Medience Corporation (Tokyo, Japan).

**Statistical analysis.** The data were presented as mean±standard deviation (SD). Statistical analyses were carried out with the Sigma Stat 2.03 (Systat Software Inc., California, USA). Statistical analysis was performed using the Student’s t-test for parametric variables and the Mann-Whitney rank sum test for non-parametric variables to determine differences between young and elderly women. To determine the associations between REE and adipocytokines, partial correlation coefficients were used after adjusting for the potential confounding influence of FM (kg), FFM (kg), E₂ (pg/ml), and T₃ (ng/dL). For all the statistical analyses, the level of significance was defined as a p value of less than 0.05.

**RESULTS**

Table 1 presents comparisons of characteristics, whole body composition, and blood biochemical profiles. Ht was significantly lower in the elderly women than in young women. However, no significant difference in BW was noted between the two groups. The elderly women had significantly higher levels of % body fat and FM, and lower levels of FFM than the young group. REE (kcal/d and kcal/kg BW/d) in the elderly group was significantly lower than in the young group (Table 2). When REE is expressed in terms of kcal/kg FFM/d, however, no significant difference in REE was found between the two groups. No significant difference in plasma adiponectin concentration was noted between the two groups, whereas serum leptin concentration was significantly higher in elderly women than in young women.

There was no significant relationship observed between REE (kcal/d, kcal/kg BW/d and kcal/kg FFM/d) and plasma adiponectin concentration in young women (Fig. 1, a-1–a-3). In contrast, in elderly women, REE (kcal/d) was significantly and inversely correlated with plasma adiponectin concentration (r = −0.386, p<0.001, Fig. 1, b-1), but REE expressed per kilogram BW and FFM were not significantly correlated (Fig. 1, b-2, b-3). Significant and inverse relationships between REE adjusted by BW and concentrations of serum leptin were observed in the two groups (young: r = −0.318, elderly: r = −0.426, p<0.001, respectively, Fig. 2, a-2, b-2). When REE was expressed relative to FFM, however, no significant relationships were obtained for either group (Fig. 2, a-3, b-3).

A partial correlation coefficient calculated after adjusting for the confounding influence of FM (kg), FFM (kg), E₂ (pg/mL), and T₃ (ng/dL) did not demonstrate any significant relationship between REE and concentrations of plasma adiponectin and serum leptin (Table 3).
DISCUSSION

The present study demonstrates that plasma adiponectin and serum leptin concentrations are not associated with REE in either young or elderly women when confounding factors, such as FM and FFM, are taken into account. Elderly women recruited in this study were at least 3 y post-menopause. Although no significant difference in BW was noted between the young and elderly women, the elderly women had significantly higher levels of % body fat and FM, and lower levels of FFM than women, the elderly women had significantly higher levels of % body fat and FM, and lower levels of FFM than young group (Table 1). These results are consistent with previous studies, the results of which indicated that adipose tissue mass is controlled by steroid hormones and that menopause is also associated with REE in terms of kcal/kg FFM/d in all subjects (young: r=0.493, elderly: r=0.385, all subjects: r=0.456, p<0.001, respectively). In the young group but not the elderly group, serum E2 concentration significantly correlated with REE (kcal/kg FFM/d), which was measured during the follicular phase of the menstrual cycle (r= 0.222, p<0.05). These results suggest that concentrations of serum T3 and E2 may have a role in the regulation of REE in adult women.

Previous animal studies demonstrated that adiponec-

Table 1. Physical and biochemical characteristics in young and elderly women.

<table>
<thead>
<tr>
<th></th>
<th>Young (n=115)</th>
<th></th>
<th>Elderly (n=71)</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>22.3±2.1</td>
<td>(19.1–29.5)</td>
<td>63.4±6.5†</td>
<td>(50.2–77.0)</td>
</tr>
<tr>
<td>Ht (cm)</td>
<td>161.3±6.7</td>
<td>(142.2–181.0)</td>
<td>133.8±5.2*</td>
<td>(141.3–164.7)</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>55.4±6.5</td>
<td>(41.7–73.7)</td>
<td>54.2±6.0</td>
<td>(41.4–72.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3±1.9</td>
<td>(18.5–26.6)</td>
<td>22.9±2.3*</td>
<td>(19.4–28.9)</td>
</tr>
<tr>
<td>% body fat</td>
<td>24.0±4.4</td>
<td>(14.3–35.7)</td>
<td>30.2±4.8*</td>
<td>(18.6–38.9)</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>13.3±3.0</td>
<td>(7.5–24.8)</td>
<td>16.5±4.0*</td>
<td>(8.2–26.7)</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>42.1±5.5</td>
<td>(31.4–57.5)</td>
<td>37.7±3.5†</td>
<td>(28.2–38.9)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>87±5</td>
<td>(69–100)</td>
<td>94±8†</td>
<td>(78–115)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.8±0.3</td>
<td>(3.9–5.7)</td>
<td>5.1±0.3†</td>
<td>(4.6–6.0)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>178±26</td>
<td>(121–249)</td>
<td>216±27*</td>
<td>(158–282)</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>69±13</td>
<td>(40–100)</td>
<td>66±15</td>
<td>(34–107)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>60±24</td>
<td>(25–182)</td>
<td>93±44†</td>
<td>(34–280)</td>
</tr>
<tr>
<td>E2 (pg/mL)</td>
<td>75±60</td>
<td>(10–295)</td>
<td>11±3†</td>
<td>(10–29)</td>
</tr>
<tr>
<td>T3 (ng/dL)</td>
<td>108±12</td>
<td>(61–150)</td>
<td>112±18</td>
<td>(80–160)</td>
</tr>
</tbody>
</table>

Values are means±SD (range; minimum–maximum). Ht: height; BW: body weight; BMI: body mass index; FM: fat mass; FFM: fat free mass; HbA1c: glycosylated hemoglobin; E2: estradiol; T3: total triiodothyronine, *p<0.001 vs. young group (Student’s t-test), †p<0.001 vs. young group (Mann-Whitney rank sum test).

Table 2. Resting energy expenditure and adipocytokines in young and elderly women.

<table>
<thead>
<tr>
<th></th>
<th>Young (n=115)</th>
<th></th>
<th>Elderly (n=71)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>REE (kcal/d)</td>
<td>1,190±154</td>
<td>(830–1,622)</td>
<td>1,085±109†</td>
<td>(913–1,459)</td>
</tr>
<tr>
<td>(kcal/kg BW/d)</td>
<td>21.5±1.9</td>
<td>(17.8–27.5)</td>
<td>20.1±1.9*</td>
<td>(16.2–25.1)</td>
</tr>
<tr>
<td>(kcal/kg FFM/d)</td>
<td>28.4±2.3</td>
<td>(24.0–33.7)</td>
<td>28.9±2.4</td>
<td>(23.5–35.7)</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>9.9±3.8</td>
<td>(2.9–22.1)</td>
<td>9.9±4.1</td>
<td>(2.6–20.8)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>6.1±3.1</td>
<td>(1.1–19.8)</td>
<td>7.8±4.4††</td>
<td>(1.5–25.7)</td>
</tr>
</tbody>
</table>

Values are means±SD (range; minimum–maximum). REE: resting energy expenditure, *p<0.001 vs. young group (Student’s t-test), †p<0.001 and ††p<0.05 vs. young group (Mann-Whitney rank sum test).
Adiponectin increases β-oxidation through AMP-kinase activation, suggesting that adiponectin plays crucial and central roles in the regulation of energy expenditure (32). In contrast, we demonstrate that a significant inverse link exists between plasma adiponectin concentrations and REE in elderly women \((r = -0.386, p < 0.001, \text{Fig. 1, b-1})\). This is consistent with the results observed in overweight and obese males and females (14). These results might provide the possibility that protection by adiponectin against obesity-related disorders is especially important for human subjects with low RMR, and it is tempting to suggest plasma adiponectin as a valuable predictor for RMR, or vice versa.

We previously demonstrated that both FFM and FM are important predictors of REE in elderly people (20, 21). Thus, the inverse association between REE (kcal/d) and concentrations of plasma adiponectin in the present investigation in the elderly group may reflect spurious correlations. Therefore, for the purpose of determining the precise relationship between plasma adiponectin concentrations and REE, we adopted a partial correlation coefficient after adjusting for confounding influences of FM (kg), FFM (kg), E2 (pg/mL), and T3 (ng/dL). As a consequence, we have demonstrated that REE is not significantly correlated with plasma adiponectin concentrations (Table 3). These results suggest that adiponectin is not involved in the regulation of REE in young or elderly women.

Jørgensen et al. (33) demonstrated that serum leptin was a strong positive determinant for RMR in men. However, in their study, no adequate adjustment of RMR was made for either FFM or FM. In the present
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Investigation, no significant relationship between leptin and REE adjusted by body composition was observed (Table 3). This result is consistent with the report of Neuhäuser-Berthold et al. (34), suggesting that leptin might not have a significant role in the regulation of REE.

Our investigation has a few limitations. First, we did not test middle-aged (30–49 y) adults. Second, we did not include male subjects. Third, although adipocytokines such as adiponectin and leptin were related to the levels of body fat mass, we did not observe for lean and obese subjects. Future studies are needed to investigate this association in lean and obese adults and middle-aged adults.

In conclusion, the present investigation provides evi-

Table 3. Partial correlation coefficient to REE in female subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young (n=115)</th>
<th>Elderly (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REE (kcal/d)</td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>0.138 0.148</td>
<td>-0.200 0.104</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>-0.006 0.947</td>
<td>0.067 0.588</td>
</tr>
</tbody>
</table>

β: partial correlation coefficient: controlling for FM (kg), FFM (kg), E2 (pg/mL), and T3 (ng/dL).
idence to suggest that adipocytokines, such as adiponectin and leptin, do not influence REE in adult women.

Acknowledgments
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