Oxidative Stress Does Not Modulate Metabolic Rate or Skeletal Muscle Sympathetic Activity with Primary Aging in Adult Humans

CHRISTOPHER BELL, PAMELA P. JONES, AND DOUGLAS R. SEALS

Support of resting metabolic rate (RMR) by the β-adrenergic receptors of the sympathetic nervous system is attenuated with age and contributes to declines in RMR. This may be mediated by an age-associated increase in oxidative stress that can suppress β-adrenergic responsiveness and/or modulate sympathetic activity. To address these issues, RMR was determined in 12 young (23 ± 1 yr, mean ± SE) and 21 older (68 ± 3 yr) adults before and during systemic infusion of ascorbic acid (bolus, 0.06 g/kg fat free mass [FFM]; drip, 0.02). Ascorbic acid increased plasma concentrations similarly in young (72 ± 5 to 1107 ± 114 μmol/liter) and older (70 ± 6 to 1022 ± 63 μmol/liter) adults, and reduced (P = 0.001) plasma concentrations of isoprostanes (young, −82.8 ± 47; older, −107 ± 29 pg/ml). Baseline RMR_{FFM} was lower (5719 ± 215 vs. 6703 ± 328 kJ/d; P = 0.001) and muscle sympathetic nerve activity (MSNA) was greater (MSNA, 28 ± 2 vs. 23 ± 3 bursts/min; P < 0.05) in older compared with young. However, neither RMR_{FFM} (young, +117 ± 63; older, +163 ± 48 kJ/d; P = 0.14) or MSNA (young, 0 ± 2; older, −1 ± 1 bursts/min; P = 0.71) changed in either age group during ascorbic acid infusion compared with saline control. These results indicate that increased oxidative stress: 1) is not a mechanism contributing to decreases in RMR with primary aging; and 2) does not modulate MSNA in healthy adult humans. (J Clin Endocrinol Metab 88: 4950–4954, 2003)

Subjects and Methods

Subjects

We studied 33 healthy adult males and females: 12 young (19–33 yr; 7 male, 5 female) and 21 older (61–79 yr; 12 male, 9 female). All subjects were healthy, as assessed by medical history and fasting plasma glucose and insulin concentrations. In addition, older subjects underwent a physical examination with resting electrocardiogram (ECG) as well as ECG and blood pressure assessments during graded treadmill exercise to exhaustion. Subjects were nonsmokers and were not regularly taking any medications or vitamin supplements. The nature, purpose, and risks of the study were explained to each subject before written informed consent was obtained. The experimental protocol was approved by the Human Research Committee at the University of Colorado at Boulder.

Experimental procedures

All measurements were made in the morning after a 12-h fast. Subjects were studied under quiet resting conditions in the semirecumbent position.
position. Measurements were performed between 0700 and 0900 h in a dimly lit room at a comfortable temperature (±23 C). RMR was measured before and during either: 1) iv ascorbic acid administration (American Regent Laboratories Inc., Shirley, NY; priming bolus of 0.06 g/kg FFM dissolved in 100 ml of saline, infused at 5 ml/min (20-min infusion) and drip infusion of 0.02 g/kg FFM dissolved in 30 ml of saline administered over 55 min at 0.345 ml/min); or 2) saline infusion at the same rates. Subjects were instrumented for measurement of heart rate (ECG) and blood pressure (Dinamap XL Vital Signs Monitor, Johnson & Johnson, Arlington, TX). A catheter was placed in an antecubital vein and was kept patent with heparin. After a 30-min rest period following instrumentation, baseline RMR was measured, as previously described (2, 26).

The first 15 min were considered an habituation period after which oxygen consumption and carbon dioxide production were averaged each minute for 30 min using a ventilated hood indirect calorimetry system (DeltaTrac Metabolic Monitor, SensorMedics Corp., Yorba Linda, CA). RMR was calculated from the average of the 30-min collection using the Weir formula (27). The hood then was removed while an iv bolus was given of either ascorbic acid or saline. After a 5-min habituation period, RMR was measured again during continuous infusion of ascorbic acid or saline. Blood was sampled at three time points for determination of plasma ascorbic acid concentrations: during the baseline determination of RMR, immediately following the bolus, and at min 35 during the second measurement of RMR. Blood was also sampled for plasma concentrations of isoprostanes (28, 29), a marker of oxidative stress, during baseline and second determination of RMR (see Fig. 1).

Recordings of multiunit skeletal MSNA were obtained from the peroneal nerve using microneurography as previously described (30, 31). The microneurography electrode was inserted before the commencement of the first determination of RMR and remained in position until completion of the second determination of RMR. Neural activity was amplified, filtered (700–2,000 Hz), full-wave rectified, and integrated (time constant 0.1 s) (Nerve Traffic Analyzer, model 662c-3, University of Iowa Bioengineering). Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria (32, 33). MSNA was expressed as bursts of integrated activity per minute. The same investigator (P.P.J.) analyzed all neurograms and was blind to the experimental condition during which the recordings were made.

These measurements were made before and during administration of vitamin C and saline.

Dietary intake of antioxidants (vitamins C and E), together with macro- and micronutrients, were estimated from food diaries maintained for 4 consecutive days (3 weekdays and 1 weekend day). Subjects kept accurate and complete diet records and were provided with a diet scale (Scaleman, Target Corp., Minneapolis, MN) to weigh all food. A registered dietician subsequently analyzed all of the food diaries using standard computer-assisted procedures (ESHA-The Food Processor, version 4.1). Habitual physical activity was estimated using the Modifiable Activity Questionnaire (34–36).

Statistical analysis

Before statistical analysis, all RMR values were adjusted for FFM using analysis of covariance (relation between RMR and FFM: r = 0.72, P < 0.0001). Two-way ANOVA with repeated measures on one factor was used to examine differences in RMR between the groups at baseline, and changes in RMR in response to ascorbic acid and saline. Multiple comparisons of factor means were performed using the Neuman-Keuls test. Two-way ANOVA was also used to compare plasma concentration of ascorbic acid and markers of oxidative stress between young and older adults across time. Relations between variables of interest were determined by simple correlation analysis. The level of statistical significance was set at P < 0.05. Data are expressed as mean ± se.

Results

Selected subject characteristics are presented in Table 1. Compared with the young controls, the older adults had greater percentage of body fat, body mass index, diastolic blood pressure, total cholesterol, low-density lipoprotein cholesterol, fasting blood glucose, and plasma concentrations of norepinephrine (P < 0.05). Mean daily dietary intake is presented in Table 2. Dietary intake of vitamin C did not differ between young and older adults (P = 0.52).

Plasma concentrations of ascorbic acid at baseline and during the bolus and drip infusions are presented in Fig. 2. There were no differences between the young and older adults at baseline or during the bolus or drip infusions. Plasma concentrations increased in all subjects during the infusions, establishing that we were able to increase and maintain plasma concentrations of ascorbic acid at supraphysiological levels in both young and older adults. Baseline plasma concentrations of isoprostanes were not different (P > 0.05) between young and older adults (358 ± 45 vs. 385 ± 64 pg/ml). All subjects demonstrated a decrease in isoprostane concentration during the infusion of ascorbic acid (P = 0.001, Fig. 3), establishing that we were able to reduce oxidative stress in both groups. The decreases in plasma isoprostane concentrations during ascorbic acid infusion were related to baseline isoprostane levels, indicating that the reduction in oxidative stress was greatest in those subjects who demonstrated the greatest baseline levels.

Absolute values for baseline RMR were lower in the older (5566 ± 215 kJ·d−1) than in the young (6855 ± 328 kJ·d−1) adults (P < 0.01). This difference remained following adjustment for FFM (Fig. 4). RMR did not change significantly from baseline levels (P = 0.14) in either age group during

![Fig. 1](https://example.com/fig1.png)

* Blood Draw
TABLE 1. Baseline physiological characteristics of subject population

<table>
<thead>
<tr>
<th></th>
<th>Young adults</th>
<th>Older adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>7/5</td>
<td>12/9</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>23 ± 1</td>
<td>68 ± 1⁶</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>70.4 ± 3.4</td>
<td>77.2 ± 3.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 ± 2</td>
<td>170 ± 2</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>23.7 ± 0.7</td>
<td>26.6 ± 0.8⁵</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>19.9 ± 1.6</td>
<td>33.4 ± 1.6⁴</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>52.6 ± 2.7</td>
<td>48.5 ± 2.4</td>
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<tr>
<td>Physical activity (MET-h·wk)</td>
<td>64.4 ± 12.1</td>
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<tr>
<td>Resting heart rate (beats·min⁻¹)</td>
<td>55 ± 5</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>113/64 ± 4/2</td>
<td>116/69 ± 3/2²</td>
</tr>
<tr>
<td>Total cholesterol (mmol·liter⁻¹)</td>
<td>3.9 ± 0.2</td>
<td>4.9 ± 0.2⁸</td>
</tr>
<tr>
<td>HDL-C (mmol·liter⁻¹)</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>LDL-C (mmol·liter⁻¹)</td>
<td>2.2 ± 0.2</td>
<td>3.1 ± 0.1a</td>
</tr>
<tr>
<td>Triglyceride (mmol·liter⁻¹)</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Insulin (pmol·liter⁻¹)</td>
<td>38.6 ± 3.1</td>
<td>42.7 ± 4.7</td>
</tr>
<tr>
<td>Glucose (mmol·liter⁻¹)</td>
<td>5.2 ± 0.1</td>
<td>5.5 ± 0.1b</td>
</tr>
<tr>
<td>Epinephrine (pmol·liter⁻¹)</td>
<td>162.7 ± 29.5</td>
<td>151.8 ± 12.0</td>
</tr>
<tr>
<td>Norepinephrine (pmol·liter⁻¹)</td>
<td>1.51 ± 0.12</td>
<td>1.68 ± 0.15²</td>
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</tbody>
</table>

HDL-C, High-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. MET, Metabolic Equivalent (oxygen consumption of 3.5 ml/kg/min). Data are mean ± se. ‡ Denotes difference to young adults (P < 0.001). * Denotes difference to young adults (P < 0.05). Cholesterol, 1 mg/dl = 0.0259 mmol·liter⁻¹. Triglyceride, 1 mg/dl = 0.0113 mmol·liter⁻¹. Insulin, 1 μU/ml = 6.945 pmol·liter⁻¹. Glucose, 1 mg/dl = 0.0555 mmol·liter⁻¹. Epinephrine, 1 pg/ml = 5.46 pmol·liter⁻¹. Norepinephrine, 1 pg/ml = 0.00591 mmol·liter⁻¹.

TABLE 2. Daily dietary intake

<table>
<thead>
<tr>
<th></th>
<th>Young adults</th>
<th>Older adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kJ)</td>
<td>8765 ± 741</td>
<td>7918 ± 596</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>296 ± 26</td>
<td>231 ± 21</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>62 ± 6</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>72 ± 7</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>146 ± 33</td>
<td>124 ± 17</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>14 ± 6</td>
<td>11 ± 1</td>
</tr>
</tbody>
</table>

Data (mean ± se) are estimated from food diaries maintained for 4 consecutive days (3 weekdays and 1 weekend day). Energy: 1 kcal = 4.1868 kJ.

Ascorbic acid infusion (young, +117 ± 63; older, +163 ± 48 kJ·d⁻¹) compared with saline control (Fig. 4).

MSNA was greater in the older (28 ± 2 bursts/min) compared with the young (23 ± 3 bursts/min) adults at baseline (P < 0.05), and was unchanged in both age groups during ascorbic acid infusion (young, 0 ± 2; older, −1 ± 1 bursts/min). Heart rate and mean arterial pressure were unchanged (P > 0.05) during ascorbic acid administration in both young (from 55 ± 2 to 56 ± 2 beats/min; from 80 ± 2 to 82 ± 2 mm Hg) and older (from 52 ± 1 to 53 ± 1 beats/min; from 85 ± 2 to 88 ± 2 mm Hg) adults compared with saline control.

Discussion

The present study produced at least two novel findings of both physiological and clinical significance. First, increased oxidative stress does not appear to be an important mechanism mediating the reduction in RMR with primary aging in adult humans that is not dependent on body composition. Second, oxidative stress does not obviously modulate MSNA in healthy adult humans. Thus, increased oxidative stress does not appear to be an important mechanism contributing to the increase in MSNA with age in healthy adults (37–40).

The age-associated reduction in β-AR sympathetic support of RMR appears to be mediated by decreased β-AR tissue responsiveness (2) because basal sympathetic activity increases with age (37–40). This idea also is consistent with observations of attenuated increases in energy expenditure in response to β-AR stimulation in older compared with young adults (41). We reasoned that because oxidative stress 1) typically increases with age even in healthy adults (15–19); and 2) can impair β-AR tissue responsiveness (20), it might contribute to the reduction in RMR with primary adult aging that is not associated with body composition. Our results, however, demonstrate that this is not the case.

One explanation for this finding is that increased oxidative stress does not physiologically act to suppress β-AR stimulation of energy metabolism. Previous work demonstrated that ascorbic acid infusion improved tonically impaired β-AR stimulation of left ventricular contractility (20). It is
possible that oxidant modulation of these two peripheral tissue functions is fundamentally different. Alternatively, it is possible that increased oxidative stress may indeed influence β-AR control of energy metabolism but that this effect is observed only under conditions of heightened β-AR sympathetic nervous system stimulation (20).

We did not measure β-AR sympathetic nervous system stimulation directly; rather, we used MSNA as an estimate of whole body sympathetic activity. Several studies have described the tight relation between MSNA and cerebral, cardiac, and renal norepinephrine spillover supporting its use as an indicator of whole-body sympathetic activity (42–45). However, in this case we cannot be certain that the lack of change observed in MSNA during ascorbic acid administration was representative of sympathetic stimulation to other tissues contributing to β-AR-mediated thermogenesis. Several studies have used methodology (e.g., microdialysis, catheterization to calculate arterial-venous difference, etc.) that has provided description of organ-specific changes of sympathetic activity in response to various stimuli (44–52). Use of these methodologies would have strengthened our study but would also have placed a significantly greater burden on the study participants.

Another potential limitation of our study pertains to the effectiveness of an acute systemic administration of ascorbic acid on alleviating end-organ oxidative stress. Although decreased plasma isoprostane concentrations may be reflective of reduced oxidative stress in tissues primarily responsible for producing these compounds, we have no direct evidence that oxidative stress was reduced in tissues responsible for β-AR mediated thermogenesis.

We have reported on the lack of effect of acute ascorbic acid administration on RMR and MSNA. We might speculate a similar lack of effect with long-term oral administration of ascorbic acid. Plasma concentrations of ascorbic acid following long-term oral supplementation have been shown repeatedly to be considerably less than those achieved with acute iv infusion (53, 54), presumably reflecting attenuated ability to reduce oxidative stress. It is possible that a more comprehensive antioxidant intervention (e.g., using a combination of vitamins C and E) may demonstrate a modulatory effect of oxidative stress on RMR and/or MSNA.

To account for the age-associated decline in plasma volume (55), our iv dosing regime of ascorbic acid was based on FFM. Previously, we have reported (56) on the strong relation between FFM and plasma volume in adult humans of varying ages. Administration of the same absolute dose of ascorbic acid to young and older adults may have resulted in greater plasma concentrations in older adults, thus limiting our conclusions regarding age-associated differences on the influence of oxidative stress. The constant in our dosing regime calculation (bolus, 0.06 g/kg FFM) was based on previous studies that have administered an absolute dose of 2–3 g of ascorbic acid (54, 57–59) and on FFM data previously collected in our laboratory from adult humans with similar physiological characteristics to those participating in the present investigation (2). Finally, we added a drip infusion (0.02 g/kg FFM) to maintain plasma concentrations of ascorbic acid during the 55-min period following completion of the bolus administration.

In conclusion, our findings suggest that increased oxidative stress may not be a mechanism contributing to either the decrease in RMR or the increase in skeletal muscle sympathetic nerve activity with primary aging in adult humans.

Acknowledgments

We thank Benjamin L. Garvey and Jeff Groff for administrative and technical assistance, and Estes Park Positive Eating for dietary analysis.

Received March 17, 2003. Accepted June 16, 2003.

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This research was supported by NIH Grants AG06537, AG00828, 1 P30 DK48520, and AHA 0225438Z.

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