Whole body, adipose tissue, and forearm norepinephrine kinetics in lean and obese women

Simon W. Coppack, Jeffrey F. Horowitz, Deanna S. Paramore, Philip E. Cryer, Henry D. Royal, and Samuel Klein. Whole body, adipose tissue, and forearm norepinephrine kinetics in lean and obese women. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E830–E834, 1998.—We evaluated whole body and regional (subcutaneous abdominal adipose tissue and forearm) norepinephrine (NE) kinetics in seven lean (body mass index 21.3 ± 0.5 kg/m²) and six upper body obese (body mass index 36.4 ± 4.0 kg/m²) women who were matched on fat-free mass. NE kinetics were determined by infusing [3H]NE and obtaining blood samples from a radial artery, a deep forearm vein draining mostly skeletal muscle, and an abdominal vein draining subcutaneous abdominal fat. Mean systemic NE spillover tended to be higher in obese (2.82 ± 0.49 nmol/min) than in lean (2.53 ± 0.40 nmol/min) subjects, but the differences were not statistically significant. Adipose tissue and forearm NE spillover rates into plasma were greater in lean (0.91 ± 0.08 pmol·100 ml tissue⁻¹·min⁻¹ and 1.01 ± 0.09 pmol·100 ml tissue⁻¹·min⁻¹, respectively) than in obese (0.26 ± 0.05 pmol·100 g tissue⁻¹·min⁻¹ and 0.58 ± 0.11 pmol·100 ml tissue⁻¹·min⁻¹, respectively) subjects (P < 0.01). These results demonstrate that adipose tissue is an active site for NE metabolism in humans. Adipose tissue NE spillover is considerably lower in obese than in lean women, which may contribute to the lower rate of lipolysis per kilogram of fat mass observed in obesity.

catecholamines; obesity; energy metabolism

The sympathetic nervous system (SNS) is an important regulator of energy metabolism because of its effect on both energy expenditure and energy mobilization. Therefore, SNS activity may be important in the etiology and pathophysiology of obesity. A decrease in SNS activity contributes to the onset of obesity in certain animal models (6) and may also contribute to the onset of obesity in humans (34). It is not clear whether SNS activity is altered in persons who are already obese, because of conflicting results from more than 40 different studies (38). However, the data from most studies suggest that whole body SNS activity, measured as plasma norepinephrine (NE) concentration, urinary NE excretion, or systemic NE spillover rate into plasma, is either the same in lean and obese subjects or increases with increasing adiposity. Skeletal muscle SNS activity, measured by microneurography, increases with increasing body fat mass (14, 15, 27, 31). Therefore, it is possible that regional heterogeneity in SNS activity may be responsible for some of the variability observed between studies.

A better understanding of regional SNS activity may have important clinical implications in obesity. An increase in SNS activity in specific tissues may be involved in the pathogenesis of some of the medical complications of obesity, such as hypertension (36) and cardiovascular disease (24). A decrease in adipose tissue SNS activity could be responsible for the lower rates of lipolysis per kilogram of adipose tissue reported in obese compared with lean subjects (18). Alterations in regional SNS activity are likely to occur during obesity therapy, which usually involves decreasing energy intake and increasing physical activity. Hypocaloric dieting or fasting decreases muscle SNS activity, which conserves energy (21, 29, 39), whereas exercise increases SNS activity, which stimulates the release of free fatty acids and glycerol from adipose tissue to provide fuel for working muscles and gluconeogenic precursors for the liver (17, 26).

The SNS influences metabolic processes through nerves that innervate different body tissues. NE is synthesized and stored in sympathetic nerve endings and is the neurotransmitter involved in SNS signal transmission (4). Although most of the NE released from sympathetic postganglionic neurons is cleared locally by neuronal reuptake and effector cell metabolism, a portion of released NE spills over into the bloodstream. Therefore, whole body NE spillover rate into plasma has been used as an index of SNS activity. However, whole body NE spillover represents total spillover from sympathetic neurons in many tissues and NE release into plasma from the adrenal medullas and, thus, does not provide information on SNS activity in specific tissues. The use of tracer methodology in conjunction with arteriovenous balance permits the assessment of regional NE spillover by individual tissues.

In the present study, we evaluated whole body and regional (subcutaneous abdominal adipose tissue and forearm) NE kinetics in lean and obese women by infusing [3H]NE and catheterizing the radial artery, a deep forearm vein draining mostly skeletal muscle, and an abdominal vein draining subcutaneous abdominal fat. We hypothesized that whole body and forearm NE spillover rates would be greater, whereas adipose tissue NE spillover rates would be lower, in obese than in lean subjects.
MATERIALS AND METHODS

Subjects. Seven lean (28 ± 2.1 yr) and six upper body obese (waist-to-hip circumference ratio >0.85 and waist circumference >100 cm; 38 ± 3.1 yr) white women (Table 1) participated in this study, which was approved by the Institutional Review Board, the Radiological Drug Research Committee, and the General Clinical Research Center of Washington University School of Medicine. All subjects gave informed written consent. Lean and obese women were matched for fat-free mass (FFM), as determined by dual-energy X-ray absorptiometry (Hologic QDR 1000/W, Waltham, MA) 5 to 7 days before the isotopic infusion study. Subjects were nonsmokers, were weight stable for at least 2 mo before the study, and were not taking any medications. All subjects performed normal daily activities, such as shopping, driving, and walking short distances, but none participated in regular aerobic exercise, such as walking, jogging, or cycling. Subjects were considered to be healthy after a history and physical examination, blood tests, a 2-h oral glucose tolerance test, and an electrocardiogram.

Study protocol. Subjects were admitted to the General Clinical Research Center in the evening before the isotopic infusion study within the first 2 wk of the follicular phase of the menstrual cycle of each subject. All lean subjects consumed an evening meal containing 12 kcal/kg body wt, and all obese subjects consumed an evening meal containing 12 kcal/kg adjusted body wt, where adjusted body weight was calculated as ideal body weight + [(actual body weight – ideal body weight)(0.25)]. At precisely 2000, subjects ingested a meal containing 12 kcal/kg body wt, and all were not taking any medications. All subjects performed normal daily activities, such as shopping, driving, and walking short distances, but none participated in regular aerobic exercise, such as walking, jogging, or cycling. Subjects were considered to be healthy after a history and physical examination, blood tests, a 2-h oral glucose tolerance test, and an electrocardiogram.

At 0900, a constant infusion (10 nCi·kg⁻¹·min⁻¹) of levo-[ring-2,5,6-²H]NE (40–60 Ci/mmol; New England Nuclear, Boston, MA) was started and continued for 30 min. An arterial blood sample was obtained before isotope infusion to determine background NE specific activity. Blood samples were obtained from artery and deep forearm and abdominal veins simultaneously every 5 min between 0920 and 0945 (25, 20, 25, and 30 min of labeled NE infusion). Two minutes before each deep forearm venous blood sample, a wrist pressure cuff was insufflated to 180 mmHg to exclude hand blood flow from the arm flow measurement, and the arm cuff was inflated to 40 mmHg to occlude venous outflow during measurement. The four flow measurements were averaged to determine overall forearm blood flow.

Analyses. Plasma NE concentration was determined by a single isotope derivative radioenzymatic method, and plasma ²H]NE specific activity was determined after organic extraction (described previously [30]).

Calculations. Physiological and isotopic steady states were present during the last 15 min of isotope infusion as determined by constant NE concentration and ²H]NE specific activity, so Steele’s equation for steady-state conditions was used to calculate whole body NE kinetics (32).

NE spillover₁₃³ = [²H]NE infusion rate (dpm/min)

where NE spillover₁₃³ is the rate of NE spillover (appearance) into the systemic circulation (in nmol/min). SANE is the specific activity of arterial NE, and dpm is disintegrations per minute.

The systemic metabolic clearance rate for NE (in ml/min) was calculated as NE spillover₁₃³ divided by plasma NE concentration.

Local tissue NE clearance rate (in ml · unit of tissue⁻¹ · min⁻¹) was calculated as local NE tissue extraction times plasma flow (37) and assumes that NE is only carried in plasma (11):

\[
\frac{(NE_a)(SA_a) - (NE_v)(SA_v)}{(NE_a)(SA_a)} \times \text{plasma flow}
\]

where SAₐ and SAᵥ are NE specific activities in arterial and venous plasma, respectively, and NEₐ and NEᵥ are NE concentrations in arterial and venous plasma, respectively.

The calculation of NE clearance underestimates endogenous NE production because venous NE specific activity is greater than intracellular NE specific activity.

Subcutaneous ATBF was determined by measuring the rate of removal of ¹³³Xe from adipose tissue by using a monoexponential decay and an adipose tissue-to-blood partition coefficient for Xe (between 1 ml of blood and 100 g of tissue) of 10.2 ± 0.3 ml/g (1). Therefore, blood flow is expressed as milliliters of blood per 100 g of adipose tissue per minute.

Subcutaneous adipose tissue plasma flow was calculated as ATBF × (1 – hematocrit). Forearm blood flow was determined by plethysmography, which measures the rate of change in limb volume and is expressed as milliliters of blood per 100 ml of forearm per minute. Forearm plasma flow was calculated as forearm blood flow × (1 – hematocrit).

Local NE spillover from adipose tissue and forearm was calculated as (11):

\[
(NE_{extract} \times NE_a + (NE_v - NE_a)) \times \text{plasma flow}
\]

where NE_extract is NE fractional extraction.

Table 1. Characteristics of the study subjects

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<tr>
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<th>Lean Women</th>
<th>Obese Women</th>
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<tr>
<td>Body mass index, kg/m²</td>
<td>23.1 ± 0.5</td>
<td>36.4 ± 0.4</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>58.3 ± 2.1</td>
<td>96.2 ± 2.6</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>24.0 ± 1.7</td>
<td>52.3 ± 1.7</td>
</tr>
<tr>
<td>Total fat mass, kg</td>
<td>14 ± 1.2</td>
<td>50.3 ± 1.8</td>
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<tr>
<td>Fat-free mass, kg</td>
<td>42.0 ± 1.6</td>
<td>43.5 ± 1.8</td>
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Values are means ± SE.
Statistical analyses. Student’s two-tailed t-test for independent samples was used to test the significance of differences between lean and obese groups. A P value of ≤0.05 was considered to be statistically significant. All data are means ± SE.

RESULTS

Systemic NE kinetics. Arterial plasma NE concentrations and systemic NE kinetics are shown in Table 2. Mean systemic NE spillover was similar in lean and obese subjects, and the slight trend toward increased systemic NE spillover in the obese group was not statistically significant (P = 0.66).

Adipose tissue NE kinetics. Mean subcutaneous adipose tissue venous plasma NE concentrations were similar to arterial values in both lean (0.80 ± 0.08 and 0.88 ± 0.08 nmol/ml for abdominal vein and artery, respectively) and obese (0.73 ± 0.07 and 0.93 ± 0.09 nmol/ml for abdominal vein and artery, respectively) subjects. ATBF was greater in lean than obese subjects (3.99 ± 0.71 and 1.54 ± 0.22 ml·100 g tissue⁻¹·min⁻¹, respectively; P < 0.05). Subcutaneous adipose tissue NE spillover was threefold greater in lean (0.91 ± 0.08 pmol·100 g tissue⁻¹·min⁻¹) than in obese (0.26 ± 0.05 pmol·100 g tissue⁻¹·min⁻¹) subjects (P < 0.01) (Fig. 1). Clearance of plasma NE by adipose tissue was also greater in lean (1.41 ± 0.13 ml·100 g tissue⁻¹·min⁻¹) than obese (0.61 ± 0.12 ml·100 g tissue⁻¹·min⁻¹) subjects (P < 0.05; Fig. 1).

Forearm NE kinetics. Deep venous plasma NE concentrations were greater than arterial values in all subjects, demonstrating net NE release by forearm tissue. Forearm blood flow was greater in lean than obese subjects (2.31 ± 0.29 and 1.06 ± 0.14 ml·100 ml tissue⁻¹·min⁻¹, respectively; P < 0.05). Forearm NE spillover was greater in lean (1.01 ± 0.09 pmol·100 ml tissue⁻¹·min⁻¹) than in obese (0.58 ± 0.11 pmol·100 ml tissue⁻¹·min⁻¹) subjects (P < 0.01) (Fig. 2). Clearance of plasma NE by forearm tissue was also greater in lean (0.92 ± 0.11 ml·100 ml⁻¹·min⁻¹) than in obese (0.42 ± 0.05 ml·100 ml⁻¹·min⁻¹) subjects (P < 0.001) (Fig. 2).

DISCUSSION

In the present study, we evaluated whole body and regional (subcutaneous abdominal adipose tissue and forearm) NE kinetics in vivo in lean and obese women, who were closely matched for fat-free body mass. The most important new finding of this study is the marked difference in adipose tissue NE metabolism found in lean and obese women; NE spillover and clearance rates in subcutaneous abdominal adipose tissue (expressed per 100 g of fat mass) were considerably lower in obese than in lean subjects. This decrease in adipose tissue NE kinetics (an index of SNS activity) may be an important mechanism for downregulating basal adipose tissue lipolysis in obese persons. Compared with lean subjects, lipolytic rates per kilogram of fat mass are decreased in obese subjects, whereas whole body lipolysis or lipolytic rates per kilogram of lean body mass are often increased because of the large increase in body fat mass (13, 18). Therefore, decreased adipose tissue lipolysis in obese persons may have clinical benefits by preventing even greater increases in whole body lipolytic rates. Excessive lipolysis and free fatty acid release into plasma could have adverse metabolic effects on hepatic very low density lipoprotein production and plasma lipid concentrations (20), hepatic glucose production (9), and peripheral glucose uptake (35).

In comparison with the lean group, forearm NE spillover rate, an index of skeletal muscle SNS activity, was lower in the obese subjects. These results differ from previous studies (14, 15, 27, 30), which found muscle sympathetic nerve activity increased with increasing adiposity. It is possible that differences in methodologies between these studies and ours may be responsible for the different results. The previous studies determined leg muscle SNS activity by microneurog-

Table 2. Whole body plasma NE concentration and kinetics

<table>
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<tr>
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<th>Lean Women</th>
<th>Obese Women</th>
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<tr>
<td>Plasma NE, nmol/l</td>
<td>1.00 ± 0.10</td>
<td>1.09 ± 0.17</td>
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<tr>
<td>Systemic NE spillover, nmol/min</td>
<td>2.53 ± 0.40</td>
<td>2.82 ± 0.49</td>
</tr>
<tr>
<td>Systemic NE clearance, l/min</td>
<td>2.63 ± 0.14</td>
<td>2.61 ± 0.22</td>
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Values are means ± SE. NE, norepinephrine.
would be statistically significant had we studied more subjects. Indeed, data from a recent study (16) suggest that results obtained by microneurography may correlate poorly with regional skeletal muscle NE spillover. Moreover, in the two studies that evaluated skeletal muscle SNS activity by microneurography in women, values for obese subjects who had similar body mass index or percent body fat as our obese group were within the range of values reported among women who had the same body mass index or percent body fat as our lean group (15, 27). It is also likely that the presence of increased forearm subcutaneous and intermuscular fat contributed to the low rate of forearm NE spillover measured in our obese subjects because of the low rate of NE spillover in fat tissue.

We used subcutaneous abdominal adipose tissue NE spillover as an index of body fat SNS activity and forearm NE spillover as an index of skeletal muscle SNS activity. However, sympathetic outflow to different peripheral tissues is not uniform (38), and it is also likely that heterogeneity in SNS activity also exists within similar tissues located at different sites. Although skeletal muscle SNS activity measured by microneurography during basal conditions has been found to be similar in arm and leg muscle beds (3, 23, 33), Karlsson et al. (16) found that muscle SNS activity measured by NE spillover was twofold greater in arms than in legs. SNS activity in adipose tissue depots has not previously been studied. Nonetheless, if we assume that our regional results are representative of similar tissues throughout the body, our data suggest that skeletal muscle and adipose tissue combined account for only a small portion of whole body NE spillover rate. Therefore, most of the NE released into the circulation comes from other tissues. Other investigators have found that NE spillover from the lungs, kidneys, heart, and splanchnic bed accounted for approximately two-thirds of total spillover (8), whereas both upper and lower extremities constituted only 7% of total spillover (16).

Systemic (whole body) NE spillover rates in our obese subjects were similar to values observed in the lean group. We are aware of three other studies that have evaluated the relationship between adiposity and systemic NE spillover in humans (5, 22, 28). One study (5) found marginally lower systemic NE spillover rates in obese compared with lean subjects, whereas the other two studies (22, 28) found a direct positive correlation between adiposity and systemic NE spillover. It is possible that by carefully controlling lean body mass we minimized differences in systemic NE spillover between our lean and obese groups. Although we cannot exclude the possibility that the trend toward an increase in systemic NE spillover rate in the obese group would be statistically significant had we studied more subjects, the possibility of a type II statistical error is unlikely. Approximately 180 subjects would be needed in each group to make the differences in systemic NE spillover rate that we observed between lean and obese women statistically significant at a P value of 0.05 with a power of 0.8.

In a review of more than 40 studies that evaluated SNS activity in lean and obese subjects, considerable diversity was found in results between studies (38). As noted above for data on whole body NE spillover, measures of plasma NE and epinephrine concentration and urinary NE and epinephrine excretion were lower, the same, and higher in obese compared with lean subjects. Some of the variability in these studies may be related to confounding variables that influence SNS activity. In the present study, careful attention was made to eliminate as many factors as possible that are known to affect SNS activity (38). Lean and obese subjects were matched by gender, antecedent diet, physical activity history, and lean body mass. In addition, no subject had hypertension or abnormal glucose tolerance, which is associated with alterations in SNS activity (2, 3).

The use of tracer methodology to determine whole body NE spillover measures a summation of NE released into plasma from SNS activity in different tissues and from NE secretion by the adrenal medullas. The combined use of tracer methodology and arteriovenous balance eliminates the contribution of NE from the adrenal medullas. However, regional NE entering plasma represents only a portion of NE released by sympathetic neurons. Most of the NE is cleared by sympathetic neuronal reuptake and storage or metabolism by monoamine oxidase and by effector cell uptake and metabolism by catechol-O-methyltransferase (4). Spillover of NE from tissue into plasma depends on the number and firing rate of local sympathetic nerve terminals, capillary permeability, and the efficiency of neuronal reuptake and effector cell clearance mechanisms (cf. Ref. 11). In addition, regional blood flow can influence NE spillover because it can affect the concentration gradient between interstitial and plasma NE. Therefore, it is possible, but we believe unlikely, that differences in tissue blood flow between our lean and obese subjects contributed to the differences we observed in local NE spillover. Regional adipose tissue and forearm NE kinetics were still significantly different between lean and obese subjects when we recalculated our data by using Chang's equation for regional plasma NE appearance rate (7), which is not affected by tissue blood flow. Finally, because the anatomic distribution of sympathetic neurons within adipose and forearm tissues is not known, we cannot determine whether NE released into plasma reflects activity of sympathetic neurons innervating predominantly adipocytes in adipose tissue, myocytes in forearm tissue, local vascular structures in each tissue, or a combination of these effector cells.

In summary, the results of the present study demonstrate that subcutaneous adipose tissue is an active site for NE metabolism in humans. Moreover, adipose tissue NE spillover was considerably lower in obese than in lean subjects, which may be an important contribu-
tor to the lower rate of lipolysis per kilogram of fat mass observed in obesity.

We thank Renata Braudy and the nursing staff of the General Clinical Research Center for help in performing the experimental protocols and Dr. Guohong Zhao and Wei-qing Feng for technical assistance.

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