Prediction of basal metabolism from organ size in the rat: relationship to strain, feeding, age, and obesity

P. C. EVEN, V. ROLLAND, S. ROSEAU, J.-C. BOUTHEGOURD, AND D. TOMÉ

Unité Mixte de Recherche, Physiologie de la Nutrition et du Comportement alimentaire, Institut National de la Recherche Agronomique, 75005 Paris, France

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Even, P. C., V. Rolland, S. Roseau, J.-C. Boutegourd, and D. Tomé. Prediction of basal metabolism from organ size in the rat: relationship to strain, feeding, age, and obesity. Am J Physiol Regulatory Integrative Comp Physiol 280: R1887–R1896, 2001.—Use of the weight of various organs and tissues together with their specific metabolic activity for prediction of basal metabolism (BM) seems to be promising. In this study we compared the use of this method with those based on simple or multiple regression analyses. We observed that 97.4% of differences in BM in a group of nine adult male Wistar rats weighing 273–517 g could be accounted for by changes in tissue and organ weights. BM measured in lean Zucker and Sprague-Dawley rats did not diverge from the prediction of the model by >1.6%. According to the organ-based model as well as multiple regression analyses, but not simple regression analyses, BM was increased 18–21% in young rats, decreased 6–7% in food restricted/refed rats, and decreased 19–21% in aged rats. Only with obese rats did the predictions of the two methods diverge. The main reason for this discrepancy seems to be the way adipose tissue size and metabolism are taken into account.

body composition; metabolic activity; predictive model; energy expenditure

CORRECTING BASAL METABOLISM (BM) for differences in body weight and body composition is a major challenge (1, 8, 10, 12, 17, 19, 25, 32, 38, 48). The relationship between BM and body weight is not linear when different species of grossly different sizes are compared (8, 25, 32), but also within species, whether the difference was due to age (9, 55) or to under- or overfeeding or obesity (14, 45, 54). Dimensional analysis of the size of animals (25, 32, 38, 48) has been demonstrated to account for variations in BM between subjects of different species and large differences in body size, but cannot be reliably used within species (25, 39).

A major focus of the efforts to circumvent this problem has been the use of the concept of fat-free mass (FFM) or lean body mass (LBM) (26), and, until recently, normalization by FFM seemed to be the best available predictor of basal metabolic rate, because, in most cases, it enabled correction for variations induced by age, sex, and weight (37). However, the use of FFM to normalize BM may introduce a bias in many instances, because FFM is not homogenous but instead includes tissues with very different specific metabolic activities (12, 22). Therefore, the use of LBM reaches limitations when changes in body composition affect the ratio of LBM to fat mass or, within the LBM component, change the relative size of the various tissues.

In theory, whole body BM can be estimated from the sum of weight of tissues and organs multiplied by their specific metabolic rate. Elia (12) has been able to determine the specific metabolism of the various tissues and organs in vivo. Therefore, if one can assess body composition precisely, it is theoretically possible to predict BM with a much higher degree of accuracy than from body weight or any other single component of body weight. The potential efficacy of such an approach to correct BM for body size and body composition has been demonstrated in healthy human adults of normal body weight (20). The present study aimed to more precisely evaluate in the rat model the efficacy and robustness of the use of predictive models, which include various parameters known to affect BM such as whole body mass, height, LBM, fat mass, body surface, age, and various combinations of these parameters to normalize BM of subjects in different experimental conditions and to account for the wide differences in body size and body composition between subjects. For that purpose, BM in adult male Wistar rats used as a reference group was compared with BM in young Wistar rats, food restricted/refed adult Wistar rats, adult lean Zucker rats, adult obese Zucker rats, adult Sprague-Dawley rats, and aged Sprague-Dawley rats.

METHODS

Animals

Seven groups of male rats were used in these experiments. They were all fed a standard laboratory maintenance diet (Extralabo M25, Pietrement, France, 3.2 kcal/g) and were assigned to seven groups designed to measure the effect of age, obesity and food restriction on BM. Group 1 comprised nine Wistar rats weighing 270–520 g. This group was used as the reference control group. Group 2 comprised seven Wistar
rats initially weighing 233–304 g and submitted to 3–4 wk of food restriction to ~70% of spontaneous food intake (17 g/days). Four of the rats were used while food restricted and weighed 230–304 g, and three of the rats were used after 4 days of refeeding and weighed 400–410 g. This group was designed to measure the effect of long-term mild food restriction and refeeding on BM. Group 3 comprised six young Wistar rats weighing 180–225 g. This group was used to compare BM between young growing rats and adult rats. Group 4 had 10 lean Zucker rats weighing 290–380 g. This group served as the control for group 5. Group 5 contained 14 obese Zucker rats weighing 500–750 g. This group was used for measurement of BM in obese subjects. Group 6 had seven adult Sprague-Dawley rats weighing 350–520 g to serve as the control group for group 7. Group 7 was made up of six old Sprague-Dawley rats aged >18 mo and weighing 550–615 g. This group was used for measurement of the effects of aging on BM.

**Measurement of BM**

BM was measured by indirect calorimetry using a metabolic device described in previous publications (13, 15). Temperature in the metabolic chamber was maintained at 26–27°C. BM was recorded after the following procedure in all rats. Food was removed from the home cage of the rat the previous day at 1800. The rats were housed in the metabolic chamber at 1200 with only 12 g of food available. With this restricted amount of food in the cage and the previous overnight food restriction, the food allotment in the metabolic cage was terminated before 0200. Thereafter basal metabolism decreased progressively and reached a stable value by 0700 in the morning. Recording of respiratory exchange was terminated at 1100. BM was taken as the average resting metabolism measured during the last 3 h of recording. Food was not withdrawn from the food-restricted rats the day before measurement.

**Measurement of Body Composition**

Body composition of the rats was measured by means of dissection and weighing of the fresh weight of the main organs and tissues of the body. Immediately after measurement of BM, the rat was weighed, anesthetized (pentobarbital sodium, 60 mg/kg, Sanofi Santé), and then exsanguinated by removal of blood from the abdominal aorta. This led to collection of 8–15 ml of blood depending on the weight of the rat. Then the main organs and tissues were removed, blotted dry, and immediately weighed to the nearest 0.1–0.01 g. The main organs dissected and weighed were heart, liver, spleen, kidney, brain, abdominal adipose tissues (mesenteric, retroperitoneal, epididymal), skin, subcutaneous abdominal adipose tissue, tail, and head. After completion of the dissection, the remainder of the body, i.e., muscle mass plus skeleton (excluding tail and head), was weighed and classified as “carcass.” Muscle weight was computed from carcass weight assuming that in all rats, skeleton accounted for 13.5% of carcass weight. This value was calculated from direct measurement of skeleton weight in 10 carcasses of adult Wistar rats.

For the purpose of analysis, three other components were derived: organ and tissues with a high metabolic activity (brain, liver, kidney, heart) were grouped under the component “High,” tissues with a low metabolic activity (adipose depots, skin + subcutaneous fat, tail) were grouped under the component “Low,” the residual organs that were not individually weighed (lungs, testis, etc.) were grouped under the component residual (Res). LBM in this study was taken as body weight minus the component Low.

**RESULTS**

**Body Weight and Body Composition**

Table 1 gives the weight of each of the main components of body weight in the different groups, in absolute terms as well as relative to LBM. Because large differences in body weight in the various groups make it difficult to compare directly the data in absolute terms, the comparison in the following sections refers to the data expressed relative to LBM.

**Comparison in the three control groups (adult Wistar rats, adult lean Zucker rats, and adult Sprague-Dawley rats).** Despite the fact that the three groups of rats were adults of normal body weight, important differences in body composition were observed between them. Compared with the Wistar rats, liver weight as well as weight of the component High were lower in the lean Zucker rats, whereas in contrast, muscle mass was greater. In SD rats, the weights of the liver, brain, kidney, and the component High were lower. Muscle mass in SD rats also tended to be higher than in Wistar rats, but the significance remained borderline (P = 0.051). In contrast with the differences observed on the metabolically more active organs, the weight of the component Low (adipose tissue + skin + tail) was very similar in the three groups (between 37 and 39% of LBM).

**Comparison between Wistar rats: young vs. adult and food restricted vs. ad libitum fed.** In young rats, body composition included relatively less tissue with a low metabolic activity (muscles and the component Low) and more tissues with a higher metabolic activity (components Res and High) than in adult rats. In food-restricted rats, the most important change was due to a large decrease in the weight of the adipose tissue leading also to a decrease in the weight of the component Low. In contrast, for all the other tissues and organs, including muscle mass, no significant changes were observed by comparison with ad libitum-fed rats.

**Comparison between lean and obese Zucker rats.** Nearly all of the components of body composition differed between lean and obese Zucker rats. In obese rats, the adipose stores, but also several of the body components with a high metabolic activity (Res, High, liver, heart), were enlarged. In contrast, muscle mass was much smaller. Comparison of obese Zucker rats with adult Wistar rats led to the same conclusions.

**Comparison between old and adult Sprague-Dawley rats.** In this experiment, skin and subcutaneous adipose tissues were weighed together so that separate values for adipose tissue and skin are not available in Sprague-Dawley rats. Old Sprague-Dawley rats were characterized by an increase in the weight of the component Low and a decrease in brain weight. Weights of the other organs and tissues were not different. Comparison of the old Sprague-Dawley rats with the adult Wistar rats led to the same conclusions.
Comparison of Basal Metabolism Between the Group of Control Wistar Rats and the Other Groups

Normalization of basal metabolism according to body weight, muscle mass, LBM, High, and Res. The correlation between BM and body weight established in the group of control Wistar rats was used to predict BM expected in the other rats according to their own weight. The differences between the predicted and measured BM values were then computed for each group (Fig. 1A and Table 2). This study showed that measured BM values in the SD control rats were close to those predicted according to their body weight (−3.56%). In contrast, measured BM values were larger than predicted in young Wistar rats (+30.53%) and lower than predicted in the lean Zucker rats (−10.77%) as well as in the food-restricted Wistar rats (−5.93%), the obese Zucker rats (−27.25%), and the old Sprague-Dawley rats (−26.19%).

Following the same procedure, BM was compared between the Wistar rats and the other groups after adjustment of BM with various parameters of body weight (muscle mass, LBM, High, Res) The results of these studies are summarized in Table 2 and Fig. 1, B-E. They showed that BM values derived in the various groups from the above simple regression analyses differed depending on which body component was used to adjust BM. As a result, taking BM in the adult Wistar rats as reference, adjusted BM varied from 1.7 to 15.8% in lean Zucker rats, from 1.7 to 7.1% in control Sprague-Dawley rats, from 1.2 to 2.1% in young Wistar rats, from 2.7 to 27.25% in obese Zucker rats, and from 2.6 to 26.19% in old Sprague-Dawley rats.

Table 1. Weight of the main components of body weight in the various groups

<table>
<thead>
<tr>
<th></th>
<th>Wistar (n = 9)</th>
<th>ZL (n = 10)</th>
<th>SD (n = 7)</th>
<th>Young (n = 6)</th>
<th>FR (n = 7)</th>
<th>ZO (n = 14)</th>
<th>Old (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expressed in g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>390.1±0.33</td>
<td>337.3±0.34</td>
<td>410.4±0.47</td>
<td>208.1±0.26‡</td>
<td>326.5±0.66</td>
<td>550.6±0.66</td>
<td>593.8±0.77‡</td>
</tr>
<tr>
<td>AT (g)</td>
<td>83.1±1.7</td>
<td>66.1±1.4</td>
<td>—</td>
<td>42.2±0.53‡</td>
<td>62.1±0.11</td>
<td>153.0±0.18</td>
<td>—</td>
</tr>
<tr>
<td>Muscle (g)</td>
<td>180.1±4.8</td>
<td>165.1±3.2</td>
<td>194.7±3.9</td>
<td>91.5±0.55</td>
<td>156.1±1.65</td>
<td>150.6±1.65</td>
<td>262.9±2.05‡</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>14.3±0.3</td>
<td>8.0±0.4‡</td>
<td>12.4±0.4</td>
<td>8.4±0.47†</td>
<td>11.2±0.12</td>
<td>17.4±0.53</td>
<td>17.3±0.54</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>1.9±0.1</td>
<td>1.9±0.2</td>
<td>1.6±0.4</td>
<td>1.7±0.44</td>
<td>1.8±0.19</td>
<td>1.9±0.26</td>
<td>1.7±0.47</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>1.1±0.1</td>
<td>0.9±0.2</td>
<td>1.1±0.4</td>
<td>0.7±0.44†</td>
<td>0.9±0.12</td>
<td>1.2±0.28</td>
<td>1.4±0.21</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>2.8±0.2</td>
<td>2.2±0.2</td>
<td>2.6±0.4</td>
<td>2.0±0.44†</td>
<td>2.4±0.12</td>
<td>2.7±0.33</td>
<td>3.7±0.34</td>
</tr>
<tr>
<td>Low (g)</td>
<td>109.8±2.4</td>
<td>91.3±1.9</td>
<td>115.4±2.7</td>
<td>53.9±0.39‡</td>
<td>80.4±0.39</td>
<td>283.5±8.5</td>
<td>195.1±15.4</td>
</tr>
<tr>
<td>High (g)</td>
<td>20.2±1.7</td>
<td>13.2±0.9</td>
<td>17.9±1.1</td>
<td>14.6±0.53‡</td>
<td>18.5±0.53</td>
<td>26.1±1.3</td>
<td>24.3±1.3</td>
</tr>
<tr>
<td>Res (g)</td>
<td>51.8±2.9</td>
<td>41.4±2.6</td>
<td>51.6±3.5</td>
<td>48.5±0.81†</td>
<td>69.4±1.91</td>
<td>69.9±1.92</td>
<td>69.9±1.92</td>
</tr>
</tbody>
</table>

**Expressed in percent of LBM**

<table>
<thead>
<tr>
<th></th>
<th>Wistar (n = 9)</th>
<th>ZL (n = 10)</th>
<th>SD (n = 7)</th>
<th>Young (n = 6)</th>
<th>FR (n = 7)</th>
<th>ZO (n = 14)</th>
<th>Old (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (%)</td>
<td>139.3±1.7</td>
<td>137.0±1.0</td>
<td>138.8±1.6</td>
<td>134.9±1.2†</td>
<td>132.3±1.0</td>
<td>207.1±1.6</td>
<td>149.1±1.6†</td>
</tr>
<tr>
<td>AT (%)</td>
<td>30.3±0.3</td>
<td>26.8±0.1‡</td>
<td>—</td>
<td>27.3±1.29‡</td>
<td>25.0±1.08</td>
<td>58.7±1.17</td>
<td>—</td>
</tr>
<tr>
<td>Muscle (%)</td>
<td>64.2±0.4</td>
<td>67.0±0.5‡</td>
<td>65.9±0.2</td>
<td>59.3±1.28‡</td>
<td>63.2±0.36</td>
<td>56.8±0.20</td>
<td>65.9±1.28*</td>
</tr>
<tr>
<td>Liver (%)</td>
<td>5.0±0.1</td>
<td>3.2±0.1‡</td>
<td>4.2±0.14‡</td>
<td>5.4±0.65</td>
<td>4.6±0.57</td>
<td>6.5±0.26‡</td>
<td>4.3±0.64‡</td>
</tr>
<tr>
<td>Brain (%)</td>
<td>0.4±0.01</td>
<td>0.3±0.01</td>
<td>0.05±0.01</td>
<td>1.1±0.44‡</td>
<td>0.7±0.33</td>
<td>0.4±0.44‡</td>
<td>0.3±0.38</td>
</tr>
<tr>
<td>Heart (%)</td>
<td>0.4±0.01</td>
<td>0.4±0.01</td>
<td>0.04±0.01</td>
<td>0.4±0.44†</td>
<td>0.3±0.01</td>
<td>0.4±0.45‡</td>
<td>0.3±0.38</td>
</tr>
<tr>
<td>Kidney (%)</td>
<td>1.0±0.01</td>
<td>0.9±0.11†</td>
<td>1.1±0.21</td>
<td>1.3±0.97</td>
<td>0.9±0.12</td>
<td>1.0±0.03</td>
<td>0.9±0.005</td>
</tr>
<tr>
<td>Low (%)</td>
<td>39.3±0.3</td>
<td>37.0±0.3</td>
<td>38.8±0.6</td>
<td>34.9±1.28‡</td>
<td>32.3±1.37</td>
<td>107.1±1.66</td>
<td>17.5±0.86</td>
</tr>
<tr>
<td>High (%)</td>
<td>7.1±0.1</td>
<td>5.3±0.1‡</td>
<td>6.1±0.10‡</td>
<td>8.4±0.83</td>
<td>6.6±0.76</td>
<td>8.7±0.25‡</td>
<td>6.1±0.24‡</td>
</tr>
<tr>
<td>Res (%)</td>
<td>18.5±0.2</td>
<td>17.0±0.2</td>
<td>17.6±0.3</td>
<td>22.9±0.69</td>
<td>20.0±0.29</td>
<td>25.4±0.99</td>
<td>17.5±0.86</td>
</tr>
</tbody>
</table>

Values are means ± SE (in parentheses). Weight of adipose tissue (AT) is not available in Sprague-Dawley (SD) and Old rats because subcutaneous AT was weighed together with skin. LBM, lean body mass; ZL, Zucker lean; FR, food restricted; ZO, Zucker old; Low, low-metabolic activity tissues; High, high-metabolic activity tissues; Res, residual organs. See METHODS for more complete descriptions of groups. *P < 0.05; †P < 0.01; ‡P < 0.001 vs. Wistar (unpaired t-test).
+30.5% in young rats, from −16.3 to +9.5% in food-restricted rats, from −27.2 to +4.0% in obese Zucker rats, and from −4.8 to −26.2% in aged Sprague-Dawley rats (Fig. 2). This large variability was due to the differences in the weights of the tissues and organs relative to each other in the various groups. For example, obese Zucker rats have a much higher weight of the Res component relative to LBM than adult Wistar rats.

Table 2. Differences between predicted and observed BM in the various groups resulting from the parameters of the simple linear regression analysis performed in control Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>ZL</th>
<th>SD</th>
<th>Young</th>
<th>FR</th>
<th>ZO</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>−10.769</td>
<td>−3.556</td>
<td>30.528</td>
<td>−5.927</td>
<td>−27.253</td>
<td>−26.188</td>
</tr>
<tr>
<td></td>
<td>(2.17)‡</td>
<td>(2.23)</td>
<td>(2.65)‡</td>
<td>(2.64)</td>
<td>(1.93)‡</td>
<td>(2.97)‡</td>
</tr>
<tr>
<td>Muscle</td>
<td>−15.827</td>
<td>−7.091</td>
<td>28.032</td>
<td>−9.956</td>
<td>17.315</td>
<td>−22.528</td>
</tr>
<tr>
<td></td>
<td>(2.122)‡</td>
<td>(2.315)*</td>
<td>(3.318)‡</td>
<td>(2.424)†</td>
<td>(3.425)‡</td>
<td>(2.061)‡</td>
</tr>
<tr>
<td>LBM</td>
<td>−12.625</td>
<td>−4.669</td>
<td>22.669</td>
<td>−10.835</td>
<td>5.620</td>
<td>−19.600</td>
</tr>
<tr>
<td></td>
<td>(2.005)‡</td>
<td>(1.924)</td>
<td>(2.63)‡</td>
<td>(2.76)†</td>
<td>(2.947)</td>
<td>(2.466)‡</td>
</tr>
<tr>
<td></td>
<td>(2.56)*</td>
<td>(3.451)</td>
<td>(1.976)</td>
<td>(2.471)*</td>
<td>(2.05)†</td>
<td>(5.995)</td>
</tr>
<tr>
<td></td>
<td>(3.558)</td>
<td>(2.401)</td>
<td>(6.958)</td>
<td>(5.289)*</td>
<td>(4.81)†</td>
<td>(6.681)</td>
</tr>
</tbody>
</table>

Values are means ± SE (in parentheses) in %. BM, basal metabolism. *P < 0.05; †P < 0.01; ‡P < 0.001 vs. Wistar (unpaired t-test).
rats and a smaller muscle mass. Thus when BM was adjusted relative to Res, BM seemed to be reduced (−18.5%). In contrast, when BM was adjusted relative to muscle mass, BM seemed to be strongly increased (+17.31%). The same kind of error occurred in the other groups with the source of error depending on which component of body weight was increased or decreased in the group.

These results demonstrate that it is essential to take into account the relative weights of the main components of total body weight to correct BM for changes in body composition adequately. The aim of the next study was to use this methodological approach.

Normalization of BM according to organ-tissue weights and to their specific metabolic activity. Inasmuch as no full set of data was available to assign a specific metabolic activity to the various tissues and organs of the rats, we adjusted for the rat the data proposed by Elia (12) for humans. This was done by multiplying the specific metabolic activity of the various tissues and organs in humans by a constant that allowed the best fit for the value of BM predicted from the weight of the tissues and organs to the BM values actually measured in the group of adult Wistar rats. The multiplicative factor that allowed the best fit of the organ-based predictive model to the experimental results was adjusted empirically by a “trial and error” approach and was found to be 4.879 (Table 3).

Figure 3 shows the relative weight and energy production of the four main components of body weight predicted by the model in the different groups. This figure shows the large differences in size of the various tissues between the various groups and the large differences that can occur between tissue size and energy expenditure.

After multiplication of the specific activity of the organs as established in humans by 4.879, it appeared that the organ-based model accounted for 97.4% of the differences of BM among adult Wistar rats (Fig. 4). In the other groups, BM actually measured in these rats diverged from the prediction of the model by only −0.187% (P = 0.93) in lean Zucker rats and 1.570% (P = 0.48) in SD rats (Table 4). Thus, according to this procedure of normalization of BM, all three groups of adult rats, Wistar, Sprague-Dawley, and lean Zucker, appeared to have the same BM. In contrast, BM appeared to be increased in young Wistar rats only and appeared decreased in all the other experimental groups (Table 4).

Normalization of BM by stepwise multiple regression analysis: adjustment of the model. As the organ-based model showed that adult Wistar, lean Zucker, and Sprague-Dawley rats could be considered to have a similar BM per unit of metabolically active mass, we pooled the rats of these three groups into a single larger group of 26 rats from which we tested the possibility of predicting BM from stepwise regression analyses. We considered it useful to attempt this study to test 1) whether a statistical model could validate the incorporation of the Wistar, lean Zucker rats, and Sprague-Dawley control rats into a single homogenous group and 2) whether the differences among groups observed after this statistical procedure agreed with the differences reported from the organ-based model.

Stepwise regression analysis using BM as the dependent variable and Low, muscle, liver, brain, heart, kidney, and Res as independent variables led to a model that finally included only muscle and liver as significant predictors of BM (Table 4). According to this model, BM measured in the three control groups clustered tightly along the regression line, confirming that BM in these three groups was the same after differences in muscle and liver weight were taken into account (Fig. 5A, Table 4). Among the experimental groups, the statistical model also closely matched the conclusions drawn from the organ-based model except in the obese Zucker rat in which the stepwise regression analysis suggested that BM was normal (+0.27%),
whereas the organ-based model suggested that BM was decreased (−13.31%; Table 4, Fig. 4 vs. 5).

Stepwise regression analyses was also used to test a simplified model using only the four main components of body weight, i.e., muscle, High, Low, Res. This led to a model that finally included only muscle and High as significant predictors of BM (Fig. 5B, Table 4). This model led to results that were very close to the ones derived from the previous stepwise regression analysis.

The results of the stepwise regression analysis thus confirmed that the three control groups could be considered to have the same BM after differences in body composition were taken into account, but raised the question of why the two methods diverged so strongly only with the obese Zucker rats. We suspected that the discrepancy was due to the fact that in obese Zucker rats the large increase in body weight is primarily related to the accumulation of subcutaneous adipose tissue (Table 1) (6).

Enlargement of the adipose stores in the obese Zucker rats is the result of both hyperplasia and hypertrophy of the adipose cells (5, 7, 29, 51). Hypertrophy of adipose cells is mostly due to the accumulation of inert lipid droplets, increasing adipocyte size by an average of 4.3 times (7) and therefore decreasing its specific metabolic activity by the same order of magnitude. Because in the obese Zucker rats average mass of the adipose tissue was 153.08 ± 7.87 g, whereas the average mass of the overall Low component was 283.58 ± 9.03 g, we estimated that the average metabolic activity of the component Low in obese rats was 2.3 times smaller than in control rats. By introducing this correction in the organ-based model, we observed that the decrease in BM was reduced from 13.3 to 6.84% (Table 4). Despite the fact that the difference remained significant, this correction reduced the discrepancy observed between the stepwise regression analysis procedures and the organ-based method.

DISCUSSION

The inability to normalize BM by simply correcting its value according to body weight soon appeared to be related to the fact that in many cases, body composition changes together with either body size or age (2, 16, 23,
thus altering the proportions of the contribution to energy production of the various tissues. Major research groups thus consider that, for predicting basal metabolism accurately, fat mass and FFM must be taken into account, but also that FFM should be subdivided into its main components (21, 37, 43, 50, 59). The main goal of the present study was to verify whether such a method 1) was usable in rats and 2) was able to deal with changes in body composition that occur in relation to various parameters such as strain, age, feeding status, and obesity. The results showed that only the methods taking into account the weight of various organs and tissues of the body seemed to be able to provide correct adjustment of BM between the various groups. Indeed the relationship between BM and body weight in adult Wistar rats was linear and very tightly correlated within a given interval of body weight, but the data from young rats, older rats, and food-restricted rats were far apart in this relationship. The same was true for obese Zucker rats in which BM appeared excessively low after correction for body weight.

### Specific Metabolism of the Various Tissues and Organs

To adjust in the rat the specific metabolic activity of the various tissues and organs established in humans, we proposed the hypothesis that the metabolic rate of the various tissues relative to each other was the same in rats and in humans. This hypothesis was made possible because the data available in various animal species ranging from the mouse to the horse show that the metabolism of the organs relative to each other does not exhibit any specific metabolic change with body size (from H. A. Krebs, 1950, quoted and discussed in Ref. 30). Accordingly, we observed that it was possible to accurately predict BM in adult Wistar rat by increasing 4.879 times the specific metabolic activity of the organs and tissues.

### Table 4. Differences between predicted and observed BM in the various groups resulting from the prediction of BM based on the specific metabolic activity of the various tissues and organs

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>ZL</th>
<th>SD</th>
<th>Young</th>
<th>FR</th>
<th>ZO</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ-based model</td>
<td>0.000</td>
<td>−0.187</td>
<td>1.570</td>
<td>21.587</td>
<td>−6.034</td>
<td>−13.308</td>
<td>−19.233</td>
</tr>
<tr>
<td></td>
<td>(1.393)</td>
<td>(2.234)</td>
<td>(2.250)</td>
<td>(1.611)</td>
<td>(2.259)</td>
<td>(1.987)</td>
<td>(2.438)</td>
</tr>
<tr>
<td>Stepwise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle-liver</td>
<td>0.561</td>
<td>−1.580</td>
<td>0.265</td>
<td>19.844</td>
<td>−6.684</td>
<td>0.267</td>
<td>−21.492</td>
</tr>
<tr>
<td></td>
<td>(0.861)</td>
<td>(2.133)</td>
<td>(2.482)</td>
<td>(0.570)</td>
<td>(2.228)</td>
<td>(2.472)</td>
<td>(4.281)</td>
</tr>
<tr>
<td>Muscle-high</td>
<td>0.527</td>
<td>−2.093</td>
<td>1.130</td>
<td>18.448</td>
<td>−6.791</td>
<td>−0.177</td>
<td>−21.194</td>
</tr>
<tr>
<td></td>
<td>(0.902)</td>
<td>(2.139)</td>
<td>(2.483)</td>
<td>(0.638)</td>
<td>(2.013)</td>
<td>(2.452)</td>
<td>(4.546)</td>
</tr>
<tr>
<td>Organ-based adjusted model</td>
<td>————</td>
<td>————</td>
<td>————</td>
<td>————</td>
<td>————</td>
<td>————</td>
<td>————</td>
</tr>
</tbody>
</table>

Values are means ± SE (in parentheses) in %. *P < 0.05; †P < 0.01; ‡P < 0.001 vs. Wistar (unpaired t-test).
account well for the size difference between humans and rats. The other result in favor of the physiological validity of the procedure is the fact that BM predicted from organ size did not differ from the BM actually measured in all three strains of adult rats fed ad libitum. Such a coincidence observed simultaneously between predicted and measured BM values in the three strains could not be observed with any of the methods that used one single component of body weight to normalize BM but was confirmed by stepwise regression analysis. Finally, the capacity of this method to deal with changes in various components of body weight is indicated by the fact that lean Zucker and Sprague-Dawley rats differed in their body composition relative to Wistar rats in different ways, lean Zucker rats having a relatively larger muscle mass and a smaller liver mass, Sprague-Dawley rats having no differences at the level of muscle mass, but smaller brains, livers, and kidneys. The improvements brought by the ability to predict BM from the size and weight of the main organs and tissues constituting the body offer the possibility to shed some new light on several longstanding questions on whether or not BM changes during food restriction, aging, and obesity.

**Basal Metabolism and Food Restriction, Aging, or Obesity**

**Food restriction.** Whole body BM declines with food restriction. The question remains whether BM is still decreased if changes in body size and body composition are adequately taken into account, and no definitive answers have been given (3, 14, 27, 35, 36). In the present study, BM appeared more reduced after adjustment for LBM (−10.8%) than for body weight (−5.9%), certainly because LBM decreased less than whole body weight. Adjustment according to the organ-based model also suggested that BM was decreased after food restriction. The decrease (6%) was similar to that suggested after correction for whole body weight and after correction by stepwise regression analyses (−6.7 to −6.8%). The convergence of the results brought by these different methods is not surprising because the main tissue affected by food restriction was the adipose one whose metabolic activity accounted for only a very small part of BM. Taken together, the present results confirm that BM is indeed slightly reduced during food restriction. In addition, because the decline in BM (measured according to the organ-based method) was similar in the four rats used while food restricted (−5.4 ± 3%) and the three rats that were previously refed during 4 days (−6.87 ± 1.85%), the present results confirm recent observations that the decrease in BM induced by food restriction is maintained several days during refeeding (11, 34).

**Age.** The fact that BM can be affected by age is shown by the many equations derived for use in humans that include the parameter age (1, 58). The changes in body composition throughout the span of life are complex, involving at various degrees all the tissues and organs of the body. This clearly explains why adjustments of BM founded on one single component of body weight led to such variable results. In contrast, because adjustment of BM from organ size was able to account completely for complex differences in body composition between Wistar, lean Zucker, and Sprague-Dawley rats, it is clear that it was best fitted to take into account the complex changes in body composition from youth to aging. Thus the present study suggests with reasonable confidence that BM is increased in young growing subjects and decreased in aged but healthy subjects. This confirms an array of previous results (4, 18, 42, 43) and further suggests two points. First, it seems that the decrease of BM from youth to adulthood is rapid because the difference in body weights and LBMs between the older young rats and the younger adults was small. Such an observation was also done in humans in the study of Bitar and colleagues (4). Second, it seems that the decrease in BM during aging is more progressive. Indeed, in this study, five of the aged rats were taken from retired reproducers (Iffa-Credo, 69592 l’Arbresle Cédez, France) and were 18–20 mo old. In these five rats, BM assessed from the organ-based model appeared reduced by 16 ± 1%. The two other rats, remaining from a previous experiment done in the laboratory, were >2 years old, and one of them was already losing some weight. In these rats, the decrease in BM was larger, respectively, 24.6 and 30%. Therefore the decrease in BM observed during aging is the result of a decrease in both the weight of the metabolically active tissues and their specific metabolic activity. Which organ and tissues are more particularly involved remains to be investigated.

**Obesity.** It is repeatedly suggested that a decrease in BM may be an important factor leading to obesity (24, 44, 47). The difficulty of correcting BM for body size and composition in obese subjects is primarily due to the fact BM must be corrected more specifically in relation to the hypertrophy of the adipose tissue, whereas muscle mass and, more generally, lean tissues are relatively less affected. It is clear thus that any adjustment of BM according to body weight or LBMs will introduce large errors. This was clearly demonstrated in this study where, depending on the reference used, BM in obese rats ranged from −27% (correction according to body weight) to +5.6% (correction according to LBM). The other problem that was revealed in this study is that in obesity adipocytes are hypertrophied. This phenomenon induces a decrease in the specific metabolic activity of the adipose tissue. In this study, we attempted to take this problem into account by assuming that the specific metabolic activity of the adipose tissue was reduced 4.3 times in the obese Zucker rats. By doing so we reduced the differences with the stepwise regression analyses, but it is clear that to be able to establish a really precise measure of BM in obese Zucker rats, specific studies should be undertaken to get precise information about the specific metabolic activity of the various tissues, not only adipose tissue, but also liver, muscles, and skin that may include more fat than in control animals.
Nevertheless, the data presently provided by the organ-based model as well as by the stepwise regression analysis converge to suggest that BM in the obese Zucker rat is probably not reduced as much as previously thought. Such a result is in line with the most recent reports (24, 56) and questions the hypothesis that a reduced basal rate of energy expenditure may participate in the etiology of obesity.

Conclusion and Perspectives

The present study reveals the potential of the use of the weights and specific metabolic activities of the organs and tissues constituting the body for predicting BM and in this way confirm and extend the observation of Gallagher et al. (20). This method appears to be able to deal with large changes in body size and body composition as long as it can be assumed that no significant changes occur in the composition of the various organs and tissues making up the body. This limitation was found for the obese rats of the present study in which the extremely large variations in the size and composition of the adipose tissue challenged the method. The same may be true in other experimental conditions or pathologies in which the level of hydration of the cells or the composition of specific organs such as lipid inclusion in muscular dystrophy or steatosis due to cirrhosis, ketogenic diets, or liver diseases may vary. One possible solution to this limitation of the method may be to use additional or alternative parameters such as the dry weight of the tissues, the weight of the defatted tissue, or the DNA content per unit of weight of tissue. With these improvements, and possibly with further adjustments of the specific metabolic activity attributed to each organ, there is no doubt that this method will soon allow us to perform appropriate adjustments of BM whatever the differences in body size and composition. A precise measurement of organ and tissue sizes can be done from carcass analysis in animals. A precise measurement of organ and tissue contribution to metabolic rate. In: Energy Metabolism, Tissue Determinants and Cellular Corollaries. New York: Raven, 1992: 61–77.


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