No insulating effect of obesity

Alexander W. Fischer,1,2 Robert I. Csikasz,1 Gabriella von Essen,1 Barbara Cannon,1 and Jan Nedergaard1
1Department of Molecular Biosciences, The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, Stockholm, Sweden; and 2Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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Fischer AW, Csikasz RI, von Essen G, Cannon B, Nedergaard J. No insulating effect of obesity. Am J Physiol Endocrinol Metab 311: E202–E213, 2016. First published May 17, 2016; doi:10.1152/ajpendo.00093.2016.—The development of obesity may be aggravated if obesity itself insulates against heat loss and thus diminishes the amount of food burnt for body temperature control. This would be particularly important under normal laboratory conditions where mice experience a chronic cold stress (at ≈20°C). We used Scholander plots (energy expenditure plotted against ambient temperature) to examine the insulation (thermal conductance) of mice, defined as the inverse of the slope of the Scholander curve at subthermoneutral temperatures. We verified the method by demonstrating that shaved mice possessed only half the insulation of non-shaved mice. We examined a series of obesity models [mice fed high-fat diets and kept at different temperatures, classical diet-induced obese mice, ob/ob mice, and obesity-prone (C57BL/6) vs. obesity-resistant (129S) mice]. We found that neither acclimation temperature nor any kind or degree of obesity affected the thermal insulation of the mice when analyzed at the whole mouse level or as energy expenditure per lean weight. Calculation per body weight erroneously implied increased insulation in obese mice. We conclude that, in contrast to what would be expected, obesity of any kind does not increase thermal insulation in mice, and therefore, it does not in itself aggravate the development of obesity. It may be discussed as to what degree of effect excess adipose tissue has on insulation in humans and especially whether significant metabolic effects are associated with insulation in humans.

obesity; insulation; ob/ob

DESPITE THE PRESENT INTEREST IN METABOLISM in connection with the global obesity epidemic, there is little knowledge concerning the extent to which obesity as such affects metabolism. There is widespread interest in issues such as the release of adipokines from adipose tissue depots, but many basic issues, such as the ability of adipose tissue to affect metabolism physically, have been little examined. One of these issues is the question of the ability of adipose tissue to function as a thermal insulation barrier, decreasing the heat loss from the organism and in this way decreasing the amount of energy needed to keep the organism warm, thereby increasing the amount of excess energy prone to storage in the form of (additional) fat.

Whether an insulating effect of obesity exists is of significance both for humans and for animal models of obesity. However, with regard to experimental animals, the issue of insulation is of further interest. This is because most metabolic experiments are conducted with mice kept under conditions (standard laboratory conditions of ≈21°C) that are principally cold for the mouse (10, 19, 27, 38, 42, 44) and thus where a high proportion of total metabolism (nearly half) is devoted to counteracting the resulting heat loss. This is a condition very different from that which is relevant for the metabolic physiology of most humans (living in a thermoneutral environment). Thus, under the current experimental conditions for mice, an insulating effect of increasing obesity would successively diminish the heat loss and thus enhance, in a self-amplifying way, the further development of obesity. Additionally, the adaptation to living at a relatively cold temperature may in itself promote additional insulation (via fur), affecting metabolic rate.

Despite these possible profound influences of alterations in thermal insulation, data on the effects of obesity on insulation are scarce and only found for a few specific rodent models in studies concerned mainly with other issues (1, 5, 17, 21, 28). We have failed to find studies either in mice or in humans that systematically empirically quantify the effect of a series of different obesities on insulation. Therefore, in this study, we examine the possible influence of obesity on metabolism as mediated via alterations in insulation.

METHODS

Animals

Study 1 (effect of fur). Male C57BL/6NCrl mice were purchased at 12 wk of age from Nova (Sweden) and kept at 21°C with free access to chow diet (~10% calories from fat; Lactamin R70) and water on a 12:12-h light-dark cycle. One day before the start of the experiment, one group of mice was anesthetized using 4% isofluorane anesthesia and shaved using a commercial electrical shaver (T-Edjer II). The control group was anesthetized as well, and a running shaver was placed next to them while the mice were kept in anesthesia. After this, the mice were immediately returned to their home cages and placed in metabolic chambers and allowed to adapt to the chambers overnight at 30°C with ad libitum access to chow diet and water. Measurements and calorimetry were performed as described below.

Study 2 (diet and acclimation temperature). Male C57BL/6NCrl mice were purchased at 12 wk of age from Nova (Sweden). The mice were housed in single cages at room temperatures of 30, 21, or 4°C (4°C after a 1-wk acclimation period at 18°C) on a 12:12-h light-dark cycle. The mice were supplied with a cardboard house and wood-wool nesting material. Thus, the experienced temperature in the nest was most likely higher than the room temperature. The mice had free access to water and were fed ad libitum with either chow diet (~10% calories from fat; Lactamin R70) or high-fat diet (45% of calories from fat; Research Diets D12451). After 6 mo of acclimation to the respective diet and temperature,
the mice were placed in metabolic chambers and analyzed as described below.

**Study 3 (DIO mice).** Sixteen-week-old male diet-induced obese (DIO)-control and DIO mice (C57BL/6NJTac mice) were purchased from Taconic and housed at room temperature (21°C) and fed either a control low-fat diet (=10% of calories from fat; NIH31) or high-fat diet (60% of calories from fat; Research Diets D12492). Feeding of the respective diets had started at 6 wk of age. The mice were housed in single cages with ad libitum access to food and drinking water on a 12:12-h light-dark cycle. The experiments were performed after 6–7 mo of diet feeding.

**Study 4 (ob/ob mice).** Fifteen-week-old male C57BL/6 Lepob/JRj mice and lean littermates (Janvier Laboratories, Saint-Berthevin, France) were used in this study. The mice were fed a chow diet and housed at an environmental temperature of 21°C. Note that the mice and the raw data analyzed here are the same as in Fischer et al. (21). The mice had been implanted with metal-containing telemetry transponders (E-Mitter G2; Respirationics) 2 wk before the experiment. Thus, no MRI analysis of body composition could be performed. The mice were analyzed as described below.

**Study 5 (strain dependence).** Male 129S and C57BL/6 mice from the animal breeding facility at Stockholm University were housed at room temperature (21°C) and fed either a chow diet (Lactamin R70) or high-fat diet (Research Diets D12451) for 1 mo starting at 12 wk of age. The mice were housed in single cages with ad libitum access to food and drinking water and a 12:12-h light-dark cycle. Metabolic experiments were performed as described below before the start of the feeding period and after 1 mo of feeding the respective diet.

**Measurements**

Body composition and body weight of the mice were measured 2 days before (studies 3 and 5) or the day after indirect calorimetry was performed (studies 1 and 2). The body composition was measured by an in vivo magnetic resonance imaging (MRI) technique using the EchoMRI-100 (Echo Medical Systems, Houston, TX). The resulting lean body mass does not include bone, fur, etc., and thus is not equivalent to fat-free dry mass. Examination of the performance of the instrument concerning determination of a lipid mass of 10 g (as relevant for the present study) yielded an accuracy value of =0.5% and a precision of 0.3% (mean deviation from the mean for 6 repetitive measurements).

**Indirect Calorimetry**

To measure oxygen consumption and carbon dioxide production in an indirect calorimetry system (INCA; Somedic, Hörby, Sweden), the mice, in their home cages but without house and nesting material, were transferred to a sealed chamber (5.6 liters) with a temperature controller to maintain a stable, adjustable temperature. After calibration of the oxygen sensors, oxygen consumption and carbon dioxide production were measured every second minute. In studies 1 and 2, the mice were allowed to adapt to the chambers overnight at 30°C. Afterward, the temperature in the chambers was raised to 33°C and gradually decreased every second hour via 30, 27, 20, and 15°C to 5–8°C. In studies 3 and 5, the mice were allowed to adapt for 1 h at 30°C, and afterward the temperature was gradually decreased every 1.5 h from 30°C via 25, 20, 15, and 10°C to 5–8°C. In study 4, the mice were allowed to adapt overnight at 21°C. After this, the temperature was increased to 33°C and then gradually decreased every second hour via 30, 27, 24, and 20°C to 16°C. During the experiments, the mice had free access to water and food. Routinely, the actual chamber temperatures were measured individually for each chamber and set temperature, and the measured temperatures were those used for plotting the curves.

**Calculations and Statistics**

The energy expenditure in watts (W) was calculated using a modified Weir equation (57a):

\[
\text{Energy expenditure [W]} = \left(0.2716 \frac{W \times \text{min}}{\text{ml}} \times V(O_2)_{\text{consumed}} \left[ \frac{\text{ml}}{\text{min}} \right] + 0.07616 \frac{W \times \text{min}}{\text{ml}} \times V(CO_2)_{\text{produced}} \left[ \frac{\text{ml}}{\text{min}} \right] \right)
\]

The average energy expenditure during the last hour at each temperature was used for the analysis (see RESULTS). During this hour, the mice may display different levels of physical activity, and the energy expenditure of this activity would necessarily be included in the average energy expenditure. However, as observed elsewhere and discussed in general (22, 42, 50), and as demonstrated by Virtue et al. (55) specifically for physical activity, at subthermoneutral temperatures such extra energy for physical activity does not necessarily add to the total energy expenditure, as the heat production for thermoregulation would be correspondingly diminished. Therefore, we have used total energy expenditure data for the calculations. However, analysis of the data using only the lowest energy expenditure values (5 consecutive 2-min periods; resting metabolic rates) yielded final results qualitatively similar to those obtained here with total values (i.e., no insulating effect of obesity) (not shown).

The energy expenditure at each temperature was plotted vs. the environmental temperature (“Scholander Plots”; see Ref. 48). The slopes of the Scholander plots at and below 27°C, representing the thermal conductance (W/°C), were calculated using the best linear fit. The insulation was calculated as the inverse (°C/W) of the slope of the Scholander curves. The basal metabolic rate was defined here as the energy expenditure at 30°C. Analyses were performed using Microsoft Excel and GraphPad Prism. Statistical significance was examined using Student’s t-test and ANOVA as indicated; P ≤ 0.05 was considered to be statistically significant. All means shown are average values ± SE.

**RESULTS**

To quantify the general effects of body composition on the insulation of mice, we used several widely used mouse models for obesity. We calculated the insulation of the mice from Scholander plots, as described below and in METHODS. In addition to analyzing the effect of obesity in these models [long-term HFD feeding, classical diet-induced obese (DIO) mice and genetically obese ob/ob mice], we also analyzed the interaction of housing temperature and of mouse strain on the insulation.

**Experimental Setup**

To determine the insulation of mice, Scholander experiments were performed as exemplified in Fig. 1. As seen in Fig. 1A, a wild-type mouse [fed a chow diet and housed at 21°C (standard laboratory conditions)] was gradually exposed to colder temperatures, starting at 33°C and going down to ~7°C. Each drop in chamber temperature resulted in a strong initial increase in the metabolic rate, probably as a result of increased stress and activity due to the sudden increased cold stress. This increase peaked after ~15 min, and after 30–60 min the metabolism stabilized at a new, higher level. These stable levels were markedly increased from 30 (thermoregulation) to 7°C, representing the increased need for heat production to compensate for the increased heat loss. For analysis, the average energy expenditure during the last hour at each temperature was used. As seen in Fig. 1B, the pattern was highly

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Fur Has a Significant Insulating Capacity

To validate the method for quantification of insulation, we examined the effects of shaving the mice, i.e., removing an important part of their insulation. As seen in Fig. 2A, no effect of shaving on the metabolic rate was visible at 33°C, but with decreasing temperatures the difference between control mice and shaved mice became more pronounced, with shaved mice displaying markedly elevated metabolic rates. Already at 27°C, shaved mice displayed distinctly higher metabolism than control mice (Fig. 2A), implying a change in their thermoneutral zone. When cold stress was further increased, both groups further increased their metabolism. However, at the lowest temperature, the difference between shaved mice and control mice became smaller, presumably because of an inability of the shaved mice to further increase their metabolism, as they would have reached their maximum thermogenic capacity (they were preacclimated only to 21°C; Fig. 2A) (39). The energy expenditure values obtained in the last hour at each temperature were plotted against the actual chamber temperature (Scholander plots; Fig. 2B). The slope of the Scholander curve at subthermoneutral temperatures is a reflection of the insulation of the animal, as a higher insulation will result in a reduced need to produce heat, thereby decreasing the slope of the curve. Thus, the inverse slope of the Scholander curve represents the insulation of the animal. The calculated insulation of the shaved mice (Fig. 2C) was ~40% lower than the insulation of control mice. Thus, using this experimental approach, we were clearly able to detect differences in insulation.

No Effects of Acclimation Temperature or Obesity on Insulation

To manipulate the body lipid content of mice so as to evaluate its significance for heat loss, mice were exposed to
Table 1. Characteristics of the mice used to examine the effects of housing temperature and diet

<table>
<thead>
<tr>
<th></th>
<th>30°C</th>
<th>HFD</th>
<th>21°C</th>
<th>HFD</th>
<th>4°C</th>
<th>HFD</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>35.9±0.8</td>
<td>51.8±0.9***</td>
<td>32.6±1.2#</td>
<td>48.7±1.2***</td>
<td>31.5±1.3#</td>
<td>48.8±1.2***</td>
</tr>
<tr>
<td>Lipid mass, g</td>
<td>11.1±0.4</td>
<td>24.2±0.7***</td>
<td>6.8±0.9###</td>
<td>21.3±0.7***#</td>
<td>5.7±0.9###</td>
<td>20.5±0.5***###</td>
</tr>
<tr>
<td>Lean mass, g</td>
<td>20.9±0.4</td>
<td>25.0±0.4**</td>
<td>21.4±0.2</td>
<td>22.9±0.5</td>
<td>21.8±0.5</td>
<td>22.6±0.4*</td>
</tr>
<tr>
<td>BMR, W</td>
<td>0.30±0.02</td>
<td>0.40±0.01***</td>
<td>0.34±0.03</td>
<td>0.36±0.01#</td>
<td>0.35±0.01#</td>
<td>0.39±0.02</td>
</tr>
<tr>
<td>BMR, mW/g LM</td>
<td>14.3±0.9</td>
<td>17.3±0.3**</td>
<td>16.0±1.5</td>
<td>15.8±0.8</td>
<td>16.2±0.7</td>
<td>17.1±0.5</td>
</tr>
<tr>
<td>BMR, mW/g BW</td>
<td>8.3±0.5</td>
<td>7.7±0.2</td>
<td>10.7±1.3</td>
<td>7.4±0.4###</td>
<td>11.2±0.5###</td>
<td>8.0±0.4*</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>2.9±0.05</td>
<td>2.3±0.06</td>
<td>4.5±0.05###</td>
<td>3.0±0.04###</td>
<td>7.2±0.1### &amp;</td>
<td>4.3±0.06### &amp;</td>
</tr>
</tbody>
</table>

All values are means ± SE; n = 6–7. HFD, high-fat diet; BMR, basal metabolic rate; LM, lean mass; BW, body weight. Final body weight, body lipid content, lean mass, and average daily food intake during weeks 5–8 of acclimation as well as the BMR (expressed per animal, per LM, and per BW) of the mice are shown.

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different conditions (diet and temperature) for 6 mo. The food intake was markedly different between the mice living at different temperatures (Table 1), confirming the large effect of ambient temperature on metabolism. Using this approach, mice with very different degrees of obesity (>4-fold difference in lipid mass) were obtained (Table 1). The mice were analyzed as in Figs. 1 and 2, and the resulting Scholander plots are shown in Fig. 3, A–C, based on total energy expenditure per mouse. As seen in Fig. 3, A–C, HFD-fed mice consistently displayed slightly higher levels of energy expenditure per mouse than did lean mice (see below). Surprisingly, there was no effect of either diet or housing temperature on the insulation of the mice, as is visible in the slopes of the Scholander plots in Fig. 3, A–C, and as is calculated in Fig. 3D. Plotting insulation vs. either lean (Fig. 3E) or lipid mass (Fig. 3F) revealed no effect of obesity. Thus, we were unable to detect insulating effects of adiposity despite profound differences in the degree of lipid mass in the different groups. Also, in contrast to expectations (but see Refs. 23 and 39), cold acclimation in itself did not increase the insulation of the animals (see Discussion).
Normalization of Energy Expenditure to Lean Mass Does Not Effect the Outcome

The calculation of insulation based on the total energy expenditure levels per mouse revealed no effect of obesity on insulation but a small effect on total metabolic rates. However, the entire mouse is not metabolically active, as the lipids themselves (triglycerides), as well as bone and fur, etc., are metabolically inert (i.e., the corresponding metabolic activity is localized to the lean mass around them). Therefore, we also calculated the insulation based on energy expenditure per lean mass (Fig. 4). As shown in Fig. 4, A–C, the normalization to lean mass virtually eliminated the slight differences in energy expenditure levels between lean and obese mice (cf. Fig. 3). This difference was thus mainly due to the increase in lean mass associated with the expansion of the adipose tissue. However, the slopes of the Scholander curves remained the same in lean and obese mice. Calculation of insulation (Fig. 4D) revealed no effects of housing temperature or diet on insulation, even when calculated based on energy expenditure normalized to lean mass. Correspondingly, correlation analysis clearly showed no correlation between insulation and lean mass (Fig. 5E). Thus, even when energy expenditure was normalized to lean mass to account for differences in metabolically active tissue, no insulating effect of obesity was seen.

The Misleading Effect of Normalization to Body Weight

Normalization to body weight is often used to “correct” for differences in body size. As pointed out in many articles (9, 10, 24, 42, 53), in obesity research this approach must necessarily lead to misleading conclusions, as the increase in body weight in obesity is due largely to an increase in the amount of metabolically inert lipids (Table 1). To examine whether such a traditional normalization approach would affect the apparent outcome of the insulation measurement, we performed the same data analysis as described in Figs. 3 and 4, using energy expenditure values normalized to body weight. In contrast to the previous ways of analyzing, normalization to body weight had a qualitative effect on the interpretation of the influence of obesity on the (apparent) insulation of the mice. As shown in Fig. 5, A–C, normalization to body weight necessarily yielded energy expenditure levels in the HFD-fed mice that were much lower than in the chow-fed mice, irrespective of acute ambient temperature. Because this apparent hypometabolism in the HFD-fed mice was proportional throughout the temperature range, it necessarily resulted in a change in the slope of the curves, yielding lower slopes in HFD-fed mice. Thus, strikingly, normalization to body weight resulted in a misleading impression of significantly higher insulation in HFD-fed mice than in chow-fed mice (Fig. 5D). Even so, notably, there was no effect of acclimation temperature on insulation. Correlation analysis resulted here in an apparently significant correlation between insulation and lean mass (Fig. 5E) and lipid mass (Fig. 5F). Thus, division of energy expenditure by body weight (as is commonly done) may misleadingly give an impression of higher insulation in obese mice.

Fig. 4. Effects of housing temperature and diet; calculation of insulation based on energy expenditure per lean mass. The same data as in Fig. 3 were analyzed with energy expenditure normalized to lean mass (LM). A–C: Scholander plots. D: insulation. E and F: correlation between insulation and lean mass (E) and lipid mass (F). E: \( r^2 = 0.0001 \); F: \( r^2 = 0.002 \) (both not significant). All values are means ± SE; \( n = 41 \) in E and F, and \( n = 6–7 \) in A–D.
Table 2. Characteristics of the DIO mice

<table>
<thead>
<tr>
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<th>Control Diet</th>
<th>DIO</th>
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<tr>
<td>BW, g</td>
<td>37.5 ± 1.3</td>
<td>48.3 ± 0.3*</td>
</tr>
<tr>
<td>Lipid mass, g</td>
<td>9.0 ± 0.6</td>
<td>20.3 ± 0.3*</td>
</tr>
<tr>
<td>LM, g</td>
<td>21.3 ± 0.2</td>
<td>22.9 ± 0.2*</td>
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All values are means ± SE; n = 7–9. DIO, diet-induced obese. BW, body lipid content, and LM of the mice used in Fig. 6 are shown. *P ≤ 0.001 between diets.

Fig. 5. Effects of housing temperature and diet: misleading calculation of insulation based on energy expenditure per body weight. The same data as in Fig. 3 were analyzed with energy expenditure normalized to body weight (BW). A–C: Scholander plots. D: insulation. Two-way ANOVA showed significant effect of diet (P ≤ 0.001) but no effect of temperature and no interaction. E and F: correlation between insulation and lean mass (E) and lipid mass (F). E: r² = 0.12 (P ≤ 0.05); F: r² = 0.42 (P ≤ 0.001). All values are means ± SE; n = 41 in E and F, and n = 6–7 in A–D. *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001 between diets at each acclimation temperature (Student’s t-test). Note that the gray correlation lines in A, B, C, E, and F and striped colored bars in D (compared with the solid colored bars in Figs. 3D and 4D) are used to indicate that these data result from erroneous normalizations.

**Standard Diet-Induced Obese Mice Are Not Better Insulated than Lean Mice**

To verify our finding of obesity not affecting insulation in mice, we performed the same experiments using commercially available diet-induced obese (DIO) mice (see Table 2 for characteristics of the mice used). DIO mice and mice fed the corresponding low-fat diet were analyzed using either whole mouse energy expenditure or energy expenditure normalized to lean mass or to body weight (Fig. 6). Similarly to the obese mice analyzed in Fig. 3, DIO mice showed slightly but constantly elevated metabolic rates at all environmental temperatures (Fig. 6A), principally in accord with the findings of Abreu-Vieira et al. (1). However, again, the slope of the Scholander curves and the resulting calculated insulation (Fig. 6B) were similar in DIO and lean mice. No positive correlation between insulation and lean mass (Fig. 6C) or lipid mass (Fig. 6D) was found.

Normalization to lean mass did not affect the slope or the calculated insulation (Fig. 6, E and F), and there was no significant correlation between insulation and either lean or lipid mass (Fig. 6, G and H). Normalization to body weight again misleadingly reduced the slope of the Scholander curves in the DIO mice (Fig. 6I), thereby implying an increase in the apparent insulation of the animals (Fig. 6J) but, even so, only with a nonsignificant trend toward positive correlation between insulation and lipid mass (and no correlation with lean mass; Fig. 6, K and L). Thus, the results seen in the mice used in Figs. 3–5 were principally reproduced in this experiment, and again no effect of obesity on insulation was found.

**Genetic Obesity Does Not Lead to Increased Insulation**

To further confirm the lack of effect of obesity on insulation, we performed Scholander experiments on genetically obese (leptin-deficient) ob/ob mice and their lean littermates. These mice are characterized by developing massive obesity (Table 3). Similarly to the case in DIO mice (Figs. 3 and 6), and as pointed out earlier (21, 31), the total metabolic rates of ob/ob mice were higher than in the wild-type mice during the entire Scholander experiment (Fig. 7A). However, the slopes of the Scholander curves were similar. Thus, calculation of insulation revealed no effect of genetic obesity on insulation (Fig. 7B). Normalization of the energy expenditure to body weight again led to the misleading impression of increased insulation in obese mice.
Fig. 6. Effects of standard diet-induced obesity on insulation. Standard diet-induced obese (DIO) mice and mice fed the corresponding low-fat control diet were purchased and analyzed as described in Figs. 1–5. The effects of different types of normalization are shown: total energy expenditure per mouse (A–D), normalization of energy expenditure to lean mass (LM; E–H), and normalization to body weight (BW; I–L). A, E, and I: Scholander plots. B, F, and J: insulation. C, G, and K: correlation between insulation and lean mass and between insulation and lipid mass (D, H, and L). C: \( r^2 = 0.27 \) \( (P \leq 0.05) \); D: \( r^2 = 0.07 \); G: \( r^2 = 0.13 \); H: \( r^2 = 0.01 \); K: \( r^2 = 0.0001 \); L: \( r^2 = 0.14 \). All values are means ± SE; \( n = 16 \) for the correlations in C, D, G, H, K, and L, and \( n = 7–9 \) in A, B, E, F, I, and J. (*) \( P \leq 0.1 \) between diets.

(Fig. 7C). Thus, irrespective of the cause of obesity (diet/ genetics), the resulting obesity does not influence insulation.

No Evidence for Strain Dependence of the Absence of Insulating Effect of Obesity

Whereas mice of the C57BL/6 strain are generally described as obesity prone, mice on the 129S background are characterized by being resistant to diet-induced obesity. To examine whether a defect in insulation in 129S mice, leading to increased metabolism, could be responsible for their lean phenotype, we measured the insulation of BL/6 and 129S mice. The mice were analyzed before and after a 4-wk period of high-fat diet (HFD) feeding starting at 12 wk of age. As shown in Table 4, the BL/6 mice on HFD gained much more weight than the 129S mice. However, even in the 129S, feeding a HFD led to a doubling in lipid weight. As shown in Fig. 8, A and B, the 129S mice displayed elevated metabolic rates, expressed here per lean mass, compared with C57BL/6 at all temperatures, but the slope of the Scholander plots was similar in BL/6 and 129S mice. HFD feeding reduced the difference in energy expenditure levels between BL/6 and 129S, whereas the slopes were again similar (Fig. 8C). Thus, the calculated insulation was not significantly different between BL/6 and 129S mice, and HFD feeding again did not affect insulation (Fig. 8D). Correlation analysis revealed no significant positive correlation between insulation and lipid mass in either 129S mice (Fig. 8E) or BL/6 mice (Fig. 8F). Thus, the resistance to obesity in the 129S strain cannot be ascribed to a lower insulation.

Table 3. Characteristics of the ob/ob mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ob/ob</th>
</tr>
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<tbody>
<tr>
<td>BW, g</td>
<td>27.4 ± 0.5</td>
<td>48.1 ± 0.9***</td>
</tr>
<tr>
<td>BMR, W</td>
<td>0.24 ± 0.01</td>
<td>0.36 ± 0.02**</td>
</tr>
<tr>
<td>BMR, mW/g BW</td>
<td>8.82 ± 0.41</td>
<td>7.47 ± 0.33*</td>
</tr>
</tbody>
</table>

All values are means ± SE; \( n = 6 \). BW, BMR, and BMR normalized to BW of the mice used in Fig. 7 are shown. Note that the mice have already been described by Fischer et al. (21). * \( P \leq 0.05 \), ** \( P \leq 0.01 \), and *** \( P \leq 0.001 \) between genotypes.
and after feeding HFD for 4 wk (16 wk) are shown. *
P
LM, g 18.4
Lipid mass, g 2.7

In the present investigation, we analyzed the effects of obesity and of housing temperature on insulation in mice. Despite the obvious significance of insulation for analysis of metabolic data, we are unaware of earlier publications systematically quantifying the insulating role of obesity in laboratory mice. Although an insulating effect of fur as such was easily demonstrated, we found that neither any type of obesity nor any acclimation temperature had any impact on the insulation.

**Estimation of Insulation Based on Scholander Plots**

We have used Scholander plots here to quantitate insulation. For these plots, the extra heat production needed to counteract heat loss at different cold ambient temperatures is plotted against these ambient temperatures. This is in principle similar to the determination of insulation (or thermal conductance) in physical structures and presumes that the insulator is passive, a prerequisite that may not be correct in animals. However, the resulting Scholander plots will normally still be linear; i.e., the insulation part of the animal behaves as if it was a single physical structure. Because the outcome is linear, the slope of the curve (principally the insulation) can be determined without knowledge of the “true” body temperature, given that the energy expenditure is measured at different ambient temperatures and that the resulting data are proportional to the change in ambient temperature. This means that the mouse defends a “functionally defined” body temperature that may or may not coincide with a measured body temperature. For the Scholander analysis, it is thus essential to use the “functionally defined” body temperature. This is what we have done here by determining the insulation directly from the slope of the Scholander curves.

**Fur is Responsible for Half the Insulation of Mice**

We found that fur removal almost halved the insulation of the mice despite mouse fur being very short. This not only demonstrated the significance of fur for protection against heat loss in mice but also validated the method used here and indicated that alterations in insulation in the order of less than 20% can be detected by this method.

Although no direct quantification of the significance of fur for mouse insulation was reported earlier, there are several indications that fur is quantitatively important. These indications derive mainly from measurements of a higher rate of oxygen consumption at a single ambient temperature in shaved mice (26, 33). From such single ambient temperature oxygen consumption studies (26), theoretical Scholander curves may be constructed (42). Such curves come out close to the curves experimentally obtained here. Also, in genetically nude mice, higher rates of oxygen consumption were observed (15, 26, 29, 41, 51), but the outcome is more difficult to evaluate in the genetically nude mice, as other factors may be associated with the nude phenotype. Also food intake is higher at “normal” laboratory temperatures in shaved mice and in genetically nude mice (40).

The result of the shaving experiment also demonstrates that the mouse has no further means to physiologically increase insulation after the fur is removed; i.e., full physiological insulation is already achieved (through vasoconstriction, etc.). Although it may be said that insulation is not only physical (e.g., through the fur) in the mouse but also physiological (1),

**Table 4. Characteristics of 129 and BL6 mice**

<table>
<thead>
<tr>
<th></th>
<th>12 Wk</th>
<th>16 Wk</th>
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</thead>
<tbody>
<tr>
<td>BW, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>129 Chow</td>
<td>23.8 ± 0.6</td>
<td>24.6 ± 0.7</td>
</tr>
<tr>
<td>BL6 Chow</td>
<td>25.2 ± 0.2*</td>
<td>27.9 ± 0.6**</td>
</tr>
<tr>
<td>Lipid mass, g</td>
<td>2.7 ± 0.1</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>129 Chow</td>
<td>28.1 ± 0.1</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>BL6 Chow</td>
<td>20.1 ± 0.2**</td>
<td>21.1 ± 0.3***</td>
</tr>
<tr>
<td>LM, g</td>
<td>18.4 ± 0.4</td>
<td>18.2 ± 0.5</td>
</tr>
<tr>
<td>129 Chow</td>
<td>20.1 ± 0.2**</td>
<td>21.1 ± 0.3***</td>
</tr>
<tr>
<td>BL6 Chow</td>
<td>20.4 ± 0.6*</td>
<td>20.4 ± 0.6*</td>
</tr>
</tbody>
</table>

All values are means ± SE; n = 5–6 for 16-wk-old mice and n = 11 for 12-wk-old mice. BW, LM, and lipid mass of the 129 and BL6 mice before (12 wk) and after feeding HFD for 4 wk (16 wk) are shown. *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001 between genotypes.
this component is apparently already fully utilized and cannot be further recruited when the animal is challenged.

Our data also underline the tenet that alteration (reduction) of the insulation capacity of the fur is in itself sufficient to cause profound metabolic alterations (42). As seen in Fig. 2, furless mice examined at “standard” laboratory temperatures (18–20°C) have a need for extra (i.e., above basal metabolic rate) heat production (to compensate for the extra heat loss) that is nearly twofold higher than that of normal mice. This thus constitutes a need for a higher rate of thermogenesis and will in itself lead to activation of brown adipose tissue (BAT) and, with time, to recruitment of BAT and browning of white fat. The extra energy consumed for heat production will thus protect against diet-induced obesity. Therefore, several “browning agents” (agents that recruit BAT and that “brown” white adipose tissue, with implied anti-obesity effects) may work by decreasing the insulation of the mice, e.g., through alterations of the amount or quality of the fur, similar to the shaving used here. These mice will be subject to an increased heat loss at any ambient temperature below thermoneutrality; i.e., the mice will experience the ambient temperature as colder than that at which they are formally housed. Indeed, several “browning agents” (including genetic modifications) result in mice without fur or with visibly altered fur, and several others may, in other ways, decrease insulation (reviewed in Ref. 42) and thus induce “browning”. Sometimes this explanation for published browning effects is recognized (25, 26, 57), but often it is not.

The Obesity Resistance of 129S is Not Explainable By Increased Heat Loss

The 129S mouse strain, as well as, e.g., the AJ mouse, is generally considered to be resistant to diet-induced obesity (4, 6). The metabolic background for this is not understood. It could be reasoned that the resistance is due to low insulation so that at any subthermoneutral temperature the 129S mice would experience more cold than the C57BL/6 mice. This would also explain the higher degree of BAT recruitment (20) and browning of the white fat (56) seen in these mice. However, we did not observe any difference in insulation between the strains, so there is no difference in heat loss cannot explain the leaner phenotype.

Fig. 8. Strain dependence of insulation. Scholander experiments were performed on 12-wk-old 129S and C57BL/6 mice and on the same mice after an additional 4 wk of feeding HFD/chow diet. A: Scholander plot of 12-wk-old mice fed chow diet. B: Scholander plot of 16-wk-old mice fed chow diet. C: Scholander plot of 16-wk-old mice fed HFD for 4 wk. In A–C, ambient temperature is the set chamber temperature. D: insulation. One-way ANOVA revealed no significant differences. E and F: correlation between insulation and lipid mass in 129S mice (E) and BL6 mice (F). E: r^2 = 0.154; F: r^2 = 0.1 (both not significant). All values are means ± SE; n = 22 in E and F, and n = 5–6 in A–D. LM, lean mass.
Acclimation Temperature Does Not Affect Insulation

Surprisingly, cold acclimation of the mice did not affect their thermal insulation. This finding is in contrast to a general anticipation of the effects of acclimation to cold and to implications from several earlier studies (see below). However, Meyer et al. (39) also reported unchanged thermal conductance (insulation) when mice acclimated to temperatures similar to those used here were examined under subthermoneutral conditions.

With regard to insulation, cold could potentially recruit several mechanisms. One would be to increase the thickness of fur. Indeed, several studies have shown effects of cold acclimation on hair coat development and hair thickness in mice housed at −3°C (7) or that of the offspring of mice that had been housed at −3°C for multiple generations (8). Similarly, rats housed at 17°C from birth developed a greater coat mass than rats reared in the warm (54). In the above-mentioned studies, either the animals were exposed to cold shortly after birth or the parental generations had been living in the cold, but Al-Hilli and Wright (2) showed that even mice exposed to cold (8°C) at 23 days of age developed longer and thicker hair than control animals. However, even if such changes in fur should have occurred here in our experiments, we see no effects on insulation.

Alternatively, cold acclimation could increase the thickness of an insulating fat layer. Although much fat is found deeper in the body of mice, mice display a layer of fat in the skin presently referred to as dermal adipose tissue (3, 18, 34). Indeed, the absence of this fat layer (caused by ablation of the syndecan-1 gene) makes mice susceptible to cold, and the thickness of this fat layer is doubled by acclimation to 23 vs. 30°C (32). There are thus clear indications, both through fur growth and through more dermal fat, that mice would become more insulated following cold acclimation. However, our data and those of Meyer et al. (39) clearly demonstrate that no increased insulation is actually observed.

Obesity Does Not Insulate Mice

Probably as an extrapolation of the idea that the blubber fat layer found under the skin of seals and whales acts as thermal insulation [and it may even be discussed if this is the case, as the blubber may have energy storage as a primary purpose (36)], it has generally been considered that fat depots in humans and in experimental animals (mice) could play a role in thermal insulation (but see Ref. 1). Following this reasoning, the fat in obese persons has been discussed to possess an insulating function, protecting the obese against heat loss and in this way diminishing caloric combustion and therefore adding to the development of obesity.

However, we were unable to observe any insulating effect of obesity in several models of mouse obesity (Figs. 3, 4, and 6–8). Only if the energy expenditure data were analyzed by dividing by body weight would an effect of obesity be apparent (Figs. 5–7), but this is a misleading representation, as the whole obese mouse does not lose less heat (Figs. 3 and 7).

Some insulating effect of the fat may nevertheless be consistent with the data obtained here. This is because heat is lost via and thus proportional to the surface of the mouse, and the surface area of a mouse is principally a function of body weight. Geometrically for a sphere, surface area is proportional to body volume (≈weight)^0.67. The most obese mice used here were ≈50 g vs. the leanest, ≈25 g, yielding proportionality factors of 13.6 vs. 8.6. Because the mice are not spheres, the values have to be adjusted with the empirical Meeh factors, which have been established to be 8.3 for obese mice and 9.8 for normal mice (11). The heat loss factors should thus be 115 vs. 84, i.e., indicating some 36% greater surface area and heat loss in the obese mice at a given subthermoneutral ambient temperature. Because we see no increase in heat loss (increased metabolism) per mouse between lean and obese mice, the implication is that insulation has indeed been increased due to obesity, rather surprisingly to an extent that exactly counteracts the increase in surface area. Thus, at the whole mouse level, there would be no effect on metabolism.

One reason that no effect of obesity on insulation is observed may be the localization of the adipose depots. A major part of the fat in the mouse body is not found in a layer that could be discussed anatomically as having an insulating function. Rather, many of the depots are found deep in the body (e.g., mesenteric), and even those found more peripherally (often termed subcutaneous) are not distributed as a uniform layer wrapped around the body but rather consist of several separated depots (13, 16). Thus, much of the fat could anatomically be considered in retrospect to be unable to perform any insulating task. Only the dermal fat has a distribution appropriate for an insulating function, and indeed, loss of this adipose tissue (through ablation of the syndecan-1 gene) is reported, as noted above, to result in higher cold sensitivity of the mice (32). However, the alterations in dermal fat thickness caused by acclimation to different temperatures (32) do not seem to alter insulation (Fig. 3), and the high cold sensitivity of the syndecan-1-ablated mice could be secondary to further structural skin or fur problems caused by the loss of the dermal adipocytes or by other effects of the absence of syndecan-1.

Although not documented, it is clear from dissection of obese mice that even the subdermal adipose tissue is increased in obesity, and an influence on thermoregulation would be expected in these mice. Again, we were unable to observe such an effect in any of the obesity models tested here.

All of the above experiments have been performed with animals exposed to cold air. Because of the large difference between the thermal conductivity of air and water (52), the data here cannot be directly considered to also be relevant for animals exposed to cold water.

Does Obesity Insulate Humans?

Full quantitative insulation studies such as those performed here on mice have not been performed on humans. Because of the human size and surface/volume ratio, and thus high thermic inertia, such experiments are not easily performed. Nonetheless, the general connotation has been that obese persons are better insulated than lean persons, and this would tend to aggravate the obesity. Based on the mouse data presented here, the question may be raised as to whether this is really the case. Just as in mice, human obesity is may be associated with central rather than peripheral lipid accumulation, and as in mice, increased subcutaneous fat may not necessarily lead to better insulation.

However, there are reports that obese women have a lower abdominal skin temperature when exposed, lightly dressed, to
subthermoneutral temperatures (20–25°C) (12, 30, 46, 47). The connotation that fat insulates in humans seems experimentally to be based primarily on this type of observation. There are some studies examining possible higher cold-induced heat production in lean and obese persons, but the outcome is not fully clear, e.g., because the effect is not of obesity but of not being lean (46), values are normalized with a factor akin to dividing by body weight (14), or the obese group was very heterogeneous (58). Thus, despite the ability of humans to carry much thicker layers of fat than mice can, it has not been firmly established that obese persons empirically are more insulated than normal persons.

However, as implied in the introduction, in any case, this may not be an important issue in human metabolic research. This is because of the different thermic environments of human beings vs. experimental mice. In contrast to mice at 20°C that constantly have to use energy to counteract heat loss to the environment, humans normally live de facto under thermoneutral conditions (housing plus clothing). Therefore, it is doubtful that an insulating effect of obesity, even if it existed, would in any discernable way affect the development or maintenance of human obesity. At thermoneutrality, no extra food combustion is required to counteract heat loss, and the degree of insulation would thus not play any role for the metabolic balance equation for normal life humans.

Thus, in contrast to common views, we demonstrate here that at least in mice obesity is not associated with increased insulation, and obesity thus does not in this way affect the metabolism of mice. Whereas it may not be possible to extrapolate our findings directly to humans, it may also be understood that, even in human metabolism, obesity would not reinforce itself due to a possible insulating effect, as humans are not living under cold stress.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
A.W.F., B.C., and J.N. conception and design of research; A.W.F., R.C., and G.v.E. performed experiments; A.W.F., B.C., and J.N. interpreted results of experiments; A.W.F. prepared figures; A.W.F. and G.v.E. analyzed data; A.W.F., B.C., and J.N. edited and revised manuscript; A.W.F., R.C., G.v.E., B.C., and J.N. approved final version of manuscript.

REFERENCES

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