Effect of a high or low ambient perinatal temperature on adult obesity in Osborne-Mendel and S5B/Pl rats

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White, Christy L., H. Doug Braymer, David A. York, and George A. Bray. Effect of a high or low ambient perinatal temperature on adult obesity in Osborne-Mendel and S5B/Pl rats. *Am J Physiol Regul Integr Comp Physiol* 288: R1376–R1384, 2005. First published January 27, 2005; doi:10.1152/ajpregu.00162.2004.—Perinatal environment is an important determinant of health status of adults. We tested the hypothesis that perinatal ambient temperature alters sympathetic activity and affects body composition in adult life and that this effect differs between SSB/Pl (S5B) and Osborne-Mendel (OM) strains of rat that were resistant (S5B) or susceptible (OM) to dietary obesity. From 1 wk before birth, rat litters were raised at either 18 or 30°C until 2 mo of age while consuming a chow diet. Rats were then housed at normal housing temperature (22°C) and provided either high-fat or low-fat diet. OM rats initially reared at 18°C gained more weight on both diets than those reared at 30°C. Perinatal temperature had no effect on body weight gain of the SSB rats on either diet. At 12 wk of age, OM and SSB rats reared at 18°C had higher intakes of the high-fat diet than those reared at 30°C but lower intakes of the high-fat diet than those reared at 30°C. Lower perinatal temperature differentially affects body weight and metabolic rate in response to the sympathetic 3-agonist CL-316243, was greater in both OM and SSB rats reared at 18°C than in those reared at 30°C. Perinatal temperature differentially affects body weight in OM and SSB rats while having similar effects on food intake, response to a 3-agonist, and BAT 3-AR and UCP-1. The data suggest that OM rats are more susceptible to epigenetic programming than SSB rats.

Body weight; food intake; metabolic rate; dietary obesity; neonatal environment; fetal programming

Obesity has become a major health concern. Worldwide, 7% of the adult population is estimated to be obese (43). In the United States, the prevalence increased 5.6% just from 1999 to 2000 (31). Childhood obesity is on the rise (17), and obese children are at higher risk of becoming obese adults (44) with an increased risk of early mortality in adulthood (15). The consequences of obesity, diabetes, cardiovascular disease, hypertension, and some forms of cancer (46) present as a medical problem that has been described as “an epidemic that threatens global well being” (21).

Both genetic and environmental factors play a role in the development of obesity. Whereas single gene mutations causing obesity are well known in rodents, they are rare in humans. Multigenic interactions are more relevant to human obesity, for which the genetic contribution has been estimated to account for 25–80% of the variability in obesity (40, 52). More importantly, genes affect how a phenotype responds to the environment. The environmental influence on obesity is also strong, as illustrated by the differences between Pima Indians living in the U.S. and those still residing in their native Mexico (40). Meal size, dietary fat, and a sedentary lifestyle are all thought to be environmental factors that contribute to obesity (1, 3, 17, 38, 50). Animal studies have shown that a high-fat diet will lead to obesity (51) and leptin resistance (28).

The perinatal environment is particularly important to development. Influences during this time program the plasticity of the nervous system and may cause permanent changes that are carried on into adulthood and across generations (30). For example, in rodents, neonatal overfeeding, caused by small litter size or by insulin injections into neonatal rats, may lead to obesity, diabetes, and altered sympathetic activity in adult animals (9, 16, 35, 53). Food restriction during the first 2 wk of pregnancy leads to obesity that is affected by the composition of the diet (18, 19). Studies in humans have demonstrated that both maternal smoking and maternal diabetes during gestation will lead to adult obesity in the offspring (8, 37, 45).

Studies in Sprague-Dawley rats demonstrate the importance of the interaction between the perinatal environment and genotype. Some Sprague-Dawley rats are prone to diet-induced obesity (DIO), whereas others are diet resistant (DR) (27), and a substrain of DIO and DR rats can be created, suggesting polygenic inheritance (25). Furthermore, maternal obesity has been related to increased obesity and alternations in brain monoamine function in obesity-prone offspring (24, 26).

Environmental temperature is also a significant factor during the neonatal period. Rat pups raised at low ambient temperatures (16 or 18°C) for the first 1 or 2 mo of life have higher sympathetic activity than pups raised at elevated ambient temperatures (28 or 30°C) (2, 32, 33, 56). Activation of the sympathetic drive to brown adipose tissue (BAT) in response to cold is enhanced in mice that have previously been acclimated to the cold (20). Rats initially raised at 18°C also have higher body weights when eating either rat chow or a high-sucrose diet than those raised at 30°C (55). In addition, a recent study in human beings has shown that women have increased prevalence of coronary heart disease when born during the coldest months of the year (23). There are many examples of rodents that do not become obese eating a high-fat diet [SWR and A/J mice and SSB/Pl (S5B) rats] and of rodents that do become obese eating a high-fat diet [C57BL/6J and AKR mice, Osborne-Mendel (OM) rats]. However, body fat is also higher in OM rats than in SSB rats when both are eating a low-fat diet (42). The OM rat prefers fat, whereas the SSB rat prefers carbohydrates (34), and when only the high-fat diet is available do OM rats eat more and become obese (10). The SSB rat has greater stimu-

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luation of the sympathetic nervous system to interscapular brown fat than OM rats in response to a high-fat diet, as illustrated by their higher norepinephrine concentration and turnover rate in BAT (11). Given that the activity of the sympathetic nervous system has been related to feeding behavior and that both are fundamental to the control of energy balance, the reduced level of activity of the sympathetic nervous system in OM rats may enhance their susceptibility to the fattening consequences of eating a high-fat diet. To test this hypothesis, we reared OM and S5B rats at either 18 or 30°C for their first 2 mo of life and then exposed them to either a high-fat or a low-fat diet as adults while maintaining them at an environmental temperature of 22°C.

MATERIALS AND METHODS

Animal maintenance and breeding. OM and S5B rats were obtained from the Pennington Center Vivarium. A separate breeding colony was set up for these experiments. One male was paired to one female for 1 wk for breeding purposes. One week before the females were to give birth, they were moved out of the breeding colony into temperature-controlled chambers (Powers Scientific, Pipersville, PA). One half of the group of dams of each strain went into a 30°C chamber, and the other half into an 18°C chamber. The chambers had glass doors, so illumination was provided by room lighting, which was set at a 12:12-h light-dark cycle with lights coming on at 0700. Within 24 h of birth, the dams were limited to 7–8 pups each. Animals were housed in plastic shoebox containers and fed chow (Rodent Chow 50001; Purina Mills, St. Louis, MO) ad libitum. Tap water was available in water bottles. Pups were weaned at 3 wk of age and housed two per cage in shoebox cages at that time. Because of the possibility of sexual dimorphism, only the male pups were utilized in the studies reported in this article.

At 60 days of age, pups were removed from the temperature-controlled chambers and singly housed in hanging wire-bottomed stainless steel cages in a room maintained at 22°C (±1°C). Water was available ad libitum through an automated watering system. There were 25–26 animals per group through 8 wk of age except for the metabolic chambers, in which 6 representative animals per group were studied. Eight rats from each group were killed at 8 wk of age. The remaining 17–18 animals from each group were then divided into a high-fat diet group and a low-fat diet group with 8–9 animals per group. The Institutional Animal Care and Use Committee approved all the experimental protocols, which were consistent with the National Institutes of Health guidelines for the use of experimental animals.

Diets. Pups were weaned onto a rat chow diet (Rodent Chow 50001; Purina Mills) at 3 wk of age. At 8 wk of age, when rats were removed from the chambers, they were switched to pelleted high- or low-fat diets (Research Diets D01080901 and D080902, respectively; New Brunswick, NJ). The high-fat diet consisted of 55% fat energy, 21% carbohydrate energy, and 24% protein energy and had an energy density of 4.84 kcal/g (Table 1). The low-fat diet consisted of 10% fat energy, 66% carbohydrate energy, and 24% protein energy with an energy density of 3.71 kcal/g. These diets were fed ad libitum unless otherwise noted.

Body weight and body composition analysis. Body weight was measured when pups were 1 wk of age, at weaning, and then weekly thereafter. The Lee index of adiposity (the cube root of body weight divided by the nasaonal length) was calculated at 15 wk of age. Rats were anesthetized with isoflurane, and the nasaonal length was measured. Eight rats in each group were killed at 8 wk of age for tissue weight analysis immediately before all remaining rats were moved from the chambers into a 22°C housing environment. The remaining rats were killed at 16 wk of age. Tissue weights were taken at that time.

Food intake measurements. Daily food intakes were measured over the course of 4 days at 10, 12, and 15 wk of age. Food remaining in the hopper and the spillage below the cage were measured every 24 h. An average of food intake over the 4-day period was then taken.

Measurement of energy expenditure. Energy expenditure (VO2) was measured using an Oxymax indirect calorimeter (Columbus Instruments, Columbus, OH). Rats were studied at 7 wk of age, before the special diets were started, and again when they were 13 wk of age, 5 wk after the special high- or low-fat diets were started. Rats were put in the metabolic cages at 1600 and fasted overnight, but they had free access to water throughout the experiment. The metabolic rate of 7-wk-old rats was assessed at the same ambient temperature at which they were being housed (either 18 or 30°C). The 13-wk-old rats were tested at the thermoneutral ambient temperature of 27°C. After adapting to the metabolic chambers overnight, rats were injected intraperitoneally with a β3-agonist, CL-316243 (Wyeth-Ayerst, Philadelphia, PA), at a dose of 1 mg/kg delivered in 1 ml/kg saline, and VO2 was measured for the next 4 h. Data from the 2 h before injection were used as baseline values.

Respiratory quotient (RQ) was analyzed as percent relative cumulative frequency (PRCF), a method described by Liu et al. (29). RQs were combined from 8–9 animals in each group to compile each curve, which was made up of a total of 650–730 measurements taken over a 22-h period. The benefit of PRCF is that it simplifies interpretation of large data sets and illustrates slight shifts in the curves.

Assays. Insulin, corticosterone, and leptin were analyzed with radioimmunoassay kits (Linco Research, St. Louis, MO).

RNA analysis. mRNA was isolated from BAT via the modified guanidinium-isothiocyanate method (4) using TRIzol (GIBCO BRL, Gaithersburg, MD). Samples were quantified by spectrophotometry, checked for quality on an agarose-formaldehyde gel, and then treated with DNase. The mRNA levels were determined using quantitative real-time RT-PCR in the ABI-Prism 7900 HT Sequence Detection system (Applied Biosystems, Foster City, California) with cyclophilin as an internal standard. Primers are listed in Table 2.

Data analysis. Data are presented as means ± SE. Statistical analysis was performed using repeated-measures analysis of variance (ANOVA) for body weight, weight gain, and food intake. A two-way ANOVA was used for tissue weights and metabolic chamber data at 8 wk, whereas a three-way ANOVA was used at 16 wk for tissue weights, metabolic chamber data, the Lee index, serum assays, and RNA data. Bonferroni’s adjustment was used in the post hoc analysis to preserve an overall P < 0.05 level.

RESULTS

Body weight and body composition analysis. Body weight for rats up to 8 wk of age is shown in Fig. 1. There were significant effects of strain [F(1,100) = 197.44, P < 0.0001]
The effects of perinatal temperature on weights of selected tissues are shown in Tables 3 and 4. At 8 wk of age (Table 3), there was a significant effect of temperature for interscapular BAT \([F(1,128) = 56.19, P < 0.0001]\) and of strain for gastrocnemius muscle \([F(1,128) = 63.06, P < 0.0001]\) and the retroperitoneal fat pad \([F(1,128) = 4.45, P = 0.0439]\). There was also a significant interaction between strain and temperature in the gastrocnemius muscle \([F(1,128) = 4.58, P = 0.0412]\). Interscapular BAT weight was significantly increased in animals reared at 18°C compared with those of the same strain reared at 30°C. Gastrocnemius weight was also significantly different between the two temperatures in OM rats. The gastrocnemius was the only tissue that was significantly different between the two strains, and this difference was observed at both ambient temperatures.

Tissue weights at 16 wk of age (Table 4) demonstrated significant main effects of strain \([F(1,63) = 6.37, P = 0.0142]\) and temperature \([F(1,63) = 9.83, P = 0.0026]\) for interscapular BAT. OM rats reared at 30°C eating a high-fat diet had significantly higher interscapular BAT weight than OM rats reared at 18°C. The weight of the gastrocnemius muscle showed a significant main effect of strain \([F(1,63) = 99.44, P < 0.0001]\). The gastrocnemius muscle weighed significantly more in OM rats than in SSB rats in each diet-temperature group. The retroperitoneal fat pad showed significant effects of strain \([F(1,63) = 78.99, P < 0.0001]\), temperature \([F(1,63) = 10.70, P = 0.0017]\), and diet \([F(1,63) = 61.41, P < 0.0001]\) as well as a significant interaction between strain and diet \([F(1,63) = 5.02, P = 0.0285]\). Weight of the retroperitoneal fat pad was significantly increased in OM rats reared at 18°C eating a high-fat diet compared with OM rats reared at 30°C. There was also a significant difference between OM and SSB rats in each diet-temperature group, with the fat pads from OM rats weighing more. Animals eating a high-fat diet in each strain-temperature group had significantly increased retroperitoneal fat pad weights compared with animals eating a low-fat diet. Weight of the retroperitoneal fat pad was also calculated as a percentage of the total body weight, and there was essentially no difference between this and the fat pad weights not corrected for body weight (data not shown).

The Lee index values of rats measured at 15 wk of age are shown in Table 4. There were significant effects of strain \([F(1,63) = 16.27, P = 0.0002]\) and diet \([F(1,63) = 52.76, P < 0.0001]\) and a significant interaction between temperature and diet \([F(1,63) = 4.75, P = 0.0331]\). The difference between the high-fat and low-fat diets was significant in each strain-
Table 3. Effect of perinatal temperature on body weight and tissue weights for OM and S5B rats at 8 wk of age

<table>
<thead>
<tr>
<th></th>
<th>OM</th>
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<th>S5B</th>
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<tr>
<td></td>
<td>18°C</td>
<td>30°C</td>
<td>18°C</td>
<td>30°C</td>
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<tr>
<td>n</td>
<td>25</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>254.6±4.1*</td>
<td>264.9±5.0†</td>
<td>207.2±3.0</td>
<td>197.5±2.0</td>
</tr>
<tr>
<td>Tissue weights, g</td>
<td></td>
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<tr>
<td>Interscapular BAT</td>
<td>0.41±0.04‡</td>
<td>0.21±0.03</td>
<td>0.46±0.03§</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>Retroperitoneal fat pad</td>
<td>0.72±0.01</td>
<td>0.87±0.17</td>
<td>0.62±0.08</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td>Gastrocnemius muscle</td>
<td>1.28±0.05*‡</td>
<td>1.46±0.06‡</td>
<td>0.99±0.03</td>
<td>0.94±0.05</td>
</tr>
</tbody>
</table>

Data are expressed in grams as means ± SE. *P < 0.05, difference between strains at 18°C. †P < 0.05, difference between strains at 30°C. ‡P < 0.05, difference between temperature in Osborne-Mendel (OM) rats. §P < 0.05, difference between temperature in S5B/pl (S5B) rats.

EFFECT OF A COLD PERINATAL ENVIRONMENT IN TWO RAT STRAINS

R1379

**Fig. 2.** Effect of perinatal temperature on body weight gain for 2 mo while OM or S5B rats were housed at room temperature and consumed either a high-fat (HF) or low-fat (LF) diet. Data are expressed as changes in mean weekly body weights (± SE) after rats were removed from temperature-controlled chambers. *P < 0.05, difference between temperatures in HF group. †P < 0.05, difference between temperatures in LF group. ‡P < 0.05, difference between diets in animals reared at 18°C. §P < 0.05, difference between diets in animals reared at 30°C.

温度群 ville except for the OM rats reared at 18°C. Additionally, there was a significant difference between the OM and S5B rats reared at 30°C eating a low-fat diet. The nasoanal length (Table 4) showed significant main effects of strain \[ F(1, 63) = 211.75, P < 0.0001 \] and temperature \[ F(1, 63) = 4.44, P = 0.0391 \] and a significant interaction between diet and temperature \[ F(1, 63) = 4.00, P = 0.0498 \]. OM rats were significantly longer than S5B rats in each temperature-diet group.

**Food intake measurements.** The effects of perinatal temperature on daily food intake are shown in Table 4. Food intake measurements were performed when rats were 10, 12, and 15 wk of age, but only the 12-wk data are shown for clarity. There were significant effects of strain \[ F(1, 63) = 74.55, P < 0.0001 \], temperature \[ F(1, 63) = 15.00, P = 0.0003 \], and diet \[ F(1, 63) = 28.30, P < 0.0001 \]. OM rats ate significantly more than S5B rats in every temperature-diet group. This effect of perinatal temperature was greater than intake of the low-fat diet in every strain-temperature group except for S5B rats reared at 30°C. In addition, OM rats reared at 18°C eating a low-fat diet had a significantly increased metabolic rate compared with those reared at 30°C in both strains at all time points, except for OM rats at 0–1 and 3–4 h after administration of CL-316243. S5B rats reared at 30°C had a significantly greater response than rats reared and tested at 18°C in both strains at all time points, except for OM rats reared at 0–1 and 3–4 h after administration of CL-316243 compared with OM rats reared at 30°C. At 13 wk, when all rats were tested at thermoneutrality, there was a significant effect of perinatal temperature \[ F(1, 63) = 16.05, P = 0.0002 \] on the percent increase in energy expenditure after administration of a CL-316243, as shown in Fig. 4. Rats reared at 18°C eating a low-fat diet had a significantly increased metabolic rate compared with those reared at 30°C in both OM and S5B strains. This effect of perinatal temperature in response to CL-316243 was not seen in rats fed the high-fat diet. Although not significant, there was a trend in animals
reared at 30°C to show a difference between the high-fat and low-fat diets that was not seen in animals reared at 18°C.

PRCF curves are shown in Fig. 5. Both strains showed a shift to the right toward a higher RQ in animals reared at 30°C compared with those reared at 18°C. This shift indicates that a higher frequency of higher RQ values is present in those groups. OM rats reared at 30°C also showed a shift to the right in animals fed the low-fat diet compared with those fed the high-fat diet. This difference with diet was not seen in the other strain-temperature groups.

Assays. The effects of perinatal temperature on serum levels of corticosterone, insulin, and leptin are shown in Table 6. There was a significant main effect of strain \( [F(1,62) = 6.07, P = 0.0165] \) on serum corticosterone levels. There were no significant individual differences. Analysis of serum insulin showed main effects of strain \( [F(1,63) = 30.22, P < 0.0002] \) and temperature \( [F(1,63) = 5.90, P = 0.0180] \) and an interaction between strain and diet \( [F(1,63) = 4.15, P = 0.0457] \). Serum insulin was higher in OM rats than in S5B rats in all the groups except for OM and S5B rats reared at 18°C eating a high-fat diet. Serum leptin showed significant main effects of strain \( [F(1,63) = 84.09, P < 0.0001] \) and diet \( [F(1,63) = 50.06, P < 0.0001] \) and an interaction between strain and diet \( [F(1,63) = 10.98, P = 0.0015] \). Serum leptin levels of OM rats were higher than those of S5B rats in every temperature-diet group. In addition, of the OM rats, those eating the high-fat diet reared at both 18 and 30°C had higher serum leptin levels compared with those eating the low-fat diet. S5B rats reared at 30°C eating a high-fat diet also had higher serum leptin levels than those eating the low-fat diet.

RNA analysis. The effects of perinatal temperature on gene expression in BAT are shown in Table 6. There was a significant main effect of temperature \( [F(1,51) = 16.72, P = 0.0002] \) on BAT \( \beta_3 \)-adrenergic receptor (\( \beta_3 \)-AR) mRNA levels. S5B rats reared at 30°C and eating a high-fat diet had higher \( \beta_3 \)-AR levels than those reared at 18°C. Levels of BAT uncoupling protein-1 (UCP1) mRNA showed significant main effects of temperature \( [F(1,58) = 21.82, P = <0.0001] \) and diet \( [F(1,58) = 6.42, P = 0.0140] \). Both OM and S5B rats eating a high-fat diet had increased UCP1 mRNA levels when reared at 30°C compared with those reared at 18°C. BAT UCP2 mRNA levels had a significant main effect of temperature \( [F(1,48) = 8.49, P = 0.0054] \). OM rats reared at 18°C and eating a high-fat diet had significantly higher UCP2 mRNA

| Table 4. Effect of perinatal temperature on OM and S5B rats at 12–16 wk of age while being housed at room temperature and consuming either a high-fat or low-fat diet |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | OM              | SSB             | OM              | SSB             |
|                                | 18°C            | 30°C            | 18°C            | 30°C            |
| n                               | 9               | 8               | 9               | 8               |
| Weight gain, g                  | 196.8 ± 11.2*†‡ | 168.5 ± 3.5*†‡  | 171.6 ± 6.9*†‡  | 126.5 ± 6.7     |
| Food Intake, kcal/day           | 87.5 ± 3.0*†‡   | 71.2 ± 1.3*     | 77.2 ± 2.7*†‡  | 67.1 ± 1.9*     |
| Nasoal length, cm               | 25.3 ± 0.26*†‡  | 25.1 ± 0.14*    | 24.9 ± 0.24*    | 24.9 ± 0.21*    |
| Lee index                       | 298.6 ± 1.8     | 293.4 ± 1.1     | 299.7 ± 1.4†‡  | 291.0 ± 2.0*    |
| Tissue weights, g               |                 |                 |                 |                 |
| Interscapular BAT               | 0.45 ± 0.07‡     | 0.47 ± 0.08     | 0.73 ± 0.15     | 0.54 ± 0.06     |
| Retroperitoneal fat pad         | 4.56 ± 0.38*†‡  | 2.61 ± 0.2*     | 3.61 ± 0.34*†‡ | 2.22 ± 0.21*    |
| Gastrocnemius muscle            | 2.72 ± 0.23*     | 2.78 ± 0.19*    | 2.85 ± 0.13*‡   | 2.76 ± 0.11*    |

Values are means ± SE. Weight gain and tissue weights were measured at 16 wk; food intake was measured at 12 wk; nasoanal length was measured at 15 wk; and the Lee index was measured at 15 wk and expressed as the cube root of body weight (grams)/nasoanal length (mm). *P < 0.05, strain difference in the same temperature-diet group. †P < 0.05, Diet difference in the same temperature-diet group. **P < 0.05, strain difference in the same temperature-diet group.

| Table 5. Effect of perinatal temperature on baseline metabolic rate for OM and S5B rats at in 7 and 13 wk of age |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | OM              | SSB             | OM              | SSB             |
|                                | 7-wk-old rats   |                 | 13-wk-old rats  |                 |
| VO₂BW⁰.⁷⁵                      |                 |                 |                 |                 |
| n                               | 6               | 6               | 6               | 6               |
| HF                               | 1,495.2 ± 30*   | 1,342.3 ± 83    | 1,537.3 ± 39*   | 1,241.7 ± 31    |
| LF                               |                 |                 |                 |                 |
| 18°C                            |                 |                 |                 |                 |
| HF                               | 680.1 ± 60      | 655.1 ± 31      | 683.9 ± 44      | 562.2 ± 79      |
| LF                               | 9               | 8               | 9               | 9               |

Seven-week-old rats were tested at their housing temperature, and 13-week-old rats were tested at a thermoneutral temperature. Baseline values of VO₂ (mL·kg⁻¹·h⁻¹) were averaged over a 2-h period and are expressed as a function of body weight (BW) raised to the 0.75 power (VO₂/BW⁰.⁷⁵). *P < 0.05, difference between 18 and 30°C animals.

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levels than those reared at 30°C. Levels of BAT UCP3 mRNA showed significant main effects of temperature \( F(1,55) = 13.85, P = 0.0005 \). S5B rats reared at 30°C and eating a high-fat diet had higher UCP3 mRNA levels than those reared at 18°C. Intriguingly, all of the significant effects observed with temperature were seen only in animals eating a high-fat diet, not in any of the low-fat diet groups.

The effects of perinatal temperature on gene expression in retroperitoneal white adipose tissue (WAT) are shown in Table 6. There was a main effect of strain \( F(1,50) = 7.74, P = 0.0076 \) on levels of \( \beta_3 \)-AR mRNA. However, there were no individual strain differences. UCP1 mRNA also had a significant main effect of strain \( F(1,47) = 16.49, P = 0.0002 \). There was a significant difference between OM and S5B rats reared at 30°C and eating a low-fat diet. There were no significant main effects observed for UCP2 mRNA. There was a significant main effect of strain for UCP3 mRNA \( F(1,53) = 8.81, P = 0.0045 \). There was a significant difference between OM and S5B rats reared at 30°C and eating a high-fat diet.

DISCUSSION

These experiments have explored the hypothesis that ambient temperature during pregnancy and the neonatal period has a significant effect on food intake and body fat in adult life and that these effects differ between the OM and S5B strains of rats. The major observation reported in this article is that perinatal temperature affected the weight gain of OM rats on both high-fat and low-fat diets, whereas it had no effect on the weight gain of S5B rats eating either diet. OM rats reared at 18°C gained more weight than those reared at 30°C during adult life irrespective of the diet on which they were placed. In addition, both OM and S5B rats raised at 18°C had an enhanced response to the \( \beta_3 \)-agonist CL-316243 compared with rats raised at 30°C when tested at 13 wk at thermoneutrality. Interestingly, this effect of perinatal temperature was greater in rats eating the low-fat diet rather than in those eating the high-fat diet in both strains. In contrast to the metabolic rate data, animals raised at 30°C had higher levels of \( \beta_3 \)-AR, UCP1, and UCP3 mRNA in BAT than those raised at 18°C, and this effect was seen only in animals eating a high-fat diet. OM and S5B rats had similar BAT UCP1 and \( \beta_3 \)-AR levels, responses to \( \beta_3 \)-agonist, and food intake levels, but only the OM rats gained weight when born and reared at the lower temperature. Although these results do not explain this difference, they do suggest that the animals were programmed in the perinatal period by environmental cold to alter their responses to dietary fat and \( \beta_3 \)-adrenergic agonists.

The effects of ambient perinatal temperature on weight gain were seen after the animals were removed from the environmentally controlled chambers and when eating the high- and low-fat diets. Three questions can be asked: 1) What is the composition of the weight gain? 2) What are the mechanisms underlying the response to neonatal rearing temperature in OM rats? 3) Why was this response not seen in S5B rats? We do know that while the OM rats are longer than the S5B rats, body length is not different because of the perinatal temperature. It is tempting to suggest that the increased body weight of OM rats reared at 18°C was due to an increase in body fat. However, evidence for this is not convincing. In the groups eating the high-fat diet, there was only a small increase in the weight of the retroperitoneal fat pad and no change in the Lee

Fig. 3. Effect of perinatal temperature on metabolic rate in 7-wk-old OM and S5B rats. Baseline values were averaged over a 2-h period. CL-316243 was then injected, and values for the next 4 h were taken. Values are expressed as percent increases over baseline values of VO\(_2\) (means ± SE). \( ^aP < 0.05 \), difference between strains at 30°C. \( ^bP < 0.05 \), difference between temperatures in OM rats. \( ^cP < 0.05 \), difference between temperatures in S5B rats.

Fig. 4. Effect of perinatal temperature on metabolic rate in 13-wk-old OM and S5B rats. Baseline values were averaged over a 2-h period. CL-316243 was then injected, and values for the next 4 h were taken. Values are expressed as percent increases over baseline values of VO\(_2\) (means ± SE). \( ^aP < 0.05 \), difference between temperatures in LF group.
Fig. 5. Effect of perinatal temperature on percent relative cumulative frequency (PRCF) of respiratory quotient (RQ) in 13-wk-old OM and SSB rats eating a high-fat or low-fat diet.

Table 6. Effect of perinatal temperature on mRNA levels of UCP1, UCP2, UCP3, and β3-AR in BAT and WAT and of serum levels of corticosterone, insulin, and leptin all in 16-wk-old OM and SSB rats eating a high-fat or low-fat diet

<table>
<thead>
<tr>
<th>Gene expression levels</th>
<th>OM</th>
<th>SSB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>LF</td>
</tr>
<tr>
<td>BAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7–9</td>
<td>6–8</td>
</tr>
<tr>
<td>β3-AR</td>
<td>0.52±0.3</td>
<td>0.36±0.2</td>
</tr>
<tr>
<td>UCP1</td>
<td>8.81±2.6</td>
<td>10.11±1.5</td>
</tr>
<tr>
<td>UCP2</td>
<td>1.57±0.5</td>
<td>0.93±0.2</td>
</tr>
<tr>
<td>UCP3</td>
<td>7.77±2.9</td>
<td>10.3±3.2</td>
</tr>
<tr>
<td>WAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7–8</td>
<td>6–8</td>
</tr>
<tr>
<td>β3 Adrenergic receptor</td>
<td>0.40±0.1</td>
<td>0.54±0.2</td>
</tr>
<tr>
<td>UCP1</td>
<td>0.81±0.3</td>
<td>0.97±0.4</td>
</tr>
<tr>
<td>UCP2</td>
<td>0.72±0.1</td>
<td>0.51±0.1</td>
</tr>
<tr>
<td>UCP3</td>
<td>4.76±1.4</td>
<td>3.93±1.3</td>
</tr>
<tr>
<td>Serum levels, ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>284.7±37</td>
<td>384.7±71</td>
</tr>
<tr>
<td>Insulin</td>
<td>3.6±0.24</td>
<td>4.3±0.31</td>
</tr>
<tr>
<td>Leptin</td>
<td>9.3±0.97</td>
<td>5.2±0.51</td>
</tr>
</tbody>
</table>

Values are means ± SE. Gene expression data are expressed as ratios of the gene to cyclophillin. *P < 0.05, strain difference in the same temperature-diet group. †P < 0.05, diet difference in the same temperature-strain group. ‡P < 0.05, temperature difference in the same strain-diet group.
available potential for response. Both strains increased their metabolic rate after administration of CL-316243; however, because rats reared at 30°C had a higher response potential, they showed a greater increase.

When metabolic rate was measured at thermoneutrality at 13 wk of age, there were no differences in baseline values associated with rearing temperature between the rats. However, animals reared at 18°C showed a greater increase in their metabolic rate after the β3-AR agonist administration of CL-316243 compared with animals reared at 30°C. This effect of perinatal temperature was greater in rats eating the low-fat diet than in those eating the high-fat diet and, unlike the body weight changes, was present in both OM and SSB rats. These data suggest that the high-fat diet attenuates the effects of rearing temperature on the response of the adrenergic receptors.

The effect of diet composition on activity of the sympathetic nervous system is an important component needed for interpreting these experiments. Young and Landsberg (54) showed that high-carbohydrate diets stimulate the sympathetic nervous system. In contrast, high-fat diets suppress the expression of β3-ARs in adipose tissue (5, 39). The ability of high-carbohydrate diets to enhance the sympathetic nervous system and of high-fat diets to impair the response to sympathetic activation probably explains why the differential response to the β3-agonist CL-316243 was attenuated in rats reared at 18 and 30°C when eating the high-fat diet.

The mechanism(s) of these temperature-induced effects and the reason(s) for the absence of these effects on body weight of SSB rats remain unclear. Previous reports by Young and colleagues (32, 56) suggested that cold exposure during the neonatal period produces a long-term increase in the activity of the sympathetic nervous system in rodents. Our data do not support this suggestion. Indeed, the higher level of expression of both β3-AR mRNA and UCP1 mRNA in BAT in rats reared at 30°C compared with those reared at 18°C suggests that the rats raised at 30°C maintain a higher sympathetic activation when rehoused at 22°C than do animals exposed to a perinatal temperature of 18°C. Thus the lower response to the β3-AR agonist CL-316243 in rats exposed to the higher perinatal temperature probably reflects the higher state of sympathetic activation in these animals. At 13 wk of age, the animals reared at a perinatal temperature of 30°C have induced more β3-AR, UCP1, and UCP3 mRNA in BAT and would show a smaller response to the exogenous β3-AR agonist when tested at 22°C, as they did in the case of both the OM and SSB rats, presumably reflecting enhanced sympathetic activity. In contrast, when tested at 7 wk of age, when rats were housed at their ambient temperature of 18 or 30°C, the opposite result was obtained, indicative of the increased sympathetic activation in the rats housed and tested at 18°C.

There were no differences in weight gain in SSB rats at either ambient temperature. The differences in WAT may be the key to explaining the differences observed between the strains. SSB rats had increased levels of mRNA for β3-AR, UCP1, and UCP3 in WAT compared with OM rats. Although not significant, these levels were also higher in animals reared at 18°C compared with those reared at 30°C. Previous work in our laboratory (49) has shown that SSB rats also have increased induction of UCP1 mRNA expression in WAT after injection with a β3-agonist, an effect that was not observed in OM rats.

Work by Kozak and colleagues (14, 22) suggests that this induction of brown adipocytes in WAT depots may be linked to a physiological adaptation to resist obesity and that it is genetically controlled. Administration of a β3-agonist and cold adaptation both induce brown adipocytes in WAT (7, 12, 14). These new cells have sympathetic innervation (13) and cause a BAT-like thermogenesis. The increased sympathetic activity in WAT may protect the SSB rats from developing obesity when reared at 18°C.

Previous results in rats reared at 18 and 30°C for the first 2 mo of life and then raised at 22°C for 2 mo showed increased UCP1 mRNA expression in BAT (56). One big difference between our study and those performed by Young and colleagues (32, 56) was that their rats were not placed in the temperature-controlled chambers until 1 day of age. In contrast, the dams in our study were placed in the chambers 1 wk before giving birth. The differences between in utero versus postnatal exposure obviously had a huge impact on levels of sympathetic activity in the offspring.

The concept of neonatal programming is very clearly supported by these studies. It is known that cold exposure increases metabolic rate (47), and it also has been shown to cause hyperphagia in rats (36, 41). The effects of this early-life programming persisted even after the rats were transferred to an ambient temperature of 22°C. Both the increase in metabolic rate in response to the β3-agonist CL-316243 and the increase in food intake remained higher weeks after the end of the cold exposure. Thus, although there were no temperature-dependent differences in body weight in SSB rats, there were still differential responses to the exogenous β3-AR agonist, increases in BAT β3-AR and UCP1 expression, and a greater intake of food in SSB rats eating a high-fat diet and raised at 18°C than in those raised at 30°C. Early environmental influences are thought to induce epigenetic changes in gene regulation through histone methylation that alters the transcriptional activity of genes (48). Precisely which genes are responsible for the temperature-dependent changes observed in OM rats is unclear at this time. However, the differential response of OM and SSB rats to the early exposure to different environmental changes suggests either that the epigenetic responses differ between the two strains or that the SSB rat is able to compensate as an adult to prevent the phenotypic differences in body weight that were observed in the OM rats.

GRANTS
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REFERENCES
1. Astrup A. Dietary fat is a major player in obesity—but not the only one. *Obes Rev* 3: 57–58, 2002.
EFFECT OF A COLD PERINATAL ENVIRONMENT IN TWO RAT STRAINS


46. Talan MI, Tatelman HM, and Engel BT. Cold tolerance and metabolic heat production in male C57BL/6J mice at different times of day. Physiol Behav 50: 613–616, 1991.


