Effects of 2-G exposure on temperature regulation, circadian rhythms, and adiposity in UCP2/3 transgenic mice

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Fuller, Patrick M., Craig H. Warden, Sean J. Barry, and Charles A. Fuller. Effects of 2-G exposure on temperature regulation, circadian rhythms, and adiposity in UCP2/3 transgenic mice. J Appl Physiol 89: 1491–1498, 2000.—Altered ambient force environments affect energy expenditure via changes in thermoregulation, metabolism, and body composition. Uncoupling proteins (UCPs) have been implicated as potential enhancers of energy expenditure and may participate in some of the adaptations to a hyperdynamic environment. To test this hypothesis, this study examined the homeostatic and circadian profiles of body temperature (Tb) and activity and adiposity in wild-type and UCP2/3 transgenic mice exposed to 1 and 2 G. There were no significant differences between the groups in the means, amplitudes, or phases of Tb and activity rhythms at either the 1- or 2-G level. Percent body fat was significantly lower in transgenic (5.2 ± 0.2%) relative to the wild-type mice (6.2 ± 0.1%) after 2-G exposure; mass-adjusted mesenteric and epididymal fat pads in transgenic mice were also significantly lower (P < 0.05). The data suggest that 1) the actions of two UCPs (UCP2 and UCP3) do not contribute to an altered energy balance at 2 G, although 2) UCP2 and UCP3 do contribute to the utilization of lipids as a fuel substrate at 2 G.

uncoupling proteins; thermogenesis; lipids; hyperdynamic; energy expenditure

HOMEOTHERMS EXPOSED TO HYPERDYNAMIC fields (2 G) exhibit profound changes in thermoregulation (9, 10, 12–14). These changes in temperature regulation have been documented in mice, rats, and primates (10, 12, 21). In addition, evidence from studies in rats suggests that changes in both heat production and heat loss occurs after hypergravity exposure (15). Mice exposed to a hyperdynamic environment (2 G) via centrifugation show an immediate ~6°C decline in mean daily body temperature (Tb) followed by a recovery to a new steady state ~1–2 wk later (21). The amplitude of the circadian rhythm of Tb is highly attenuated after exposure to 2 G in a 12:12-h light-dark cycle; recovery from this occurs in ~2 wk (21). Mean daily Tb and the circadian Tb amplitude after exposure to a 2-G environment exhibit classic triphasic homeostatic responses (initial transient, recovery, and new steady state).

Metabolic rate, as measured by oxygen consumption, increases in chronic hypergravity (22). In 2 G, small mammals increase oxygen consumption linearly with total body weight (24, 25). Moreover, in several species, maintenance nutritional requirements have been shown to be directly related to the level of acceleration, within the limit of chronic G tolerance (30, 31). A corresponding decrease in metabolic energy expenditure was documented in rhesus monkeys exposed to microgravity spaceflight environments (Cosmos 2044 and 2229) (11, 32). In these monkeys, total metabolic energy expenditure, as measured by turnover of doubly labeled water, was reduced by ~40% (11, 32). These findings represent the most direct demonstration of the metabolic cost of gravity. Unfortunately, the constancy of the Earth’s gravitational field masks this important relationship between the metabolic cost of living and the gravitational environment.

The proportional distribution of body mass between fat and fat-free components has also been studied in hyperdynamic environments. The first few days of chronic centrifugation are accompanied by decreased body mass (3) and altered glucose and fat metabolism (6, 7). During exposure to a hyperdynamic environment, a loss of body fat has been observed in mice (23), rats (34), rabbits (19), and chickens (3). This reduction in the body fat component of body mass can be quite large; for example, chickens decrease from 30% body fat at 1 G to 3% at 3 G (3). The decrease in body fat suggests a fundamental shift in substrate utilization during exposure to a hyperdynamic environment. Furthermore, such a metabolic shift may signify an altered functional role for adipocytes and myocytes in regulating intermediary metabolism and energy homeostasis during 2 G.

Mitochondrial uncoupling proteins (UCPs) have been implicated as potential enhancers of energy expenditure. The in vivo physiological role of UCPs is not

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known; however, UCPs putatively function to both increase tissue thermogenesis and regulate the use of lipids as a fuel substrate (29). The first UCP described was UCP1, located in brown adipose tissue. More recently, UCP2 and UCP3, homologues of brown adipose UCP1, have been described in central and peripheral tissues, including homeostatic brain centers (16, 28), as well as skeletal muscle, white adipose tissue, liver, spleen, and heart (2, 8). Both UCP2 and UCP3 have in vitro uncoupling properties similar to UCP1 (2, 8). The creation of the UCP2/3 transgenic line of mice provides an opportunity to test possible in vivo roles of UCP2 and UCP3. The mice in this study were derived from a transgenic line that overexpressed UCP2 in the spleen, hypothalamus, gastrocnemius, and white adipose tissue and UCP3 in the gastrocnemius muscle. The construct is thought to contain all of the promoter elements for expression in the normal, natural tissues. Furthermore, Northern blots have confirmed that UCP2 and UCP3 are made in all the normal tissues and not in any others.

The 2-G experimental paradigm provides a unique tool for investigating the in vivo physiological role of UCP2 and UCP3 in thermoregulation and adiposity. Because of the potential roles of UCP2 and UCP3 in mediating thermoregulatory thermogenesis through increased basal (metabolism) or facultative (increased heat production) tissue thermogenesis, exposure to 2 G may elicit a differential response in \( T_b \) in transgenic and wild-type mice. Moreover, because UCP2 and UCP3 have been implicated as regulators of lipids as a fuel substrate, the transgenic population may demonstrate differential adipose metabolism relative to non-transgensics during exposure to 2 G. In this context, UCP2/3 may serve a metabolically adaptive role for certain tissues during periods of increased fatty acid mobilization. Such a putative role has been previously proposed for UCP2/3 (4).

Thus this study tests the hypotheses that UCP2/3 overexpression will 1) alter the profile of \( T_b \) regulation at 2 G and 2) increase the utilization of fat as assessed by changes in body composition and adipose pad masses.

**METHODS**

**Transgenics.** The transgenic mice were made using an 80-kb human bacterial artificial chromosome (BAC) clone. The BAC clone was isolated by Genome Systems by hybridization with a human UCP2 cDNA probe. This clone contains all of UCP2 and UCP3. These are organized UCP3 and then UCP2 in the 5' to 3' orientation. In addition, there is 8 kb 3' of UCP2 and ~40 kb 5' of UCP3. This was determined by direct sequencing of UCP2 containing subclones, by demonstrating that UCP3 is 8 kb 5' of UCP2, and from published information on the gene structure and intron sizes of human UCP2 and UCP3. Transgenic mice were verified by PCR and with tissue expression, using Northern blot analysis. Care of the mice in the experiment met the standards set forth by the National Institutes of Health (NIH) in their *Guide for the Care and Use of Laboratory Animals* and was approved by the University of California Davis Institutional Animal Care and Use Committee.

**Animals and biotelemetry.** Eight male adult (28–33 g) UCP2/3 transgenic mice and nine male adult (29–32 g) non-transgenic littermate (wild-type) (*Mus musculus*) were implanted intraperitoneally with biotelemetry transmitters (VM-FH disc; Minimitter, Sunriver, OR) to record \( T_b \) and activity. A surgical plane of anesthesia was initiated and maintained with the use of 3% isoflurane in pure medical-grade oxygen, administered by an adjustable isoflurane vaporizer (Viking Medical Products, Medford Lakes, NJ). With the use of aseptic techniques, a midline celiotomy was performed and a sterilized transmitter was inserted into the peritoneal cavity. All incisions were sutured and treated with lidocaine and a topical antibiotic. The mice recovered on a heating pad, with \( T_b \) constantly monitored via a colonic probe.

**Housing and centrifuge.** After 10 days of recovery, the mice were placed on a 4.6-m-diameter centrifuge. The animals were individually housed in standard plastic mouse cages with food (Lab Diet) and water ad libitum. Each cage was placed on top of a telemetry receiver interfaced to a microcomputer data acquisition system (Data Sciences). \( T_b \) values were recorded at 5-min intervals, and activity data were collected in 5-min bins. The cages were housed inside centrifuge modules, which provided ventilation, a 12:12-h light-dark cycle, an ambient temperature of 25 ± 1°C, and visual isolation. Modules containing the cages were mounted with one degree of freedom, thereby ensuring that the net G field was always perpendicular to the cage floor. A 2-wk period of 1 G was used to establish baseline levels, after which the mice were exposed to 2 G via centrifugation for 8 wk. Centrifugation was interrupted twice weekly for ~15- to 20-min periods required for animal husbandry.

**Adipose and body composition.** At the end of the 2-G exposure, the mice were removed from the centrifuge and killed immediately. Adipose pads (mesenteric, retroperitoneal, femoral, and epididymal) were removed and weighed. Body composition was determined by the method of Bell and Stern (1). Briefly, carcasses were prepared by evisceration and freeze-dried for 7 days (or until 2 consecutive daily weighings differed by no more than 2%) and then weighed to obtain dry mass and percent body water. Lipids were then ether extracted for 7 days and acetone extracted for 5 days. The carcass was then re-freeze-dried for 24 h and weighed to determine percent body fat.

**Statistics.** Phase, mean, and activity of the \( T_b \) and activity rhythms were determined with the use of a phase-fitting (least-squares harmonic regression analysis) program that utilized a Fourier-based algorithm. Repeated-measures ANOVA was used to compare gravitational conditions [1 G (control), early 2 G (adaptation), and late 2 G (recovery)]. Specific mean comparisons were made using a post hoc Tukey's test (SPSS). For adipose mass, comparisons between the experimental and control groups were analyzed by unpaired \( t \)-test. Level of significance of \( P < 0.05 \) was used for all tests.

**RESULTS**

\( T_b \). We compared the daily \( T_b \) data collected for 5-day intervals during 1 G (days 3–8 of 1 G), early 2 G (days 2–6 of 2 G), and late 2 G (days 44–48 of 2 G). Figure 1 shows the \( T_b \) data for individual wild-type (left) and transgenic (right) mice during 1 G (Fig. 1, A and D), early 2 G (Fig. 1, B and D), and late 2 G (Fig. 1, C and F). During 1 G, the circadian rhythm in \( T_b \) is robust, with a range (maximum to minimum) of ~2.5°C (Fig. 1,
No difference in the 24-h mean $T_b$ was shown between the wild-type and transgenic mice (Table 1). Furthermore, the mean $T_b$ during the light and dark periods did not differ between groups (Table 1). During early 2 G (Fig. 1, B and E), mean $T_b$ was depressed and the circadian $T_b$ amplitude was highly attenuated in both groups. The mean 24-h $T_b$ was $\sim 1^\circ$C lower for both the wild-type and transgenic mice (Table 1). Moreover, the mean $T_b$ during the light and dark periods were similarly depressed ($\sim 0.7$ and $1.4^\circ$C, respectively) in both groups (Table 1). No differences in the mean 24-h $T_b$ during light and dark periods were shown between the wild-type and transgenic groups. During late 2 G (Fig. 1, C and F), both mean $T_b$ and the circadian $T_b$ amplitude ($\sim 2.5^\circ$C) recovered to values shown at the 1-G control level. No difference in the 24-h mean $T_b$ was shown between the wild-type and transgenic mice (Table 1). The $T_b$ means during the light and dark periods also did not differ between groups (Table 1).

The average daily mean rhythm amplitude (calculated as the mean to maximum of the best-fit Fourier function) and phase (calculated as the time of the maximum of the best-fit Fourier function) of $T_b$ for each group are shown in Fig. 2. During 1 G, there were no differences between the wild-type and transgenic groups.

### Table 1. Body temperature and activity in 1-G, early 2-G, and late 2-G environments

<table>
<thead>
<tr>
<th>Parameter/Group</th>
<th>1 G</th>
<th>Early 2 G</th>
<th>Late 2 G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>L</td>
<td>D</td>
</tr>
<tr>
<td>$T_b$, °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>37.2 ± 0.2</td>
<td>36.5 ± 0.2</td>
<td>37.7 ± 0.02</td>
</tr>
<tr>
<td>Transgenic</td>
<td>37.1 ± 0.1</td>
<td>36.5 ± 0.3</td>
<td>37.7 ± 0.01</td>
</tr>
<tr>
<td>Activity, counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>22.2 ± 1.3</td>
<td>4.5 ± 0.8</td>
<td>30.9 ± 1.2</td>
</tr>
<tr>
<td>Transgenic</td>
<td>21.0 ± 1.5</td>
<td>5.0 ± 1.2</td>
<td>30.2 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. 24 h, Daily mean; L, light period mean; D, dark period mean; $T_b$, body temperature.
groups in $T_b$ mean (Fig. 2A), $T_b$ amplitude (Fig. 2B), or $T_b$ phase (Fig. 2C). There were no differences in mean daily $T_b$ did not differ between the transgenic and wild-type groups at 1 G, early 2 G, or late 2 G (repeated-measures ANOVA, Table 2). Furthermore, the circadian $T_b$ amplitude did not demonstrate a statistically significant difference between groups at 1 G, early 2 G, or late 2 G (repeated-measures ANOVA, Table 2). Lastly, phase of the $T_b$ rhythm did not differ between the groups for any treatment (Fig. 2C). There were no differences in $T_b$ mean during the light and dark periods between the groups (not shown).

There were significant differences in both the wild-type and transgenic daily mean $T_b$ across the three 5-day intervals (repeated-measures ANOVA, Table 2). During early 2 G, the mean daily $T_b$ of both groups was significantly lower than during both 1 G and late 2 G (post hoc Tukey’s test, $P < 0.001$). Circadian $T_b$ amplitude in both groups was also significantly depressed during early 2 G relative to 1 G and late 2 G (post hoc Tukey’s test, $P < 0.001$).

**Activity.** The daily activity data were compared for the 5-day intervals during 1 G (days 3–8 of 1 G), early 2 G (days 2–6 of 2 G), and late 2 G (days 44–48 of 2 G). Figure 3 shows the activity data for individual wild-type (left) and transgenic (right) mice during 1 G (Fig. 3, A and D), early 2 G (Fig. 3, B and E) and late 2 G (Fig. 3, C and F). During 1 G, the circadian rhythm in activity is robust, with a range (maximum to minimum) of ~150 counts (Fig. 3, A and D). There were no differences in the 24-h mean activity between the wild-type and transgenic mice (Table 1). Moreover, the mean activity during the light and dark periods were similarly depressed (~2 counts and 25 counts, respectively) in both groups (Table 1). There were no differences in the 24-h mean activity during the light and dark periods between the wild-type and transgenic groups. During late 2 G (Fig. 3, C and F), both mean activity and the circadian activity amplitude (~100 counts) established new steady states, which were lower than those at 1-G control levels. The 24-h mean activity levels were not different between the wild-type and transgenic mice (Table 1). The mean activities during the light and dark periods also did not differ between groups (Table 1).

The average daily mean rhythm amplitude (calculated as the mean to maximum of the best-fit Fourier function) and phase (calculated as the time of the maximum of the best-fit Fourier function) of activity for each group are shown in Fig. 4. During 1 G, there were no differences between the wild-type and transgenic groups in activity mean (Fig. 4A), activity amplitudes (Fig. 4B), or activity phase (Fig. 4C). Mean daily activity did not differ between the wild-type and transgenic groups in 1 G, early 2 G, or late 2 G (repeated-measures ANOVA, Table 2). Furthermore, the circadian activity amplitude did not demonstrate a statistically significant difference between groups in 1 G, early 2 G, or late 2 G (repeated-measures ANOVA, Table 2). Lastly, phase of the ACT rhythm did not differ between groups in any treatments (Fig. 4C). There were no differences in

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### Table 2. Repeated-measures ANOVA for $T_b$ and activity

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F-Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_b$ Means, °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>0.03</td>
<td>1, 15</td>
<td>0.03</td>
<td>0.43</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>G level</td>
<td>41.74</td>
<td>2, 30</td>
<td>20.87</td>
<td>621.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Group $\times$ G level</td>
<td>0.03</td>
<td>2, 30</td>
<td>0.01</td>
<td>0.41</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>$T_b$ Amplitude, °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>0.00</td>
<td>1, 15</td>
<td>0.00</td>
<td>0.23</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>G level</td>
<td>1.69</td>
<td>2, 30</td>
<td>0.84</td>
<td>335.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Group $\times$ G level</td>
<td>0.00</td>
<td>2, 30</td>
<td>0.00</td>
<td>0.21</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Activity Means, Counts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>0.00</td>
<td>1, 15</td>
<td>0.00</td>
<td>0.00</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>G level</td>
<td>1131.00</td>
<td>2, 30</td>
<td>565.00</td>
<td>377.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Group $\times$ G level</td>
<td>1.03</td>
<td>2, 30</td>
<td>0.51</td>
<td>0.34</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Activity Amplitude, Counts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>0.02</td>
<td>1, 15</td>
<td>0.02</td>
<td>0.03</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>G level</td>
<td>666.00</td>
<td>2, 30</td>
<td>333.00</td>
<td>552.00</td>
<td>0.000</td>
</tr>
<tr>
<td>Group $\times$ G level</td>
<td>0.60</td>
<td>2, 30</td>
<td>0.30</td>
<td>0.19</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

SS, sum of squares; DF, degrees of freedom; MS, mean square.
There were significant differences in daily mean activity for both wild-type and transgenic mice when comparing the three 5-day intervals (repeated-measures ANOVA, Table 2). During early 2 G, the mean daily activity of both groups was significantly lower than during 1 G and late 2 G (post hoc Tukey's test, \( P < 0.001 \)). Circadian activity amplitude in both groups was also significantly depressed during early 2 G relative to 1 G and late 2 G (post hoc Tukey's test, \( P < 0.001 \)). During late 2 G, the mean daily activity of both groups was significantly lower than that during 1 G and higher than that at early 2 G (post hoc Tukey's test, \( P < 0.01 \)), indicating that activity levels did not recover to their 1-G levels, similar to that occurring for \( T_h \). Circadian activity amplitude in both groups was also significantly depressed during late 2 G relative to 1 G; however, it was significantly higher than that at early 2 G (post hoc Tukey's test, \( P < 0.01 \)), indicating that circadian activity amplitude did not recover similar to that occurring for \( T_h \).

Body composition and adiposity. Transgenic and wild-type mice did not differ in mean body mass at 1 G (32.5 ± 1.3 and 31.2 ± 0.8 g, respectively). After 8 wk at 2 G, both groups had significantly smaller body masses (\( P < 0.001 \)) relative to 1 G (28.3 ± 0.7 and 27.3 ± 0.8 g, respectively). Thus, at the end of the 2-G exposure, both groups were ~13% smaller; however, there was no significant difference in body mass between the groups after 2-G exposure.

Figure 5 shows the body composition analysis data. Percent body fat was significantly lower in transgenic (5.2 ± 0.2%) relative to wild-type mice (6.2 ± 0.1%) after 8 wk of 2-G exposure (\( P < 0.05 \), Fig. 5A). Conversely, percent fat-free mass was significantly higher in transgenic mice (94.8 ± 0.2%) relative to wild-type mice (93.8 ± 0.1%) after 8 wk of 2-G exposure (\( P < 0.05 \), Fig. 5B). Percent body water was not, however, different between transgenic (50 ± 0.6%) and wild-type mice (49.9 ± 0.5%) after 8 wk of 2-G exposure (Fig. 5C). In addition to percent body fat, after 8 wk at 2 G, the epididymal, mesenteric, retroperitoneal, and femoral fat pads in transgenic mice were smaller than those in wild-type mice; however, this was statistically significant only in the mass-adjusted epididymal (\( P < 0.05 \)) and mesenteric fat pads (\( P < 0.01 \)) (Fig. 6).
DISCUSSION

The major finding of this study was the effect of the 2-G environment on the body composition of UCP2/3 transgenic mice compared with that of wild-type mice. The leaner transgenic mice also showed smaller epididymal and mesenteric fat pads compared with the wild-type mice. These findings are consistent with a role for UCP2 and/or UCP3 in participating in fat metabolism at 2 G. In contrast, no differences were noted between the measured levels of Tb and activity at either 1 or 2 G. These findings suggest that UCP2 and UCP3 may not play a role in basal or facultative systemic thermogenesis, regardless of the ambient force environment. These results are in agreement with previous studies that document upregulation of UCP2 and UCP3 gene expression in skeletal muscle during food deprivation at thermoneutrality, conditions that reduce thermoregulatory thermogenesis (28). Moreover, these results are in agreement with studies showing that UCP3 knockout mice have normal circadian rhythms of Tb and motor activity (14a).

Body composition. The findings that the transgenic mice demonstrated a leaner phenotype after 2-G exposure are in contrast to those for the 1-G phenotype (unpublished observations). At 1 G, no difference in percent body fat or mesenteric, femoral, or retroperitoneal fat pad masses was demonstrated between the transgenic mice and the nontransgenic littermates on a low-fat diet. However, at 1 G, transgenic mice did demonstrate a significantly smaller epididymal fat pad mass. Importantly, these observations are from mice that were on a different diet and ~1 mo younger. Unfortunately, the limited availability of UCP2/3 transgenic mice precluded our study design from including a diet and age-matched 1-G group for body composition analysis. However, because both the 1- and 2-G groups were mature, we would not expect a 1-mo age difference between groups to significantly affect the results. The average mass decrease and final body weight were not significantly different between groups. However, the transgenic mice had a higher percent of fat-free mass. These results suggest that overexpression of UCP2 and/or UCP3 may alter calorie partitioning, that is, the percent of calories stored as fat or lean mass, although the mechanism remains unknown.

UCP2 upregulation occurs during both obesity (high lipogenesis) and during starvation (high lipolysis), suggesting a complex role for UCP2 in fatty acid metabolism (4). For example, a role for UCP2 in titrating ATP production and regulating NAD(+) to NADH ratios has been suggested (4). This increased expression of UCP2 may occur when available substrate exceeds cellular energy demands, decreasing the efficiency of ATP production by oxidation. Increased redox pressure associated with abundant substrate supply has been suggested as a means of increasing the production of reactive oxygen species (ROS), such as superoxide (O2•−) (5). UCP2 may therefore help control the generation of unwanted ROS under conditions of high substrate by providing an alternative substrate oxidation pathway, which is not coupled to ATP production. The putative role for UCP2/3 in constraining mitochondrial ROS production may implicate the UCPs in physiological functions as diverse as apoptosis (33) and even in a possible role in the immune system.

Very little is known about the mechanisms for uncoupling and the physiological substrates for UCP2 and UCP3 when expressed in mammals (18). Recently, a regulator of UCP2 activity was described. This activator, retinoic acid, appears to stimulate UCP2 activity in a pH-dependent manner (27). However, the physiological situations that lead to increased intracellular retinoic acid production are unknown. Furthermore, it is unclear whether retinoic acid exerts its effect via transactivation of the UCP2 gene via nuclear retinoic acid receptors or via a direct interaction between retinoic acid and UCP2 (27).

The differential fat pad response to 2 G is consistent with previous findings in rats (L. E. Warren, personal communication). It appears that adipose tissue pads are differentially regulated, although the functional significance of this finding is not understood. Differential adipose metabolism has also been demonstrated in animals at 1 G. For example, mice fed diets with varying quantities of eicosapentaenoic, docosahexaenoic, and linolenic acids demonstrate decreases in only the epididymal fat pad (17). Moreover, in mice, which exhibited the greatest loss of epididymal fat, UCP2 levels were increased 2.7-fold in white adipose tissue (17). This diet-induced upregulation of UCP2 with concomitant adipose metabolism supports our findings that UCP2 and UCP3 do contribute to the increased fat metabolism and utilization at 2 G.

Tb and activity. This study clearly demonstrates the ability of the UCP2/3 transgensics to maintain Tb, with activity levels comparable between groups. However, these data do not preclude altered metabolic responses of the transgenic animals. Although the transgenic mice demonstrated activity levels comparable to those of the wild-type mice, it is possible that there were differing relative contributions of thermogenesis (heat
production) and thermolysis (heat loss). To date, no heat balance studies have addressed alterations in $T_b$ regulation, metabolism, or rhythmicity in either micro- or hypergravity. Consequently, the relative contribution of heat production vs. heat loss to these changes is not known.

In addition to active physical mechanisms of heat production and heat loss, passive changes such as redistribution of body fluids and altered physical convection may contribute to the apparent changes in $T_b$ regulation in hypergravity and microgravity. Furthermore, the attenuated activity levels consequent of increased G may negatively affect thermal balance. In this case, thermogenesis may be increased to augment the loss of activity-generated metabolism. Conversely, the increased postural muscle load at 2 G may, in part, compensate for the decrease in ambulatory activity-generated heat. The underlying contributory role of these changes in activity levels to altered thermal balance remains to be fully elucidated; however, altered activity levels may have profound influences on heat production and heat loss at 2 G, particularly in small homeotherms such as mice. This study suggests that increased tissue thermogenesis via the action of UCP2 and UCP3 does not contribute to the thermoregulatory changes documented during hypergravity exposure.

Importantly, however, a role for UCP2 and UCP3 in local temperature control has not been ruled out. For example, a recent study has suggested that UCPs may play an important role in modulating neurotransmission in homeostatic brain centers via increased local brain mitochondrial uncoupling activity and heat production (16).

The results of this study suggest that UCP2 and UCP3 do not contribute to an altered energy balance at 2 G. It does appear, however, that UCP2 and/or UCP3 may contribute to the utilization of fat as a fuel substrate during 2-G exposure. Furthermore, the results suggest that UCP2 and/or UCP3 may alter, via unknown mechanisms, calorie partitioning.

An important future study would be to examine the effects of 2 G on UCP2 and UCP3 knockouts. A UCP2 and UCP3 knockout model would help quantify the contributory roles of UCP2 and UCP3 to adipose metabolism at 2 G. In addition, because hepatocytes play a major role in regulating intermediary metabolism and energy homeostasis, it will be important to evaluate the role of UCPs in the liver at 1 and 2 G.

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REFERENCES


32. Stein TP, Dotenko MA, Korolkov VI, Griffin DW, and Fuller CA. Energy expenditure in rhesus monkeys (Macaca mulatta) during spaceflight using doubly labeled water (\(^{2}H_2^{18}O\)). *J Appl Physiol* 81: 201–207, 1996.
