In a hypergravity environment neonatal survival is adversely affected by alterations in dam tissue metabolism rather than reduced food intake

Laura M. Lintault,1 Elzbieta I. Zakrzewska,1 Rhonda L. Maple,1 Lisa A. Baer,2 Theresa M. Casey,1 April E. Ronca,2 Charles E. Wade,2 and Karen Plaut1,2

1Department of Animal Science, University of Vermont, Burlington, Vermont; and 2National Aeronautics and Space Administration-Ames Research Center, Moffett Field, California

Submitted 11 September 2006; accepted in final form 20 February 2007

Exposure of rat dams to hypergravity during pregnancy is associated with increased pup mortality, reduced food intake, and decreased rates of glucose oxidation and lipogenesis in mammary tissue. We hypothesized that increased pup mortality is due to changes in maternal metabolism and not to reduced food intake of dams. Effects of hypergravity on rate of glucose oxidation and lipogenesis in mammary, liver, and adipose tissue were measured in rat dams centrifuged at 2.0 G [hypergravity (HG)], kept at 1.0 G (control), or fed to match the intake of HG rats (pair fed) from gestation until G21 or postpartum day 3 (P3). Body weight, percent body fat, metabolizable energy, and nitrogen balance were significantly less in HG dams compared with controls (P < 0.05); however, these factors were not different between HG and pair-fed dams. By P3, 100% of control and pair-fed pups survived, while only 49% of HG pups survived. At G21, rates of glucose oxidation and lipogenesis in mammary and adipose tissue were less in HG than in control and pair-fed dams (P < 0.1 and P < 0.05). In liver, at G21, the rate of lipogenesis was greater in HG than control and pair-fed dams (P < 0.01); at P3, lipogenesis was greater in control than HG and pair-fed dams (P < 0.05). Gene expression of ATP citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase increased in liver from pregnancy to lactation in control dams, with increased pup mortality, reduced food intake, and decreased rates of glucose oxidation and lipogenesis in mammary tissue. We hypothesized that increased pup mortality is due to changes in maternal metabolism and not to reduced food intake of dams. Effects of hypergravity on rate of glucose oxidation and lipogenesis in mammary, liver, and adipose tissue were measured in rat dams centrifuged at 2.0 G [hypergravity (HG)], kept at 1.0 G (control), or fed to match the intake of HG rats (pair fed) from gestation until G21 or postpartum day 3 (P3). Body weight, percent body fat, metabolizable energy, and nitrogen balance were significantly less in HG dams compared with controls (P < 0.05); however, these factors were not different between HG and pair-fed dams. By P3, 100% of control and pair-fed pups survived, while only 49% of HG pups survived. At G21, rates of glucose oxidation and lipogenesis in mammary and adipose tissue were less in HG than in control and pair-fed dams (P < 0.1 and P < 0.05). In liver, at G21, the rate of lipogenesis was greater in HG than control and pair-fed dams (P < 0.01); at P3, lipogenesis was greater in control than HG and pair-fed dams (P < 0.05). Gene expression of ATP citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase increased in liver from pregnancy to lactation in control and pair-fed dams but not HG dams. Thus reduced food intake and body mass due to hypergravity exposure cannot explain the dramatic decrease in HG pup survival.

In a hypergravity environment neonatal survival is adversely affected by alterations in dam tissue metabolism rather than reduced food intake.

Increased pup mortality is due to changes in maternal metabolism rather than reduced food intake of dams. Effects of hypergravity on rate of glucose oxidation and lipogenesis in mammary, liver, and adipose tissue were measured in rat dams centrifuged at 2.0 G [hypergravity (HG)], kept at 1.0 G (control), or fed to match the intake of HG rats (pair fed) from gestation until G21 or postpartum day 3 (P3). Body weight, percent body fat, metabolizable energy, and nitrogen balance were significantly less in HG dams compared with controls (P < 0.05); however, these factors were not different between HG and pair-fed dams. By P3, 100% of control and pair-fed pups survived, while only 49% of HG pups survived. At G21, rates of glucose oxidation and lipogenesis in mammary and adipose tissue were less in HG than in control and pair-fed dams (P < 0.1 and P < 0.05). In liver, at G21, the rate of lipogenesis was greater in HG than control and pair-fed dams (P < 0.01); at P3, lipogenesis was greater in control than HG and pair-fed dams (P < 0.05). Gene expression of ATP citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase increased in liver from pregnancy to lactation in control and pair-fed dams but not HG dams. Thus reduced food intake and body mass due to hypergravity exposure cannot explain the dramatic decrease in HG pup survival.

Glucose oxidation; metabolizable energy; mammary

TREMENDOUS CHANGES in physiological, metabolic, and physical demands on an animal are associated with pregnancy and lactation. Total nutrient requirements during late gestation are ~75% greater compared with a nonpregnant animal of the same body weight (1). During this time, the dam must supply nutrients for the fetuses that are growing exponentially and for mammary development to prepare for lactation (14, 15, 28). Homeostatic and homeorhetic signals regulate the altered demands on the dam. The homeostatic and homeorhetic changes that occur during pregnancy and lactation include neuroendocrine responses that stimulate mammary growth and development and drive maternal behavior, feeding, and appetite to enable nourishment of the neonate, as well as tissue metabolism (12).

Similar to alterations in other environmental stimuli, such as temperature and light, changes in gravitational force affect mammalian metabolism. For the National Aeronautics and Space Administration (NASA) to reach its ultimate goal of establishing and maintaining sustained human space exploration to the Moon, Mars, and other destinations within our solar system (5), the effects of gravity on animal metabolism need to be determined. Studies investigating the effects of gravity on developmental biology, in particular the metabolism of pregnant and lactating rats and the development of their young, are pertinent to provide NASA with information about mammalian reproduction and development in altered gravity.

Inertial forces are increased in hypergravity. Animals need to overcome these forces to maintain homeostasis. Wade et al. (35) noted that rats subjected to hypergravity have a higher resting energy expenditure than their stationary counterparts. To keep energy balance in a state of equilibrium, an animal exposed to hypergravity must either increase its energy intake or decrease its energy expenditure to avoid losing body mass (35). In general, rats exposed to a hypergravity environment are characterized by smaller body mass and reduced body fat reserves compared with 1-G controls (21–23, 32, 34). The reduction in body mass and fat reserves is, in part, due to the reduction in energy intake, a consequence of reduced behavioral activity and food and water intake, during the first few days of exposure to increased gravitational loading (4, 30). After ~4 days, the animals increase their food and water intake and gain body mass but consistently have a lower body mass than the stationary controls (30).

Previously, we have shown that the rate of glucose oxidation and incorporation into lipids (lipogenesis) increased in mammary tissue from rats exposed to microgravity and decreased in rats exposed to hypergravity (24, 25). When these data were evaluated relative to a common reference point and subjected to curve fitting, a continuum of metabolic response was revealed that was highly significant (r² = 0.99) across five gravitational loads, indicating that mammary metabolic activity decreases as G load increases at a constant rate (25). It is unclear whether the hypergravity-induced changes in metabolism are specific to the mammary gland, and if a decline in food intake is a major contributor to the hypergravity effect. In the present study we measured the rate of glucose oxidation and incorporation into lipids in mammary, liver, and adipose tissue in pregnant and lactating rats. A group of rats kept at 1 G and fed to match the intake of hypergravity-exposed rats was used to determine if the decrease in metabolic activity is due to reduced food intake. Gene expression of enzymes (ATP citrate

http://www.jap.org
lyase, acetyl-CoA carboxylase, and fatty acid synthase) in the liver that are involved in lipid metabolism and respond to changes in nutritional status was also analyzed. The effects of hypergravity and food intake on metabolizable energy, nitrogen balance, and body composition were also measured. We hypothesized that increased pup mortality is due to a combination of changes in maternal metabolism, hormone concentrations, and behavior in hypergravity and not to the reduced food intake of dams.

MATERIALS AND METHODS

Animals. The experimental protocol was approved by the NASA Ames Research Center and University of Vermont Institutional Animal Care and Use Committees. The protocol was conducted in accordance with the guidelines issued by the committees. Eighty-four primiparous Sprague-Dawley rats were obtained from Taconic Laboratories (Germantown, NY) on day 2 of pregnancy. Dams were maintained under standard colony conditions: 12:12-h light-dark cycle; 21 ± 1°C; 30–50% humidity. On gestation day 4 (G4), rats were matched by weight, and 28 were placed on the 24-ft-diameter centrifuge (2 G, 20.1 rpm) at NASA Ames Research Center (Moffett Field, CA) [hypergravity (HG) treatment]. Control dams (n = 28) were housed in the same room but held at 1 G (control). An additional group of rats were housed in similar conditions as the controls and fed to match the food intake of the HG group (pair fed; n = 28). All rats were housed in metabolic cages (19) from G6 to G18 or G21 (1 day before parturition). On G18, 10 rats per treatment were euthanized for metabolizable energy (ME) analysis, and on G21, 8 rats per treatment were removed from the centrifuge or control cages and anesthetized with isoflurane, and tissues were collected for analysis. The remaining rats (n = 10 per treatment) were moved to vivarium cages on G21 and placed back on centrifuge (HG) or control environments (control and pair fed) to allow for parturition and nursing until postpartum day 3 (P3), when these rats were anesthetized for tissue collection. Importantly, litters were randomized within treatments on P0 and reassigned to the dams in equal numbers as described by Ronca et al. (29).

Experimental diets/sample collection. The centrifuge was stopped for 1 h each day for animal health checks and daily data collection. Feces and urine were collected from all treatments, immediately weighed, and stored at −20°C until analysis. Dams were weighed daily. Rats were fed a standard Purina rat chow diet (Purina no. 5102) in the form of ground pellets, containing 3.4 kcal/g energy and 22.5% protein. Water was available ad libitum for all rats. Food and water intake were measured daily by subtracting the refused food/water from the total offered. Control and HG rats were fed ad libitum. For rats removed from treatment on G21, the average food consumption per day of HG rats was calculated and fed to the pair-fed rats on the following day. For rats removed from treatment on P3, as well as the ME rats, the pair-fed rats were fed the average amount of food the HG rats consumed during previous studies on that gestation day.

Body composition. Dams used for ME were anesthetized, and body composition was measured using TOBEC (total body electrical conductivity) instrumentation (EM-SCAN, Springfield, IL) just before tissue removal and euthanization. Briefly, the TOBEC instrument measures energy absorption in the presence of a radio-frequency electromagnetic field, calibrated to match the response of a conductivity sensing element. Energy is more absorbed in somewhat conductive materials (i.e., hydrated, lean tissue) than in highly resistive materials (i.e., fur, body fat) (3, 13, 27).

ME and nitrogen balance. Fecal and urine samples and food intake data were used for analysis of ME and nitrogen balance. Fecal samples were freeze-dried and ground through a 2-mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). Dry matter was determined by drying the feces in a forced draft oven at 100°C. Aliquots of urine samples (1-ml) were dried for 24 h at 45°C and immediately weighed. Gross energy content of all samples was determined by bomb calorimetry (model 1281, Parr, Moline, IL) according to the procedure described by Tyrrell et al. (33). For ME calculations, urinary energy was corrected for protein loss because the protein lost from the body originated in the tissues, not in the food simultaneously consumed (16). To calculate ME, food intake from one day was matched with urinary and fecal excretions from the following day to allow time for digestion and rate of passage. Urinary urea was measured by an automated Dade Behring Dimension X machine (Deerfield, IL). Urinary nitrogen (N) was calculated from the formula established by Ortiz and Wade (20) for rats using duplicate determinations of nitrogen and urea: urinary N (mg/ml) = urea (mg/ml) × 1.067 − 0.302. Nitrogen intake was calculated as protein content divided by 6.25. Fecal nitrogen was assumed a constant value of 60 mg/day. Nitrogen balance was calculated as the difference between N intake and N excretion (urinary and fecal).

Behavioral observations. Dams were time lapse- videotaped (12:1 record/playback ratio) for 24 h before tissue collection on P3. Overall behavioral activity (duration) was quantified during playback of the videotapes. Visualization during the dark phase of the cycle was enabled by red lighting. Data were analyzed as described by Ronca et al. (29).

Insulin analysis and metabolic assay. Blood samples were obtained by cardiac puncture from P3 dams anesthetized with isoflurane. Plasma was prepared and immediately frozen for later analysis. Insulin levels were determined using ELISA kits from Linco Research (Irvine, CA). Blood glucose levels were measured using the Dade Behring Dimension XpandT Clinical Chemistry System (Dade Behring, Newark, DE).

Abdominal no. 4 mammary glands, livers, and visceral adipose tissue were removed from anesthetized dams. Mammary and liver tissues were kept in 25 mmol/l Tris, 0.25 mol/l sucrose, 1 mmol/l EDTA (pH 7.3) on ice, and adipose tissue was kept in saline at 37°C to maintain tissue viability during the time between tissue removal and incubation for metabolic analysis. Metabolic activity of the tissues was measured as described by Bauman et al. (2) and modified by Plaut et al. (26). Tissue slices from each animal were placed in metabolism flasks in Krebs-Ringer bicarbonate buffer (0.154 mol/l NaCl, 0.154 mol/l KCl, 0.110 mol/l CaCl2, 0.154 mol/l KH2PO4, 0.154 mol/l MgSO4, 0.154 mol/l NaHCO3) containing 1 µg/ml insulin (Sigma Chemical), 10 mmol/l glucose, and [U-14C]glucose (1 µCi/flask; ICN). Flasks were incubated in a shaking water bath at 37°C for 2, 3, and 4 h for adipose, mammary, and liver tissue, respectively. Following incubation, 0.5 mol/l sulfuric acid was injected into the media to stop metabolic activity, and CO2 was trapped as NaHCO3 on filter paper wet with 1 mol/l hyamine hydroxide. Filter papers were removed and counted in Bio-Safe II Counting Cocktail (Research Products International, Mt. Prospect, IL) in a LS6500 Multipurpose Scintillation Counter (Beckman Instruments, Fullerton, CA). Tissues were removed from the media, saponified, and extracted with petroleum ether before counting in Bio-Safe NA (Research Products International, Mt. Prospect, IL) in the scintillation counter. Glucose oxidation to CO2 and glucose incorporation into lipids were calculated and expressed as nanomoles of glucose utilized per 100 mg tissue per 1 h of incubation.

Lipolysis. Lipolysis was measured in adipose tissue at P3. Glycerol release was measured as described by Zakrzewska et al. (37). Briefly, tissue slices were incubated for 2 h in Krebs-Ringer bicarbonate buffer containing 3% fatty acid-free BSA, in the absence of insulin. Glycerol released into the media was measured using an enzymatic assay (R-Biopharm, catalog no. E0148270). To account for differences in adipose cell number and size among tissue slices, adipose tissue was preserved in osmium tetroxide and digested in 8 mol/l urea to isolate adipocytes (38). After isolation, adipocytes were viewed under a dissecting microscope and photographed. They were counted and measured using threshold-based image segmentation, connectivity analysis, and segment labeling as described by Zakrzewska et al.
considered significant at Statistical Analysis Systems Software (Cary, NC). Differences were ment differences on particular days. Lipolysis data were analyzed using a repeated-measures design with contrast statements to examine treat- and nitrogen balance data were all analyzed using an unstructured, function to normalize the variance. The food intake, body mass, ME, and body composition data were all analyzed using ANOVA and the method used the Image Processing Toolbox of Matlab (The Mathworks).

Real-time quantitative PCR analysis. Liver tissue from dams was used for gene expression analysis. Real-time quantitative PCR analysis (qRT-PCR) was used to measure gene expression levels in the following enzymes that respond to changes in nutritional status: ATP citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS). For qRT-PCR analysis, total RNA was extracted from frozen liver tissue using Trizol Reagent (Invitrogen, Carlsbad, CA). Three times the recommended volume of Trizol was used per 50–100 mg tissue to obtain satisfactory purity as measured by the 260/280-nm ratio. The remainder of the extraction procedure was performed according to the manufacturer’s instructions. Samples were DNase treated using the DNA-free (Ambion, Austin, TX) kit. RNA was reverse transcribed into cDNA using the GeneAmp kit (Applied Biosystems, Foster City, CA), and qRT-PCR analysis was performed using the ABI Prism 7700 (Applied Biosystems) and a unique Taq-Man Assays-on-Demand Gene Expression kit (Applied Biosystems) specific for rat: ATP-citrate lyase (ACYL) (Rn00566411_m1), acetyl Co-A carboxylase (ACAC) (Rn00573474_m1), fatty acid synthetase (FAS) (Rn00589037_m1), and lipoprotein lyase (LPL) (Rn00561482_m1), and β2 microglobulin (Rn00560865_m1), which was used as the housekeeping gene.

Relative gene expression was calculated according to the following equations:

\[ \Delta C_T (\text{individual animal}) = C_T (\text{target gene}) - C_T (\text{housekeeping gene}) \]  
\[ \Delta \Delta C_T (\text{individual animal}) = \Delta C_T (\text{individual animal}) - \Delta C_T (\text{mean G21 control = calibrator}) \]

Relative expression = \[ 2^{- \Delta \Delta C_T [\text{individual animal}] } \]

where \( C_T \) is cycle threshold. The mean relative expression (RQ) for G21 control dams was used as the "calibrator," such that the RQ of each dam was divided by the mean RQ of G21 control dams. Data were expressed as mean RQ of each gene within a treatment group ± SE.

Statistical analysis. The metabolic, gene expression, behavioral, and body composition data were all analyzed using ANOVA and the Bonferroni t-test for multiple comparisons when the overall \( F \) was significantly different (36). Main effects were treatment and day of pregnancy or lactation. Data were transformed using the square root function to normalize the variance. The food intake, body mass, ME, and nitrogen balance data were all analyzed using an unstructured, repeated-measures design with contrast statements to examine treatment differences on particular days. Lipolysis data were analyzed using the Genmod procedure. All data were analyzed using the Statistical Analysis Systems Software (Cary, NC). Differences were considered significant at \( P < 0.05 \). Values in the text are means ± SE.

RESULTS

Food intake. Food intake of HG dams initially decreased when exposed to 2 G. As the rats became acclimated to the hypergravity environment, they resumed their food intake to ~17 g/day but never approached the food intake of the control dams. Food intake of pair-fed rats matched that of the HG rats. Food intake of both HG and pair-fed rats was significantly less than control rats on G11–G18 (control mean = 24 g/day; \( P < 0.001 \); Fig. 1).

Body mass. Body mass of HG dams initially decreased when exposed to 2 G. After acclimation to the hypergravity environment, HG rats gained body mass at a similar rate as the control dams (7.70, 10.64, 14.61, and 16.36 g/day on G15–G18 for control and 7.75, 9.22, 9.78, and 16.62 g/day on G15–G18 for HG) but never attained the same final body mass as the control group. Body mass of pair-fed rats matched that of the HG rats. Body mass of both HG and pair-fed rats was significantly less than control rats on G12–G18 (\( P < 0.01 \); Fig. 1).

Body composition. Body composition in terms of percent fat of the dams on P1 was 26.0 ± 1.2, 19.0 ± 1.6, and 23.0 ± 1.7% for control, HG, and pair-fed rats, respectively, and was significantly less in HG dams compared with control dams (\( P < 0.001 \)) but not different between HG dams and pair-fed rats (\( P < 0.1 \)).

ME. ME of the HG dams followed the same pattern as absolute food intake; it decreased with the reduction in food intake and increased to ~49 kcal/day after acclimation to hypergravity was achieved. ME of pair-fed dams followed the same pattern. Although dams from all treatments gained weight for the duration of the study, the ME of the HG and pair-fed rats was significantly less than that of the control group (control mean = 69 kcal/day; \( P < 0.01 \); Fig. 2).

Nitrogen balance. Nitrogen balance of HG dams decreased by ~110% when first exposed to hypergravity and then increased as they became acclimated to the environment. Nitro-
gen balance of pair-fed dams followed the same pattern as HG dams. Nitrogen balance of HG and pair-fed dams was less than that of control dams for the duration of the study (P < 0.05), except pair-fed was not different from control or HG on G15. In addition, nitrogen balance was greater in HG dams compared with pair-fed on G17 and G18 (P = 0.05; Fig. 2).

Behavior/pup survival. Although reduced in both the HG and pair-fed groups, the total amount of time the dams were active in the 24-h period beginning on P2 did not differ among treatments (31,172 ± 3,912, 24,311 ± 2,396, and 24,663 ± 3,030 s for control, HG, and pair fed, respectively).

Forty-one percent of the HG pups died within the first 24 h after birth. After adjusting the litter sizes, the survival rate of pups from HG litters was 66% for the next 24 h (48 h after birth) and 49% by P3. In contrast, there were no deaths in pair-fed or control groups.

Insulin and glucose levels. Although plasma insulin levels were less in HG dams compared with control, the differences were not significant among treatments with control, pair-fed, and HG concentrations being 0.60 ± 0.08, 0.052 ± 0.01, and 0.44 ± 0.09 ng/ml, respectively. Plasma glucose concentrations were not significantly different between control (217 ± 8.6 mg/dl) and HG dams (198 ± 10.4 mg/dl); however, in pair-fed dams the levels (173 ± 7.4 mg/dl) were decreased (P = 0.002) compared with control.

Tissue metabolism. On G21, glucose oxidation to CO2 in mammary tissue was decreased by 27% in HG rats compared with control rats (P < 0.10). In adipose tissue, glucose oxidation to CO2 in HG dams was less than half of the rate of oxidation for the control and pair-fed dams (93, 44, and 118 nmol·100 mg−1·h−1 for control, HG, and pair-fed, respectively, P < 0.01). Liver glucose oxidation to CO2 was not different among treatments (Fig. 3A). Glucose incorporation into lipids in the mammary tissue was ~32% of that observed in HG dams compared with the control and pair-fed groups (P = 0.10). In adipose, glucose incorporation into lipids was 35, 8, and 68 nmol·100 mg−1·h−1 for control, HG, and pair-fed dams, respectively, and was significantly reduced in the HG rats compared with both the control and pair-fed groups (P < 0.05). The rate of glucose incorporation into lipids in liver tissue was very low, yet significantly different among treatments (1.20, 3.23, and 0.54 nmol·100 mg−1·h−1 for control, HG, and pair-fed rats, respectively), with HG rats significantly greater than pair-fed rats (P < 0.01; Fig. 3B).

In P3 HG dams, glucose oxidation to CO2 and incorporation into lipids in mammary tissue, while greatly reduced, were not different among treatments (Fig. 3, A and B). The rate of glucose oxidation to CO2 in liver was 98, 74, and 98 nmol·100 mg−1·h−1 for control, HG, and pair-fed dams, respectively, and was significantly less in the HG rats compared with control and pair-fed rats (P < 0.05; Fig. 3A). Glucose incorporation into lipids in liver was significantly different among treatments (4.76, 1.09, and 2.32 nmol·100 mg−1·h−1 for control, HG, and pair fed, respectively), with control rats significantly greater than HG and pair-fed rats (P < 0.05; Fig. 3B). Since lipolysis is the major form of lipid metabolism in adipose tissue during lactation, glycerol release was measured. It was 1,236 ± 133, 765 ± 149, and 1,125 ± 135 nmol glycerol/million adipocytes per hour (±SE) for control, HG, and pair-fed dams, respectively, and was significantly less in HG dams compared with the control and pair-fed groups (P = 0.05).

**Gene expression.** To further explore the effects of hypergravity on liver lipid metabolism, gene expression of enzymes that respond to changes in nutritional status were measured using qRT-PCR. In G21 dams, relative gene expression of ACL was 1.1, 2.1, and 0.5 for control, HG, and pair-fed dams, respectively, and was significantly less in pair-fed dams compared with HG dams (P = 0.05; Fig. 4A). Additionally, gene expression was less in pair-fed rats compared with both control and HG rats for ACC (1.0, 1.1, and 0.4 for control, HG, and pair fed, respectively; P < 0.01; Fig. 4B) and FAS (1.3, 2.2, and 0.3 for control, HG, and pair fed, respectively; P < 0.05; Fig. 4C).

In P3 dams, ACC and FAS expression were not different among treatments. Gene expression was significantly less in HG rats compared with control for ACL (1.1, 0.5, and 0.8 for control, HG, and pair fed, respectively; P < 0.05), while expression in pair-fed dams was not different from the control or HG groups (Fig. 4A).

When gene expression of P3 dams was compared with that of G21 dams, ACL and FAS increased more than fourfold in
control and pair-fed dams \((P < 0.05)\) but did not change in HG dams (Fig. 4, A and C). ACC, which was low in pair-fed rats, increased to levels similar to control \((P < 0.01)\). ACC did not increase from pregnancy to lactation in control or HG rats but did increase in pair-fed rats (Fig. 4B).

When gene expression of control, HG, and pair-fed dams was plotted vs. the rate on glucose incorporation into lipids, a positive correlation \((r = 0.74)\) was evident for ACL but not for ACC or FAS. When control or pair-fed gene expression and rate of glucose incorporation into lipids were plotted alone, the correlation coefficient increased to 0.81 and 0.86, respectively (Table 1). In addition, a positive correlation \((r = 0.75)\) between the rate of glucose incorporation into lipids and FAS expression was found when control data were plotted alone (Table 1). There was little correlation between the rate of glucose incorporation into lipids and gene expression for the HG dams.

**DISCUSSION**

Similar to the previous report of our laboratory (30), food intake and body mass of the HG dams decreased on exposure to hypergravity. Once the rats became acclimated to the environment, food intake and body mass increased but did not reach the level of the controls. As expected, food intake and body mass of the pair-fed dams matched that of the HG dams for the duration of the study. Furthermore, no difference in overall activity of the dams was found among treatments. Therefore, the weight loss observed in hypergravity-exposed dams may be explained by reduced food intake, since body weight, adiposity, (percent body fat) and ME were not different between pair-fed and HG-treated dams. In addition, as expected in conditions of reduced food intake, nitrogen balance was generally neutral to positive in HG rats, with pair-fed rats showing a similar pattern, and both were lower than nitrogen balance of controls.

Importantly, these findings show that the reduced food intake and body mass due to hypergravity exposure cannot explain the dramatic decrease in neonatal survival in HG-treated litters. Only 49% of the pups in the HG treatment survived to P3, while 100% of the pups in the pair-fed treatment survived to P3, irrespective of similarities in food intake and body mass for pair-fed and HG dams. Further, fetuses and pups from HG dams have reduced body weight compared with control and pair-fed animals (data not shown), and while dam behavior was altered in HG it did not apparently cause the hypergravity associated increased pup mortality (39). These data suggest that hypergravity exposure alters the dam’s ability to nurture her young. In the remainder of this study,

<table>
<thead>
<tr>
<th>Group</th>
<th>ACL (r(P))</th>
<th>ACC (r(P))</th>
<th>FAS (r(P))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary control</td>
<td>0.81 ((P &lt; 0.001))</td>
<td>0.36 (NS)</td>
<td>0.75 ((P &lt; 0.01))</td>
</tr>
<tr>
<td>HG</td>
<td>0.31 (NS)</td>
<td>0.23 (NS)</td>
<td>0.19 (NS)</td>
</tr>
<tr>
<td>Pair fed</td>
<td>0.86 ((P &lt; 0.001))</td>
<td>0.52 (NS)</td>
<td>0.58 ((P &lt; 0.05))</td>
</tr>
</tbody>
</table>

HG, hypergravity; ACL, ATP citrate lyase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase.
we test the hypothesis that the homeorhetic changes in mammary, adipose, and liver tissue metabolism necessary for a successful lactation are altered in hypergravity and account for the reduced pup survival.

The rate of glucose oxidation and lipogenesis in mammary tissue was reduced in pregnant HG rats compared with the control. These results are similar to previous findings in our laboratory (25). Glucose oxidation to CO₂ in mammary tissue of the pair-fed dams was intermediate between the control and the HG dams, indicating that reduced food intake may contribute to the hypergravity effect. In contrast, glucose incorporation into lipids in HG dams was markedly less (70%) than both pair-fed and control rats. The fact that rates of lipid synthesis from glucose were the same in the pair-fed and control groups supports the concept that changes in metabolism experienced in HG dams are not the result of a reduction in food intake (Fig. 3). In mammary tissue from P3 dams, the rates of glucose oxidation and incorporation into lipids were reduced, but not different among treatments for either measure of metabolism (Fig. 3). While not significant, this magnitude decrease is consistently observed in hypergravity and, therefore, is biologically significant. Smaller litter sizes may have contributed to the lack of differences in glucose utilization on P3. Since 50% of the HG pups died within 48 h after birth, litters were normalized to account for neonatal death in all treatments, and thus dams needed to nourish much smaller litters. Significantly smaller litters likely did not push the HG dams to their maximum lactation capacity on P3, and thus lactation was not as great a stress on mammary metabolism as it would have been with a full litter.

Glucose utilization in adipose tissue of G21 HG dams was less than half of the rate in the control and pair-fed dams (Fig. 3), and lipolysis was significantly less in P3 HG rats compared with control and pair-fed dams. Smaller adipocytes were also observed in visceral adipose tissue from HG dams compared with control and pair fed (data not shown). Etherton and Allen (7) have showed a positive relationship between the rate of lipolysis and adipocyte size. We do not know whether smaller adipocytes resulted in reduced lipolysis or vice versa. Additionally, the fact that the rate of lipolysis in pair-fed rats was the same as the rate of lipolysis in control rats indicates that the lower level of glycerol release in HG dams is not due to reduced food intake. These data suggest that hypergravity is negatively affecting homeorhetic mechanisms necessary for lactation. The adaptations in rates of lipogenesis and lipolysis in adipose tissue at the transition from pregnancy to lactation are a good example of homeorhesis, as it allows the dam to adapt to supply nutrients to the fetuses to prepare for parturition (1, 14, 15, 28).

Glucose oxidation to CO₂ in the liver of G21 dams was not different among treatments. Glucose incorporation into lipids was three- to sixfold greater in the HG dams compared with the control and pair-fed groups, respectively (Fig. 3). It is possible that liver lipid metabolism increases in HG dams to send more triglycerides to other tissues, such as mammary and adipose, for storage in preparation for the upcoming lactation. This is supported by work done by Feller et al. (8), who found that prolonged exposure (12 mo) of female rats to hypergravity (4.7 G) significantly increased in vitro acetate incorporation into lipids in liver tissue while the total lipid content of the liver significantly decreased. This suggests that the liver is converting more acetate to lipids for export from the liver to other tissues, thus leaving fewer lipids stored in the liver itself. In contrast to pregnancy, liver metabolism was reduced in lactating HG dams. Glucose oxidation to CO₂ was significantly less in HG dams compared with both control and pair-fed dams, and glucose incorporation into lipids was reduced in HG and pair-fed dams compared with control (Fig. 3). Again, the difference in glucose metabolism in liver between HG and pair-fed dams indicates that reduced food intake does not account for the hypergravity effect. The switch in liver lipid metabolism in pregnant dams compared with lactating dams exemplifies the changes in overall metabolism that occur to support the demands of milk production. During lactation, lipolysis is predominant in the liver, and triglycerides are shunted to the mammary gland for milk fat synthesis. The increased energy demands on the dams and the large de novo synthesis of lipids in the mammary gland also do not allow for the higher level of liver lipid metabolism.

To further explore the effects of hypergravity and food intake on liver lipid metabolism, we analyzed gene expression of three enzymes in the liver that are known to change in response to nutritional status. ACL, ACC, and FAS are involved in de novo synthesis of long-chain fatty acids. Levels of these enzymes are increased by carbohydrate-rich diets, insulin, and thyroid hormone and are decreased by starvation and glucagon (11). Gene expression of ACL, ACC, and FAS in the liver matches the changes found in the metabolic data. This was expected since glucose incorporation into lipids in liver tissue is a measure of lipid synthetic rate. Expression of ACL, ACC, and FAS in the livers of pair-fed dams was significantly less than in the control and HG dams at G21 (Fig. 4), thus indicating that a reduction in food intake reduces gene expression, but cannot account for the changes observed in the liver of rats exposed to hypergravity. If the reduction in food intake caused the changes observed in hypergravity, gene expression of those enzymes involved in lipid synthesis would have also been reduced in HG dams.

Expression of ACL and FAS increased more than 350% from pregnancy to lactation in control and pair-fed dams. Expression of ACC also increased in pair-fed dams (Fig. 4). This supports the increase in glucose utilization by the liver to support lactation. However, Smith (31) measured enzymatic activity of key lipogenic enzymes and found that the activity of ACL was reduced by 30% in liver from pregnant rats compared with unmated controls and did not increase until peak lactation and involution. The increase in gene expression found in our study may, therefore, be in preparation for increased enzymatic activity during peak lactation. In contrast to the increase in gene expression observed in pair-fed and control rats as they transitioned from pregnancy to lactation, expression of lipid synthesis genes did not change in HG dams. Clearly, liver lipid synthesis is altered in hypergravity; however, it is not simply a change in gene expression but includes changes in transcriptional regulators, gene stability, and/or activation.

When the rates of lipid synthesis were plotted against gene expression, ACL expression was highly correlated with lipid synthesis (r = 0.74). If each treatment was plotted separately, both control and pair-fed rats had very strong correlations between lipid synthesis and expression of ACL (r = 0.81 and 0.86, respectively; Table 1). On the other hand, lipid synthesis was poorly correlated with gene expression in the liver of HG
dams ($r \leq 0.31$; Table 1). ACC expression would also be expected to be correlated with lipid synthesis since it is the rate-limiting step in fatty acid synthesis. However, the relationship was not as clear. Hormones, such as insulin and glucagon, may impact ACC expression, therefore decreasing its reliability in predicting lipid synthesis. Expression of FAS, which is involved in fatty acid chain elongation, was also positively correlated to lipid synthesis in control and pair-fed rats ($r = 0.75$ and 0.58, respectively; Table 1). The lack of correlation among the data in the HG dams indicates that other factors are influencing gene expression and/or liver lipid metabolism when the rats are exposed to the hypergravity environment.

Pregnancy is accompanied by the development of insulin resistance in a variety of species (6, 9, 10). Alterations in insulin sensitivity are also associated with changes in the metabolism in specific tissues over the course of pregnancy. However, there were no significant changes in insulin or plasma glucose concentrations with exposure to hypergravity. In male rats, our group and others have previously shown a dose-related reduction in insulin levels during hypergravity exposure with no change in glucose concentrations, suggesting an increase in insulin sensitivity (17, 18). These changes were not observed in the present study of pregnant animals.

The majority of changes observed in tissue metabolism of pregnant and lactating rats exposed to hypergravity were due to the increase in G load and not the reduction in food intake. Alterations in tissue metabolism related to lipid synthesis coordinate with changes in expression of enzymes related to lipid synthesis in the liver. However, genes involved in lipid synthesis did not increase when HG animals transitioned from pregnancy to lactation, and the correlation between lipid synthesis and gene expression disappeared, indicating a disruption of the gene expression pathway. Thus it is hypergravity and not changes in food intake that significantly alters mammary, adipose, and liver function. This suggests that the mammary gland is not sufficiently geared toward de novo lipogenesis, and the white adipose tissue and liver are not poised to coordinate with changes in expression of enzymes related to lipid synthesis from rats exposed to chronic radial acceleration.


ACKNOWLEDGMENTS

We gratefully acknowledge Tim Hunter, Scott Tighe, and Mary Lou Shane from the Vermont Cancer Center DNA Analysis Facility for running RNA quality analysis and performing the real-time quantitative RT-PCR reactions. We also thank Dr. Michael Van Amburgh and Debbie Ross from Cornell University for the use of their bomb calorimeter, as well as for tremendous help with running the samples and trouble-shooting the machinery. Finally, we sincerely thank Kathryn Coughlan, Tanya Mulvey, and Gina Roberts for superb assistance with data collection and analysis.

Present address of C. Wade and L. Baer: MTR Branch, US Army Institute of Surgical Research, Fort Sam Houston, TX, 78234.
Present address of A. Ronca: Dept. of Obstetrics and Gynecology, Wake Forest Univ. School of Medicine, Winston-Salem, NC, 27157.

GRANTS

This research was supported by National Aeronautics and Space Administration Grants NCC2-1373 and EPSCoR NCC5-581.

REFERENCES

8. Feller DL, Neville ED, Oyama J, Averkin EG. Chemical and metabolic changes of hepatic lipids from rats exposed to chronic radial acceleration.


