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# Effects of spaceflight and thyroid deficiency on hindlimb development. I. Muscle mass and IGF-I expression

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**Adams, G. R., S. A. McCue, P. W. Bodell, M. Zeng, and K. M. Baldwin.** Effects of spaceflight and thyroid deficiency on hindlimb development. I. Muscle mass and IGF-I expression. *J. Appl. Physiol.* 88: 894–903, 2000.—Thyroid deficiency (TD) in neonatal rats causes reduced growth of skeletal muscle that is disproportionately greater than that for other tissues (G. R. Adams, S. A. McCue, M. Zeng, and K. M. Baldwin. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 276: R954–R961, 1999). TD depresses plasma insulin-like growth factor I (IGF-I) levels, suggesting a mechanism for this effect. We hypothesized that TD and exposure to spaceflight (SF) would interact to reduce skeletal muscle growth via a reduction in IGF-I levels. Neonatal rats were flown in space for 16 days. There was a similar, nonadditive reduction in the growth of the body (~50%) and muscle weight (fast muscles, ~60%) with either TD or SF. In the soleus muscle, either SF or TD alone resulted in growth reductions that were augmented by SF-TD interactions. There were strong correlations between *1*) muscle mass and muscle IGF-I levels and *2*) circulating IGF-I and body weight. These results indicate that either hypothyroidism or exposure to SF will limit the somatic and muscle-specific growth of neonatal rats. The impact of these perturbations on skeletal muscle growth is relatively greater than the effect on somatic growth. The mechanisms by which either TD or SF impact growth appear to have a common pathway involving the control of plasma and muscle IGF-I concentrations.

skeletal muscle; microgravity; unweighting; fast twitch; slow twitch; hypothyroidism; insulin-like growth factor I

IN RATS, THE FIRST month of postnatal life encompasses a period of rapid growth and development during which both the total body and skeletal muscle mass of neonates increase exponentially and the adult pattern of muscle contractile protein expression becomes fully established (6). During this time, growth-related signals such as thyroid hormone levels are increasing to coordinate both growth and the maturation process (13, 20, 24, 26, 30). Experimentally induced hypothyroidism has been shown to impede both general and muscle-specific growth and maturation (e.g., Refs. 16 and 25). We recently reported that the imposition of a thyroid-deficient state on neonates starting at 7 days postpartum resulted in a retardation in the growth of the heart and skeletal muscles of these animals (6). Interestingly, the growth-inhibiting effect of hypothyroidism on skeletal muscle was disproportionately greater than that for other tissues and organs. Clearly, skeletal muscle developmental processes are strongly dependent on the thyroid axis of control.

One possible mechanism for the effect of hypothyroidism on muscle growth is suggested by reports that indicate that thyroid hormone regulates components of the growth hormone (GH)-insulin-like growth factor I (IGF-I) system. For example, Nanto-Salonen et al. (24–26) have reported that hypothyroidism in neonatal rats disrupts normal developmental patterns of IGF-I and -II peptide expression and the IGF binding proteins. Consistent with this notion, we found that thyroid deficiency resulted in declining levels of circulating IGF-I at a time when euthyroid neonates were experiencing a developmentally critical "upsurge" in plasma IGF-I (6). IGF-I is thought to mediate many of the physiological effects of GH (15), and disruption of the IGF-I axis during critical developmental periods appears to have powerful effects on processes related to growth and development.

In addition to hormonal influences, skeletal muscles are known to be sensitive to changes in loading state. In adult rats, spaceflight for periods as short as 6–9 days causes atrophy of both fast- and slow-twitch skeletal muscles as well as functionally significant alterations in muscle contractile performance (e.g., Refs. 8, 10, and 11). In general, the atrophy response to unloading is more pronounced in antigravity muscles expressing greater proportions of the slow myosin heavy chain (MHC) isoform (2, 7, 11). Although the effects of muscle unloading have been studied extensively in mature mammals, the importance of muscle loading during development has not been comprehensively addressed. In one of the few such studies, Elder and McComas (14) found that chronic hindlimb unweighting in weanling rats (e.g., 18 days of age) resulted in significantly lower muscle mass and delayed myofiber phenotype development in the soleus through 18 wk of age.

In rat neonates, the period from 7 to 30 days postpartum is crucial for the development of hindlimb locomotive patterns (12). Starting at postpartum *day 6–7*, neonates begin pelvic weight-bearing activity, and by *day 10* they have initiated true quadrapedal locomotion (12). Intermittent hindlimb unweighting of rat neonates from postpartum *day 14* to *30* results in persistent disruption of gaiting patterns in adulthood (i.e., after 30 days of recovery) (31). To our knowledge, there are few or no data that address the extent to which factors known to regulate aspects of skeletal muscle

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growth, such as thyroid hormone, GH, and IGF-I, interact with and/or may respond to the loading conditions encountered during the time period when the neonatal rats become ambulatory.

On the basis of the foregoing information, we developed the following hypotheses. *1*) The unloading of neonatal rat muscles during the developmental period from 7 days postpartum through ~20 days of age results in a significant reduction in skeletal muscle growth. *2*) The same duration of unloading would have less pronounced effects on skeletal muscle growth when imposed on older neonates (e.g., ~2 wk old) whose muscles were further along the developmental cascade. *3*) The imposition of a hypothyroid state would predominate over an unloading-induced reduction in muscle development. *4*) Reductions in IGF-I expression mediate alterations in somatic and muscle-specific growth in response to unloading and/or the hypothyroid state.

To test these hypotheses, it was necessary to impose a chronic (i.e., continuous) unloaded state on both normal and thyroid-deficient neonatal rats. The most common method used to study the effects of unloading on freely moving limb muscles is the rat hindlimb suspension model (e.g., Ref. 4). Although this model has been applied to smaller animals such as mice and neonatal rats, the implementation of continuous hindlimb suspension in neonates is problematic due to the need for nursing at regular intervals, thereby providing a discontinuous or episodic unloading stimulus (31). Spaceflight provides the opportunity to maintain a dam and neonates together while providing continuous unloading of the limb muscles. Accordingly, we performed the studies reported herein as part of the National Aeronautics and Space Administration (NASA)/National Institutes of Health Space Life Sciences Neurolab mission, which occurred during April-May of 1998. This and the accompanying study (5) report the results of studies conducted on euthyroid and hypothyroid neonatal rats that were exposed to spaceflight for 16 days. The findings indicate that there were significant, apparently unloading-specific effects on the development of rat skeletal muscles and that hypothyroidism resulted in similar derangements that were in some cases additive with the unloading effect. In particular, both hypothyroidism and unloading significantly impacted the plasma and muscle IGF-I levels in neonatal rats.

## METHODS

*Litter formation and experimental design.* Timed pregnant dams were obtained from Taconic Farms (Germantown, NY) and housed in standard rodent cages in the vivarium at Kennedy Space Center in Florida. Shortly after birth, each litter was adjusted to $n = 8$ pups and matched for gender distribution. Those litters assigned to the series of integrated experiments designated as the Mammalian Development projects in the Neurolab Mission were selected on the basis of *1*) the pups demonstrating normal body growth during the first 5 days of age, *2*) the corresponding dams exhibiting normal water and food consumption profiles, and *3*) the dams demonstrating effective maternal behaviors such as neonate retrieval. A cohort of litters from this pool was randomly assigned to the following experimental groups for this particular project: *1*) 7-day euthyroid vivarium control, *2*) 7-day euthyroid asynchronous ground control (AGC), *3*) 7-day thyroid-deficient (TD7) vivarium control, *4*) TD7-AGC, *5*) 7-day euthyroid flight based, and *6*) thyroid-deficient flight based. The flight groups for this component of the project were launched into space at 7 days of age.

Three additional litters of animals from timed-pregnant rats were randomly assigned to experimental groups representing animals that were launched at ~14 days of age and were designated as *1*) 14-day euthyroid vivarium control, *2*) 14-day euthyroid AGC, and *3*) 14-day euthyroid flight based. There were six neonates per litter in these groups. Because there were insufficient housing facilities (see below) for the older neonatal groups during spaceflight, ground-based and flight-based thyroid-deficient groups were not used in the experiments involving the older neonatal groups.

Three additional groups of neonates were killed at 7 days of age (basal) to provide baseline values for variables of interest. All experimental protocols were approved by both the NASA and University of California Irvine Institutional Animal Care and Use Committee.

*Procedures to induce thyroid deficiency in neonatal rats.* The experimental design involved the delivery of propylthiouracil (PTU) to the neonates via the dam's milk. However, technical constraints associated with the flight hardware precluded the delivery of PTU to the dam via either food or water supplies. Accordingly, PTU was administered to the dam via the implantation of mini-osmotic pumps (Alzet 2ML4), which were filled with a sterile PTU solution (56 mg/ml) under aseptic conditions. Pumps were prepared 4 h before implantation and maintained in physiological saline at 37°C to initiate flow. On the basis of nominal pump performance (2.24 ± 0.09 μl/h), this concentration of PTU delivered ~12 $mg \cdot kg^{-1} \cdot day^{-1}$ to the dam (~250 g body wt) for at least 28 days. This PTU dose is approximately three times that required to completely block the conversion of L-thyroxine ($T_4$) to 3,5,3′-triiodothyronine ($T_3$) (18). The results from pilot studies indicated that this protocol rendered both the dam and nursing neonates hypothyroid (see RESULTS and Ref. 6). To validate the effectiveness of PTU delivery via the dam's milk, ground-based pilot studies were conducted that compared direct, daily PTU injections to the neonates (12 $mg \cdot kg^{-1} \cdot day^{-1}$) with the effects of PTU delivery via the dam as described above.

Two days before launch, when the neonates designated for the TD7 group were 5 days old, the thyroid-deficient dam was anesthetized with Metofane (Pitman-Moore, Mundelein, IL) administered via a nose cone. After anesthetic induction, the abdominal area on one side was shaved and swabbed with Betadine, and the dam was then placed in a sterile field. An ~1.5-cm incision was made 2.5 cm from the bottom of the rib cage on the side of the dam to avoid interference with the mammary glands. An incision was then made in the underlying muscle to gain access to the peritoneal cavity. The osmotic pump containing PTU was inserted into the peritoneal cavity. The muscles of the peritoneal wall and skin were then closed sequentially with suture. The dams were fully recovered and returned to the neonates in 14.9 ± 3.6 min (mean ± SD). On subsequent days, additional dams were implanted, and their neonates were designated as TD7 ground-based controls.

*Cage facilities for flight- and ground-based groups.* The flight groups that were launched at 7 days of age were housed in research animal holding facility (RAHF) cages that were fitted into a rack located in the spacelab module, which was carried in the payload bay of the orbiter. These cages were modified to provide a "nursing" area for the dam and litter as well as a "free" area for the dam. The corresponding ground-

based AGC groups were housed in modified vivarium cages of similar dimensions to the RAHF cages, whereas the ground-based vivarium controls were housed in standard vivarium cages. The older (e.g., *day 14*) flight group was housed in an animal enclosure module (AEM) cage designed to fit into a rack located in the middeck of the orbiter. The corresponding AGC animals were housed in cages closely approximating the configuration of the AEMs, and the ground-based vivarium controls were housed in standard vivarium cages. Two days before launch, all litters were moved to their respective flight- and ground-based cage facilities, and, 24 h before launch, the flight groups were loaded into the space shuttle. These experiments were temporally staggered so that, although the ground-based AGC and vivarium groups were processed 48 and 96 h, respectively, after that of the flight groups, the ages of each of the flight- and ground-based groups representing the "younger" and "older" neonatal groups were identical at the time of tissue procurement.

*Tissue processing.* Sixteen days after launch, the rats returned to Kennedy Space Center, which served as the orbiter landing site. Five hours after they landed, euthanasia (50 mg/kg Nembutal) and dissection of the rats commenced. As part of this process, blood was withdrawn from the left ventricle, the hematocrit was determined, and then the sample was centrifuged at 1,000 *g* for 10 min at 4°C. The resulting plasma was stored at −80°C until analyzed for IGF-I. The ventricles, soleus, plantaris, medial gastrocnemius (MG) and lateral gastrocnemius, the three vasti [intermedius (VI), medialis, and lateralis], and tibialis anterior (TA) muscles were then rapidly removed, trimmed of connective tissue, weighed, and snap frozen. All tissue and plasma samples were stored at −80°C for subsequent analyses. The tissue and plasma samples were shipped to the University of California, Irvine, on dry ice where the analytic procedures described below and in the accompanying study (5) were performed. The amount of muscle tissue, and in particular slow-twitch anti-gravity muscles, obtained from the flight groups was limited (see Table 2). Due to the apportionment of these tissues for various analyses, we chose to focus on two representative fast-twitch muscles for the complete suite of analyses presented herein. As detailed in the accompanying study (5), samples of cardiac muscle were prepared and analyzed for MHC content as a verification of the hypothyroid state.

*Plasma and muscle IGF-I content.* The IGF-I extraction was performed as described previously (3). Briefly, muscle samples from the TA and MG were pulverized under liquid nitrogen, and the resultant powder was transferred to tared, precooled microcentrifuge tubes for acid/ethanol extraction (9). Plasma samples were also extracted via the acid/ethanol method. The IGF-I RIA was conducted according to the manufacturer's instructions using a rat-specific RIA kit (DSL, Webster, TX).

*DNA determination.* The muscle DNA concentration was measured as a gross measure of continuing cellular proliferation during development. Muscle DNA was measured in whole muscle homogenates using a fluorometric assay for the DNA binding of fluorochrome bisbenzimide H-33258 (Calbiochem, San Diego, CA). Calf thymus DNA was used as a standard (22).

*Statistical analysis.* All values are reported as means ± SE. For each time point, treatment effects were determined by ANOVA with post hoc testing (Student-Newman-Keuls) using the Prism software package (Graphpad). Pearson's correlation analyses of relationships were performed using the Prism package. For all statistical tests, the 0.05 level of confidence was accepted for statistical significance.

## RESULTS

*General observations.* Spaceflight presented the lactating dams with a number of challenges. One of the primary concerns appears to have been the retention of the neonates in the nesting/nursing area. As a result of these stresses, a number of the younger neonates were either abandoned or destroyed by the dams in an apparent attempt to maximize the survival of the remaining offspring. During the flight, distressed or abandoned neonates were euthanized by flight crew members. After landing, NASA veterinarians examined each neonate and disqualified any apparently unhealthy animals from further analysis. As a result, the number of flight animals available for analyses was somewhat reduced (see Table 2).

In general, rats from the two ground-based control groups (i.e., vivarium control and AGC) had similar values for the measured variables whether they were housed in standard vivarium cages or cages similar to those used for the flight. To simplify the presentation of results, the data for these two groups have been combined into a single ground-based group. Accordingly, for reporting purposes, the group designations as indicated in Table 1 are as follows: NC7 ground and NC7 flight (7 days old at launch) NC14 ground and NC14 flight (14 days old at launch) for euthyroid (normal control) animals and TD7 ground and TD7 flight (7 days old at launch) (Table 1) for the thyroid-deficient groups.

In ground-based pilot studies, we determined that the delivery of PTU via the dam's milk resulted in a depression in plasma $T_3$ and $T_4$ similar to that seen when the PTU dose (12 $mg \cdot kg^{-1} \cdot day^{-1}$) was administered via direct injection to the neonates (Fig. 1). In the primary study, the plasma recovered from flight neonates was reserved for IGF-I analysis, as very little plasma was obtained from these small animals. However, as is evidenced by the decrease in body growth (see Table 2) and by a complete reversal of the MHC isoform profile of the ventricles from the TD7 vs. NC7 animals [data presented in the accompanying study

Table 1. *Litter and treatment characteristics*

| Age at Launch | Litter Assignment | Analysis Group Designation |
|---|---|---|
| | *Euthroid* | |
| 7 Days | Basal | Basal |
| | VC7 | NC7 ground* |
| | AGC7 | |
| | Eu-FLT7 | NC7 FLT |
| 14 Days | VC14 | NC14 ground* |
| | AGC14 | |
| | Eu-FLT14 | NC14 FLT |
| | *Thyroid deficient* | |
| 7 Days | TD7 VC | TD7 ground* |
| | TD7 AGC | |
| | TD7 FLT | TD7 FLT |

VC, standard vivarium cages; AGC, flight analog cages (asynchronous ground control); NC, normal (euthyroid) control; TD, thyroid deficient; Eu, Euthyroid; FLT, flight. *VC and AGC groups were combined (see RESULTS).

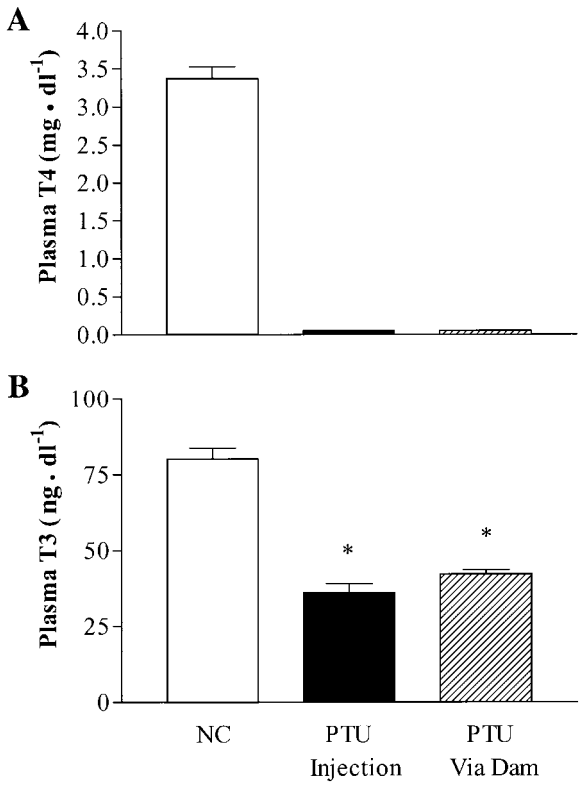


Fig. 1. Effects of propylthiouracil (PTU) administration on the plasma L-thyroxine ($T_4$; *A*) and 3,5,3′-triiodothyronine ($T_3$; *B*) levels of rat neonates. In a ground-based validation study, the effectiveness of PTU delivery via the dam's milk vs. direct daily PTU injection to the neonates (see METHODS) on plasma $T_4$ and $T_3$ concentrations was found to be equivalent. $*P < 0.05$ vs. 7-day-old normal control (euthyroid) group (NC7); $n = 6$ per group.

(5)], the delivery of PTU from dam to neonate was sufficient to impose a significant hypothyroid state during the spaceflight. In addition, the suppression of α-MHC expression (<10% α-MHC) seen in the TD7 flight and TD7 ground rats (5) indicates that these animals were nursing (and therefore receiving PTU) throughout the flight period.

As is delineated in the accompanying study (5), cardiac MHC expression is extremely sensitive to nutritional status (17, 23). The cardiac MHC profiles of the NC7 and NC14 (both flight and ground) neonates clearly establish that these animals were also receiving adequate nutrition [see accompanying study for data (5)].

The blood hematocrit values for the 7-day-old rats were similar across groups, with a slightly lower value in the TD7 ground vs. NC7 ground group (Fig. 2). The 14-day-old flight rats had a small but significant increase (3%) in hematocrit levels. However, none of the hematocrit values varied substantially, indicating that all the animals were sufficiently hydrated (Fig. 2).

*Somatic growth.* Exposure to spaceflight resulted in a significant reduction in the general growth of neonatal rats (NC7 and NC14) (Fig. 3*A*). The imposition of a thyroid-deficient state resulted in a reduction in body mass growth that was similar in effect to spaceflight exposure and was not significantly altered further by the combination of the two interventions (Fig. 3*A*).

The reductions in growth, evidenced by the body weight measurements, were paralleled by decreased levels of plasma IGF-I (Fig. 3*B*) such that there was a significant correlation between plasma IGF-I levels and body weight among the experimental groups (Fig. 3*C*).

*Skeletal muscle growth.* The general decrease in somatic growth was also reflected in ventricular and in lower limb muscle weights from the flight vs. age-matched ground-based rats (Table 2). Because of the differences in body weight, a more meaningful presentation of these data can be obtained from the normalization of muscle weight to the individual body weight. In addition to accounting for generalized differences in somatic growth, normalized muscle weight depicts the physiologically relevant relationship between muscle mass and the potential load (body weight) imposed on limb muscles. The normalized weights of three representative muscles, in order of increasing responsiveness to spaceflight, are presented in Fig. 4. The TA is an ankle flexor that does not directly oppose gravity in normal use. Compared with the mass of the NC7 ground group, this muscle demonstrated a small but significant decline in growth as a result of exposure to spaceflight (−15%) and to hypothyroid treatment (−34%) and a combination of these two (−41%) interventions. In the older neonates, the growth of the TA muscle was not significantly retarded (Fig. 4). The MG is a extensor of the ankle and thus would normally oppose gravity during movement. It has an MHC profile that is similar (primarily fast) to the TA (4). The MG muscles of 7-day-old rats demonstrated a 30% decrease in growth from exposure to spaceflight, whereas growth of the MG muscles from thyroid-deficient rats was depressed by 42 and 47% in the TD7 ground and TD7 flight groups, respectively (Fig. 4). The patterns of response seen in other predominantly fast MHC expressing

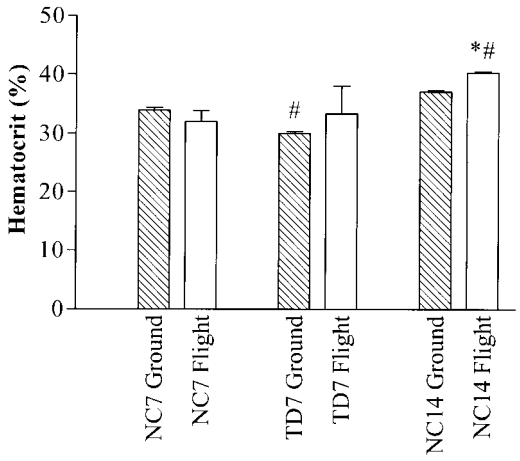


Fig. 2. Blood hematocrit values of flight and ground control neonates following 16 days of spaceflight. $*P < 0.05$ vs. ground group; $\#P < 0.05$ vs. NC7 ground; *n* values are given in Table 2. TD7, 7-day-old thyroid deficient (hypothyroid); NC14, 14-day-old normal control (euthyroid). Ground, age-matched ground-based controls; flight, neonates exposed to spaceflight for 16 days.

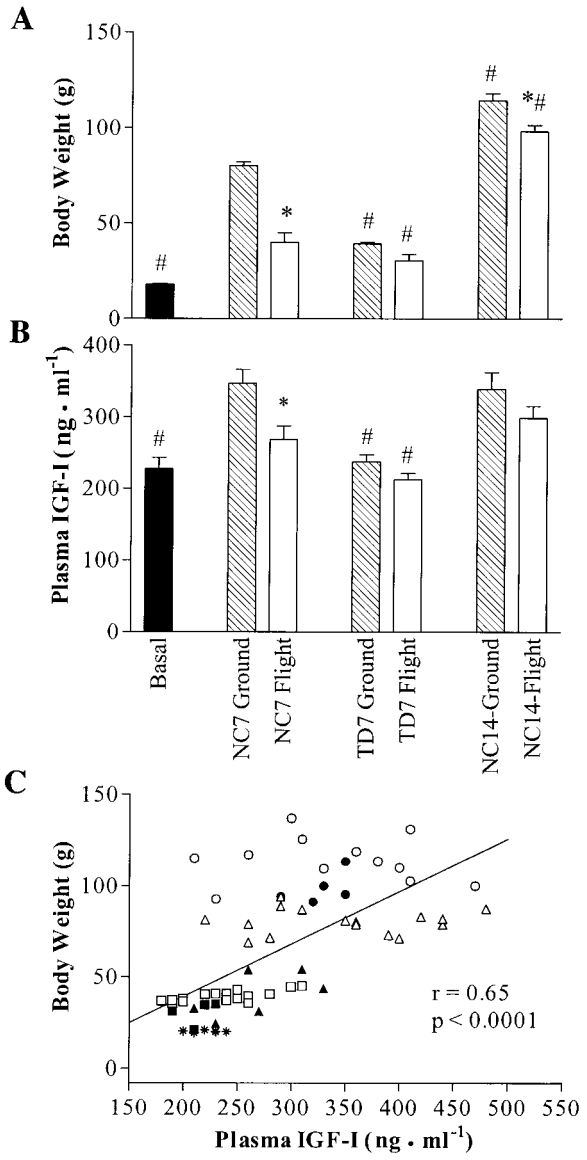


Fig. 3. Effects of thyroid deficiency and/or spaceflight on neonate body weight and plasma insulin-like growth factor I (IGF-I) concentration. Basal, 7-day-old neonates. *A*: body weight. *B*: plasma IGF-I concentration. *$P < 0.05$ vs. ground group; #$P < 0.05$ vs. NC7 ground; *n* values are given in Table 2. *C*: correlation between body weight and plasma IGF-I concentration with best fit line. *, Basal; △, NC7 ground; ▲, NC7 flight; □, TD7 ground; ■, TD7 flight; ○, NC14 ground; ●, NC14 flight.

muscles from the knee extensor and ankle extensor groups were quantitatively similar to that of the MG muscle (Table 2). The soleus is a slow-twitch MHC-expressing antigravity muscle that also contributes to ankle extension during locomotor activity and is also thought to function to maintain posture in rats. In euthyroid rats, spaceflight depressed the growth of the soleus by 50% in the 7-day-old and by 32% in the 14-day-old neonates (Fig. 4). PTU treatment resulted in a 30% decrease in soleus growth (vs. NC7 ground). The combination of thyroid deficiency and spaceflight resulted in an additional 30% decrease (e.g., total −61%) in soleus growth.

Two representative muscle types were selected for more complete biochemical analysis: the MG, a representative fast-twitch antigravity limb muscle, and the TA, a fast-twitch, non-weight-bearing muscle. Because of the extremely small size of the soleus and VI (see Table 2), there was insufficient tissue from either of these slow-twitch muscles to allow for the complete battery of analyses reported herein.

Growth-related increases in MG muscle protein and DNA content were significantly reduced by either exposure to spaceflight or imposition of thyroid deficiency (Fig. 5). In each case (e.g., protein or DNA content) spaceflight plus thyroid deficiency did not have a significant additive effect relative to the TD7 ground group. As with muscle mass, the DNA and protein content of the older neonates (NC14) was relatively insensitive to spaceflight in the MG muscle (Fig. 5). The DNA and protein content of the TA muscles was quantitatively similar to the MG (data not shown). The DNA per muscle and protein per muscle were highly correlated, suggesting that the retardation of growth in the MG (Fig. 5*C*) and TA (data not shown) muscles did not disrupt the apparent coordination between changes in these measures.

*IGF-I and muscle growth.* Changes in muscle IGF-I peptide levels for the TA and MG muscles were associated with the retardation seen in muscle weights (Figs. 4 and 6). In the NC7 ground-based neonates, an apparent developmental surge in IGF-I can be seen compared with basal values (Fig. 6, *A* and *B*). This response appears to have been blunted in the flight and TD7 rats such that their IGF-I levels corresponded

Table 2. *Body weight and muscle weight from basal, control TD, and space-flown neonatal rats*

| | *n* | Body Weight, g | Muscle Weight, mg | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ventricle | Soleus | Plantaris | MG | LG | VI | VL | VM | TA |
| Basal | 24 | 17 ± 1† | 77 ± 4† | 4 ± 0.3† | 7 ± 1† | 12 ± 1† | | | | | 15 ± 1† |
| NC7 ground | 16 | 80 ± 2 | 340 ± 13 | 31 ± 1 | 64 ± 2 | 135 ± 4 | 155 ± 6 | 19 ± 1 | 121 ± 4 | 61 ± 3 | 128 ± 4 |
| NC7 FLT | 6 | 40 ± 5* | 230 ± 36* | 7 ± 1* | 19 ± 4* | 48 ± 8* | 57 ± 1* | 7 ± 1* | 53 ± 8* | 25 ± 5* | 53 ± 6* |
| TD7 ground | 16 | 39 ± 1† | 137 ± 3† | 10 ± 1† | 20 ± 1† | 38 ± 3† | 46 ± 3† | 6 ± 0.4† | 41 ± 2† | 21 ± 1† | 42 ± 2† |
| TD7 FLT | 4 | 31 ± 3† | 104 ± 3† | 5 ± 1*† | 13 ± 2† | 27 ± 3† | 29 ± 3† | 6 ± 1† | 50 ± 9† | 14 ± 2† | 28 ± 3*† |
| NC14 ground | 12 | 114 ± 4† | 425 ± 19† | 46 ± 2† | 99 ± 3† | 216 ± 8† | 234 ± 12† | 27 ± 1† | 181 ± 7† | 100 ± 5† | 192 ± 5† |
| NC14 FLT | 6 | 98 ± 3*† | 365 ± 8* | 27 ± 2*† | 78 ± 3*† | 180 ± 8*† | 203 ± 12*† | 19 ± 1* | 163 ± 6*† | 90 ± 4† | 151 ± 6*† |

Values are means ± SE; *n* = no. of rats. Basal, neonates killed at 7 days of age; NC7, euthyroid neonates 7 days old at lift off; TD7, thyroid deficient neonates 7 days old at lift off; NC14, euthyroid neonates 14 days old at lift off; ground, ground-based neonates; FLT, space-flown neonates. MG, medial gastrocnemius; LG, lateral gastrocnemius; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis; TA, tibialis anterior. *$P < 0.05$, FLT vs. matched ground-based control; †$P < 0.05$ vs. NC ground group.

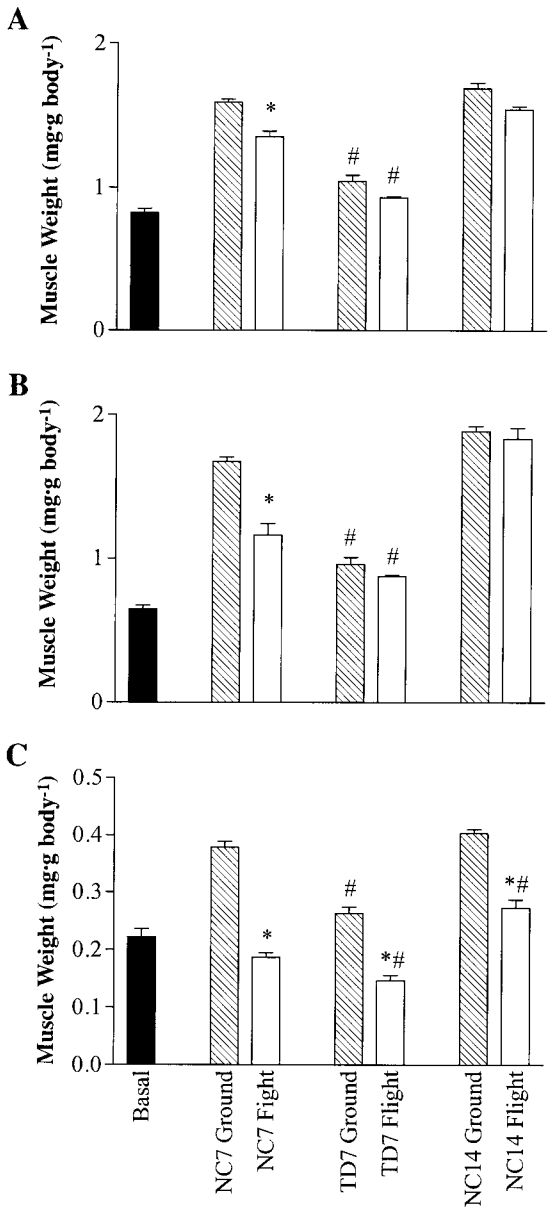


Fig. 4. Effects of thyroid deficiency and/or spaceflight on neonate muscle mass normalized to body weight. *A*: muscle weight of tibialis anterior (TA), a non-weight-bearing locomotor muscle expressing primarily fast myosin heavy chain (MHC). *B*: muscle weight of medial gastrocnemius (MG), a weight-bearing locomotor muscle expressing primarily fast MHC. *C*: muscle weight of soleus, a weight-bearing postural/locomotor muscle expressing primarily slow MHC. $*P < 0.05$ vs. ground group; $\#P < 0.05$ vs. NC7 ground.

more closely with those of the less mature basal group. There was a significant correlation between the wet weight of the these muscles and the muscle IGF-I peptide concentration (Fig. 6, *C* and *D*). Similarly, there was a significant correlation between muscle IGF-I peptide concentration and the protein or DNA content of the MG (Fig. 7) and TA muscles (data not shown). Together, these data suggest a link between IGF-I-stimulated muscle growth (Fig. 6) and the coordination of both protein and DNA accumulation in the growing muscle.

## DISCUSSION

In rats, the first month of postnatal life involves a remarkably dynamic period of growth and development that is regulated by complex hormonal mechanisms. To date, it has not been clear how (or if) these various growth-regulating mechanisms are impacted by environmental stimuli such as the weight-bearing activity of neonates.

A number of studies have found that thyroid hormone levels are increasing during the early postpartum time period (13, 20, 26, 30), and experimental evidence indicates that this process is important for the regulation of both general and muscle-specific growth and maturation (e.g., Refs. 16, 24, 25). However, the relationship between, or interaction of, thyroid state and weight-bearing activity during neonatal development

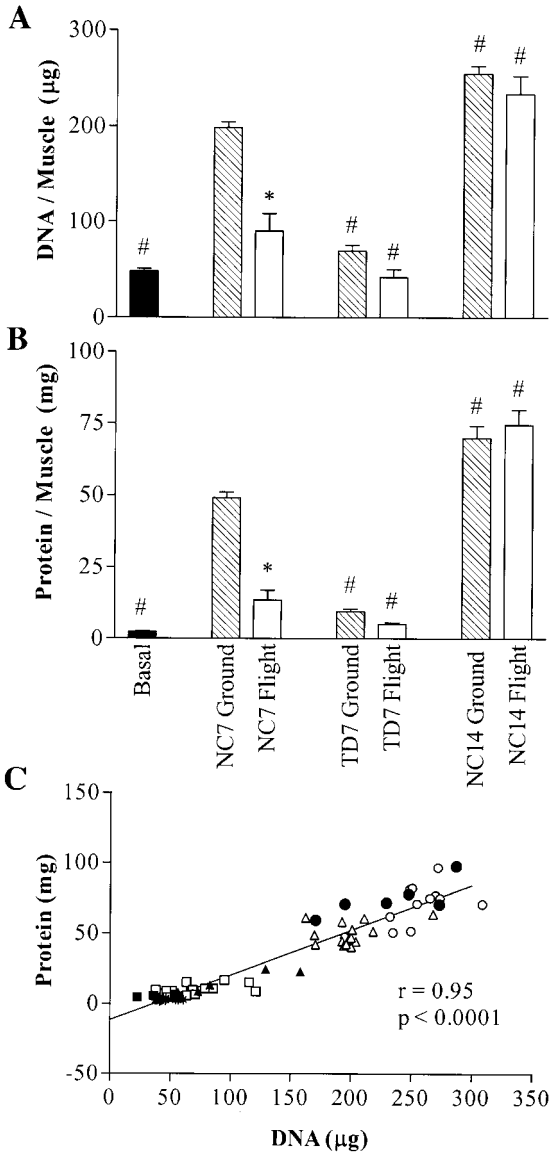


Fig. 5. Effects of thyroid deficiency and/or spaceflight on growth-related biochemical measures from neonate MG muscle. *A*: total muscle DNA. *B*: total muscle protein. *C*: correlation between total protein and total DNA with best fit line. $*P < 0.05$ vs. ground group; $\#P < 0.05$ vs. NC7 ground.

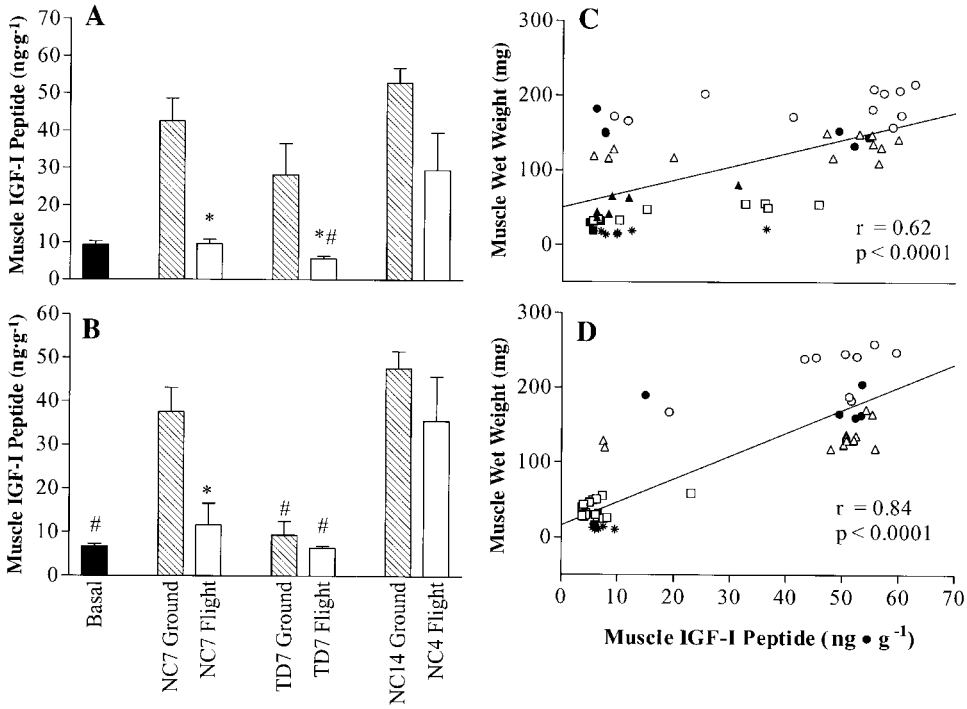


Fig. 6. Effects of thyroid deficiency and/or spaceflight on IGF-I peptide concentrations from neonate MG and TA muscles. *A*: TA IGF-I concentration. *B*: MG IGF-I concentration. *C*: correlation between muscle wet weight and muscle IGF-I peptide from all TA muscles depicted in *A*. *D*: correlation between muscle wet weight and muscle IGF-I peptide from all MG muscles depicted in *B*. *, Basal; △, NC7 ground; ▲, NC7 flight; □, TD7 ground; ■, TD7 flight; ○, NC14 ground; ●, NC14 flight. *$P < 0.05$ vs. ground group; #$P < 0.05$ vs. NC7 ground.

has not been extensively characterized. It is clear that the adult pattern of muscle contractile protein expression becomes fully established by 30 days of postnatal development and that hypothyroidism during this period will result in a retardation in the growth and phenotypic development of skeletal muscles (6). Interestingly, the growth-inhibiting effect of thyroid deficiency on skeletal muscle is disproportionately greater than that for other tissues and organs.

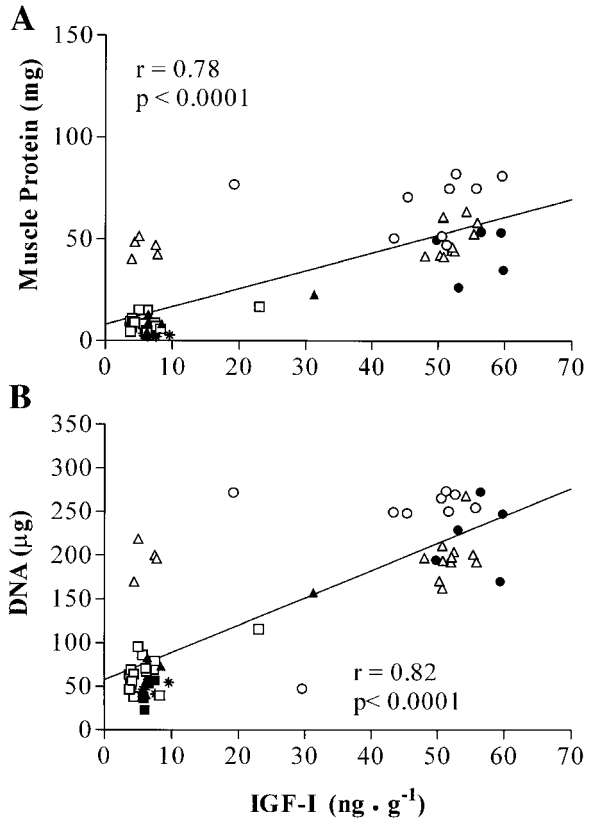


Fig. 7. Correlation between muscle IGF-I peptide concentration and muscle protein (*A*) or muscle DNA content (*B*) for MG muscles from all groups. *, Basal; △, NC7 ground; ▲, NC7 flight; □, TD7 ground; ■, TD7 flight; ○, NC14 ground; ●, NC14 flight.

The primary experimental groups in the present study consisted of neonatal rats that were 5–7 days of age at the onset of the experimental treatments. The period from 7 to 30 days postpartum is crucial for the development of hindlimb locomotive patterns (12). During this time, neonates will begin pelvic weight bearing (*day 6–7*) and initiate true quadrapedal locomotion (*day 10*) (12). Previous studies have found that intermittent hindlimb unweighting of rat neonates from *day 14* to *30* postpartum results in persistent disruption of gaiting patterns in adulthood (31). In creating the design of these studies, it was reasoned that unweighting and/or the imposition of hypothyroidism during this time period would result in a significant perturbation of the muscle developmental program, thereby helping to elucidate the importance of these two variables in this process.

*Somatic growth.* In an attempt to partition the relative impact of spaceflight vs. thyroid deficiency, we have calculated the relative somatic growth deficit imposed by each treatment relative to ground-based thyroid-deficient or euthyroid controls using the data from Fig. 3*A* to generate Fig. 8. From this analysis, it is evident that either spaceflight or hypothyroidism resulted in an ~50% decrease in somatic growth [flight effect (NC7) vs. thyroid deficiency effect] in the younger neonates. In the thyroid-deficient neonates, the separate and combined effects of thyroid deficiency and spaceflight on somatic growth were similar, as indicated by the diminished flight effect and similar thyroid-deficient and thyroid-deficient plus flight effects. (Fig.

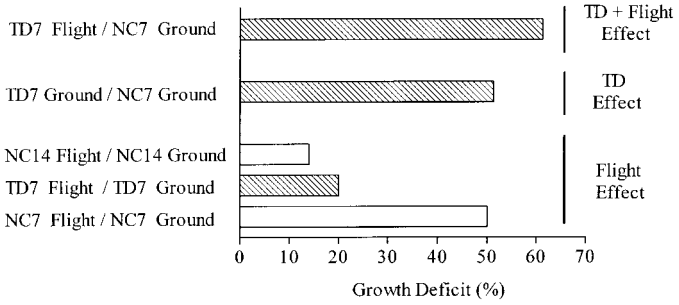


Fig. 8. Relative growth deficit imposed by the separate and combined interventions of thyroid deficiency (TD) and exposure to spaceflight calculated from the body weight data presented in Fig. 3*A*. Flight effect was calculated as NC7 flight divided by NC7 ground, TD7 flight divided by TD7 ground, and NC14 flight divided by NC14 ground. TD effect was calculated as TD7 ground divided by NC7 ground. TD + flight effect was calculated as TD7 flight divided by NC7 ground.

8). This presentation also highlights the lesser sensitivity of the older neonates (NC14) to the effects of loss of weight-bearing activity (Fig. 8, flight effect).

The controlling factors underlying the spaceflight-induced decrement in growth appear to have been different from those invoked by the hypothyroid state. This can be deduced from the fact that the NC7 flight and NC14 flight animals were not thyroid deficient and the finding that the TD7 flight animals did not experience a significantly greater degree of growth retardation than their ground-based controls. However, it does appear that the ultimate growth-retarding mechanism invoked by both thyroid deficiency and spaceflight involved a decrease in the circulating levels of IGF-I (Fig. 3, *B* and *C*). This suggests that both hypothyroidism and loss of load-bearing activity in some way impact systems that regulate plasma IGF-I levels, possibly by reducing GH production. This is further supported by the strong correlation between plasma IGF-I levels and the body weight of both the TD7 and euthyroid groups (Fig. 3*C*). However, previous studies have shown that the effect of hypothyroidism on growth has a component that does not appear to be mediated via the GH-IGF-I axis (24). Although exposure to spaceflight would be expected to, at least initially, invoke a number of stress-related responses (e.g, cortisol) in neonates, the strong correlations between growth and IGF-I and the continued coordination between muscle protein and DNA levels suggest that the primary mechanism impacting growth, and in particular muscle growth, was functioning via the IGF-I axis.

*Skeletal muscle growth.* Compared with overall somatic growth, the growth of neonatal skeletal muscle was disproportionately impacted by the removal of weight-bearing activity during this critical developmental time period (Fig. 4). As with body weight, we have used the normalized data from Fig. 4 to apportion the relative growth deficit imposed by the separate and combined treatments of hypothyroidism and spaceflight (Fig. 9). The loss of weight-bearing activity resulted in decreased muscle growth, particularly in the younger neonates (Fig. 9, NC7 group, flight effect). In general, the mass of the non-weight-bearing TA muscles appeared to be much less sensitive to the unloading imposed by spaceflight relative to weight-bearing fast- and slow-twitch muscles such as the MG and soleus (Figs. 4 and 9). In contrast to the somatic effects of these two treatments, thyroid deficiency appeared to have a greater impact on fast muscle growth than that of spaceflight (Fig. 9, TA and MG). Whereas the interaction of thyroid deficiency and spaceflight did not result in an additional significant decrease in the mass of the TA and MG (Fig. 4, TD7 flight vs. TD7 ground), this interaction was significant in the soleus. The overall trend suggests that thyroid deficiency had the most potent effect on muscle growth, whereas the interaction of thyroid deficiency and flight may have contributed to a further decrement (Fig. 9).

In response to increased loading, adult skeletal muscle myofibers will incorporate myonuclei from the available pool of satellite cells in an apparent attempt to maintain some finite DNA-to-protein ratio (reviewed in Ref. 1). During early neonatal development, the satellite cell population of individual skeletal muscle fibers goes though a period of progressive decline in number balanced by a reciprocal increase in the number of myonuclei per fiber as the myofibers progress toward the adult configuration (21, 27). This decline in satellite cell concentration appears to be a function of the differentiation of satellite cells and their incorporation

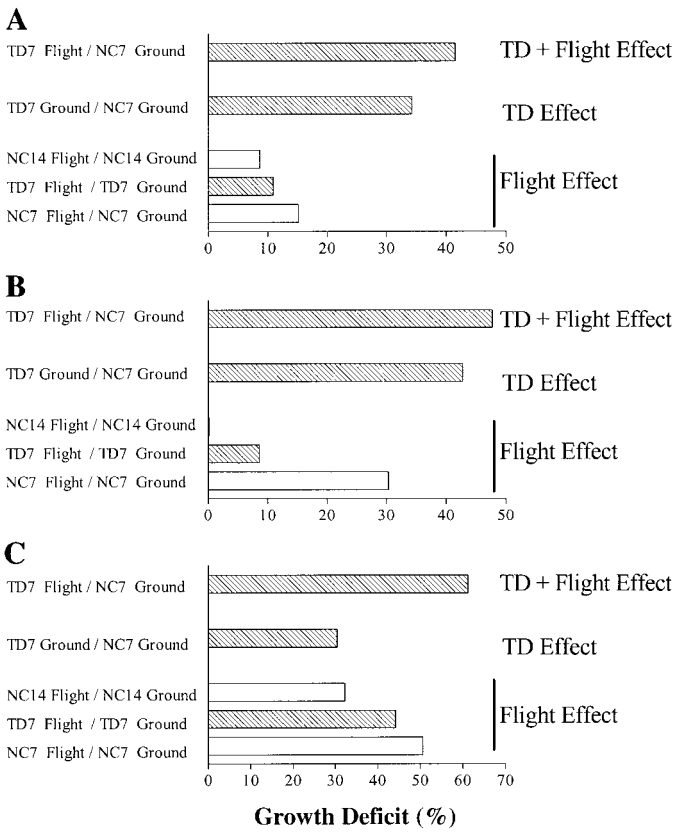


Fig. 9. Relative growth deficit imposed on TA (*A*), MG (*B*), and soleus (*C*) skeletal muscles by separate and combined interventions of TD and exposure to spaceflight calculated from the normalized muscle weight data presented in Fig. 4. Percent deficit was calculated by dividing normalized muscle weights as indicated on the *y*-axis and multiplying by 100.

into growing myofibers combined with a decrease in the proliferative activity of the remaining satellite cells (29). These events illustrate the developmental coordination of processes occurring in multiple myogenic and muscle cell types during the postnatal period. In the present study, there was a similar age-related decrease in the DNA concentration of the muscles from neonates in all of the experimental groups (data not shown). In agreement with previously published reports (19, 28), either loss of weight-bearing activity or hypothyroidism resulted in lower muscle DNA content per muscle compared with control muscles (Fig. 5*A*). The continued coordination between the amount of muscle protein and the DNA content present in the muscles of the neonates is illustrated by the data from the MG muscle (Fig. 5*C*). This suggests that the inhibition of mechanisms related to muscle growth imposed by the separate or combined effects of spaceflight and hypothyroidism ultimately affected processes that are responsible for coordinating all aspects of the growth program (e.g., protein accretion as well as satellite cell proliferation, differentiation, and fusion).

Although the IGF-I peptide levels of the TA muscles from some of the ground-based rats were essentially unchanged by thyroid deficiency (Fig. 6), there was an overall positive correlation between muscle mass and IGF-I concentration for both the TA and MG muscles from the eight experimental groups in the study (Fig. 6, *C* and *D*). In contrast to the hypothyroid state, spaceflight resulted in significant decreases in the IGF-I concentrations of both the TA and the MG muscles of the younger neonates. A number of studies have established a clear relationship between muscle loading and autocrine/paracrine IGF-I production in adult rats (reviewed in Ref. 1). The results of the present study are consistent with the hypothesis that muscle IGF-I production in response to loading state modulates muscle mass and extends this observation to include developmental processes. Furthermore, the maintenance of the muscle DNA-to-protein relationship in the experimental groups from this study strongly suggests that IGF-I signaling may be the key mechanism coordinating these growth processes.

As with somatic growth, the growth of the TA and MG muscles from the older neonates was insensitive to the removal of weight-bearing activity (Figs. 4 and 9). This suggests that there is a critical developmental time period during which the growth of these fast-twitch muscles is heavily influenced by mechanical loading.

In contrast to the pattern seen in fast-twitch MHC-expressing muscles, growth of the soleus, a postural muscle that expresses predominantly slow MHC, appeared to be particularly susceptible to loss of weight-bearing activity in all of the age groups studied (Figs. 4 and 9). In addition, the soleus muscles of the thyroid-deficient flight group appear to demonstrate an additive effect between spaceflight and hypothyroidism, a response that was not observed in any of the fast-twitch muscles studied. In general, the atrophy response to unloading is thought to be more pronounced in antigravity muscles expressing a greater proportion of the slow MHC isoform (2, 7, 11). Because of the restricted amount of tissue available, no measurements of muscle IGF-I were possible for the soleus muscles from this study. However, on the basis of the IGF-I data from the TA and MG muscle, one might speculate that the growth retardation of the soleus muscle in both the euthyroid and hypothyroid neonates may reflect a greater reliance of this muscle on autocrine/paracrine IGF-I production mechanisms that are stimulated by weight-bearing activity.

In summary, the results of this study indicate that either hypothyroidism or the loss of weight-bearing activity will limit the somatic and muscle-specific growth of neonatal rats. Furthermore, the impact of these perturbations on limb skeletal muscle growth appears to be relatively greater than the effect on somatic growth.

The mechanisms by which either hypothyroidism or unloading impact growth appear to converge on the control of plasma- and muscle-IGF-I-derived concentrations. In general, there did not appear to be significant additive effects of spaceflight exposure and thyroid deficiency, suggesting that, at the level of the mechanism controlling the IGF-I response, the repression imposed by either intervention appeared to be maximal.

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## REFERENCES

1. **Adams, G. R.** The role of IGF-I in the regulation of skeletal muscle adaptation. In: *Exercise and Sport Science Reviews*, edited by J. R. Holloszy. Baltimore, MD: Williams & Wilkins, 1998, vol. 26, p. 31–60.
2. **Adams, G. R., and K. M. Baldwin.** Age dependence of myosin heavy chain transitions induced by creatine depletion in rat skeletal muscle. *J. Appl. Physiol.* 78: 368–371, 1995.
3. **Adams, G. R., and F. Haddad.** The relationships between IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. *J. Appl. Physiol.* 81: 2509–2516, 1996.
4. **Adams, G. R., F. Haddad, and K. M. Baldwin.** Interaction of chronic creatine depletion and muscle unloading: effects on postural and locomotor muscles. *J. Appl. Physiol.* 77: 1198–1205, 1994.
5. **Adams, G. R., F. Haddad, S. McCue, P. Bodell, M. Zeng, L. Qin, A. Qin, and K. M. Baldwin.** Effects of spaceflight and thyroid deficiency on rat hindlimb development. II. Expression of MHC isoforms. *J. Appl. Physiol.* 88: 904-916, 2000.
6. **Adams, G. R., S. A. McCue, M. Zeng, and K. M. Baldwin.** The time course of myosin heavy chain transitions in neonatal rats: importance of innervation and thyroid state. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 276: R954–R961, 1999.
7. **Baldwin, K. M., R. E. Herrick, E. Ilyina-Kakueva, and V. S. Oganov.** Effects of zero gravity on myofibril content and isomyosin distribution in rodent skeletal muscle. *FASEB J.* 4: 79–83, 1990.

8. **Baldwin, K. M., R. E. Herrick, and S. A. McCue.** Substrate oxidation capacity in rodent skeletal muscle: effects of exposure to zero gravity. *J. Appl. Physiol.* 75: 2466–2470, 1993.
9. **Breier, B. H., B. W. Gallaher, and P. D. Gluckman.** Radioimmunoassay for insulin-like growth factor-1: solutions to some potential problems and pitfalls. *J. Endocrinol.* 128: 347–357, 1991.
10. **Caiozzo, V. J., M. J. Baker, R. E. Herrick, M. Tao, and K. M. Baldwin.** Effect of spaceflight on skeletal muscle: mechanical properties and myosin isoform content of a slow muscle. *J Appl. Physiol.* 76: 1764–1773, 1994.
11. **Caiozzo, V. J., F. Haddad, M. J. Baker, R. E. Herrick, N. Prietto, and K. M. Baldwin.** Microgravity induced transformations of myosin isoforms and contractile properties of skeletal muscle. *J. Appl. Physiol.* 81: 123–132, 1996.
12. **Clarac, F., L. Vinay, J. R. Cazalets, J. C. Fady, and M. Jamon.** Role of gravity in the development of posture and locomotion in the neonatal rat. *Brain Res. Rev.* 28: 35–43, 1998.
13. **Dussault, J. H., and F. Labrie.** Development of the hypothalamic-pituitary-thyroid axis in the neonatal rat. *Endocrinology* 97: 1321–1324, 1975.
14. **Elder, G. C. B., and A. J. McComas.** Development of rat muscle during short and long-term hindlimb suspension. *J. Appl. Physiol.* 62: 1917–1923, 1987.
15. **Florini, J. R., D. Z. Ewton, and S. A. Coolican.** Growth hormone and insulin like growth factor system in myogenesis. *Endocr. Rev.* 17: 481–517, 1996.
16. **Glasscock, G. F., and C. S. Nicoll.** Hormonal control of growth in the infant rat. *Endocrinolology* 112: 800–805, 1983.
17. **Haddad, F., P. W. Bodell, S. A. McCue, R. E. Herrick, and K. M. Baldwin.** Food restriction-induced transformations in cardiac functional and biochemical properties in rats. *J. Appl. Physiol.* 74: 606–612, 1993.
18. **Iino, S., T. Yamada, and M. A. Greer.** Effect of graded doses of propylthiouracil on biosynthesis of thyroid hormones. *Endocrinology* 68: 582–588, 1961.
19. **Jacobs, S. C., P. R. Bar, and A. L. Bootsma.** Effect of hypothyroidism on satellite cells and postnatal fiber development in the soleus muscle of rat. *Cell Tissue Res.* 286: 137–144, 1996.
20. **Jennische, E.** Sequential immunohistochemical expression of IGF-1 and the transferrin receptor in regenerating rat muscle in vivo. *Acta Endocrinol.* 121: 733–738, 1989.
21. **Kelly, A. M.** Satellite cells and myofiber growth in the rat soleus and extensor digitorum longus muscles. *Dev. Biol.* 65: 1–10, 1978.
22. **Labarca, C., and K. Paigen.** A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* 102: 344–352, 1980.
23. **Morris, G. S., D. G. Surdyka, F. Haddad, and K. M. Baldwin.** Apparent influence of metabolism on cardiac isomyosin profile of food-restricted rats. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 258: R346–R351, 1990.
24. **Nanto-Salonen, K., G. F. Glasscock, and R. G. Rosenfeld.** The effects of thyroid hormone on insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) expression in the neonatal rat: prolonged high expression of IGFBP-2 in methimazole-induced congenital hypothyroidism. *Endocrinology* 129: 2563–2570, 1991.
25. **Nanto-Salonen, K., H. L. Muller, A. R. Hoffman, T. H. Vu, and R. G. Rosenfeld.** Mechanisms of thyroid hormone action on the insulin-like growth factor system. *Endocrinology* 132: 781–788, 1993.
26. **Nanto-Salonen, K., and R. G. Rosenfeld.** Insulin-like growth factor binding protein expression in hyperthyroid rat is age dependent. *Endocrinology* 131: 1489–1496, 1992.
27. **Schultz, E.** Satellite cell behavior during skeletal muscle growth and regeneration. *Med. Sci. Sport Exerc.* 21, *Suppl.* 5: S181–S186, 1989.
28. **Schultz, E., K. C. Darr, and A. Macius.** Acute effects of hindlimb unweighting on satellite cells in growing skeletal muscle. *J. Appl. Physiol.* 76: 266–270, 1994.
29. **Schultz, E., and B. H. Lipton.** Skeletal muscle satellite cells: changes in proliferation potential as a function of age. *Mech. Ageing Dev.* 20: 377–383, 1982.
30. **Sieck, G. C., L. E. Wilson, B. D. Johnson, and W. Z. Zhan.** Hypothyroidism alters diaphragm muscle development. *J. Appl. Physiol.* 81: 1965–1972, 1996.
31. **Walton, K.** Postnatal development under conditions of simulated weightlessness and spaceflight. *Brain Res. Rev.* 28: 25–34, 1998.