

Gravity Plays an Important Role in Muscle Development and the Differentiation of Contractile Protein Phenotype

Experiment Team

Principal Investigator: **Kenneth M. Baldwin**

Co-Investigators: **Gregory R. Adams,
Fadia Haddad**

University of California, Irvine, USA

Authors

Gregory R. Adams, Fadia Haddad, Kenneth M. Baldwin

ABSTRACT

Several muscles in the body exist mainly to work against gravity. Whether gravity is important in the development of these muscles is not known. By examining the basic proteins that compose muscle, questions about the role of gravity in muscle development can be answered. Myosin heavy chains (MHCs) are a family of proteins critically important for muscle contraction. Several types of MHCs exist (e.g., neonatal, slow, fast), and each type is produced by a particular gene. Neonatal MHCs are produced early in life. Slow MHCs are important in antigravity muscles, and fast MHCs are found in fast-twitch "power" muscles. The gene that is "turned on" or expressed will determine which MHC is produced. Early in development, antigravity skeletal muscles (muscles that work against gravity) normally produce a combination of the neonatal/embryonic MHCs. The expression of these primitive MHCs is repressed early in development; and the adult slow and fast MHC genes become fully expressed. We tested the hypothesis that weightbearing activity is critical for inducing the normal expression of the slow MHC gene typically expressed in adult antigravity muscles. Also, we hypothesized that thyroid hormone, but not opposition to gravity, is necessary for expressing the adult fast IIB MHC gene essential for high-intensity muscle performance. Groups of normal thyroid and thyroid-deficient neonatal rats were studied after their return from the 16-day Neurolab mission and compared to matched controls. The results suggest: (1) Weightlessness impaired body and limb skeletal muscle growth in both normal and thyroid-deficient animals. Antigravity muscles were impaired more than those used primarily for locomotion and/or nonweightbearing activity. (2) Systemic and muscle expression of insulin-like growth factor-I (IGF-I), an important body and tissue growth factor, was depressed in flight animals. (3) Normal slow, type I MHC gene expression was markedly repressed in the normal thyroid flight group. (4) Fast IIB MHC gene expression was enhanced in fast-twitch muscles of normal thyroid animals exposed to spaceflight; however, thyroid deficiency markedly repressed expression of this gene independently of spaceflight. In summary, the absence of gravity, when imposed at critical stages of development, impaired body and skeletal muscle growth, as well as expression of the MHC gene family of motor proteins. This suggests that normal weightbearing activity is essential for establishing body and muscle growth in neonatal animals, and for expressing the motor gene essential for supporting antigravity functions.

INTRODUCTION

Skeletal muscles comprise the largest organ system in the body, accounting for ~50% of the body's mass. They can change their structure and function in response to (a) chronic physical activity and/or (b) various hormonal/growth factors. Muscles used extensively in either opposing the force of gravity or in performing locomotion, i.e., leg muscles, are highly sensitive to the lack of gravity, as occurs when individuals are exposed to spaceflight. In space, affected muscle fibers undergo marked atrophy and lose strength and endurance (Baldwin, 1996; Caiozzo, 1994; Caiozzo, 1996; Widrick, 1999). Collectively, this can impair performance capability. The full extent of the atrophy in space has not been delineated in humans due, in part, to the fact that astronauts perform physical exercise to counteract muscle atrophy. These activities curtail, but do not prevent, muscle atrophy. Studies on adult rodents in which no countermeasures are performed clearly show that certain muscles normally used in opposing gravity can atrophy by ~50% over several days (Baldwin, 1996; Caiozzo, 1994).

The contractile machinery of a muscle fiber, which regulates muscle contraction, is particularly sensitive to gravity. One of the major proteins comprising the contractile machinery is myosin (referred to as myosin heavy chain (MHC)). This complex protein serves as an important structural and regulatory molecule that precisely regulates the contraction process, particularly how fast or intensely it occurs. MHC proteins exist in different forms called isoforms, and each MHC isoform is the product of a specific MHC gene. Every muscle is highly organized in terms of how these genes are expressed among the muscle fibers. For example, there are clusters of muscle fibers innervated by a common neuron (called a motor unit) that express a slow type of MHC (designated as type I; see Figure 1). Motor units that express this slow MHC are more effective (e.g., they function with less energy expenditure) in enabling the muscle to oppose gravity and perform sustained movement patterns such as walking and jogging. Other motor units express faster forms of MHC in different combinations, and these fast MHCs are designated as fast IIa, fast IIx, and fast IIb in increasing order of their impact on the speed of contraction (Figure 1). Thus, motor units that express these faster MHCs contract faster and generate greater power and speed of movement compared to the slow motor units—although at the expense of greater energy expenditure. In microgravity, the slow muscle fibers lose their capacity for slow MHC gene expression; and the fibers express greater than normal amounts of the fast isoforms, while at the same time the affected fibers atrophy; e.g., they become smaller in diameter, weaker, and faster contracting (Caiozzo, 1994; Caiozzo, 1996; Ohira, 1992; Widrick, 1999).

Following birth, muscles are in an undifferentiated state in terms of their relative size, functional properties, and pattern of MHC gene expression (Adams, 1999). Instead of expressing the typical slow and fast forms of MHC in the various motor units as presented in Figure 1, in the infant state various motor units express immature forms of MHC, designated as embryonic and neonatal isoforms. These are thought

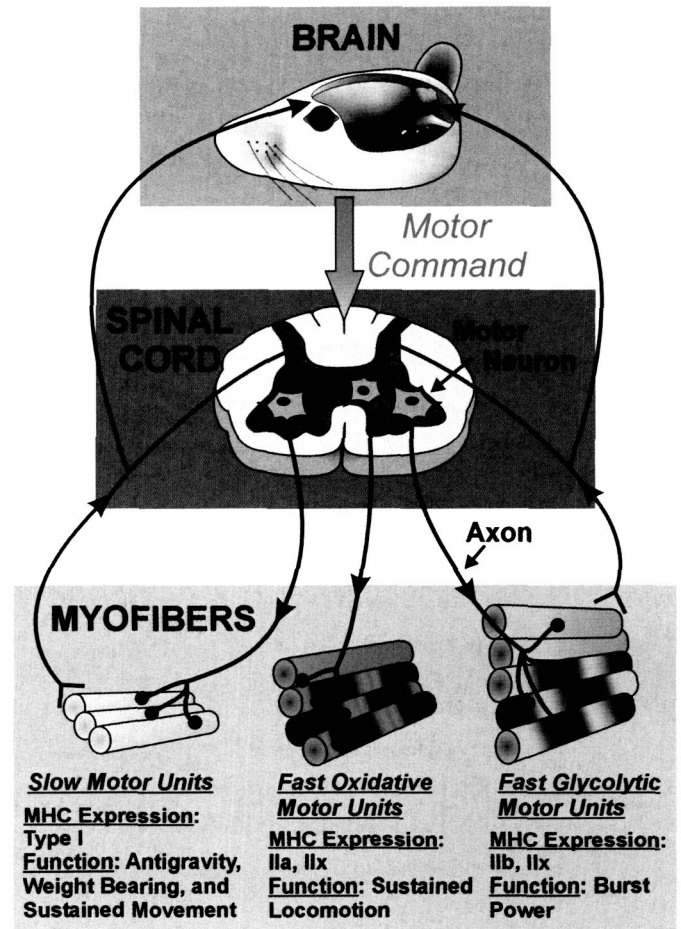


Figure 1. Functional specialization of skeletal muscle. The brain initiates the motor command for a motor neuron to fire and stimulate a group of muscle fibers to contract. A motor unit consists of a single motor neuron together with all the muscle fibers it innervates. Motor units vary in the number of myofibers included; this number can vary several hundred fold in fast vs. slow motor units. Different types of motor units express different MHC phenotypes, having a specialized function. Note that each myofiber can express either a single MHC isoform, or a hybrid mix of two or more isoforms. A skeletal muscle contains thousands of myofibers that belong to different groups of motor units.

to be ineffective in opposing gravity and producing locomotion (Adams, 1999). Therefore, we postulate that with weightbearing activity, and in combination with developmentally induced surges in factors such as growth hormone, insulin-like growth factor-I (IGF-I), and thyroid hormone (T3), muscles become stimulated to undergo marked enlargement and differentiation. This transforms the muscle system to the adult state. Furthermore, accumulating evidence strongly suggests that IGF-I treatment may be an important therapeutic strategy in the treatment of individuals with debilitating muscle-wasting diseases such as muscular dystrophy and amyotrophic lateral sclerosis (ALS). Thus, fundamental studies on the role that IGF-I might play in regulating skeletal muscle growth in infants

and adults are of clinical importance. Figure 2 depicts the proposed cascade of events impacting body growth, muscle growth, and fiber differentiation.

Thus, while gravity has been suspected to play an important role in the control of muscle structure and function, relatively little is known about its role in neonatal development. Since it is neither logistically nor ethically feasible to expose human infants to the environment of spaceflight, animal research is necessary. In animals, however, the effects of gravity (and especially its lack) on muscle growth and development have not been studied extensively. This is because ground-based models cannot continuously remove gravity or weightbearing stimuli during critical stages of development to impact the musculoskeletal system. In rats, the first month of postnatal life

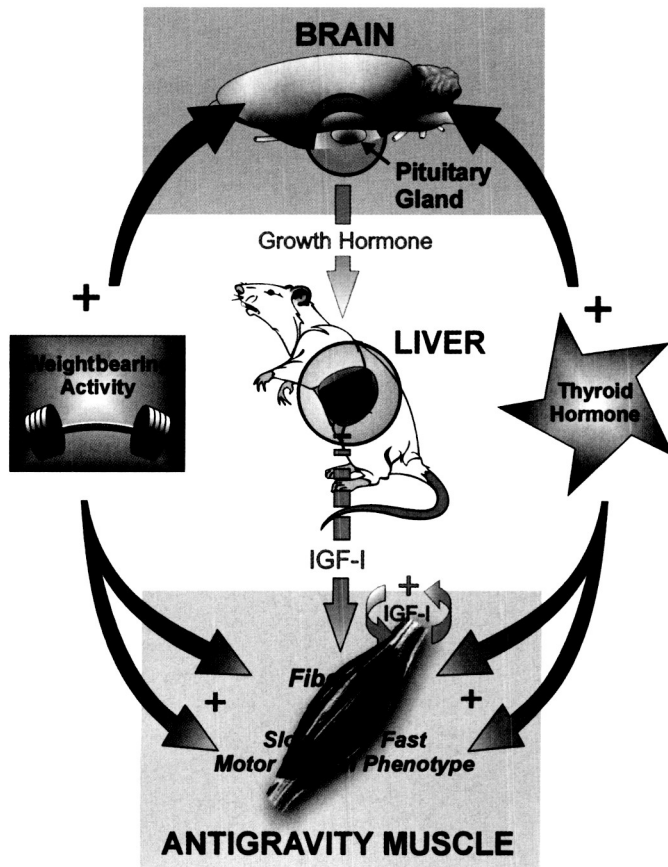


Figure 2. Proposed cascade of events impacting muscle growth and fiber differentiation. Growth hormone (GH) secreted by the pituitary gland in the brain stimulates the liver to produce systemic IGF-I, which circulates in the bloodstream. This GH-IGF-I production is stimulated by circulating thyroid hormone. Also shown is that the muscle has the capacity to express IGF-I independently of the liver. This response is influenced by the level of activity and functional demand imposed on the muscle. Thyroid hormone affects muscle differentiation and MHC phenotype. Muscle fiber size and biochemical properties (MHC phenotype) are influenced by the interaction between thyroid hormone, weightbearing activity, and growth factors such as IGF-I.

encompasses a period of rapid growth and development when these animals increase in size almost exponentially (Adams, 1999). Compared to humans, the rate of rodent development is compressed such that the first 25 days of growth roughly approximate the first 5–7 years for children. For example, starting at just 6–7 days of age, young rats begin pelvic weight-bearing and by day 10 they walk on all fours (Clarac, 1998). During this time the growth of some muscles, particularly those limb muscles that oppose gravity and bear the animal's weight, will outpace the growth of the body as a whole. Since these muscles can be expected to experience the same milieu of circulating hormones and growth factors as the other tissues in the body, the question arises as to how this differential muscle growth is controlled. The 16-day Neurolab spaceflight mission enabled us to critically examine the continuous absence of weightbearing activity during key stages in the development of rodent muscle. During this time, primitive motor genes are being turned off while adult motor protein (MHCs) genes are being turned on. Thus, the role that gravity plays in this muscle growth and differentiation process, in the context of other factors such as thyroid hormone and IGF-I, could be examined for the first time in a systematic way.

We tested the following hypotheses: (1) In the absence of either weightbearing activity or an intact thyroid state, both body and muscle growth become impaired due to reduced IGF-I expression, both systemically and intramuscularly. (2) Younger neonates are more sensitive than older neonates to unweighting interventions, because their normal regulatory mechanisms are interrupted earlier in development. (3) Weightbearing activity is essential for the normal expression of slow, type I MHC gene expression. (4) Thyroid hormone is necessary for transforming the neonatal/embryonic MHC into the fast adult MHC type of a typical fast skeletal muscle—and this process occurs independently of gravity.

METHODS

Experimental design and litter formation

To study the interactive effects of spaceflight and thyroid status on muscle development, we needed to generate groups of rats having either an intact functioning thyroid gland (designated as euthyroid) or a defective thyroid gland (designated as thyroid deficient (TD)). To accomplish this, timed-pregnant female rats were obtained from Taconic Farms (Germantown, NY), and were housed initially in standard rodent cages in the vivarium at Kennedy Space Center in Florida. The litters produced were adjusted to contain eight rats with equal gender distribution. Study litters were selected based on: (a) normal body growth during the first five days of age, and (b) normal water/food consumption and normal rat retrieval behavior among the mothers (Adams, 2000a). Litters were then randomly assigned to the following experimental groups: (1) euthyroid-ground-control (N=16); (2) euthyroid-flight (N=8); (3) TD-ground-control (N=16); and (4) TD-flight (N=8). In actuality, there were two separate groups of ground controls used for comparisons for both the euthyroid- and TD-flight animals (Adams, 2000a).

Table 1. Litter and treatment characteristics.

Age at Launch	Group Assignment
	↓ <i>Euthyroid</i>
	Basal
Eight Days	NC8-Ground*
	NC8-Flight
14 Days	NC14-Ground*
	NC14-Flight
	Thyroid Deficient
Eight Days	TD8-Ground*
	TD8-Flight

NC, normal (euthyroid) control; TD, thyroid deficient.

*: Each ground group was a pool of two separate groups that differed in that they were housed in either standard vivarium rat cages or in smaller cages that matched the configuration of cages used on the Space Shuttle.

These two groups were housed in either standard rat cages or in smaller cages matching the configuration of the cages used on Neurolab. Since the results obtained from these two groups were similar, they were combined under a single "ground" heading to simplify data presentation. The flight groups were launched into space at eight days of age.

An additional three litters from timed-pregnant rats were randomly assigned to experimental groups for launch at approximately 14 days of age. They were designated as: (1) euthyroid-ground-controls-14 (N=12) and (2) euthyroid-flight-14 (N=six). This older flight group was launched at approximately 14 days of age. As in the case for the two younger ground control groups, a single large ground control group was formed from the merging of the two separate older ground control groups as described above (Adams, 2000a). The older group did not include a TD group since there was insufficient housing on board the Shuttle. In addition to the above experimental groups, three litters also were selected randomly and studied on the day of launch (i.e., ~eight days of age) for baseline analyses. All experimental procedures were approved by both the NASA Institutional Animal Care and Use Committee (IACUC) and the University of California, Irvine IACUC. Table 1 summarizes the various assigned litters and their subsequent grouping for tissue analyses and data presentation.

Manipulations and tissue processing

Thyroid deficiency was induced in those designated litters by surgically implanting an osmotic pump into the abdominal cavity of the nursing mother rat. The pump delivered a continuous dose of the antithyroid drug, propylthiouracil (PTU), which made the young rats hypothyroid via the delivery of PTU via the mother's milk. The details of this procedure are provided elsewhere (Adams, 2000a).

Sixteen days after launch, the rats were returned to Kennedy Space Center, which was the Orbiter landing site. Five hours after landing, the rats were euthanized and dissected for tissue procurement as previously described (Adams, 2000a).

Analytical procedures

Cardiac native myosin analyses utilizing non-denaturing polyacrylamide gel electrophoresis were performed as previously described (Haddad, 1993; Swoap, 1994). Skeletal MHC isoforms were separated using an SDS-PAGE technique (Adams, 1999; Talmadge, 1993). MHC mRNA analyses utilized a semiquantitative reverse transcriptase polymerase chain reaction procedure as described previously (Adams, 2000b). Muscle and plasma IGF-I, muscle protein, and DNA content were determined as previously described (Adams, 2000a).

Statistics

All values are reported as mean and standard error of the mean. For each time point, treatment effects were determined by analysis of variance (ANOVA) with post-hoc testing using the Prism software package (GraphPad Software, Inc., San Diego, CA). Pearson's correlation analyses of relationships were performed using the Prism package. The 0.05 level of confidence was accepted for statistical significance.

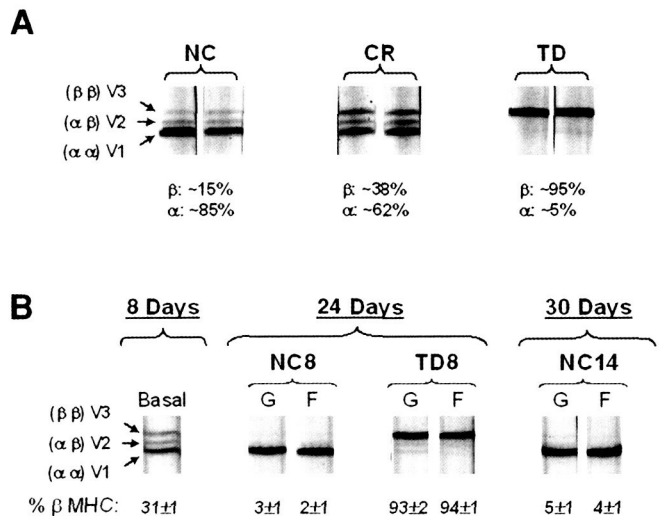


Figure 3. Heart myosin isoforms as separated by native gel electrophoresis and stained with coomassie blue. (A) Cardiac isomyosin profile of normal control (NC), 50% caloric restricted (CR), and TD adult rats. (B) Cardiac isomyosin composition in the different Neurolab groups. Eight days (basal group), 24 days (NC8, TD8), and 30 days (NC14) refer to the age of the rats at the time of tissue procurement. G: Ground, F: Flight. Also shown is the % MHC in each group as determined by laser scanning densitometry of the actual gels (Molecular Dynamics, Inc., Sunnyvale, CA).

Table 2. Body weight and muscle wet weight from basal, control thyroid-deficient, and space-flown young rats.

	N	Body	Ventricles	Soleus	Plantaris	Medial Gastroc.	Lateral Gastroc.	Vastus Inter.	Vastus Lateralis	Vastus Medialis	Tibialis Anterior
		(g)					(mg)				
Basal	24	17±1#	77±4#	4±0.3#	7±1#	12±1#	—	—	—	—	15±1#
NC8-Ground	16	80±2	340±13	31±1	64±2	135±4	155±6.	19±1	121±4	61±3	128±4.
NC8-Flight	6	40±5*	230±36*	7±1*	19±4*	48±8*	57±1*	7±1*	53±8*	25±5*	53±6*
% change (F vs. G)		-50	-32	-77	-70	-64	-63	-63	-56	-59	-58
TD8-Ground	16	39±1#	137±3#	10±1#	20±1#	38±3#	46±3#	6±0.4#	41±2#	21±1#	42±2#
TD8-Flight	4	31±3#	104±3#	5±1*#	13±2#	27±3#	29±3#	6±1#	50±9#	14±2#	28±3*#
% change (F vs. G)		-21	-24	-50	-35	-29	-37	-6	22	-33	-33
% change (TD vs. NC)		-51	-60	-68	-69	-72	-70	-66	-66	-66	-67
NC14-Ground	12	114±4#	425±19#	46±2#	99±3#	216±8#	234±12#	27±1#	181±7.#	100±5#	192±5#
NC14-Flight	6	98±3*#	365±8*	27±2*#	78±3*#	180±8*#	203±12*#	19±1*	163±6*#	90±4#	151±6*#
% change (F vs. G)		-14	-14	-41	-21	-17	-13	-30	-10	-10	-21

Basal rats studied at eight days of age; NC8, euthyroid neonates eight days old at liftoff; TD8, thyroid-deficient neonates eight days old at liftoff; NC14, euthyroid neonates 14 days old at liftoff. Ground (G), ground-based rats; Flight (F), space-flown rats. Gastroc, gastrocnemius; Inter, intermedius. *, P<0.05 Flight vs. matched ground-based control; #, P<0.05 vs. NC8-Ground group.

RESULTS

Body weights

Body weights of the euthyroid and TD ground control groups in this study closely matched the growth curve established for these two groups in previous ground-based experiments (Table 2, Adams, 1999). As is typical for TD animals, body weights of the TD rats were significantly lower than those of the euthyroid group. Body weight of the euthyroid and TD flight groups were significantly lower than their ground-based counterparts. The effect of flight on body weight in the older rats (NC14 group) was much less dramatic than in the younger group (NC8) (Table 2).

On the Neurolab mission, many of the young (eight days old at launch) rats died on the flight. One concern was that these rats received inadequate nutrition. The reduced weight gain in the flight groups, however, is less likely the result of lack of nutrition. This is based on myosin isoform profiles in the heart. The MHC genes in the heart are very sensitive to both energy intake restriction and thyroid state. Cardiac β -MHC expression becomes predominant in response to either hypothyroidism or reductions in energy intake (Figure 3A). Cardiac myosin analysis shows, however, that cardiac β -MHC is not expressed in young euthyroid rats (NC8) exposed to spaceflight (Figure 3B). If these animals were energy deprived, we would have expected to see significant β -MHC gene expression in the hearts of the euthyroid-flight group (NC8-flight). Further, since the hearts of the both the ground- and flight-based TD rats responded with almost exclusive (>93%) expression of the β -MHC isoform (which is a key marker of thyroid dependency), the flight-exposed TD rats must have received relatively the same amount of nourishment (milk containing the antithyroid drug) as compared to

their ground-based counterparts, so as to make both groups TD to the same degree.

Skeletal muscle growth

Figures 4 and 5 show a schematic representation of some key hindlimb muscles, soleus and plantaris, as they grew during the Neurolab experiment. Note the dramatic difference between the size of the muscles in the baseline controls studied at launch vs. the ground-control animals at the completion of the mission. Consistent with the body weight data, absolute muscle weights were markedly reduced in all flight groups relative to age-matched ground controls (Table 2). Also, muscle weights of the TD groups were lower than the euthyroid groups (both flight and ground control) (Table 2). In the antigravity slow-twitch soleus muscle, spaceflight exerted a greater relative growth retardation response compared to its fast-twitch counterpart, the plantaris (compare Figure 4 v. 5). When the data were normalized to body weight (mg muscle/gram body weight), it was apparent that the relative soleus muscle weight in the younger spaceflight groups did not increase beyond basal values (Figure 6). In other words, the relative muscle mass of the soleus muscle of the young spaceflight rats at 23 days of age remained essentially the same as that seen in the baseline group studied at eight days of age; i.e., just prior to the 16-day flight (Figure 6). Nonweight-bearing leg muscles such as the tibialis anterior appeared to be the least affected by spaceflight (see Table 2 and Figure 6). Also, it is important to note that the spaceflight inhibitory effects on muscle growth were less dramatic in the older rodents (NC14) than in the younger groups (NC8) (Table 2, Figure 6).

As an index of muscle growth, muscle total protein and DNA were studied in selected muscles, such as the medial gastrocnemius (MG). The data show significant decreases in total muscle protein and DNA content due to either flight or

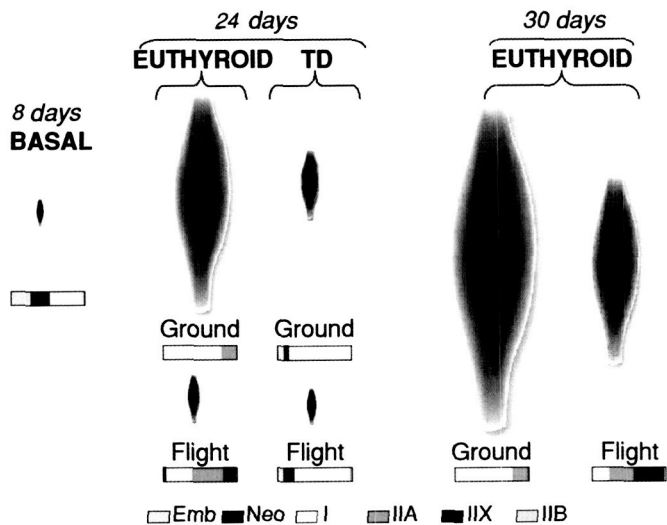


Figure 4. Soleus muscle growth and MHC protein profile across the Neurolab experimental groups. Schematic representation of the soleus muscle as it grows during the time-course of the Neurolab experiment in each of the experimental groups. Note the dramatic difference between the size of the muscle in the baseline controls studied at launch (basal) vs. the ground control animals at the completion of the mission (24 days and 30 days euthyroid). Also note the lack of growth in the soleus muscle of TD flight animals. Below each muscle is a bar representing the distribution of MHC isoform proteins in the corresponding group.

thyroid deficiency, especially in the younger neonates (NC8). These data are consistent with a flight-induced reduction in muscle growth based on muscle weight data (Table 2). Despite these differences in muscle DNA and protein content, a strong relationship was maintained between the DNA and protein content across all the experimental groups. These findings suggest a tightly coordinated relationship between muscle size and both DNA and protein content. This relationship was not disrupted by either spaceflight or thyroid deficiency.

Hormonal data: insulin-like growth factor-I

The reductions in growth, evidenced by the body weight measurements, were paralleled by the decreased levels of plasma IGF-I (Figure 7) such that there was a significant correlation between plasma IGF-I levels and body weight among the experimental groups (Figure 7C). In analyzing IGF-I expression at the muscle level, it is important to note that IGF-I was not determined on soleus muscle since there was insufficient tissue available for such analyses. However, determinations were made on the tibialis anterior and medial gastrocnemius muscles, which are relatively larger muscles than the soleus (Table 2). Results show that IGF-I peptide levels for these muscles were significantly reduced in response to both spaceflight and thyroid deficiency (Figure 8 A and B). Data analyses reveal a significant positive correlation between intramuscular IGF-I and muscle weight (Figure 8 C and D). In the ground-based neonates, an apparent developmental surge in both circulating and muscle

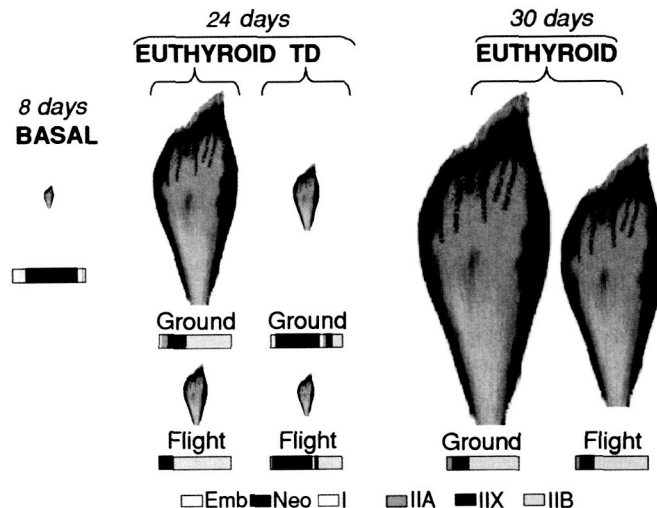


Figure 5. Plantaris muscle growth and MHC protein profile across the Neurolab experimental groups. Schematic representation of the rat plantaris muscle as it grows during the time-course of the Neurolab experiment in each of the experimental groups. Note the dramatic difference between the size of the muscle in the baseline controls studied at launch (basal) vs. the ground control animals at the completion of the mission (23 days and 30 days euthyroid). Below each muscle is a bar representing the distribution of MHC isoform proteins in the corresponding group.

IGF-I can be seen when compared to the basal values (Figures 7 and 8), especially between eight days (basal) and 24 days of age (NC8). This response appears to have been blunted, particularly in the young euthyroid flight-based animals and in both the ground-based and flight-based TD groups such that their levels corresponded more closely to those of the less mature basal group. Also, there was a significant positive correlation between muscle IGF-I peptide concentration and the protein or DNA content of the MG muscle.

Interaction between spaceflight and thyroid deficiency on growth processes

Exposure to spaceflight resulted in a significant reduction in the general growth of young rats (NC8 and NC14) (Figure 7A). The imposition of a TD 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 (Figure 7A).

The complex presentation of growth data in Figure 7 makes it difficult to discern the relative impact the different treatments imposed on the rats. To partition the impact of spaceflight vs. thyroid deficiency and to discern potential interactions between these variables, we calculated the relative body growth deficit imposed by each treatment (Flight/TD) either alone or in combination relative to the appropriate group. The data from Figure 7A were used to generate Figure 9. From this analysis, it is evident that both spaceflight (NC8-Flight vs. NC8-Ground)

and hypothyroidism (TD8-Ground vs. NC8-Ground) resulted in an $\approx 50\%$ decrease in somatic growth in the younger rats when each was imposed separately. The flight effect on somatic growth was much reduced when imposed on TD animals (TD-Flight vs. TD-Ground). Likewise, TD effects were also reduced

when taken in the context of spaceflight (TD-Flight vs. NC-Flight). The combined effects of thyroid deficiency and of spaceflight on somatic growth (TD8-Flight vs. NC8-Ground) were slightly larger (60% vs. 50%; $p > 0.05$) but not statistically different than the effects of each of the manipulations imposed

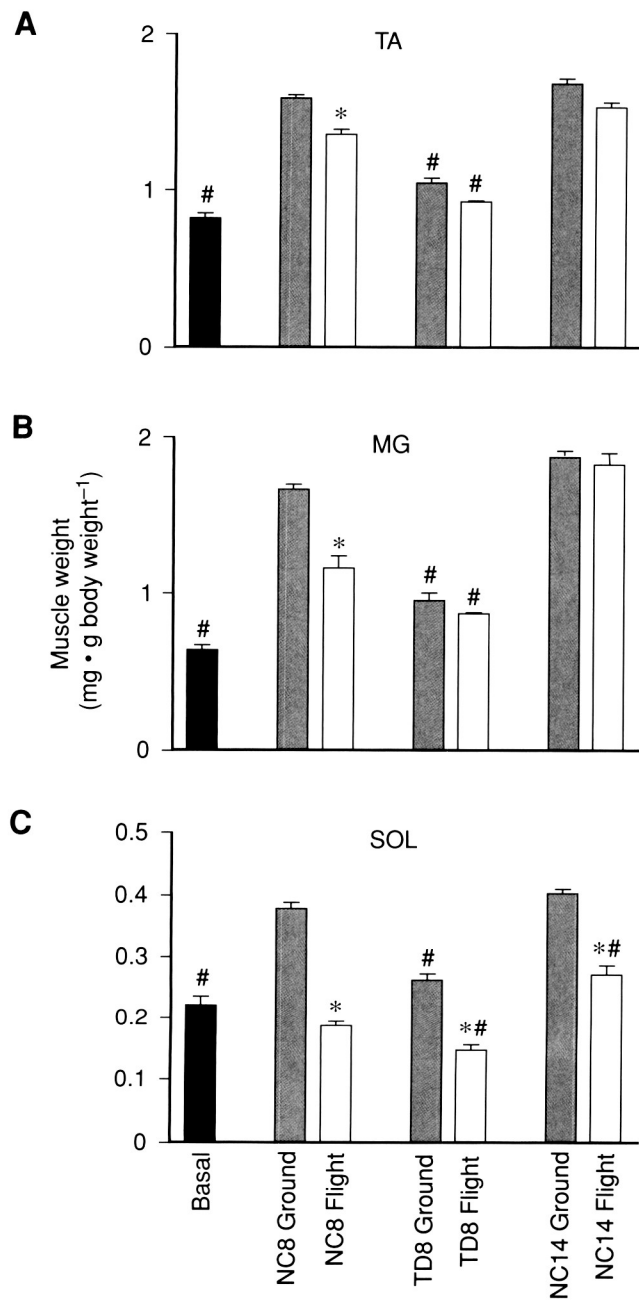


Figure 6. Muscle weight normalized to body weight: the effects of TD and/or spaceflight. (A) TA, tibialis anterior, a non-weightbearing locomotor muscle expressing primarily fast MHC. (B) MG, medial gastrocnemius, a weightbearing locomotor muscle expressing primarily fast MHC. (C) SOL, soleus, a weightbearing postural/locomotor muscle expressing primarily slow MHC. Refer to Table 2 for group designations. *, $p < 0.05$ Flight vs. Ground; #, $p < 0.05$ vs. NC8 Ground. n values as in Table 2. (From Adams, 2000a, with permission; reproduced from the *Journal of Applied Physiology*.)

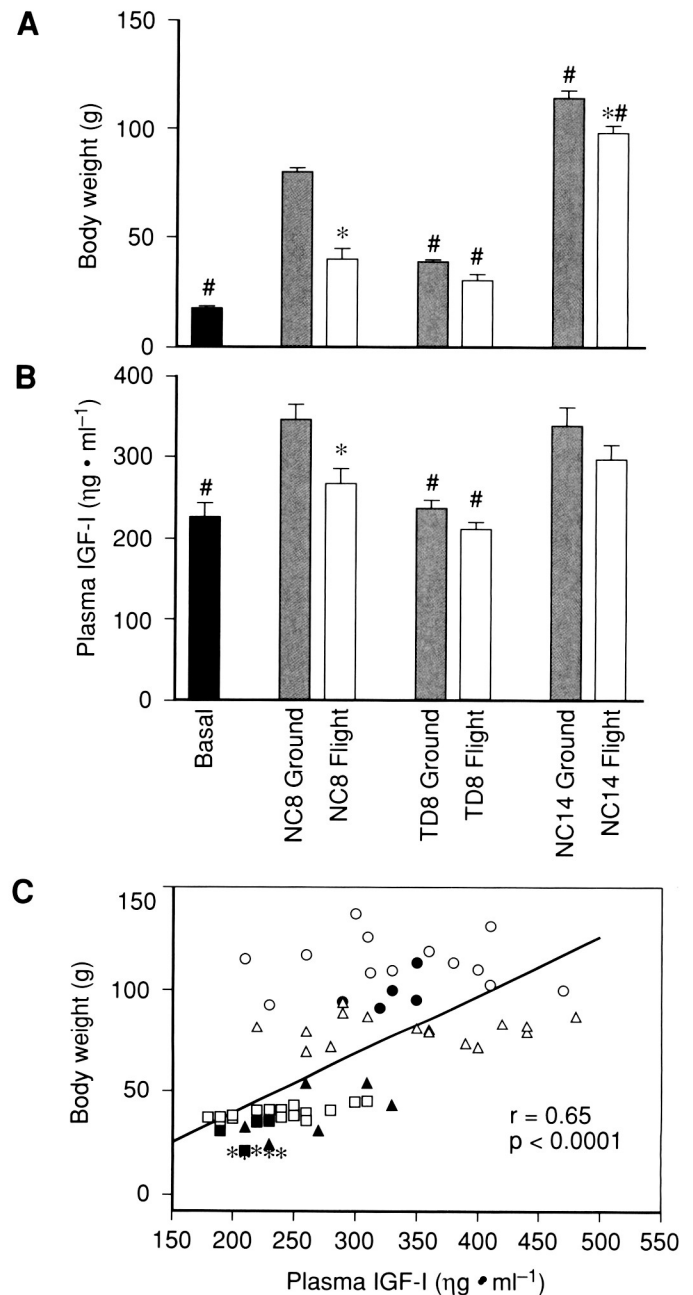


Figure 7. Body weight and plasma IGF-I concentration: the effects of TD and/or spaceflight. (A) Body weight. (B) Plasma IGF-I concentration. (C) Correlation between body weight and plasma IGF-I concentration with best-fit line. Refer to Table 2 for group designations. *, $p < 0.05$ Flight vs. Ground; #, $p < 0.05$ vs. NC8 Ground; N values as in Table 2. Symbols: *, Basal; Δ , NC8 Ground; \blacktriangle , NC8 Flight; \square , TD8 Ground; \blacksquare , TD8 Flight; \circ , NC14-Ground; \bullet , NC14 Flight. (From Adams, 2000a, with permission; reproduced from the *Journal of Applied Physiology*.)

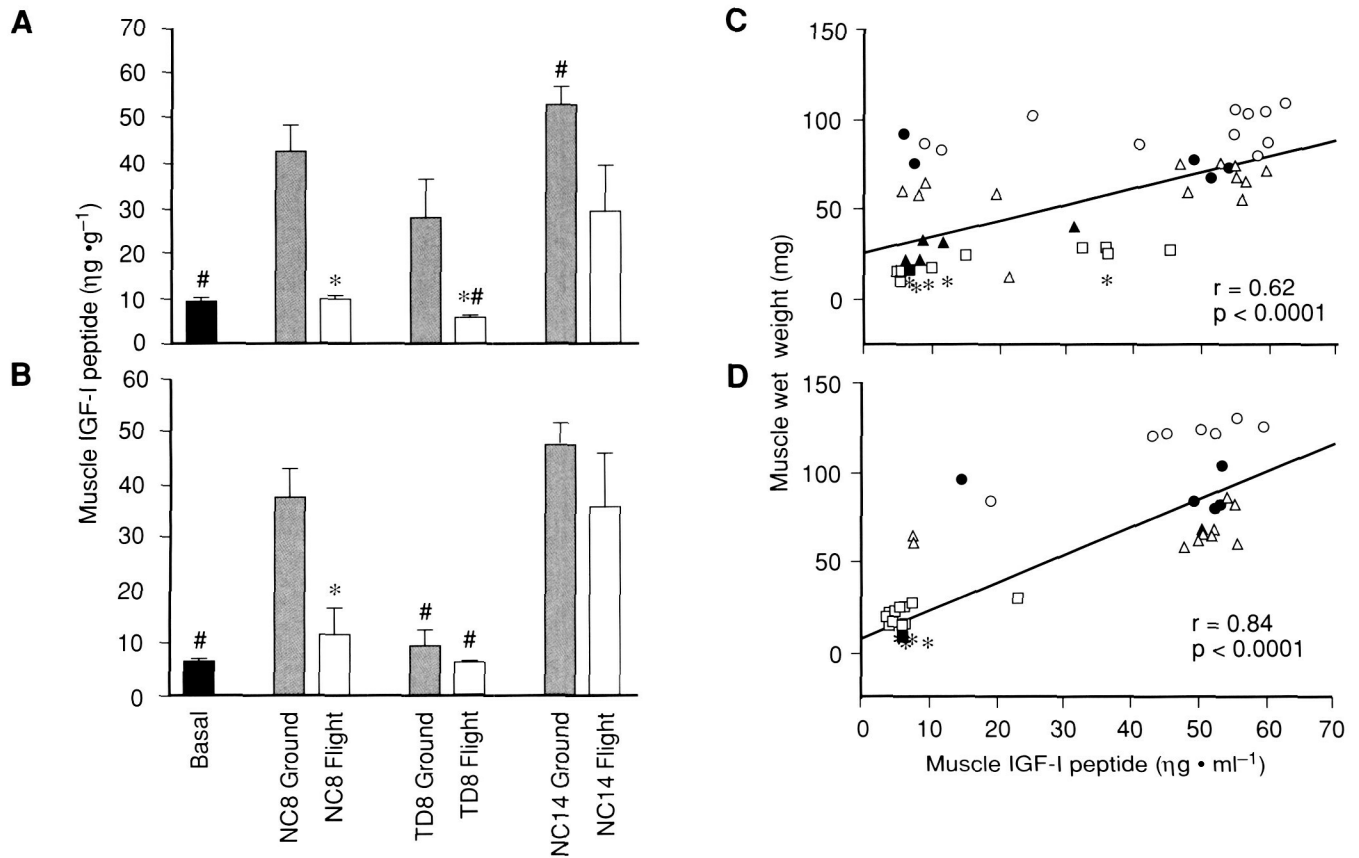


Figure 8. Muscle IGF-I peptide concentrations: effects of TD and/or spaceflight. (A) Tibialis anterior (TA) muscles IGF-I concentration. (B) Medial gastrocnemius (MG) muscles IGF-I concentration. (C) The correlation between muscle wet weight and muscle IGF-I peptide from all TA muscles depicted in A. (D) The correlation between muscle wet weight and muscle IGF-I peptide from all MG muscles depicted in B. Refer to Table 2 for group designations. *, $p < 0.05$ Flight vs. Ground; #, $p < 0.05$ vs. NC8 Ground. Symbols are as in Figure 7, n values are as in Table 2. (From Adams, 2000a, with permission; reproduced from the *Journal of Applied Physiology*.)

separately (Figure 9). This presentation also highlights the lesser sensitivity of the older rats (NC14) to the effects of loss of weightbearing activity (Figure 9, NC14-Flight vs. NC14-Ground). This age-related finding corresponds with data that show a decline in receptors for IGF-I in skeletal muscle IGF-I after about 12 days of age, indicating a potential age-related decrease in the sensitivity of muscles to this growth factor (Shoba, 1999).

The general decrease seen in body growth was also reflected in lower limb muscle weights from the flight vs. ground-based rats (Table 2). As with body weight, we have used the data from Figure 6 to apportion the relative growth deficit imposed by the separate and combined treatments of hypothyroidism and spaceflight (Figure 10). Skeletal muscles expressing primarily fast MHC isoforms (such as the medial gastrocnemius) showed decreased muscle growth in the younger (NC8-Flight vs. NC8-Ground) but not in the older group (NC14-Flight vs. NC14-Ground) (Figure 10). In an antigravity muscle such as the soleus, spaceflight resulted in a significant relative muscle growth deficit in both younger (50%) and older neonates (32%). Thyroid deficiency appeared to have a greater impact on fast-twitch muscle (TA and MG) growth than spaceflight; while

the opposite is true in antigravity slow-twitch muscle (Soleus-Sol) (Figure 10). As with body growth, there did not appear to be a notable additive effect of these treatments in any of the studied muscle types.

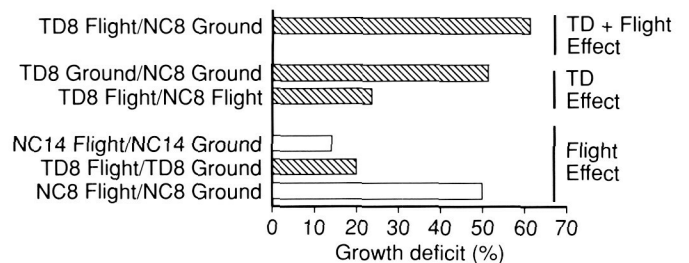


Figure 9. The relative growth deficit imposed by the separate and combined interventions of TD and exposure to spaceflight (Flight). Data are calculated from the body weight data presented in Figure 9A as follows: Flight effect: NC8-Flight/NC8-Ground, TD8-Flight/TD8-Ground, NC14-Flight/NC14-Ground; TD effect: TD7-Flight/NC7-Flight, TD7-Ground/NC7-Ground; TD+Flight Effect: TD8-Flight/NC8-Ground. (From Adams, 2000a, with permission; reproduced from the *Journal of Applied Physiology*.)

Spaceflight and thyroid deficiency effects on myosin heavy chain gene expression

Soleus Muscle – At seven days of age, the MHC profile of the soleus muscle consists primarily of embryonic (~27%), neonatal (25%), slow type I (45%), and traces of fast IIa and IIb (~2% each); i.e., the embryonic/neonatal isoforms account for ~50% of total MHC pool (Figure 4). By 24–30 days of age the soleus muscle is transformed into an adult phenotype consisting of ~80% type I MHC and 20% fast IIa MHC such that the embryonic/neonatal isoforms become repressed and replaced by increases in adult slow MHC gene expression (Figure 4). In rodents initially exposed to spaceflight at eight days of age and their muscles subsequently examined at 23 days of age (i.e., after 16 days in space), the soleus MHC profile is 3% neonatal, 36% slow, 42% fast type IIa, 16% fast type IIx, and 3% fast type IIb. Thus, spaceflight not only blunted slow, type I MHC gene expression in the developing soleus muscle, but it also created a profile typically seen in most fast muscles in which the fast MHC isoforms dominate the MHC protein pool. In contrast, thyroid deficiency caused retention of significant relative levels of both the embryonic and the neonatal MHC in the flight-based and ground-based groups, while blunting expression of all fast MHC isoforms as compared to what is typically seen in their euthyroid counterparts. Thus, regardless of gravity status, the soleus muscles of TD neonates expressed predominantly the type I isoform (76–84%) with the rest consisting of the developmental types (Figure 4).

When an MHC gene is expressed, it creates a messenger RNA (mRNA) that carries instructions on how to make the protein. These instructions are translated by the cell to make the MHC protein. The profiles seen at the protein level were essentially mimicked at the mRNA level across the various experimental groups (Figure 11). This suggests that the regulation of MHC gene expression in these developing rats is at the level of how the soleus muscle transcribes and maintains the level of mRNA (a process called pretranslational regulation) prior to translating this message into its encoded protein. Evidence for this type of regulation is the strong positive relationship that exists between MHC mRNA vs. protein for the various isoforms. One exception was that in the euthyroid-flight and ground-based groups, the expression of embryonic mRNA was still manifest throughout the developmental period (Figure 11) even though its protein product appeared to be fully repressed (Adams, 2000b). This is a unique observation, which we don't have a good explanation for at the present time; but the results suggest an uncoupling between the control of transcriptional vs. translational processing of the embryonic mRNA.

Plantaris Muscle – At eight days of age, the MHC profile of the plantaris muscle is even more undifferentiated relative to the adult state than is a slow muscle, since its MHC profile is 20% embryonic, 70% neonatal, 6% slow, and 4% fast IIb (Figure 5). This profile is essentially mirrored at the mRNA level (Figure 11), suggesting that the adult MHC genes have not been “turned on” at this early stage of development. By

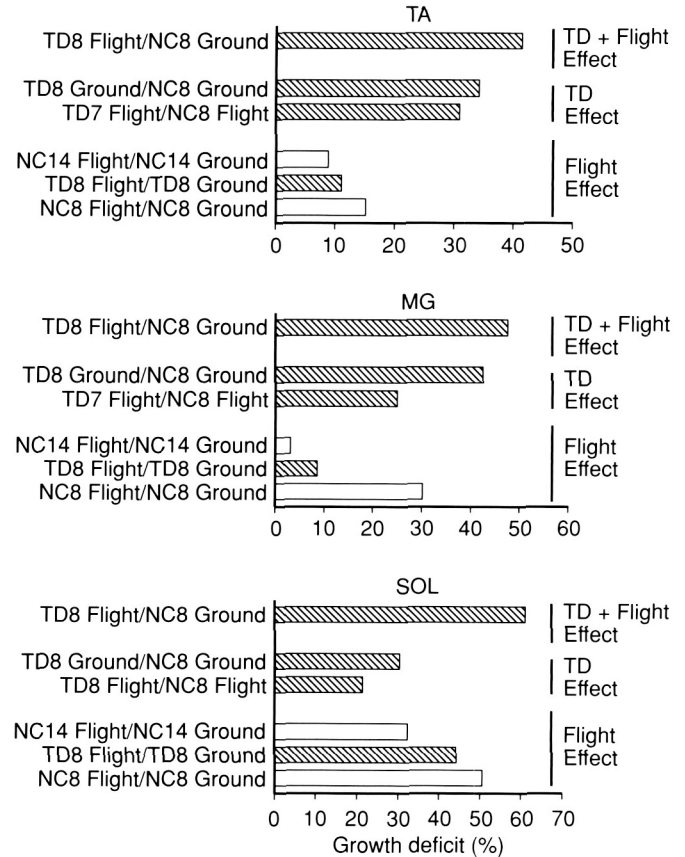


Figure 10. The relative growth deficit imposed on limb skeletal muscles by the separate and combined interventions of thyroid deficiency and exposure to spaceflight. Data are calculated from the normalized muscle weight data presented in Figure 7 (see Figure 9 legend for groupings). (From Adams, 2000a, with permission; reproduced from the *Journal of Applied Physiology*.)

23–30 days of age, the muscle becomes markedly transformed such that the profile consists of 60% fast IIb, 30% fast IIx, 5% fast IIa, and 5% slow MHC (Figure 5). Thus, this muscle, in contrast to the soleus, is characterized by a predominance of the fast type IIb and IIx MHCs. Exposure of young euthyroid rats to spaceflight at eight days of age exerts a subtle effect on this muscle by repressing expression of both the fast type IIa and slow, type I MHCs while augmenting expression of the IIb MHC. Interestingly, thyroid deficiency exerts a unique effect on the plantaris muscle by markedly blunting the transformation process, noted above, whereby the neonatal MHC isoform gene expression is repressed while that of the fast IIb MHC becomes predominant (Figure 5). In essence, thyroid deficiency maintains the plantaris muscle in an undifferentiated state, and this process occurs independently of exposure to microgravity. This process appears to be regulated by a combination of transcriptional, posttranscriptional, and translational processes based on the mRNA data profiles for the different isoforms (Figure 11).

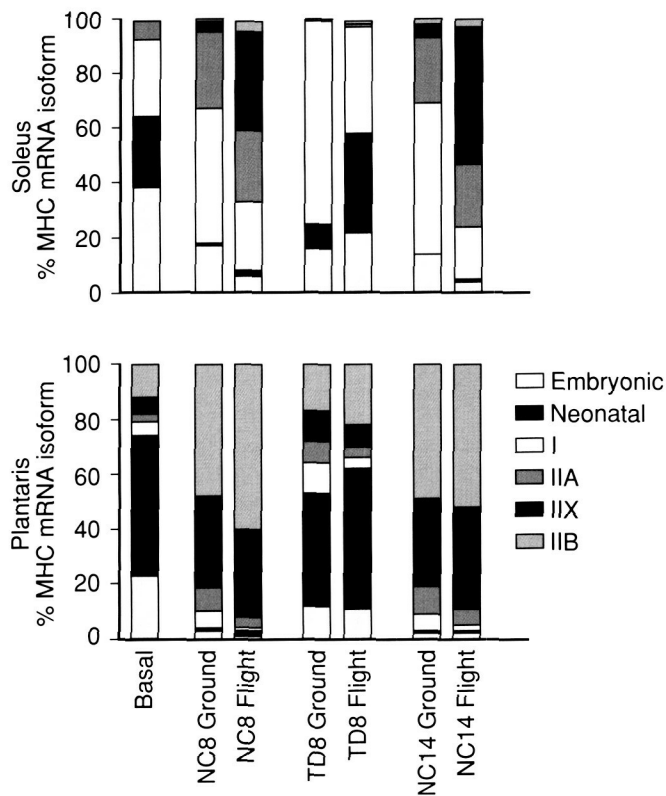


Figure 11. MHC mRNA isoform profiles in the soleus and plantaris muscles. Each MHC isoform is presented by its proportion as percent relative to the total MHC mRNA pool as determined by RT-PCR methods (see Adams, 2000b for details).

DISCUSSION

Factors impacting growth of developing skeletal muscles

The findings of this project clearly show that the separate and combined effects of unloading (as induced by the environment of spaceflight) and thyroid deficiency collectively reduce both body and muscle growth. Also, gene expression of the MHC family of motor proteins responsible for regulating muscle contractile processes is altered.

The reduction in growth appears to be more critical for younger (eight-day-old) vs. older (14-day-old) rodents. Both the body weights and normalized muscle weights of the younger rats were reduced to a markedly greater extent relative to controls compared to the older euthyroid rats exposed to spaceflight (Table 2; Figures 6, 10). This suggests that the latter group, which is further along in development when chronic weight-bearing activity was stopped, may have some protection when developing in the spaceflight environment. Furthermore, hormonal status can play a pivotal role in the growth process, since thyroid deficiency caused approximately equivalent reductions in body and muscle growth of both the ground-based and flight-based TD groups (Table 2).

Moreover, given the equivalence of the growth retardation in both the ground- and flight-based TD groups (and given the similarity of the euthyroid flight-based neonates to the TD groups

irrespective of spaceflight), some other factor(s) also may be playing a role in affecting growth across these groups. One possibility is that the young neonatal euthyroid flight-group was actually TD. We think this is unlikely since both the cardiac and soleus skeletal muscles of the euthyroid flight-group demonstrated either normal or exaggerated fast MHC profiles. This would not have been expected if these flight animals were experiencing thyroid deficiency. That is, these flight animals would have demonstrated a very high relative proportion of the β -MHC (i.e., slow, type I MHC) in both their soleus and heart muscles if they were actually TD. Such was not the case (see Figures 3, 4, 11).

Another possibility is that the euthyroid flight-group was nutritionally (calorically) compromised, which reduced their growth. While spaceflight may have had some impact on the energy intake of these animals, we do not feel that this was extensive enough to account for the growth reductions noted. Otherwise, we would have observed a relatively high level of expression of the β -MHC gene in the hearts of these animals. Instead, we observed very low to nonexistent levels of this MHC in their hearts; i.e., approximately equivalent to that seen in the young and older ground-based control groups as well as in the older euthyroid flight-based group (Figure 5). Further, if the TD flight neonates were energy deprived—i.e., getting insufficient milk and, hence, insufficient antithyroid drug—these rats would not have been hypothyroid to the same degree as their ground-based, nutritionally provided counterparts. The cardiac and skeletal MHC data suggest that they were.

Instead of the above possible scenarios, we feel that in both the younger euthyroid flight-based group and in both TD groups (flight- and ground-based), their body and muscle growth were retarded by an impairment in the normal operation of the thyroid GH-IGF-I axis (Figure 2). This is illustrated by (a) the reduction in both systemic and muscle-specific (weightbearing and nonweightbearing) IGF-I levels in these animals relative to their respective control groups; and (b) the strong positive correlation between body mass and systemic IGF-I (Figure 7). The correlations between muscle mass and muscle-derived IGF-I (Figure 8), as well as between muscle protein accumulation and muscle IGF-I levels, also support this. Taken together, these results point to a complex interaction of weightbearing activity, thyroid hormone levels, and the corresponding expression of both the systemic and muscle-specific levels of IGF-I. Together these are essential for normal body and muscle growth during critical stages of neonatal development; i.e., the first three weeks postpartum. The uncoupling of either weightbearing activity or thyroid hormone from this axis during this key stage of development clearly limits the growth potential of both the animal and the musculoskeletal system in particular.

Factors impacting the myosin heavy chain gene family during development

The MHC isoform gene family of contractile proteins represents the most abundant type of protein expressed in muscle, which accounts for ~20-25% of the protein pool in a typical adult muscle cell. Following birth, all striated muscles

(including skeletal muscle) are undifferentiated with regard to the MHC phenotype that is expressed. Those muscles used extensively for weightbearing and locomotion express primarily the embryonic/neonatal isoforms, which are ineffective in supporting the varied contractile intensities that are needed for performing high-intensity activities in the adult state.

The findings reported in this project clearly show that weightbearing activity early in the rodent's first three—four weeks following birth is required for the normal expression of slow, type I MHC, the isoform that predominates in motor units designed for opposing gravity (Figure 1). In the absence of gravity or weightbearing stimuli, full transcriptional activity and hence subsequent translation of the type I mRNA are dramatically reduced in spaceflight-exposed rats relative to their ground-based counterparts (Figures 4, 11). This reduction in slow MHC expression, in combination with the retardation in muscle growth, markedly reduces the mass of skeletal muscle (number and size of the slow motor unit pool) available to support routine antigravity activities and movement. Thus, the motor skills of the animal likely become compromised (Walton, 1998). Hence, in the absence of weight-bearing activity, and in the presence of normal circulating thyroid hormone, all the muscles of the lower extremity become biased to expressing primarily the three fast MHC isoforms (Figures 4, 5, 11). The levels of the slow, type I MHC isoform are insufficient to enable the rats to effectively oppose gravity and perform sustained locomotion.

This experiment also shows the importance of thyroid hormone (T3) in regulating both muscle mass and contractile protein differentiation during neonatal development. Thyroid hormone not only interacts with IGF-I to establish normal growth, but also appears essential to enable the rat to transform its faster contracting muscles from an undifferentiated infant state to a differentiated adult state. This transformation provides a sufficient expression of fast MHCs for the rat to perform activities of a higher intensity and power output. In the absence of sufficient thyroid hormone, while the relative abundance of slow MHC is abundantly expressed independently of weightbearing stimuli, the muscle system remains in an immature state in both size and functional capabilities. Both weightbearing activity and a normal thyroid state combine to ensure that the muscle system develops sufficiently.

One of the shortcomings of the Neurolab mission was the loss of a significant number of young rats during the flight. The reasons are not fully known, but are likely due to the housing facilities, which were not optimal for the mothers to nurture the size of the litters chosen for these experiments. That is, some of the mothers may have purposely ignored weaker rats to adequately care for the remainder of the litter. Whatever the reason, this event limited the availability of rats for analyses concerning their growth and muscle properties during recovery from spaceflight. We can only speculate that over time the young flight-exposed rats would have gained normal body and muscle mass along with a normal MHC phenotype in the absence of any therapeutic activity program. Whether these possible recovery processes would enable appropriate motor performance and exercise capacity remains uncertain.

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