Metabolic Consequences of Muscle Disuse Atrophy\textsuperscript{1,2}

T. P. Stein\textsuperscript{3} and C. E. Wade\textsuperscript{*}

Department of Surgery, University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine, Stratford, NJ 08084 and *U.S. Army Institute of Surgical Research, Fort Sam Houston, TX 78234-6315

ABSTRACT In response to decreased usage, skeletal muscle undergoes an adaptive reductive remodeling. This adaptive response has been found with disuse during human spaceflight, rat spaceflight, rat hind-limb unloading, bed rest, and aging. The reductive remodeling of skeletal muscle with disuse is largely independent of the reason for the disuse. The process involves more than a transition from slow to fast myosin fiber types. There are associated metabolic changes including a fuel shift toward glycolysis, decreased capacity for fat oxidation, and energy substrate accumulation in the atrophied muscles. Glycolysis is very effective for high-intensity short-duration acute activities, but if sustained output is needed, an energy profile where fat use is favored rather than compromised is desirable. For astronauts, there is a need to maintain as much functional capacity as possible during spaceflight for extravehicular activities. The shift toward increased activity of the glycolytic enzymes in atrophied muscle is accommodated by an increase in gluconeogenic capacity in the liver. 


KEY WORDS:  
- muscle atrophy
- liver
- space flight
- microarrays
- gluconeogenesis

In response to decreased usage, skeletal muscle undergoes an adaptive reductive remodeling. This adaptive response has been found with muscle disuse produced by human spaceflight (1,2), rat spaceflight (3,4), rat hind-limb suspension (HLS) (5,6), bed rest (7,8), and aging (9,10). As part of this adaptive remodeling process, there is also a shift in myosin isoforms from slow to fast isoforms (11–13). The muscle atrophy found with aging and disuse (spaceflight, bed rest, HLS) appears to be very similar (7,14–16), suggesting that common mechanisms are operating. Thus, findings with one situation are likely to be applicable to other disuse atrophy situations.

The changes in muscle physiology and structure with disuse have been extensively investigated. Less attention has been paid to the metabolic consequences associated with disuse atrophy, the topic of this article. Accompanying the shift in fiber type is a shift in fuel metabolism away from lipid fuels toward glucose. This metabolic shift is found with spaceflight (36), HLS (35,36), rat (Stein and Wade, unpublished observations), and aging (10,37,38).

Consequences of the fuel shift

The physiological importance of the fuel shift with disuse atrophy attracted little interest until several investigators of the human spaceflight program pointed out that this could lead to increased fatigability (7,39–43). Fast-twitch fibers are primarily dependent on intrinsic carbohydrate stores. Glycolysis is very effective for high-intensity short-duration acute activities, but if sustained output is needed, an energy profile where fat use is favored is desirable. For most of the situations described above (aging, bed rest, etc.), an inability to sustain work output is not a major cause for concern. Rather, the cause for concern is the reason for muscle disuse. The spaceflight situation is different. There is a need to maintain as much functional capacity as possible during spaceflight for extravehicular activities (space station construction and maintenance, emergency egress, and now, following the investigation of the Columbia accident, shuttle repair). These are all functions that require sustained work output. Increased fatigability in this context is counterproductive.

Another aspect of muscle disuse atrophy of potential concern to the space program is the accumulation of fat in the atrophied muscle at the expense of protein. Normal active muscle does not have extensive fat deposition. Even if the protein is not fully functional, it would be advantageous to retain it rather than lose it and have to resynthesize it once workload on the muscle is increased. Astronauts venturing to other planets will not have the luxury of a period of recuperation. Even if the actual gravity field is negligible (moon) or
two-thirds less than on Earth (Mars), moving about in cumbersome space suits is heavy work requiring functional muscles.

**Muscle fatty acid metabolism**

Most of the studies that have investigated lipid metabolism have found that fatty acid oxidation is reduced. Fatty acid oxidation is reduced with bed rest (44) and aging (45–48). With bed rest, both Ferretti et al. (49) and Hikida et al. (50) found that levels of 3-hydroxyacyl CoA dehydrogenase, a key enzyme in muscle fatty acid oxidation, decreased in the vastus lateralis muscle.

In the rat HLS model it is unclear whether fat oxidation is reduced (51,52) or unchanged (29). Two recent mRNA expression analysis studies of atrophied soleus muscle reported a downregulation of the capacity to oxidize fatty acids and an increase in glycolytic capacity (51,52). In contrast, studies on the soleus muscle in HLS rats by Grichko et al. concluded that fatty acid oxidation by muscle homogenates as measured with U-14C palmitate did not decrease with HLS although there was an increased reliance on glycolysis (13,29). After spaceflight, the ability of muscle homogenates to oxidize palmitate decreases (17). Across the various disuse models there are apparent indications of a decreased capacity for lipid utilization.

**Muscle glucose metabolism**

Numerous studies have shown that there is a greater reliance on glucose for energy in atrophied muscle (13–18,20,24,51,53–58). The observations apply to mixed, slow, and fast antigravity muscles such as the soleus and anterior digitalis longus and fast muscles such as the tibialis anterior and the extensor digitorum longus. Two single-fiber studies reported that glycolytic enzyme levels increased after spaceflight (55,59). Hexokinase and pyruvate kinase levels increased in rats after spaceflight (55,59) and HLS (20,55,60). Phosphoglycerate mutase levels increased in HLS rats (61). Phosphofructokinase levels were unchanged with bed rest (50) and spaceflight (13) but increased with HLS (54–56). Two bed rest studies reported no change in lactate dehydrogenase levels (13,50) as did one rat spaceflight study (62), although increases have been reported in space-flown rats (21,36).

**Muscle tricarboxylic acid cycle activity**

Activity of the tricarboxylic acid (TCA) cycle enzymes appears to be unchanged in atrophied muscle. Most studies reported that the activity of enzymes in the TCA cycle, such as citrate synthase (36), succinic dehydrogenase (24,53,59), and malate dehydrogenase (17), did not change. However, one study found an increase in citrate synthase activity (54). In agreement with these findings, mRNA expression analysis did not reveal any clear-cut changes in atrophied soleus muscle TCA cycle activity in the HLS rat model (51,52).

**Response of the liver**

Although many authors have commented on the fuel shift, there is no convincing explanation in the literature (13,18,40,63,64). To quote Edgerton and Roy (40), “It is not clear whether the changes in the enzymes of glycolysis of a fiber precede the myosin type change, or vice versa”; i.e., is the shift an intrinsic consequence of the change in myosin fiber type, or is the shift a response to altered fuel supply in the plasma? Interestingly, a time course HLS study by Langfort et al. (18) found that the enhanced glucose utilization preceded the reduction in muscle mass, suggesting that the enhanced glucose utilization was not directly related to the myosin fiber type shift in muscle atrophy.

If the shift is secondary to a change in fuel availability, there should be supporting changes in liver fuel metabolism processing consistent with the overall reduction in nutritional requirements of chronically inactive muscle.

In order to obtain an overview of whether there was any interrelation between the effects of HLS on muscle and liver, we compared mRNA expression analysis of the soleus muscle and liver in HLS rats using the Affymetrix microarray expression system (51). Male Sprague-Dawley-derived albino rats were randomly assigned to ambulatory control or 21 d of HLS using the tail-casting procedure of Morey-Holton and Wronsli (65). After the animals were killed, the soleus muscle and liver were removed and promptly frozen in liquid nitrogen, and aliquots were prepared for microarray analysis using the Affymetrix U34A rat genome microarray chip (Affymetrix). The results were analyzed for metabolic pathway changes with the GenMAPP program (Gladstone Foundation).

The expression analysis data for muscle showed changes in muscle fiber type expression that were in good agreement with the reported histological and biochemical findings (51). Using mRNA expression analysis, both our group (51) and Wittwer et al. (52) found decreased fatty acid β-oxidative capacity in atrophied muscle and increased glycolytic capacity. In contrast to the downregulation of fatty acid oxidation, the capacity to metabolize glucose increased. mRNA expression of hexokinase and phosphofructokinase, key regulatory enzymes for the glycolytic pathway, increased substantially (51) (Table 1). In the liver, the glycolytic pathway did not change, but there was a clear pattern of changes in the expression of the enzymes involved in gluconeogenesis. All but 1 (fructose 1,6-bisphosphatase) of the enzymes involved in the regulation of gluconeogenesis increased (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and glucose 6-phosphate; Table 1). For the other metabolic pathways, the proportion of

### Table 1

**Comparison of mRNA expression analysis for enzymes involved in the glycolytic and gluconeogenic pathways in muscle and liver of HLS rats**

<table>
<thead>
<tr>
<th>Description</th>
<th>Muscle</th>
<th>Fold</th>
<th>P</th>
<th>Liver</th>
<th>Fold</th>
<th>P</th>
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<tr>
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<tr>
<td>Hexokinase 1</td>
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<td>0.05</td>
<td>1.27</td>
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<td>1.27</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexokinase 4</td>
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<td>0.05</td>
<td>1.03</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>Phosphofructokinase</td>
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<td>0.01</td>
<td>1.29</td>
<td>0.1</td>
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<td></td>
</tr>
<tr>
<td>Aldolase A</td>
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<td></td>
<td>1.03</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldolase B</td>
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<td>0.01</td>
<td>1.10</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
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<td></td>
<td>1.04</td>
<td>NS</td>
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<tr>
<td>Phosphoglycerate mutase</td>
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<td>0.05</td>
<td>1.09</td>
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<tr>
<td>Enolase</td>
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<td></td>
<td>1.07</td>
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<tr>
<td>Pyruvate kinase</td>
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<td>1.20</td>
<td>NS</td>
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<tr>
<td><strong>Gluconeogenesis</strong></td>
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<tr>
<td>Phosphoenolpyruvate carboxykinase</td>
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<td></td>
<td>1.47</td>
<td>0.05</td>
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<tr>
<td>Glucose-6-phosphatase</td>
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</tr>
<tr>
<td>Fructose-1,6-bisphosphatase</td>
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<td></td>
<td>1.06</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>Pyruvate carboxylase</td>
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<td></td>
<td>1.20</td>
<td>0.05</td>
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</tr>
</tbody>
</table>

1. P values are by two-tailed t tests. ND, not detected; NS, not significant.
changes was smaller, and there was no discernable pattern of changes in the expression of enzymes with key roles in the intermediary metabolism.

The observations indicate that peripheral muscle disuse atrophy has a hitherto unobserved and unsuspected effect on hepatic fuel metabolism. The HLS-associated changes were primarily limited to exporting the energy-rich macronutrients, glucose, and fatty acids. There were no consistent changes in any other pathways. The shift toward increased activity of the glycolytic enzymes in atrophied muscle is accommodated by a shift toward increasing glucose availability in the liver. It appears that there is a coupling between muscle and liver glucose metabolism in this model, suggesting that the change in muscle glucose metabolism is driven by substrate availability via an increase in hepatic gluconeogenesis.

Why the increase in gluconeogenic potential?

There are 3 potential sources for increased glucose availability to the tissues. First, there could be increased glycogenolysis. Second, there could be increased Cori cycling. There are some in vitro data to suggest that an increase in Cori cycling is plausible. In vitro, lactate production is increased in atrophied muscle. Lactate dehydrogenase activity is increased with disuse atrophy (13,29,51,66,67). Third, there could be increased gluconeogenesis from amino acids.

If amino acid levels are in excess of need, gluconeogenesis will increase, thereby increasing the glucose supply to the muscles. Amino acids could be in excess because amino acid requirements are reduced for atrophied muscles. Two arguments suggest that if a major portion of skeletal muscle is essentially inactive, fewer dietary amino acids should be required.

1) About 80% of the amino acids used for protein synthesis are derived from protein breakdown. The remaining ~20% are derived from the diet (68,69). If protein turnover is reduced, there should be less need to replace amino acids that are lost from the protein turnover cycle. Protein turnover decreases with disuse atrophy (30,70–73).

2) Amino acids provide precursor substrates for the TCA cycle (68). If metabolic activity is reduced, there should be less need to replenish TCA cycle intermediates. Metabolic activity in atrophied muscle would be reduced for 2 reasons: first because there is no work being done and second because protein turnover decreases. Protein turnover is a very energy intensive process; it accounts for ~20% of the basal metabolic rate (74).

Gluconeogenesis is substrate driven. About half of any excess amino acids are gluconeogenic. Increased glucose availability would lead to an increase in the enzymes associated with the glycolytic pathways in all tissues. However, this does not occur; the fuel shift appears to be specific to atrophied muscle. Most studies that have examined the effect of supplying glucogenic precursors have been acute studies and did not find a substantive increase in glucose production (21,75,76). The situation is different when the increased availability of gluconeogenic precursors is chronic. Chronic adaptation to increased glucose intake increases the pyruvate flux through the gluconeogenic pathway via increases in the enzyme levels of phosphoenolpyruvate carboxykinase and pyruvate carboxylase in rats (77). Similarly, in humans long-term adaptation to diets high in gluconeogenic precursors (either carbohydrate or protein) leads to increased glucose production in the postabsorptive state (78,79). Both rats and humans respond to a chronic increase in the supply of gluconeogenic substrates with an increase in total glucose production in the postabsorptive state.

The consequence of increased glucose availability in HLS rats is that in the resting state the fuel mix presented to the tissues has a greater proportion of glucose than in control rats for most of the day. With time, the atrophied muscle adapts to this increased availability of glucose; hence, the pathway shift toward glycolysis and away from β-oxidation.

The situation is different with active muscles. Rats (HLS and control) are active, moving about the cage and grooming themselves. During these bursts of activity, fuel utilization and energy expenditure are increased. The increased fuel needs are met by a combination of glycogenolysis within the muscle and increased fuel supply in the plasma (primarily from fatty acid release by adipose tissue). Because of these intermittent bursts of activity, working muscle maintains normal metabolic capacity, and the integrated fuel utilization pattern over 24 h predominantly reflects the energy supply during the activity burst phases. The contribution of a moderate increase in the glucose supply in the basal state is swamped by the increased release of endogenous glucose and fatty acids during the activity bursts. Muscle is plastic, and active muscle must maintain the capacity for work, which has higher energy requirements. Thus, no change in fuel metabolism pathways is to be expected in active muscle. The bursts of activity are too short in duration to affect the time-averaged fuel pattern seen by the atrophied muscles. Thus, small changes in substrate will only affect the basal state, and atrophied muscle is permanently in the basal state.

Fat accumulation in muscle

Fat accumulation in disused muscle does not occur because overall energy availability is in excess. With humans, fat accretion occurred while subjects were in positive energy balance [bed rest, (31), energy balance bed rest (23,32), or negative energy balance (spaceflight; V. S. Oganov and A. D. LeBlanc, Baylor College of Medicine, Houston, TX, personal communication)]. As part of the 1996 Life and Microgravity Sciences Mission we measured energy balance using the combination of intake and the doubly labeled water method ([2H2O18O]). The astronauts were in serious negative energy balance (~14 kcal·kg⁻¹·d⁻¹) due to a very low dietary intake (24.6 ± 1.6 kcal·kg⁻¹·d⁻¹) (80). Protein intake, however, remained above the recommended daily allowance (80). Postflight muscle biopsies showed histological evidence of an increase in fat droplets in the muscle biopsies (R. H. Fitts, Marquette University, Milwaukee, WI, personal communication).

Researchers found the same effect after long-duration spaceflight on the Russian space station, Mir. Dietary intake was very low—low enough to affect protein turnover (81) and host defenses (82). Energy intake averaged 26 ± 2.4 kcal·kg⁻¹·d⁻¹, indicating a deficit in energy intake and negative energy balance (81). The astronauts lost weight, lost overall body fat, and had atrophied leg muscles, yet in these same muscles, a large amount of fat accumulated [(33), V. S. Oganov and A. D. LeBlanc, Baylor College of Medicine, Houston, TX, personal communication]. Thus, the triglyceride accumulation in atrophied muscle was not due to impaired mobilization of fat from fat stores or excess energy intake. The atrophied muscle was unable to use fatty acids effectively. The replacement of protein with fat is not likely to be of benefit, as it is protein, not fat, that is responsible for work by muscle.

Ruderman et al. (83) have argued that “dysregulation” of the malonyl CoA regulatory mechanism could lead to insulin...
resistance and hence fat accumulation. The argument is that a chronic oversupply of glucose leads to muscle insulin resistance and elevated malonyl CoA \((84-86)\). Malonyl CoA inhibits CPT-1, preventing long-chain fatty acid transport into the mitochondria, and so leads to fatty acid accumulation in the cytosol \((84,85,87)\). The enhanced gluconeogenic potential in the liver secondary to muscle disuse provides a source for a small but chronic oversupply of glucose.

There is no obvious physiological benefit from the accumulation of fat in atrophied muscle. Perhaps none should be expected. The gluconeogenesis-based mechanism is associated with the fed state. In the wild state, disuse atrophy is a consequence of immobility, and an immobile animal cannot forage for food and so would not survive for long. The combination of chronic muscle unloading and adequate access to food is an “unnatural” state.

**SUMMARY**

The reductive modeling of skeletal muscle with disuse is largely independent of the reason for the process. The process involves more than a transition from slow to fast myosin fiber types. There are associated metabolic changes in the atrophied muscles such as a fuel shift toward glycolysis, decreased capacity for fat oxidation, and energy substrate accumulation. These metabolic changes are important because they affect performance and may affect the recovery process.

**LITERATURE CITED**