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ORIGINAL ARTICLE

Effect of Simulated Microgravity on the Activity of Regulatory Enzymes of Glycolysis and Gluconeogenesis in Mice Liver

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Abstract Gravity supports all the life activities present on earth. Microgravity environments have effect on the biological functions and physiological status of an individual. The present study was undertaken to investigate the effect of simulated microgravity on important regulatory enzymes of carbohydrate metabolism in liver using HLS mice model. Following hind limb unloading of mice for 11 days the animal's average body weights were found to be not different, while the liver weights were decreased and found to be significantly different ($p < 0.005$) from control mice. Further, in liver the specific activity of hexokinase enzyme was reduced ($p < 0.02$) and the phosphoenolpyruvate carboxykinase activity was significantly increased in simulated microgravity subjected mice compared to control ($p < 0.003$). Immunoblot analysis show decreased phosphofructokinase-2 activity in HLS mice compared to control. Liver lactate dehydrogenase activity significantly reduced in simulated microgravity subjected mice ($p < 0.005$). Thus in our study the rodents have adapted to simulated microgravity conditions, with decreased glycolysis and increased gluconeogenesis in liver and reciprocally regulated.

Keywords Simulated microgravity · Hexokinase · PEPCK · LDH · PFK-2

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Introduction

Gravity plays an important role in adaptation of organism and supports life on earth. Microgravity is the phenomenon of weightlessness, the astronauts experience when the spacecraft moves away from the Earth's gravity and orbiting in the space. Microgravity will also be felt by the rider on a roller coaster in an amusement park (Dai et al. 2009). Microgravity environments have a total drastic effect on biological functions and physiological status that are detrimental to the health of an individual. Astronauts exposed to microgravity resulted in reduction in blood plasma volume, hemoglobin content and impairment of cardiovascular system. They also undergo bone loss and demineralization (Bikle and Halloran 1999; Dai et al. 2009). Microgravity also induces inflammatory responses, modulate immune functions and have profound effect on metabolism of bio-molecules. Animals used in space travel or exposed to simulated microgravity also show similar physiological changes observed with humans.

However, the studies on the effect of microgravity on biological functions in animals and humans in space travel are hampered because of limited opportunities, short duration and high costs (Moody and Golden 2000). Therefore extensive biological studies are required to establish safety guidelines for space travel. Many animal and cell culture models have been developed to simulate microgravity on earth. The most popular ground based rodent model for studying microgravity is Morey-Holton hind limb suspension

model (HLS model). In HLS model rodents are suspended by the tail in such a way to give a 30° head down position that compliments the human 6° head down tilt utilized in bed rest studies (Greenleaf et al. 1996; Morey-Holton and Globus 2002), which mimics the microgravity encountered during space flight. Using HLS model experiments can be scheduled on earth with low cost and manipulations can be done at different time intervals. Further, the experiments can be repeated and extended on routine basis and tissues can be collected from anesthetized animals at any time of experimentation (Morey-Holton and Globus 2002). Although several studies have been carried out on the effect of microgravity on immune responses and other biological processes, very few studies have been addressed about the effect of microgravity on carbohydrate metabolism.

Liver is an important organ developed in mammals including humans to perform the metabolism of carbohydrates, lipids, proteins and other bio-molecules. It is used in the maintenance of homeostasis of glucose in blood by glycolysis and gluconeogenesis. Glycolysis is the universal pathway used by all the organisms to extract energy from glucose. The first irreversible regulatory step in glycolysis is the conversion of glucose to glucose-6-phosphate (G6P) catalyzed by the enzyme hexokinase. Hexokinase has low Km (high affinity for glucose), permits the initiation of glycolysis even when blood glucose levels are low (Saier 1986). It is inhibited by its product G6P preventing the continuous consumption of cellular ATP. Second important reaction is phosphorylation of fructose 6-phosphate (F6P) catalyzed by phosphofructokinase-1 (PFK-1). It is the irreversible rate-limiting step in glycolysis and acts as an important control point (Kasper and Xun 1996). PFK-1 is an allosteric enzyme has the binding sites for AMP, ADP, citrate and Fructose 1, 6 bisphosphate (F-1,6-BP). When cellular energy is limited, glycolysis is upregulated and PFK-1 is allosterically activated by increased levels of AMP (Saier 1986). PFK-1 is inhibited by ATP, when cellular ATP concentrations are high and hence glycolysis is slowed down (Kasper and Xun 1996). Another allosteric activator of PFK-1 is fructose 2, 6-bisphosphate (F-2, 6-BP) and is not an intermediate of glycolytic pathway (Saier 1986). F-2, 6-BP is made from F6P by a specific kinase, phosphofructokinase-2 (PFK-2). Conversion of F6P to F-2, 6-BP is also stimulated by high levels of F6P, an example of feed forward stimulation (the opposite of feedback inhibition), which ensures that intermediates on metabolic pathways do not accumulate uselessly (Hue and Rider 1987). The third important regulatory enzyme of glycolysis is pyruvate kinase. It catalyzes the transfer of phosphate group from phosphoenol pyruvate (PEP) to ADP to form ATP and pyruvate. The enzyme pyruvate kinase is allosterically inhibited by ATP, Acetyl CoA and long chain fatty acids.

Gluconeogenesis is regularly occurring in liver to maintain the glucose level in blood to meet the demands of various tissues (Ophardt 2003). Gluconeogenesis permits the living organisms to grow at the expense of carbon as energy source other than carbohydrates and capable of synthesizing glucose from simple starting materials (Saier 1986). All the steps except the three regulating reactions in gluconeogenesis are identical to glycolysis and hence occur in reverse direction (Ophardt 2003). First regulated reaction in gluconeogenesis is ATP driven carboxylation of pyruvate to oxaloacetate catalyzed by pyruvate carboxylase. Later in the presence of GTP oxaloacetate is converted to PEP catalyzed by phosphoenolpyruvate carboxykinase (PEPCK). Other two regulated reactions are the conversion of F-1, 6-BP to F6P and G6P to glucose catalyzed by Fructose 1, 6 biphosphatase and glucose-6 phosphatase respectively. Both glycolysis and gluconeogenesis proceed largely in cytosol and control occurs in reciprocal ways. At high energy charge gluconeogenesis is stimulated and conversely at low energy charge activates the controlling steps of glycolysis, otherwise both the pathways become futile (Granner and Pilkis 1990).

Rodents subjected to simulated microgravity induce oxidative stress and lipid peroxidation causing damage to brain (Wise et al. 2005) liver (Stein et al. 2005), testis (Ding et al. 2011) and muscle loss (Fitts et al. 2000). Liver plays a major role in carbohydrate metabolism and the microgravity environment may have a profound effect, probably altering the regulatory enzyme activities of glycolysis and gluconeogenesis. Hence the present study was undertaken to investigate the effect of simulated microgravity on important regulatory enzymes hexokinase, PFK-2, PEPCK and LDH of carbohydrate metabolism in liver using HLS mice model. By studying the effect of microgravity on metabolic functions of liver in rodents, the scientists can understand and extrapolate the role played by the microgravity conditions on liver in humans. Study also help the scientists to develop counter measures, which in turn help to maintain normal biological and physiological functions of the astronauts during space journey.

Materials and Methods

Chemicals

Sodium monohydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride were of analytical grade and obtained from Fishers Scientific (Suwanee, GA). Potassium fluoride, 2-Mercapto ethanol, β-Glycerol phosphate disodium salt, Fructose-6-phosphate kinase, Phosphoenolpyruvate, 6-Phosphogluconic dehydrogenase, Glucose-6-phospate dehydrogenase (G6PDH), Malate

Dehydrogenase (MDH), 6-phospogluconate (6-PGA), 2'-Deoxyguanosine-5'diphospate sodium, B-Nicotinamide Adenine Dinucleotide H (NADH), Aldolase, Triphosphate Isomerase, Glyceraldehyde-3-phosphate dehydrogenase, D-(+)- Glucose, Dithiothreitol (DTT), Isoflurane and Adenosine 5' triphophate dipotassium salt dihydrate (ATP) were obtained from Sigma Chemical Company (St. Louis, MO). PFK-2 affinity purified goat polyclonal antibody (E-16, sc-10096) was obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA).

Animal Maintenance and Hind Limb Suspension of Mice

Six to eight week BALB/c male mice (15–20 g body weight) were obtained from Harlan Sprague Dawley (Indianpolis IN). The animals were housed in individual cages in a temperature controlled room at 22 °C and provided with standard food pellet and water available *ad libitum*. Animals were maintained on a 12 h light and 12 h dark cycle. Three days after arrival, the animals were randomly assigned as control mice group ($n = 6$) and experimental tail suspended mice group ($n = 6$). Unloading tail suspension of mice was carried out as previously described (Morey-Holton and Globus 2002). In brief the tail of the animals were cleaned and dried. A thin layer of adhesive tape was applied approximately to the middle of the tail along the medial to lateral sides and harness made of standard porous tape was pressed firmly to the glue. A paper clip was used to attach the animals tail harness to a swivel apparatus on the wire spanning the top of a cage. The height of the animal's hind limb was adjusted to prevent any contact with the cage bottom, which gives a tilt of 30 ° head downwards. The fore limb of mice maintained contact with the cage bottom allowing the animal full access to the cage. Weight of each animal was measured on a daily basis until the completion of the experiment. The standard animal protocols for maintenance of mice and HLS protocols used were approved by the Institute Animal Welfare Committee of Texas Southern University (Houston, TX).

Animal Euthanization and Liver Organ Collection

Following HLS of mice for 11 days, on the 12th day both control and HLS animals were euthanized with *isoflurane*. The liver was collected from all the animals and weights were measured. Liver samples were stored at −80 °C until further used in enzyme assays.

Liver Homogenization and Enzyme Assays

Liver samples (50 mg) were suspended in an appropriate buffer as mentioned in enzyme assay protocols, homogenized, centrifuged for 10 min at 10,000 × g at 4 °C and the supernatant was assayed for hexokinase, PEPCK and LDH the important regulatory enzymes of carbohydrate metabolism. The protein concentrations in homogenized samples were determined by Bradford's method (Bradford 1976).

Hexokinase Assay

Hexokinase catalyzes the transfer of phosphate group from ATP to glucose to form G6P. G6P is oxidized to 6-phospho gluconate in the presence of added enzyme G6PDH and $NADP^+$. Reduced NADPH formed is measured by increased absorption at 340 nm using spectrophotometer (Scheer et al. 1978). Both control and simulated microgravity mice liver tissue (50 mg) homogenates were prepared in 9 volume of ice-cold 150 mM potassium chloride, 5 mM $MgCl_2$, 5 mM EDTA, 5 mM 2-mercaptoethanol in a homogenizer for 1 min. Homogenate was centrifuged at 14,500 × g for 20 min at 4 °C and supernatant was used as the enzyme source. Tubes containing reaction mixture in a total volume of 2 ml was prepared with buffer (48 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.8 mM $NADP^+$, 5 mM 2-mercaptoethanol, 5 mM ATP, 0.5 mM glucose, 0.27 mM 6-PGA), and 2.5 units G6PDH were mixed at 30 °C. Tubes containing reaction components prepared and equilibrated as above excluding ATP and 6-PGA served as blank. Background reaction was carried out excluding ATP, glucose, and G6PDH. Reaction was started by the addition of 50 μl of liver supernatant to the above tubes, mixed and the absorption was measured at 1 min time intervals at 340 nm for 20 min at 30 °C. Using one of the control liver samples the hexokinase assay was standardized for the amount of enzyme to be used, time required, and the substrate glucose concentration required for the maximum activity. The activity of the enzyme hexokinase in all the liver samples were incubated for 20 min with protein concentration of 2 μg, and the substrate glucose used was 0.2 μ moles/L.

Phosphoenolpyruvate Carboxykinase (PEPCK) Assay

PEPCK displays a high degree of specificity for the substrate PEP in the direction of the formation of oxaloacetate. PEP in the presence of carbonate converted into oxaloacetate by PEPCK, which later reduced by added NADH + H^+ and enzyme MDH. PEPCK enzyme was assayed by measuring decrease in the absorption at 340 nm (Ash et al. 1990). Liver tissue homogenate was prepared in 9 volume of ice-cold 0.25 M sucrose, 10 mM tris HCl, pH 7.4 for 1.5 min and centrifuged at 14,500 × g for 10 min at 4 °C. The supernatant was used for PEPCK enzyme assay. The eppendorf tubes containing reaction buffer total volume of 500 μl was prepared containing 50 mM Tris-HCL, pH 7.4, 1.0 mM $MnCl_2$, 0.1 mM NADH, 2 units MDH 0.5 mM

PEP and 0.2 mM deoxygyuanosine 5′-disphosphate. The liver homogenate (10 μl) was added, mixed, the background reaction was monitored at 340 nm for 5 min at 37 °C. Reaction was started by adding 20 mM sodium bicarbonate (saturated with CO_2) to the sample, mixed and then monitored at 1 min time intervals for 5 min. Using control liver samples the PEPCK assay was standardized for the amount of enzyme to be used, time required, and the PEP concentration required for the maximum activity. PEPCK Activity in all the liver samples were measured by incubating the reaction for 30 min with protein concentration of 3 μg, and the substrate (PEP) used was 500 n moles.

Lactate Deyhydrogenase (LDH) Assay

LDH reduces the pyruvate added externally to lactate in the presence of NADH + H^+. Oxidized NAD^+ product formed was assayed by measuring decreased absorbance at 340 nm as described earlier (Holbrook et al. 1975). In brief, the Liver samples were homogenized in 20 volume of lysis buffer (Tris HCl 50 mM, pH 8, 10 % glycerol, 1 mM DTT, protease inhibitors) for 30 s at 4 °C. Homogenates were centrifuged at 10,000 × g for 10 min. Three samples containing reaction buffer (50 m mol/L Tris-HCL, pH 7.4, 1.0 m mol/L $MnCl_2$ and 0.1 m mol/L NADH) were taken in three different tubes. Liver homogenate was added to samples containing pyruvate (25 mM), mixed and the background reaction was monitored at 340 nm for 5 min at 37 °C. Reaction was started by adding liver homogenate to sample only, mixed and then monitored at 1 min intervals for 10–20 min. Using one of the control liver samples the LDH assay was standardized for the amount of enzyme to be used, time required, and the pyruvate concentration required for the maximum activity. LDH activity in all the liver samples were measured by incubating for 10 min with protein concentration of 7.5 μg, and the substrate pyruvate used was 25 μ moles/L.

Phosphofructokinase-2 (PFK-2)

The immunoblot analysis of PFK-2 enzyme protein was carried out as described earlier (Sharma and Richards 2000). Equal quantity of protein from control and hind limb suspended mice liver homogenate is separated on 10 % SDS-PAGE, transferred to Nylon membrane and blocked with 5 % milk. The membrane is probed with the PFK-2 antibody and bands were analyzed by chemiluminescence kit obtained from Amersham. Band intensity of control and microgravity subjected animals was measured by Gene Tools image analyzer.

Statistical Analysis

Body and liver weights and enzyme units were expressed as mean ± SD and statistical significance was assessed by student's *t*-test. Difference between control and simulated microgravity samples were considered significant if the level $p < 0.05$.

Results

Effect of Simulated Microgravity on Body Weight and Liver Weight

Following hind limb unloading of mice for 11 days the average body weights were obtained on a daily basis. The average body weights of animals (control and HLS mice) on the day of sacrifice were presented in Table 1. The results show that the body weights of HLS mice have all most same body weights as that of control animals and were significantly not different. However, the average liver weight of HLS mice decreased by more than 20 %, and was found to be significantly different ($p < 0.05$) from control mice (Table 1). The results suggested that the simulated microgravity has profound influence on liver which is an important organ that maintains homeostasis of glucose and other biomolecules.

Hexokinase

Hexokinase activity of both control and HLS mice of liver samples were presented in Fig. 1. The results show that the specific activity of hexokinase enzyme was significantly reduced by 49 % in HLS mice compared to control ($p < 0.02$). Being the first irreversible step of glycolysis catalyzed by hexokinase, the enzyme was inhibited due to simulated microgravity stress condition which prevents the continuous production of ATP.

Table 1 Effect of simulated microgravity on the body and liver weight of mice

Sl. No.	Body/Organ	Weight in gms ± SE (n = 6)	
		Control	HLS mice
1	Whole body weight	22.5 ± 5	22.7 ± 5
2	Liver	1.15 ± 0.05	0.93 ± 0.07*

*Significantly different from control $P < 0.005$

Average body weight and liver weight expressed as grams of HLS mice ($n = 6$) and control mice ($n = 6$). Table 1 is a representative from three experiments performed independently. The results of liver weight of HLS mice were found to be significantly different from control mice $P < 0.005$

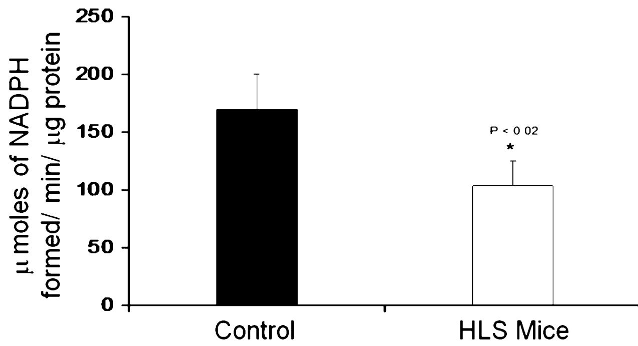

Fig. 1 Effect of simulated microgravity on specific activity of hexokinase. Both control ($n = 6$) and HLS liver ($n = 6$) tissue (50 mg) homogenates were prepared and used as the enzyme source. The results of HLS mice were found to be different from control mice ($p < 0.02$). Figure is a representative from three experiments performed independently

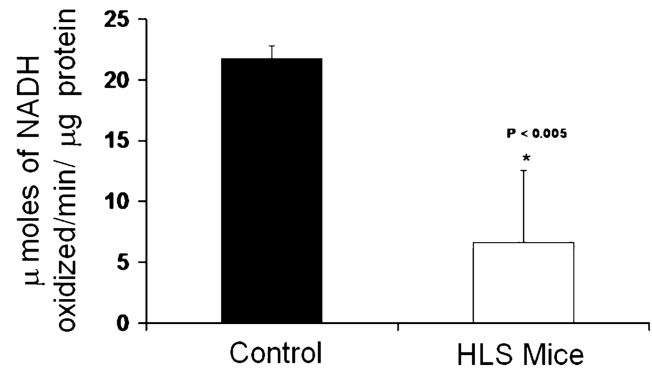

Fig. 3 Effect of simulated microgravity on specific activity of LDH. Both control ($n = 6$) and HLS liver ($n = 6$) tissue (50 mg) homogenates were prepared and used as the enzyme source. The results of HLS mice were found to be significantly decreased and different from control mice ($p < 0.005$). Figure is a representative from three experiments performed independently

Phosphoenolpyruvate Carboxykinase (PEPCK)

PEPCK activity of both control and HLS mice liver samples were presented in Fig. 2. The results show that PEPCK enzyme activity in liver was significantly increased by more than 5 fold in HLS mice compared to control ($p < 0.003$). To meet the simulated microgravity stress condition, the first regulated reaction of gluconeogenesis the ATP driven carboxylation of pyruvate to oxaloacetate, which later converted to PEP catalyzed by PEPCK was increased.

Lactate Dehydrogenase Activity (LDH)

LDH enzyme activity of both control and HLS mice liver samples were presented in Fig. 3. The results show that the LDH activity in liver was significantly reduced by 64 % in HLS mice compared to control ($p < 0.005$). Probably LDH activity was reduced so that more pyruvate was available for the synthesis of glucose via gluconeogenesis.

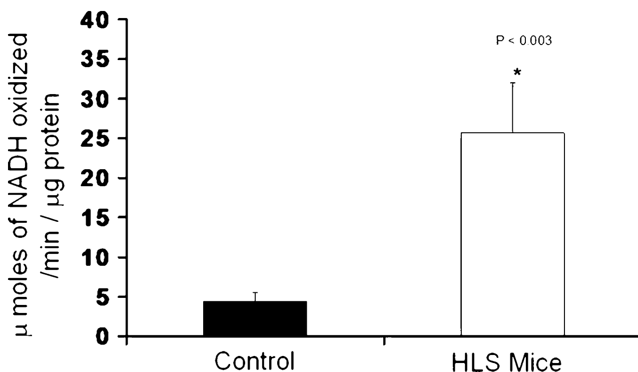

Fig. 2 Effect of simulated microgravity on specific activity of PEPCK. Both control ($n = 6$) and HLS liver ($n = 6$) tissue (50 mg) homogenates were prepared and used as the enzyme source. The results of HLS mice were found to be significantly increased and different from control mice ($p < 0.003$). Figure is a representative from three experiments performed independently

Phosphofructokinase-2 (PFK-2)

Immunoblot analysis of PFK-2 in liver samples of both control and HLS mice were presented in Fig. 4. The results show that there was 20 % decrease in the PFK-2 enzyme activity in HLS mice liver samples (lanes 3 and 4) compared to control (lanes 1 and 2). Allosteric activator of PFK-1 the F-2, 6-BP was also reduced as the PFK-2 levels decreased that confirm the reduced glycolysis in HLS mice liver.

Discussion

It is a well known phenomenon that HLS of mice induce oxidative stress with the impairment of physiology and biological functions. Stress lead to multiple signals with varied responses in different organs and subjects (Stein 2002).

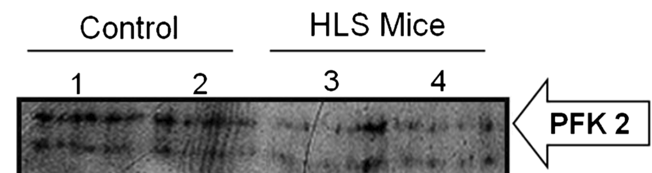

Fig. 4 Effect of simulated microgravity on Liver Phosphofructokinase-2 (PFK-2). Immunoblot analysis of protein from control and HLS mice liver homogenate. The membrane is probed with the PFK-2 antibodies obtained from Santacruz Biotechnology incorporation and bands were analyzed by chemi-luminescence kit. Lanes 1 and 2 control. Lanes 3 and 4 HLS mice liver samples

Mice subjected to HLS, induce oxidative stress, lipid peroxidation, activate NFκB damaging many regions of brain (Wise et al. 2005), and also induce apoptosis in testis (Sharma et al. 2008). In the present study the liver weight of HLS mice significantly decreased compared to control. HLS in mice probably induced oxidative stress, lipid peroxidation with changes in morphology causing damage to liver. Studies using other HLS rodent models also confirmed the impaired physiological functions and damage to many organs. In one such study, the microtubules in cells found to be gravity sensitive (Young 2006) and cytoplasm-to-myonucleus ratio was affected. Rats flown for short periods aboard in the space shuttle Discover (NASA, STS-48) show changes in skeletal muscle fibers, with significant decrease in cytoplasmic volume-to-myonucleus ratio (Kasper and Xun 1996). However, in our studies average body weight of simulated microgravity subjected mice (Hind limb suspension for 11 days) was found to be not different from control. In a previous study using rat model the simulated microgravity subjected animals lost body weights significantly compared to control (Stein et al. 2005). This difference may be due to different rodent model system used and more days of HLS treatment (21 days).

In liver and other tissues phosphorylation of glucose by hexokinase is the first regulatory step of glycolysis. The hexokinase enzyme with low Km, initiates the glycolysis even at low glucose levels (Saier 1986). To maintain homeostasis the hexokinase, together with glucose kinase helps in the effective removal of glucose even when present at high levels (Kasper and Xun 1996). Hexokinase activity of HLS mice in liver significantly decreased suggesting the reduced glycolysis. The second important reaction is phosphorylation of F6P to F-1, 6-BP catalyzed by PFK-1 is also rate limiting and irreversible step in glycolysis (Kasper and Xun 1996). High energy state of a cell [ATP] inhibits, while low energy state [AMP and ADP] upregulates PFK-1 activity. F-2, 6-BP also binds to PFK-1 and overcome the inhibiting effect of ATP (Saier 1986). F-2, 6-BP is also an important regulator of gluconeogenesis, where in low level activates and high level inhibits the process (Pilkis et al. 1988). F-2, 6-BP is made from F6P by specific PFK-2. Our immunoblot study further show the decreased PFK-2 enzyme levels, probably down regulates PFK-1 activity and reduced glycolysis. In liver three enzymes hexokinase, PFK-1, and pyruvatekinase regulates the glycolytic pathway. The levels of these enzymes are also regulated by hormones insulin and glucagon at the level of transcription. In our study we have not addressed the effect of simulated microgravity on insulin and glucagon levels in pancreas. However, such detailed analysis of carbohydrate metabolism under simulated microgravity condition needs to be investigated.

Our study show that the liver PEPCK activity significantly increased in HLS mice compared to control. In glycolysis pyruvate formed in cytosol is transported to mitochondria and is converted to oxaloacetate by pyruvate carboxylase. Oxaloacetate is reduced to malate by mitochondrial MDH and transported back to cytosol. In cytosol malate is converted back to oxaloacetate by cytosolic MDH. Oxaloacetate is converted to PEP catalyzed by PEPCK. PEPCK is the rate limiting reaction of gluconeogenesis. As PEPCK activity in HLS mice was increased significantly suggested the increased gluconeogenesis. This observation agrees well with the study carried out by Stein et al. (2005) using HLS rat on liver metabolism, where there was increased expression of pyruvate carboxylase, PEPCK and glucose 6-phosphatase of gluconeogenetic enzymes. Further, LDH activity was significantly reduced in HLS mice compared to control. This decreased activity of LDH is probably due to adoptive response of the animal to cope up with the situation of increased requirement of glucose. HLS mice probably shut down the conversion of pyruvate to lactate, and largely the pyruvate may be utilized for the synthesis of glucose via gluconeogenesis.

Glycolysis and Gluconeogenesis are reciprocally regulated. In animal tissues, AMP and F-2, 6-BP acts as allosteric activators of phosphofructoskinase, and allosteric inhibitors of fructose 1, 6-bisphosphatase (Saier 1986). Glycolysis is favored, at low concentration of ATP, while ATP when present in excess, synthesis of glucose is favored by activating gluconeogenesis. Energy state of the cell therefore regulates and directs the liver to follow either glycolysis or gluconeogenesis and avoids the futile cycle (Saier 1986).

The data presented hereby show the specific enzyme activity obtained from liver of HLS mice and suggested the adaptive response to microgravity with alteration in enzyme levels. Important finding is that glycolysis (degradation of energy fuel) was inhibited while gluconeogenesis (synthesis of energy fuel glucose) was up regulated in the liver of HLS mice. The shift towards increased activity of glycolytic enzymes in atrophied muscle of HLS rat is complemented by a shift towards increasing glucose availability in liver. There appears to be a coupling between increased glucose production in liver and increased use by atrophied muscle. It is currently believed that glycolytic fuel shift in atrophied muscle is an intrinsic consequence of change in fiber type with atrophy (Fitts et al. 2001). Our results also provide evidence, that the increased glucose availability is because of more gluconeogenesis and probably this is because of more precursors (amino acids) are available due to decreased needs of disused muscle. The previous study showed that the up regulation of glycolytic genes in disused soleus muscle preceded most of the myosin fiber type changes (Stevenson et al. 2003). Lactate levels and LDH activity are increased in atrophied muscle (Stevenson et al. 2003 and Fitts et al. 2001). Probably lactate formed in muscle enters liver and increased concentration of lactate

inhibits the activity of LDH in liver. In our study, simulated microgravity also shows decreased activity LDH in liver. Thus our result confirm that the HLS mice adapted to simulated microgravity conditions with decreased activity of regulatory enzymes of glycolysis and increased activity of regulatory enzymes of gluconeogenesis. The glycolysis and gluconeogenesis pathways were reciprocally regulated in liver.

Conclusion

Our studies suggested that, when mice were subjected to simulated microgravity conditions in liver, the glycolysis was inhibited, while gluconeogenesis was up regulated. However, the shift towards increased activity of the glycolytic enzymes in atrophied muscle is complemented by a shift towards the increasing glucose availability. Our results also provide support, that the increased glucose availability is because of more gluconeogenesis in liver and probably this is because of more precursor amino acids available due to decreased needs of disused muscle. Lactate levels and LDH activity increased in atrophied muscle. Probably this lactate enters liver and increased concentration of lactate inhibits the activity of LDH in liver. Thus, the animals were adapted to simulated microgravity conditions in liver, with decreased activity of glycolytic enzymes and increased activity of gluconeogenetic enzymes and were reciprocally regulated.

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