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EFFECTS OF ACUTE ETHIONINE-INDUCED HEPATIC ATP DEFICIENCY AT REST AND DURING EXERCISE IN FEMALE RATS

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Abstract. *The effects of an injection (ip) of ethionine (1.0 mg/g) with and without methionine (1.0 mg/g) were studied at rest and following a 30-min bout of exercise (26 m/min, 0% grade) in female Sprague-Dawley rats. Ethionine, compared to a NaCl injection, resulted in a significant ($P < 0.05$) decrease in resting levels of liver ATP, liver glycogen, and plasma glucose and in a significant increase in liver Pi/ATP ratio and plasma FFA, glycerol, glucagon, and norepinephrine concentrations. All of these responses were restored by the addition of methionine. None of these responses were amplified by the combination of ethionine and exercise stimuli. These results indicate a predominance of ethionine over the exercise stimulus and suggest that intra hepatic events related to a decreased ATP level in the liver exert a powerful regulatory influence on the metabolism.*

Key words: *liver ATP, glucagon, liver glycogen, hepatic receptors, liver inorganic phosphates*

INTRODUCTION

Ethionine, the ethyl analogue of methionine, has been used for many years as a tool to perturb hepatic metabolism. Ethionine, itself being an amino acid, is handled in much the same way as many other amino acids. It can be incorporated into protein (Wilson et al., 1981), deaminated with subsequent metabolism of the carbon skeleton (Steele and Benevenga, 1979), or it can be activated at the sulfur position and used as an ethyl donor (Pegg, 1972). In this latter respect it utilizes the same enzyme responsible for the conversion of methionine into S-adenosylmethionine.

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S-adenosylethionine is formed relatively rapidly but the failure of the cell to utilize this product to any significant degree traps adenosine and results in a very rapid depletion of hepatic ATP (Villa-Trevino et al., 1963). This in turn sets in motion a series of events which include the inhibition of protein synthesis, disruption of glucose metabolism, and the accumulation of hepatic triglycerides (Yoshizawa et al., 2002; Lyon and Kisilevsky, 1986).

In recent years, it has been reported that the fructose analogue, 2, 5-anhydro-Dmannitol elicits eating in rats (Tordoff et al., 1991). This effect has been associated with a decrease in liver ATP produced by the trapping of inorganic phosphate (Rawson et al., 1994a). The feeding response induced by the administration of 2, 5-AM was eliminated by hepatic branch vagotomy suggesting that this effect was mediated through an afferent pathway (Tordoff et al., 1991). Similarly, an ethionine-induced decrease in liver ATP has been associated with the start of feeding in male rats (Rawson et al., 1994b). Work from our laboratory has also provided evidence that the liver, through its afferent pathway, may influence the hormonal response to exercise (Cardin et al, 1991; Lavoie et al., 1989). Either the sectioning of the hepatic vagus nerve or an intra-portal infusion of pyruvate has been shown to result in an attenuation of the pancreatic hormone responses during exercise in rats (Cardin et al, 1991; Lavoie et al., 1989). There is strong evidence that the liver, through its afferent innervation, participates in the process of metabolic regulation during exercise, but the metabolic stimuli at the origin of this information remains obscure. Although different metabolic stimuli, related to glucose, lipid, and protein metabolism have been investigated (Lavoie and Cardin, 1996), the changes in the rate of energy production (ATP) in the liver during exercise have received only a limited amount of attention. In a recent study from our laboratory (Desy et al., 1999), exercise has been shown to amplify some of the metabolic and hormonal effects of the fructose analogue 2, 5-anhydro-Dmannitol, known to reduce liver ATP levels. Although these effects could not solely be attributed to the decrease in blood glucose levels during exercise, the inhibition of glycogenolysis and gluconeogenesis also resulting from the administration of 2, 5-anhydro- D-mannitol (Hanson et al., 1984), limits the use of this tool to study changes in hepatic ATP levels in an exercise situation. Administration of ethionine has been reported to result in a decrease in liver ATP levels up to 20% of that in control rats (Oshita, 2000). In contrast to 2, 5-anhydro-D-mannitol, however, liver glycogenolysis is not inhibited by the administration of ethionine (Lyon and Kisilevsky, 1986) and the acute perturbation of blood glucose homeostasis is slight.

The aim of the present study was, therefore, to gain more insight into the possible contribution of liver ATP levels to the metabolic and hormonal responses to exercise. It is hypothesized that exercise will accentuate the metabolic and hormonal effects of ethionine. This was done by using ethionine as a tool to create a perturbation in hepatic metabolism and to evaluate if this hepatic perturbation and its effects are accentuated by a 30-min exercise period. The ability of methionine to reverse the effects of ethionine was also studied in response to exercise.

MATERIALS AND METHODS

Animal care and surgery. Female Sprague-Dawley rats (Charles River Canada, St-Constant, Quebec), weighing 180-200g, were housed in individual cages and allowed

food and water ad libitum for 25 days after they were brought to our laboratory. The effects of ethionine have been shown to be more pronounced in female than in male rats (Farber et al., 1964). The lighting schedule was such that the lights were on from 07:00 A.M. until 19:00 P.M., and the room temperature was maintained between 20-23°C. During this time, rats were progressively accustomed to run on a motor-driven rodent treadmill, beginning with 15 min/day at 15 m/min and increased to 50 min/day at 28 m/min (0% grade) so that they were well accustomed to running and being handled. The habituation training program consisted of a total of eight sessions, over a 10-day period. Three days before the experiment, the rats underwent a jugular vein cannulation under sodium pentobarbital (40 mg/kg i.p.) anesthesia. The jugular catheter was implanted by a method previously described (Lavoie et al., 1989). This catheter was used for rapid anesthesia (i.v.) of rats at the end of acute exercise or resting conditions.

Groups and exercise protocol. After the last training session, rats were randomly divided into three groups injected with different substances: saline (NaCl), DL-ethionine (ETH), and ETH+DL-methionine (ETH+MET). Each group was further divided into two sub-groups sacrificed at rest or after exercise for a total of 6 groups. The exercise test consisted of running on the treadmill at 26 m/min (0% grade) for 30 min. The present mild exercise protocol was chosen to allow the possibility that additive effects of ethionine and exercise on substrate utilization could have reduced the capacity of the rats to sustain exercise. On the day of the experiment, food was removed from cages at least 3 h before the beginning of the exercise tests. Rats, weighing 240-260 g were injected (i.p.) with either DL-ethionine (1.0 mg/g body weight), DL-ethionine and DL-methionine (1.0 mg/g body weight) or an equivalent volume of saline (0.9% NaCl). The dose of ETH was chosen because it has been used successfully when injected intraperitoneally in several previous studies (Lyon et Kisilevsky, 1986; Wong et Kisilevsky, 1984). ETH and METH were obtained from Sigma-Aldrich (Winston Park, Oakville, Canada). DL-ethionine and DL-methionine were first dissolved in physiological saline (50 mg/mL) of a low pH (5.5-5.6) and the pH was thereafter adjusted to 7.0-7.4 with NaOH (1N) solution. The substances were injected two times with a two-hour interval in between. The second injection was followed by another 90-min rest period. This was done to allow the ethionine to have its effects before the beginning of the exercise period. These periods were then followed by a 30-min exercise protocol or another 30-min resting period for the rats sacrificed at rest. Immediately after completing the exercise (while they were still running) or at rest (in their cage), the rats were rapidly anesthetized with a mixture of KetamineTM (80 mg/kg) and RompunTM (10 mg/kg; xylazine) (0.02-0.05 ml; i.v.). Following complete anesthesia, the abdominal cavity was opened, and a small piece of liver (400-500 mg) from the median lobe was immediately freeze-clamped using liquid nitrogen pre-cooled aluminum tongs, then excised and immersed in liquid nitrogen (< 8 s). Thereafter, approximately 4 ml of blood was collected via the abdominal vena cava. Non-exercised rats were treated in the same manner as the exercise rats and were sacrificed at approximately the same time.

Analytical methods. Blood was collected into 5 ml syringes containing 7% EDTA (used as an anti-coagulant) and immediately separated into three fractions. The first aliquot of blood (1.5 ml) was centrifuged, and the supernatant was retained on crushed ice (4^{o}C) for glucose, lactate, insulin, free fatty acids (FFA), glycerol, and â-hydroxybutyrate analyses. The second fraction of blood (500 µl) was preserved in 50 µl of TrasylolTM, centrifuged, and the supernatant was used for glucagon determination.

The remaining part of blood (1.5 ml), used for catecholamines determinations, was transferred in tubes containing 50 ul of glutathione (60 mg/ml) and ethylene glycol-bis (â-aminoethyl ether)-N,N',N'-tetraacetic acid (90 mg/ml), kept in crushed ice and centrifuged at 4°C for 10 min within 30 min after collection. All tissue and plasma samples were stored at -79°C until analyses were performed. Plasma glucose concentrations were determined with the use of a glucose analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Insulin and glucagon levels were determined by commercially available Radioimmunoassay System Laboratory Kits (ICN Biomedicals, Costa Mesa, CA; distributed by Immunocorp, Montreal, Quebec). Glycerol, FFA and ß-hydroxybutyrate were assessed enzymatically with the use of reagent kits from Boehringer Mannheim (Distributed by Immunocorp). Catecholamines were extracted from the plasma according to the procedure described by Remie and Zaagsma (1986) and determined by means of an isocratic high-performance liquid chromatography system (HPLC; Water Division Chromatography). Liver glycogen content was determined by use of the phenol-sulfuric acid reaction (Lo and al., 1970). Adenosine triphosphate (ATP) was determined according to Lamprecht and Trautschold's method (Lamprecht and Trautschold, 1974). Enzymatic determination of ATP using freeze-clamped liver has been reported to be similar in magnitude to that observed using in vivo 31P nuclear magnetic resonance spectroscopy (Cunningham et al., 1986; Rawson and Friedman, 1994). All liver samples were rapidly weighed, then ground into a fine powder within a porcelain mortar previously frozen in liquid nitrogen and kept cold with repeated addition of liquid nitrogen. All samples were then extracted (deproteinized) with 1 w/3.5 vol of perchloric acid (0.58 N). The frozen liver powder and frozen acid were rapidly ground together and the frozen mixture transferred to a glass homogenizer while still dry and frozen. The frozen mixture was slowly allowed to thaw (30 min), while resting on crushed ice, until it was just becoming fluid and then homogenized for 10 s, while cooling on ice, and centrifuged for 10 min at 4,000 g (4°C). The supernatant was obtained and neutralized by the addition of a solution of 5M K2CO3 (1 vol/0.85 vol) (or /0.085 vol). The methyl orange was used as an indicator of pH (1 vol/ 0.0025 vol). The neutralized solution was allowed to stand for 10 min on crushed ice and centrifuged for 10 min at 4,000 g (4°C). The KClO4 precipitate was discarded and the supernatant used for ATP measurements. For liver inorganic phosphate (Pi) measurements, the liver was treated similarly as for ATP determinations, as described by Williamson et al. (1967). Inorganic phosphate was assayed by the method described by Gawehn (1974).

Statistical analyses. All the data is reported as means ± SE. Statistical analyses were performed by a two-way analysis of variance with a non-repeated measure design. Tukey's post hoc test was used in the event of a significant ($P < 0.05$) F-ratio.

RESULTS

Liver ATP levels were significantly ($P < 0.01$) decreased by ~45%, compared to saline-injected rats, following the administration of ethionine in the resting state (Fig. 1A). This decrease was ~60% in ethionine- compared to saline-injected rats following the 30-min exercise period. There were no significant ($P > 0.05$) effects of exercise on liver ATP levels, although a tendency to be decreased following exercise was observed in ETH and ETH+METH groups. The ethionine-induced decrease in liver ATP at rest and after exer-

cise was corrected by the methionine injection. Administration of ethionine did not result in a significant ($P > 0.05$) change in liver inorganic phosphate whether at rest or after exercise (Fig. 1B). There was, however, a significant ($P < 0.05$) overall increase in Pi after exercise (overall exercise effect). The ratio of Pi/ATP concentrations in the liver was significantly ($P < 0.01$) increased following the ethionine injection in rested as well as in exercised rats (Fig. 1C). As for ATP levels, there were no significant ($P > 0.05$) effects of exercise on Pi/ATP ratio, although a tendency for this ratio to increase following exercise was observed in ETH and ETH+METH groups.

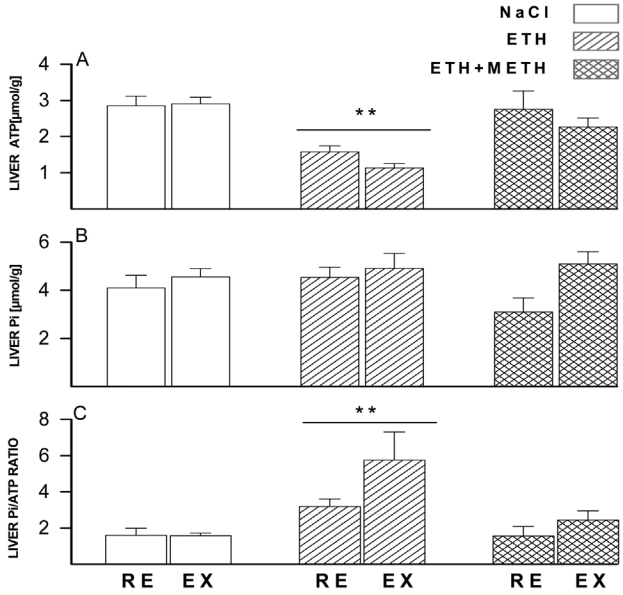

Fig. 1. Liver ATP and inorganic phosphate (Pi) concentrations and Pi/ATP ratio at rest and after exercise in saline- (NaCl), ethionine- (ETH), and ethionine+methionine- (ETH+METH) injected rats. n = 7-8, 11-12, and 9 rats in NaCl, ETH, and ETH+METH, respectively.
** $P < 0.01$ vs corresponding NaCl- and ETH+METH injected groups.

The administration of ethionine resulted in a significant ($P < 0.01$) decrease in liver glycogen and plasma glucose concentrations in rested as well as in exercised rats (Fig. 2A, B). Liver glycogen values in the ETH+METH group were also significantly ($P < 0.01$) lower than those measured in the NaCl-injected animals. There were no effects of exercise on liver glycogen and plasma glucose concentrations in any of the injected groups. Plasma lactate concentrations were significantly ($P < 0.05$) higher in ETH-injected rats only when compared to the resting values of ETH+METH-injected rats (Fig. 2C). There were no significant ($P > 0.05$) effects of exercise on plasma lactate values in any of the injected groups. The ethionine injection caused plasma FFA and glycerol to increase in both rested and exercised rats compared to the NaCl- and ETH+METH-injected rats (Fig. 3A, B). An overall effect of exercise at $P < 0.08$ was

found for FFA concentrations. β-hydroxybutyrate concentrations were also increased ($P < 0.05$) following the administration of ethionine, compared to the other injection conditions, but only in the rested state (Fig. 3C). Plasma insulin concentrations were not changed significantly ($P > 0.05$) either by the injection or by the exercise stimuli (Fig. 4A). Ethionine, as compared to NaCl and ETH+METH injections, resulted in a significant ($P < 0.01$) increase in plasma glucagon levels in both rested and exercised conditions (Fig. 4B). The same observation can also be made for the norepinephrine response but at a significant level of $P < 0.06$ (Fig. 5A). A significant ($P < 0.05$) increase in norepinephrine response following exercise was found irrespective of the injected groups. Although a tendency for plasma epinephrine concentrations to be higher following an ETH injection can be observed, there were no statistical effects of an injection or exercise on epinephrine response in all the injected groups (Fig. 5B).

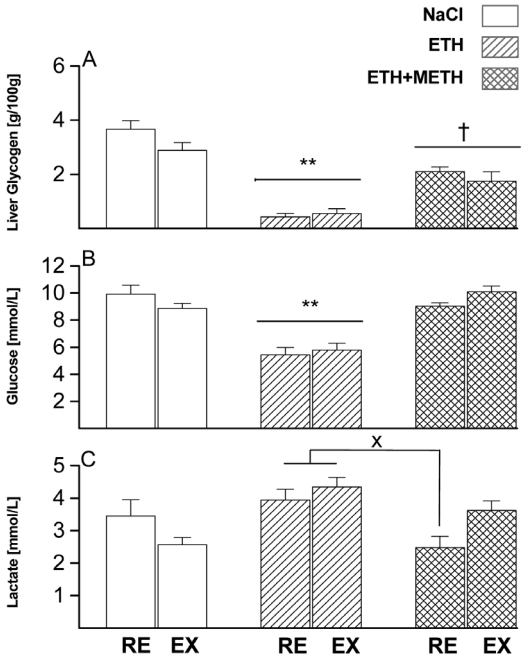

Fig. 2. Liver glycogen, plasma glucose and lactate concentrations at rest and after exercise in saline- (NaCl), ethionine- (ETH), and ethionine+methionine- (ETH+METH) injected rats. n = 7-8, 11-12, and 9 rats in NaCl, ETH, and ETH+METH, respectively.
** $P < 0.01$ vs corresponding NaCl- and ETH+METH injected groups.
† $P < 0.01$ vs corresponding saline-injected groups. X $P < 0.05$ vs resting values in ETH + METH-injected rats.

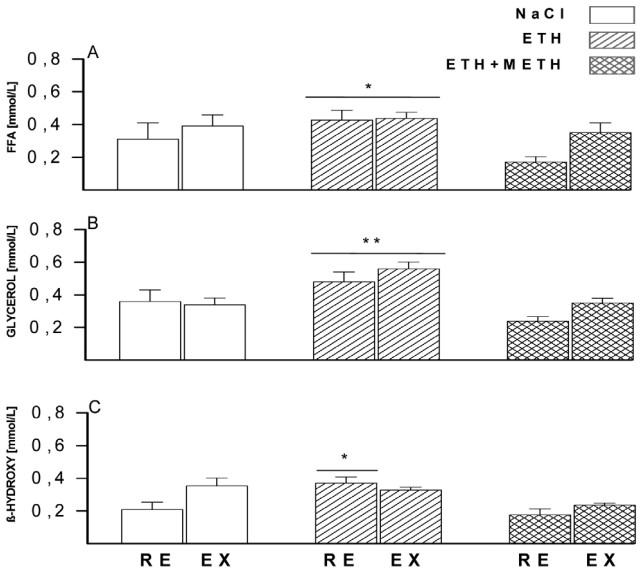

Fig. 3. Plasma free fatty acids (FFA), glycerol, and B-hydroxybutyrate concentrations at rest and after exercise in saline- (NaCl), ethionine- (ETH), and ethionine+methionine- (ETH+METH) injected rats. n = 7-8, 11-12, and 9 rats in NaCl, ETH, and ETH+METH, respectively.
** P < 0.01 vs corresponding NaCl- and ETH+METH-injected groups, * P < 0.05.

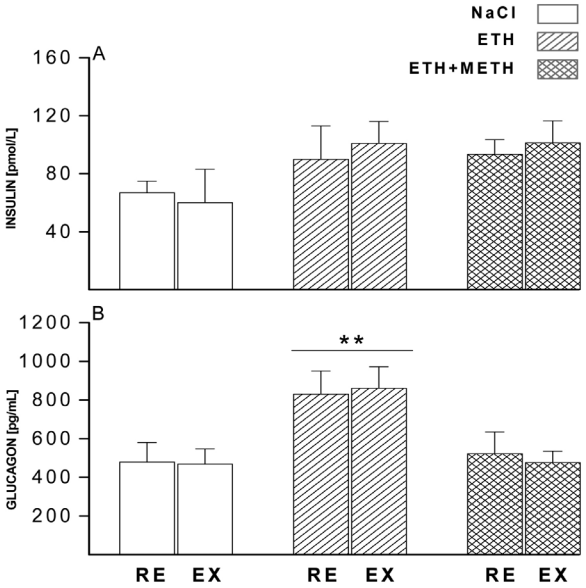

Fig. 4. Plasma insulin and glucagon concentrations at rest and after exercise in saline- (NaCl), ethionine- (ETH), and ethionine+methionine- (ETH+METH) injected rats. n = 6-8, 11-12, and 7-9 rats in NaCl, ETH, and ETH+METH, respectively.
** P < 0.01 vs corresponding NaCl- and ETH+METH-injected groups.

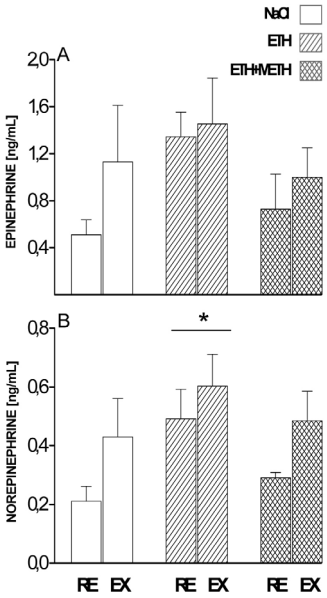

Fig. 5. Plasma norepinephrine and epinephrine concentrations at rest and after exercise in saline- (NaCl), ethionine- (ETH), and ethionine+methionine- (ETH+METH) injected rats. n = 8, 8-11, and 8 rats in NaCl, ETH, and ETH+METH, respectively. * $P < 0.06$ vs corresponding NaCl- and ETH+METH-injected groups.

DISCUSSION

In accordance with the previously reported observations using 31P NMR spectroscopy (Jeffreys et al., 1987), the administration of ethionine in the resting state resulted in an approximate 40% decrease in liver ATP concentration ~3-4 h after treatment. The decrease in liver ATP was associated with a marked decrease in liver glycogen content, a reduction in blood glucose concentration, an increase in peripheral lipid mobilization (FFA and glycerol), and an increase in plasma glucagon and norepinephrine plasma levels. The specificity of the ethionine action was shown by the observation that almost all of these responses were completely reversed by the subsequent administration of methionine. It has also been reported that liver weights are not affected by the ethionine treatment indicating that toxicity cannot be a major factor in the explanation of the ethionine effects (Tani and Ogata, 1970). The main finding of the present experiment, however, is the observation that none of the ethionine-induced metabolic and hormonal responses were amplified by the exercise stimulus. As in the resting situation, the methionine injection corrected all of the ethionine effects seen during exercise. The metabolic and hormonal responses following the administration of ethionine at rest are similar to those normally observed during a prolonged bout of exercise. Given that the exercise considerably increased energy demand, one would have expected an increased effect of ethionine administration during exercise on the metabolic and hormonal responses. The absence of at least an additive effect of ethionine and exercise indicates a predominance of the ethionine action, most likely through the reduced liver ATP levels.

It could be argued that the lack of additive effects of ethionine and exercise may be due to the rather moderate intensity and duration of the present exercise bout. The exercise resulted in significant increases in plasma norepinephrine and liver Pi levels irrespective of the injected groups. These results along with a tendency of the FFA to increase and the liver glycogen levels to decrease do indicate that exercise caused some metabolic and hormonal adaptations. Even if the exercise was mild, it is surprising to observe that the plasma glucose levels were not reduced any further in the exercising than in the resting rats injected with ethionine. This is surprising even more if one considers that liver glycogen levels were already considerably reduced by the sole injection of ethionine. That blood glucose and liver glycogen levels were not decreased any further by the combination of ethionine and exercise could be explained by a compensatory use of other substrates, such as free fatty acids and muscle glycogen. On the other hand, in a situation such as fasting, where the peripheral availability of glucose is reduced, a 30-min exercise period would have resulted in a larger decrease in blood glucose levels. That exercise did not reduce blood glucose levels any further in ethionine-treated rats strongly suggests that the ethionine effects are not related to blood glucose levels.

The observation that ethionine administration at rest results in a decrease in blood glucose levels despite the breakdown of large amounts of liver glycogen raises some questions as to what happens to liver glycogen content during ethionine administration. There is some evidence that gluconeogenesis may be suppressed in ethionine-treated rats (Tani and Ogata, 1970; Tani et al., 1973) which should increase the relative contribution of hepatic glycogenolysis. The present increase in plasma lactate in ethionine-treated rats is also in accordance with the previous finding that ethionine increases hepatic lactate (Steele and Benevenga, 1979; Tani et al., 1973). This suggests that hepatic glycolysis may be increased in ethionine-treated rats. Glucose-6-phosphate dehydrogenase activity has also been reported to increase in ethionine-treated mice (Sie and Hablanian, 1965), suggesting an increase in the pentose phosphate pathway. It is worth mentioning that the effects of ethionine on glucose-6-phosphate dehydrogenase activity and the glycogen content (profound decrease) were not alleviated by the administration of glucose (Sie and Hablanian, 1965). The reduction in blood glucose levels in ethionine-treated rats suggests a decrease in hepatic glucose production. If that is so, it did not affect the decrease in blood glucose any further during the present exercise than at rest.

In addition to a reduction in liver ATP levels, ethionine has been reported to paradoxically increase hepatic cAMP, activate glycogen phosphorylase, and, inactivate glycogen synthetase (DeRubertis and Craven, 1976; Wilson et al., 1981). The increase in hepatic cAMP levels are likely the result of hormonal actions, such as glucagon and catecholamines and imply that normal ATP availability is unlikely to be a rate-limiting factor in the expression of these hormonal actions (DeRubertis and Craven, 1976). Since the metabolic effects of ethionine could not be demonstrated in vitro (DeRubertis and Craven, 1976), it is very likely that the decrease in liver glycogen in ethionine-treated rats is mediated, at least in part, by the endogenous hyperglucagonemia. The next question then is what stimulates the glucagon response in ethionine-treated rats. The possibility that the decrease in plasma glucose is the sole factor that stimulates glucagon secretion in ethionine treated rats is unlikely for the following reasons: 1) it is uncertain that the present modest hypoglycemia could result in the relatively large increase in peripheral glucagon; 2) glucagon secretion has been reported to be incompletely suppressed by glucose infusion in ethionine-treated rats (DeRubertis and Craven, 1976); 3) in rats fed

ethionine for long periods of time (38 weeks), hyperglucagonemia persists while mild hypoglycemia was progressively corrected (Craven and DeRubertis, 1977). A direct effect of the drug on pancreatic glucagon secretion is possible (Craven and DeRubertis, 1977). On the other hand, hyperglucagonemia has been reported in a variety of situations related to a reduction in liver ATP levels such as hepatic cirrhosis (Kabadi et al., 1984), hepatic regeneration following partial hepatectomy (Lavoie et al., 1998; Morley et al., 1975) and the injection of 2, 5-anhydro-D-mannitol (Desy et al., 1999). It is thus possible that a decrease in liver ATP may be afferently related, through for instance the hepatic vagus nerve, to an increased secretion of pancreatic glucagon.

As previously reported (Jeffreys Smith et al., 1987; Lavoinne et al., 1983), hepatic inorganic phosphate levels rose concomitantly with the ATP fall. Accordingly, the Pi/ATP ratio, which is an indicator of the cell energy potential (Malloy et al., 1986), was increased in ethionine-treated rats. These results therefore, indicate a reduction of the cell phosphorylation potential in this condition. A strong tendency of a higher Pi/ATP ratio after exercise in ethionine-injected rats was observed (Fig. 1C). This suggests that exercise somewhat added to the effects of ethionine on this liver parameter. However, as stated above, exercise did not add to the metabolic and hormonal changes induced by the ethionine injection. This data indicates that if indeed the reduction of liver ATP levels is responsible for the metabolic effects of ethionine, then this stimulus is strong enough to override an added exercise stimulus.

In accordance with previous reports (Lyon and Kisilevsky, 1986; Tani et al., 1973), lipid mobilization, as seen from plasma FFA and glycerol concentrations, was increased in ethionine-treated animals. On the other hand, the modest change in β-hydroxybutyrate concentrations following ethionine administration is compatible with the report that fatty acid oxidation in liver is suppressed in animals treated with ethionine (Tani and Ogata, 1970). The FFA and glycerol increases at rest and during exercise in ethionine-injected rats might reflect the increase in norepinephrine levels. The increase in plasma FFA levels with ethionine administration is thought to be responsible for the increased liver triglycerides (Tani et al., 1973). As for the glucagon response, it is interesting to speculate on what causes the increase in FFA in ethionine-treated rats. Although the decrease in blood glucose in ethionine-treated rats has to be related to the fatty acid release (Tani et al., 1973), the mild hypoglycemia generally reported suggests that there might be other factors involved. In search of an explanation for the increase in FFA levels, it is interesting to draw a parallel between the ethionine injection approach and the partial hepatectomy approach (Lavoie et al., 1998; Morley et al., 1975). Both of these approaches lead to a typical increase in lipid mobilization, whether at rest or during exercise, which seems to be independent of a reduction in the blood glucose level (Lavoie et al., 1998). Both of them also lead to a typical decrease in liver ATP and energy charge level. It is therefore tempting to speculate that the increase in plasma FFA and glycerol levels following ethionine injection may be triggered by the decrease in liver ATP.

In summary, data from the present experiment indicates that an ethionine injection results in a decrease in resting and exercising levels of liver ATP, liver glycogen, and plasma glucose and in an increase in liver Pi/ATP ratio and plasma FFA, glycerol, glucagon, and norepinephrine concentrations. Exercise did not amplify the metabolic and hormonal effects of ethionine. It is concluded that the ethionine-induced decrease in the liver ATP level is a stimulus that can override the metabolic stimulus of a 30-min exercise period of mild intensity.

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POSLEDICE AKUTNOG ETIONINOM IZAZVANOG HEPATINOG STANJA KOD ŽENKI PACOVA PRILIKOM MIROVANJA I VEŽBANJA

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Ova studija se bavi ispitivanjem efekata ubrizgavanja (ip) etionina (1.0 mg/g) sa i bez metionina (1.0 mg/g) ženkama Sprague-Dawley pacova u stanju mirovanja i nakon 30-minutnog vezbanja (26m/min, 0% nivo). Etionin, u poredjenju sa ubrizgavanjem NaCl ima za rezultat značajno smanjenje jetrinog ATP (P<0.05), jetrinog glikogena glukoze plazme u stanju mirovanja, i značajno povećanje odnosa jetrinog Pi/ATP I plazme FFA, glikerola, glukagona, i koncentracija norepinefrina. Sve ove analize su ponovo vraćene na stari nivo dodavanjem metionina. Nijedna od ovih analiza nije bila uvećana kada je etionin bio kombinovan sa varijablom- vežbanje. Ovi rezultati ukazuju na predominantnost etionina nad stimulativnim vežbanjem i sugerišu da intra-hepatična stanja povezana sa umanjenjem nivoa ATP-a u jetri imaju snažan regulacioni uticaj na metabolizam.

Ključne reči: *jetreni ATP, glukagon, jetreni glukogen, hepatični receptori, jetreni neorganski fosfati.*