
ARTÍCULO ORIGINAL

Changes in peripheral blood mononuclear cells glutamine synthetase mRNA after exercise in healthy volunteers: exploring an alternative proposal for non hepatic ammonia metabolism

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ABSTRACT

Background. Glutamine synthetase (GS) plays a central role in the inter-organ metabolism of ammonia and hepatic encephalopathy. The main objective of the present work was to disclose the possible effect of exercise on GS mRNA expression in peripheral blood mononuclear cells (PBMC) within a group of healthy volunteers. **Material and methods.** PBMC were studied instead of skeletal muscle because of ethical concerns. Characterization of GS in lymphocytes was carried out by indirect immunofluorescence and Western blot. After a pilot trial, expression of GS mRNA in PBMC was assayed by serial measurements in healthy volunteers who had exercised on a treadmill, and on a control group who had not. Muscle mass was estimated by bioimpedance. **Results.** Cytoplasmic GS had a molecular weight of 44 kDa. Serial measurements of its mRNA demonstrated an increase in the treadmill ($n = 29$), but not in the control group ($n = 13$) ($p < 0.05$). Peak expression occurred at 1 h in males and at 6 h in females. There was a positive correlation between muscle mass and the increase of the enzyme mRNA after exercise. **Conclusion.** Exercise can increase the expression of GS mRNA in PBMC in healthy volunteers. Based on these preliminary results and on well-established physiological concepts, a hypothesis for non-hepatic ammonia metabolism is conceived. In the future could become part of the treatment of low-grade hepatic encephalopathy.

Cambios en el ARNm de células mononucleares periféricas por el efecto de glutamina sintetasa: exploración de una propuesta alternativa para el metabolismo no hepático del amonio

RESUMEN

Antecedentes. La glutamina sintetasa (GS) desempeña un papel central en el metabolismo del amonio y en la encefalopatía hepática en diferentes órganos. El objetivo principal de este estudio fue explorar el posible efecto del ejercicio en la expresión del ARNm de la GS en células mononucleares de sangre periférica (CMSP) en un grupo de voluntarios sanos. **Material y métodos.** Se estudiaron CMSP en lugar de músculo esquelético debido a consideraciones éticas. La caracterización de GS en los linfocitos se realizó mediante inmunofluorescencia indirecta y Western blot. Después de un estudio piloto la expresión del ARNm de GS en CMSP se efectuó mediante mediciones seriadas en voluntarios sanos luego de realizar una prueba de esfuerzo en banda y en un grupo control no sometido a ejercicio. La masa muscular se estimó mediante bioimpedancia. **Resultados.** Se encontró que la GS se distribuyó en el citoplasma con un peso molecular de 44kDa. Las mediciones seriadas de ARNm demostraron un aumento en el grupo sometido a ejercicio ($n = 29$), pero no en el grupo control ($n = 13$) ($p < 0.05$). La expresión pico ocurrió en 1 h en hombres y en 6 h en mujeres. Hubo una correlación positiva entre la masa muscular y el incremento de ARNm de la enzima dada

Key words. Glutamine synthetase. Ammonia metabolism. Skeletal muscle. Exercise. Hepatic encephalopathy.

INTRODUCTION

Hepatic encephalopathy (HE) and its minimal form are cumbersome complications of cirrhosis affecting up to 80% of patients and carry a dismal prognosis, with substantial costs to healthcare systems.¹⁻³ Astrocyte swelling is the common endpoint of multiple heterogeneous factors that can precipitate HE, and ammonia plays a central role on its pathogenesis.^{1,4} This occurs as failed hepatic ammonia detoxification exposes the brain to abnormally high concentrations of this metabolite, causing an increase in astrocyte oxidative stress, mitochondrial dysfunction, energy deficit, and ultimately leading to cell edema and disordered neurotransmission.⁵

The decreased ammonia detoxification that is associated with liver failure derives from a disrupted urea synthesis in periportal hepatocytes, and diminished glutamine synthesis in perivenous hepatocytes.⁶ Glutamine synthetase (GS) is the enzyme responsible for glutamine production through an ATP-dependent reaction, using ammonia and glutamate as substrates.⁷ In patients with cirrhosis it has been shown that GS catalytic activity is reduced by about 80%, paralleling a diminished urea synthesis.⁸ Additionally, experimental studies have shown that GS activity in the brain also decreases during states of hyperammonemia, whereas it is simultaneously increased in muscle.⁹

Several studies enabling a more profound characterization of ammonia metabolism have led to the development of useful compounds for the treatment of HE,¹⁰ which act by enhancing the residual urea cycle and by increasing muscle GS activity.^{11,12} Thus, it is conceivable that other tissues of the inter-organ ammonia pathway could take over hepatic ammonia metabolism in patients with liver failure, thereby mitigating HE. However, non-pharmacologic maneuvers for inducing GS have remained uninvestigated. One promising non-pharmacologic intervention is suggested by exercising models, which have shown indirect evi-

por el ejercicio. **Conclusiones.** El ejercicio puede incrementar la expresión de ARNm de GS en CMSP en voluntarios sanos. Con base en estos resultados preliminares y en conceptos fisiológicos bien establecidos se concibe una hipótesis para el metabolismo no hepático del amonio, misma que sentaría los fundamentos para el tratamiento de la encefalopatía hepática de bajo grado.

Palabras clave. Glutamina sintetasa. Metabolismo del amonio. Músculo esquelético. Ejercicio. Encefalopatía hepática.

dence for GS induction (decrease in ammonia and glutamate plasma levels), both in non-cirrhotic¹³ or cirrhotic animals.¹⁴ Although muscle GS activity is not as critical to muscle as it is to other tissues such as liver, testis, or the brain, muscle makes up 55% of total body weight and constitutes the principal source of glutamine synthesis.¹⁵⁻¹⁷ Muscle, then, should be targeted for study as a potential substitute site for ammonia metabolism in patients with liver failure, examining exercise as a plausible mechanism for inducing GS activity.^{18,19} Demonstration of the latter is a complicated project that requires a multi-step approach combining experimental and clinical research.

As a first step, we wanted to test if exercise could increase GS mRNA levels in healthy volunteers. Because of difficulties and ethical concerns with performing serial muscle biopsies, peripheral blood mononuclear cells (PBMC) were studied instead of skeletal muscle cells, since it has been shown that enzymatic changes in PBMC are paralleled by changes in skeletal muscle during exercise training.²⁰ Specifically, the present study tested whether GS is expressed in PBMC, and whether a bout of exercise can increase its mRNA levels in these cells, all in healthy volunteers.

MATERIAL AND METHODS

Study population

Healthy workers from a tertiary referral hospital were invited to participate in the study. A brief clinical history was performed and informed consent was obtained. Those lacking any contraindication to serial venopuncture or exercising and willing to participate were included. All volunteers manifested performing regular exercise to some degree or having a physically active lifestyle, and denied any history of cardiopulmonary symptoms. The study was approved by the local Institutional Board for Human Research and designed according to international guidelines.

Identification and characterization of GS enzyme in human lymphocytes

Blood from healthy donors was drawn early in the morning (before any considerable physical activity) and processed to obtain purified lymphocytes. After the blood was passed through a Ficoll gradient, only the ring of lymphocytes was retained. The purified lymphocytes were fixed with 4% paraformaldehyde, treated with 1% Triton, and blocked with a solution of 1% serum fetal bovine/1% bovine serum albumin. At this stage lymphocytes were incubated with an anti-GS antibody (1:500, Sigma), followed by incubation with an anti-rabbit IgG antibody conjugated to FITC (1:50, Sigma F1262). The signal of FITC was recorded in a Leica confocal microscopy (TCS SP5 Leica Microsystems, Wetzlar, Germany).

Western blot was performed to characterize the molecular weight of the lymphocyte GS enzyme. A sample of purified lymphocytes was solubilized in lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 1% Triton X-100 and complete protease inhibitors, Roche 04693116001), mixed with loading buffer containing 2-mercapto-ethanol as reducing agent, and then it was resolved by electrophoresis in a 10% SDS-polyacrylamide gel. The transfer of the proteins was done onto a nitrocellulose sheet, which was blocked with a solution of 5% free fat milk/0.2% Tween. Next, the membrane was incubated with the anti-glutamine synthetase antibody (1:500, Sigma), followed by incubation with an anti-rabbit IgG antibody conjugated to Alexa Fluor 488 (1:300, Invitrogen A31628). The fluorescent signal was captured in a Typhoon equipment (GE Healthcare). A brain extract from rat (Clontech, Mountain View, USA) was used as a GS positive control.

Pilot experiment for GS mRNA changes in response to exercise

Samples were taken from four volunteers early in the morning and at one-hour intervals over the course of 3 h on day one, to assess the expression of GS mRNA resulting from normal daily activities. On day two, the same individuals were submitted to two sequential exercise routines of 45 min duration, with a programmed rest period of approximately 60 min in between. Exercise consisted on jogging. Volunteers jogged for several minutes until feeling fatigued and their heart rate was documented at the end of each exercise routine. The aim was to achieve an intensity of 60% of the maximal cardiac frequen-

cy (estimated as 220 beats per minute-age in years). On day two the basal sample was taken just before the first exercise session, the third sample before the second routine of exercise; and another sample was drawn 24 h after the basal sample. All these samples were processed on the same day to isolate total RNA with the Trizol reagent, and frozen at -70 °C for further analysis.

Increase in GS mRNA expression induced by exercise

Based on the results of the previous experiments a standardized protocol was designed to further demonstrate an increase in GS mRNA in 29 healthy volunteers (treadmill group).

- **Treadmill test.** An appointment for a multistage treadmill stress test, according to standard Bruce protocol, was scheduled for each volunteer at the Heart Failure Clinic from the participating hospital. Subjects were instructed to arrive at 8:00 AM after an overnight fast. A twelve-lead electrocardiogram continuously monitored heart activity during the test and blood pressure was measured at baseline, at the third minute of each stage, at peak exercise, and each minute during recovery. All subjects were encouraged to exercise to their maximum capacity or to achieve 85% of their maximal heart rate. After the test was finished subjects were advised to perform routine labor and daily activities.
- **Blood samples.** A basal blood sample was drawn upon arrival on the day of the treadmill test (8:00 AM). After the treadmill protocol was completed blood samples were taken at 15, 60, and 120 min, 6 and 24 h. These samples were processed on the same day to isolate total RNA with the Trizol reagent, and frozen at -70 °C for further analysis.
- **Anthropometry and body composition.** On the day before the treadmill test was performed weight and height were obtained for calculation of the body mass index (BMI), in accordance with the manual reference of anthropometric standardization;²¹ and whole-body bioelectrical impedance was measured using a tetrapolar and multiple-frequencies equipment BodyStat QuadScan 4000 (BODYSTAT LTD; Isle of Man, British Isles). All measurements were made by the same investigator following the tetrapolar method reported in the existing literature.²² Fat free mass and fat mass were calculated.²³

Controlling for a possible circadian expression of GS mRNA in healthy volunteers

In order to control for a plausible circadian variation on GS mRNA expression within PBMC (which could be falsely interpreted as induction in the treadmill group) a group of 13 healthy volunteers was invited to participate to serve as a control group. Blood samples at the same time intervals used for the treadmill group were obtained, but without any exercising intervention. Performance of routine labor and daily activities was also advised. All samples were processed on the same day to isolate total RNA with the Trizol reagent, and frozen at -70 °C for further analysis.

GS mRNA expression on PBMC by real time RT-PCR

RNA was extracted from the buffy coat fraction, with Trizol reagent (Invitrogen, California, USA) according to the manufacturer's instructions. To avoid DNA contamination, RNA was treated with DNase (Roche, Pentzberg, Germany) and purified on a second step with Trizol. The quality, integrity, and concentration of RNA were determined on an agarose gel stained with ethidium bromide (0.5 µg/mL) and by 260 nm absorbance. Total RNA (500 ng) was reverse transcribed into cDNA with M-MLV Reverse Transcriptase (Promega, Wisconsin, USA) using random hexamers, following the manufacturer's recommended protocol. Real time RT-PCR was performed in a LightCycler 2.0 (Roche, Rotkreuz, Switzerland) using LNA TaqMan® probes from the Universal Probe Library Human Set (Roche, Pentzberg, Germany) in combination with specific intron spanning primers, as previously described.²⁴ Primers used to assess GS gene expression were as follows:

- *tctcgccgttagcttac* (forward), and
- *cattcggtcgttcgtg* (reverse) in combination with Universal Probe #28.

As reference, the housekeeping genes for hypoxanthine phosphoribosyl transferase (primers *tgaccttgattttgcatacc* [forward] and *cgagaagacgttcagtct* [reverse] with Universal Probe #73) and for beta-2-microglobulin (*ttctggcctggaggctac* [forward] and *tcaggaaatttgactttccatc* [reverse] with Universal Probe #42) were assayed and validated for linearity and reproducibility. Reactions were performed using 1 mL of cDNA in the LightCycler Taq-

Man Master (Roche, Pentzberg, Germany) according to the manufacturer protocol. PCR conditions were as follows: preincubation PCR step for 10 min at 95 °C; and 45 cycles for 10 sec at 95 °C, 30 sec at 60 °C, and 1 sec at 72 °C. Relative quantification of gene expression was calculated with the LightCycler Software 4.0 using the basal sample as calibrator.

Statistical analysis

Summary statistics on GS mRNA induction are shown as median ± interquartile ranges of fold changes in relation to basal expression (standardized to one). However, for a more robust analysis of serial measurements, areas under the curve (AUC) for GS mRNA were constructed according to Matthews, *et al.*²⁵ The sample taken at 24 h was not considered in the AUC given that seven subjects did not attend this follow-up visit, and inclusion of this set of measurements significantly skewed the data. The Friedman test was used to determine any difference in expression of GS across all serial blood measurements within each group of subjects (treadmill and control subjects); while Student's t-test was used for comparing the AUC between groups of subjects (treadmill *vs.* control, and males *vs.* females), after normality had been tested by means of the Kolmogorov-Smirnov test. To assess the influence of age and anthropometry on GS mRNA expression, a bivariate analysis with Spearman's rho correlation was performed. The α level adopted for significance was a two-tailed P value ≤ 0.05. All analyses were performed using the SPSS version 13.0 (SPSS Inc., Chicago, USA).

RESULTS

Identification of GS enzyme in human lymphocytes

Results on the localization and characterization of GS in lymphocytes are shown in figure 1. As in other cell types, GS was immunodetected exclusively in the cytoplasm with a strong and homogeneously distributed fluorescent signal. By means of Western blot analysis GS was identified as a band of 44 kDa, exhibiting a molecular weight similar to the GS from the brain (data not shown).

Pilot experiment for GS mRNA changes in response to exercise

Expression of GS mRNA in the 4 volunteers (ages 23 to 42 years-old, all males) subjected to the two-

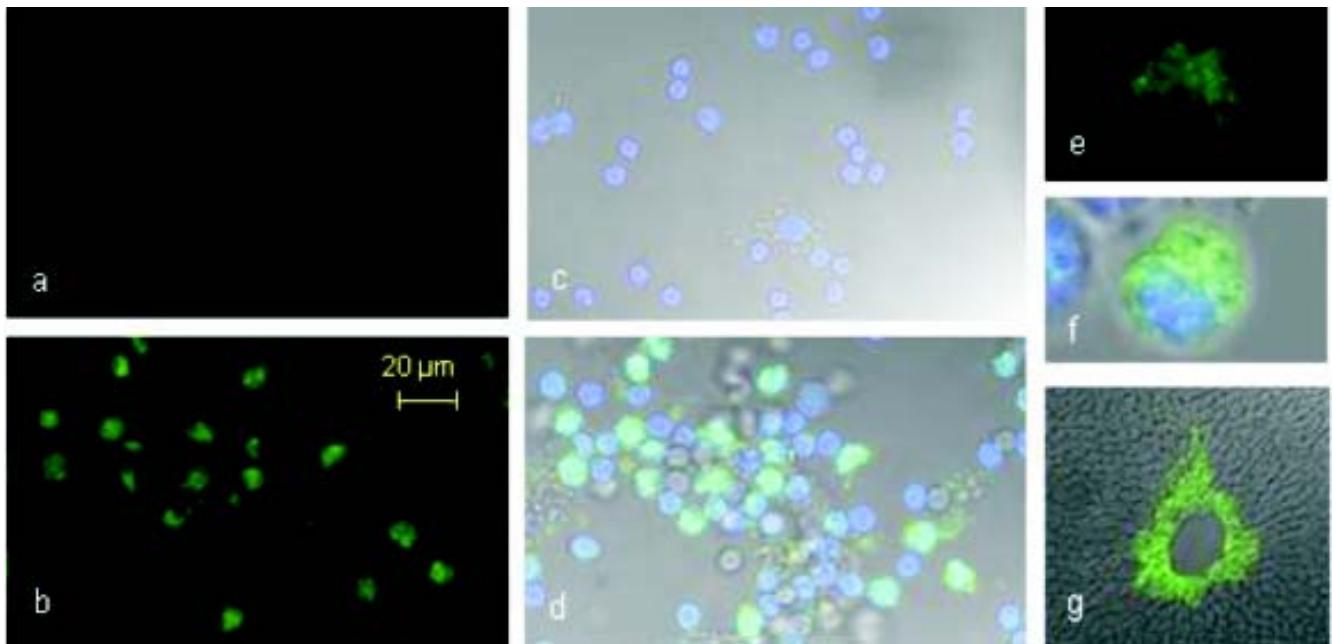


Figure 1. Localization and characterization of the GS in lymphocytes. In purified human lymphocytes the fluorescent signal corresponding to the GS protein was seen only when a specific antibody was used (B, D, E, F). Note that there was no fluorescent emission when the antibody was not added (A, C). The overlapping of the GS, nuclei (DAPI) and light microscopy images is shown in C, D and F. The magnification of the GS image alone (E) or of the overlapping described above (F) clearly shows that GS enzyme was present in the cytoplasm of the lymphocytes. In the mouse liver, GS was localized in the pericentral hepatocytes (G).

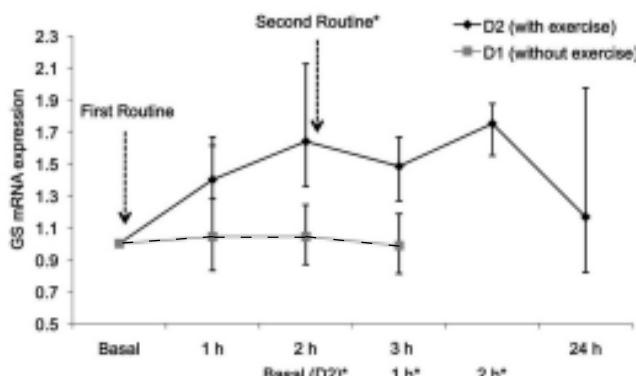


Figure 2. Pilot experiment for assessing GS mRNA changes in PBMC in relation to two sequential exercise routines. GS mRNA expression is shown as median and interquartile range of fold inductions over basal levels. Asterisks on the x-axis (bottom line) make reference to the time frame for the blood samples after the second routine. Of note, there was no 3 h sample for D2, it corresponds to 1 h*. D1: day 1. D2: day 2.

day assay is shown in figure 2. Although there was no statistically significant difference among the measurements ($p = 0.14$), three findings deserve especial mention:

- First, GS mRNA levels on day two were found to be higher after exercise routines (in relation to basal expression of day one).

- Second, the most important increase was noticed 2 h after the first bout of exercise; and
- Third, the second routine of exercising maintained gene expression, although it did not seem to improve it.

Induction of GS mRNA expression after exercise vs. circadian variation

Treadmill group consisted of 15 females (40 ± 4 years-old) and 14 males (38 ± 4 years-old), whereas in the control group there were 6 females (40 ± 6 years-old) and 7 males (34 ± 6 years-old); without differences in regards to sex and age between the two ($p = 0.5$ and $p = 0.35$ respectively). Differences in GS mRNA expression between basal levels and levels in treadmill or control groups are shown in table 1. It can be noted that an increase in GS mRNA was only observed in subjects submitted to the treadmill test (a significant difference among time-scheduled samples); that it seemed to be more pronounced in males; and that the highest levels of messenger occurred between 1 and 2 hours after exercise in males, and at 6 h in females. After construction of the AUC for the 2-h and 6-h intervals, it was clear that GS mRNA expression was higher in subjects submitted to the treadmill test, in compari-

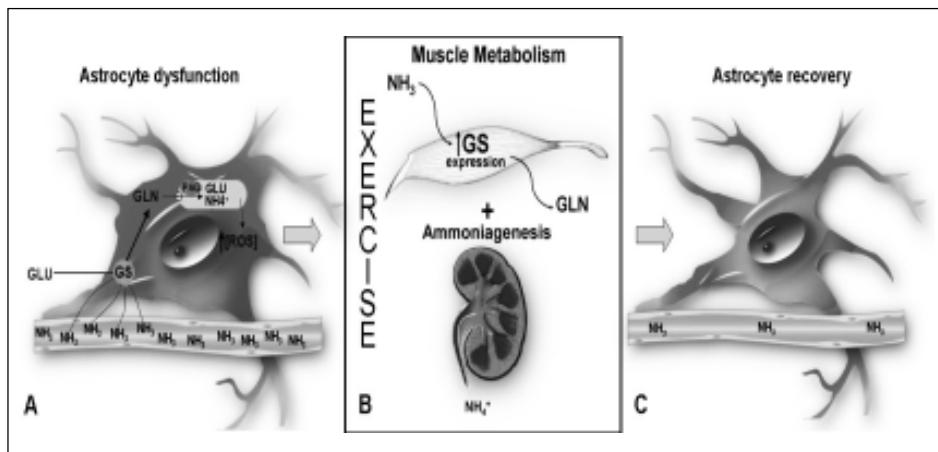


Figure 3. Mechanisms involved in the proposed exercise-induced pathway for ammonia metabolism. **A.** Astrocyte dysfunction and swelling caused by ammonia-derived oxidative stress. **B.** Induction of muscle GS expression by exercise with consequent metabolism of ammonia (NH_3) to glutamine, paralleled by an increased renal excretion of ammonium (NH_4^+) during acidosis. **C.** Improved astrocyte function caused by reduced levels of circulating ammonia. GS: glutamine synthetase. GLU: glutamate. GLN: glutamine. PAG: phosphate-activated glutaminase. ROS: reactive species.

Table 1. GS mRNA expression in PBMC in response to exercise (treadmill) or without any intervention (control).

	n	Basal	15 min	60 min	120 min	6 h*	24 h†	P‡
Treadmill	29	1	1.16 (0.85-1.36)	1.10 (0.95-1.52)	1.30 (0.86-1.50)	1.04 (0.89-1.57)	0.90 (0.64-1.30)	0.004
Males	15	1	1.27 (0.95-1.39)	1.41 (1.05-1.69)	1.35 (1.05-1.51)	0.99 (0.82-1.29)	0.99 (0.78-1.33)	ND
Females	14	1	1.05 (0.84-1.22)	1.04 (0.87-1.43)	1.10 (0.83-1.49)	1.27 (0.96-1.65)	0.83 (0.63-1.23)	ND
Control	13	1	1.07 (0.87-1.19)	1.10 (0.92-1.28)	0.97 (0.89-1.17)	1.04 (0.91-1.25)	1.02 (0.84-1.1)	0.212

GS mRNA expression is shown as median (interquartile range) of fold inductions over basal levels. *At 6 h (after exercise) 3 male subjects were lost in the treadmill group. †At 24 h, 4 additional patients were lost (2 males, and 2 females). ‡Friedman test.

Table 2. GS mRNA expression in PBMC according to AUC at 2 and 6 h.

Groups	n	AUC 2 h	AUC 6 h
Treadmill	29	148 ± 39*	453 ± 145†
Males	15	156 ± 39	436 ± 134
Females	14	141 ± 39	467 ± 158
Control	13	127 ± 18	379 ± 75

Expressed as mean ± SD of all the AUC for each involved subject. Total AUC at 2 h was constructed with 29 patients in the treadmill group, while at 6 h 26 subjects were included (3 males lost to follow-up). AUC: area under the curve. * $p = 0.021$ when compared to control, and † $p = 0.037$ when compared to control (Student's t test).

son to controls (Table 2). Furthermore, when considering only the subjects in the treadmill group, the comparisons between males and females at both time intervals did not disclose any significant difference ($p = 0.32$ and $p = 0.58$, respectively for AUC at 2 and 6 h).

Subjects in the treadmill group had a BMI of $29 \pm 4 \text{ kg/m}^2$, with a fat free mass of $64 \pm 8\%$. The BMI did not differ between genders (data not shown), but there was a higher fat free mass in

Table 3. Correlations between GS mRNA AUC at 2 h, age and body composition in the treadmill group.

Group	Age*	BMI	Fat free mass	Fat mass
Treadmill	-0.368†	-0.193	0.412†	-0.297
Males	-0.620†	-0.095	0.636†	-0.455
Females	-0.151	-0.214	0.242	-0.244

BMI: body mass index. *Age was categorized as follows: < 30, 30-50, and > 50 years-old. AUC: area under the curve. †Statistically significant at a level of $p \leq 0.05$ for Spearman's rho.

males ($69 \pm 5\%$) when compared to females ($59 \pm 6\%$), $p = 0.001$. Correlations between the AUC of GS mRNA at 2 h in the treadmill group, age, and anthropometry are shown in table 3. The same analyses performed with the AUC at 6 hours did not reveal any additional information.

DISCUSSION

The present study contributes to the characterization of GS enzyme in PBMC/lymphocytes of healthy human volunteers, and demonstrates that its

mRNA can be acutely induced by a bout of exercise, confirming our main hypothesis. Some secondary findings are worth mentioning: that this increase reaches a peak after 1-6 h of exercising, that there is no significant difference between males and females, that the increase seems to be of higher magnitude in younger people, and that it is positively correlated with fat free mass (or muscle mass). Also, it could be inferred that performing a routine of exercise twice in one day does not improve mRNA expression any further.

Whereas the existence of GS in PBMC/lymphocytes has been documented, the observed increase in gene expression due to exercise is a novelty. GS is a ubiquitous enzyme and its catalytic product, glutamine, is essential for many metabolic pathways such as amino acid, glutathione and nucleotide biosynthesis.¹⁶ The gene has already been amplified from human lymphocytes in a study of patients with congenital glutamine deficiency.²⁶ The increased levels of GS mRNA in PBMC do not directly speak to the effects of exercise on levels of GS mRNA in muscle tissue. However, it has been shown that expression of mRNA in PBMC has a good correlation with other tissues, and it has been proposed that this measurement can be used as a surrogate of tissues with a difficult access such as liver²⁷ and skeletal muscle.²⁰ Zeibig, *et al.*, could demonstrate in 6 healthy volunteers that mRNA expression of enzymes involved in fat metabolism, were correlated between lymphocytes and skeletal muscle, before and after endurance training. Through this concordant enzyme mRNA stimulation they concluded that PBMC can mimic expression profiles of muscle tissue, and therefore, they can be used to evaluate adaptive changes in muscle due to training.²⁰ PBMC consume glutamine (and glucose) for their energy requirements²⁸ and an increase in GS mRNA in relation to exercise was not expected, especially considering that the glutamine used for this purpose is derived from muscle GS activity.²⁹ As skeletal muscle constitutes the principal reservoir of GS in the organism, and given the correlation between muscle mass and the AUC of GS mRNA in the treadmill group (particularly in males, who showed an increased muscle mass), it is presumed that the increase in GS mRNA observed in PBMC mirrors a corresponding increase in GS mRNA in muscle tissue. However, this needs verification in a study including muscle biopsies.

The concept of muscle serving as an alternative tissue for liver metabolism in states of hyperammonemia is not a new one,³⁰ but one previously propo-

sed on the basis of multiple pieces of evidence. Hod, *et al.*, showed that in a model of hyperammonemia by portal vein occlusion that GS activity was considerably increased in muscle, thus decreasing ammonia levels;³¹ while Desjardins, *et al.*, found similar results in a model of portacaval anastomosis, with reduced activity in liver and unchanged in brain.⁹ Other experimental studies with infused ammonium salts³² and a model of acute liver failure³³ also support a secondary increase in muscular GS activity. However, evidence showing raised levels of muscle GS mRNA within these models has been inconsistent.^{33,34} In humans, a bedside study with 7 patients with acute liver failure also argues for this concept, as blood ammonia arterio-venous gradients showed that skeletal muscle extracts ammonia while releasing glutamine in the lower extremities.³⁵ A similar study in the upper extremities reached the same results by studying 14 patients with decompensated cirrhosis and hyperammonemia, a finding not reproduced in controls.³⁰ These studies have confirmed that in humans with liver failure skeletal muscle is capable of metabolizing ammonia, indirectly pointing to an increased activity of GS.

The consistent findings regarding muscle metabolism of ammonia in states of hyperammonemia (by means of GS) prove the relevance of dynamic inter-organ ammonia trafficking, and the importance that muscle acquires in liver failure. We speculate that exercise could become a novel treatment for patients with low grade HE (for those who are capable of exercise training) by taking advantage of this inter-organ metabolism, similarly to what has been described with the use of anti-ammonia compounds.¹² Moderate exercise, apart from preventing muscle wasting in cirrhosis, could induce muscle GS mRNA and likely activate this metabolic pathway. It is physiologically plausible that this activation would increase the levels of circulating glutamine followed by increased ammoniogenesis in the kidneys and excretion of ammonia. It is known that during hyperammonemia the kidneys can invert the rate of ammonia release into the renal vein to ammonia excretion in urine, and become an organ of net ammonia removal.¹⁸ Thus, glutamine reaching the proximal tubular cells of the nephron could be transformed by the phosphate-dependent glutaminase enzyme into ammonium (NH_4^+), to be excreted in the tubular lumen by the $\text{Na}^+ - \text{NH}_4^+$ antiporter (functioning as a $\text{Na}^+ - \text{NH}_4^+$ exchanger).³⁶ The final result would be a decrease in circulating ammonia concentration leading to improved astrocyte function and HE (Figure 3).

We are aware that the results of the present study are only the first step in this promising area of research, and that significant questions are left to explore. Does GS mRNA increase in PBMC truly correlate with muscle GS changes? Will GS mRNA induction translate into real enzymatic activity? Will this effect be the same for both men and women? And more importantly, will regular physical training give rise to a sustained induction and enzymatic activity capable of abating ammonia levels? Some evidence from healthy subjects suggests that the latter is possible^{37,38} and the final proof of concept demands the establishment of a physical training program in patients with cirrhosis. Hopefully, these results and data from research to come will translate into an alternative ammonia metabolism that could benefit patients with low-grade HE.

ACKNOWLEDGEMENTS

The authors would like to thank Arturo Meixueiro-Daza for his technical assistance.

This work was partially supported by Fundación Mexicana para la Salud Hepática (Estímulo Ángeles Espinosa Yglesias), and the Consejo Nacional de Ciencia y Tecnología (CONACyT).

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Recibido el 08 de abril 2011.

Aceptado el 05 de septiembre 2011.