Metabolomic analysis of long-term spontaneous exercise in mice suggests increased lipolysis and altered glucose metabolism when animals are at rest

Daniel Monleon,2* Rebeca Garcia-Valles,1* Jose Manuel Morales,2 Thomas Brioche,3 Gloria Olaso-Gonzalez,1 Raul Lopez-Grueso,4 Mari Carmen Gomez-Cabrera,1 and Jose Viña1

1Department of Physiology, University of Valencia, Valencia, Spain; 2Fundacion Investigacion Hospital Clinico Universitario INCLIVA, Valencia, Spain; 3Laboratory “Movement Sport and Health Sciences,” University Rennes, France; and 4Sports Research Centre, Miguel Hernandez University of Elche, Elche, Spain

Submitted 2 July 2014; accepted in final form 2 September 2014

Monleon D, Garcia-Valles R, Morales JM, Brioche T, Olaso-Gonzalez G, Lopez-Grueso R, Gomez-Cabrera MC, Viña J. Metabolomic analysis of long-term spontaneous exercise in mice suggests increased lipolysis and altered glucose metabolism when animals are at rest. J Appl Physiol 117: 1110–1119, 2014. First published September 4, 2014; doi:10.1152/japplphysiol.00585.2014.—Exercise has been associated with several beneficial effects and is one of the major modulators of metabolism. The working muscle produces and releases substances during exercise that mediate the adaptation of the muscle but also improve the metabolic flexibility of the complete organism, leading to adjustable substrate utilization. Metabolomic studies on physical exercise are scarce and most of them have been focused on the effects of intense exercise in professional sportsmen. The aim of our study was to determine plasma metabolomic adaptations in mice after a long-term spontaneous exercise intervention study (18 mo). The metabolic changes induced by long-term spontaneous exercise were sufficient to achieve complete discrimination between groups in the principal component analysis scores plot. We identified plasma indicators of an increase in lipolysis (elevated unsaturated fatty acids and glycerol), a decrease in glucose and insulin plasma levels and in heart glucose consumption (by PET), and altered glucose metabolism (decreased alanine and lactate) in the wheel running group. Collectively these data are compatible with an increase in skeletal muscle insulin sensitivity in the active mice. We also found an increase in amino acids involved in catecholamine synthesis (tyrosine and phenylalanine), in the skeletal muscle pool of creatine phosphate and taurine, and changes in phospholipid metabolism (phosphocholine and choline in lipids) between the sedentary and the active mice. In conclusion, long-term spontaneous wheel running induces significant plasma and tissue (heart) metabolic responses that remain even when the animal is at rest.

heart; positron emission tomography; glucose metabolism; adaptations to exercise; insulin sensitivity

THE BENEFICIAL EFFECTS of regular exercise have been known for a long time. We recently proposed that “exercise acts as a drug” (57). There is irrefutable evidence of the effectiveness of regular physical activity in the primary and secondary prevention of several chronic diseases (e.g., cardiovascular disease, diabetes, cancer, hypertension, obesity, depression, and osteoporosis) and premature death (51), but how and why those effects occur are not entirely clear (42). Several biological mechanisms may be responsible for these effects. For instance, exercise has been shown to improve body composition (59), enhance lipid lipoprotein profiles (58), improve glucose homeostasis, and insulin sensitivity (30), among others (1, 50, 65). Exercise is one of the major modulators of metabolism: it increases the rate of metabolic processes and modulates the concentration of different metabolic products (64). The working muscle produces and releases substances during exercise that not only mediate the adaptation of the muscle but also improve the metabolic flexibility of the complete organism, leading to adjustable substrate utilization (7). An extensive investigation of metabolic changes with exercise will yield relevant information to explain the beneficial effects of exercise (7, 41).

High-resolution nuclear magnetic resonance (NMR) spectroscopy of biofluids and tissues combined with multivariate analysis methodologies, like principal component analysis, represent a powerful technique for investigating the metabolome in the area of physiology, drug toxicology, and disease diagnosis and prognosis (2, 45, 48). NMR is one of the most efficient, robust, reproducible, and cheap methods for obtaining metabolic profiles in biological specimens without extensive sample preparation (46). In most of the biomedical applications of NMR, plasma and urine molecular profiles are investigated to obtain information on exogenous and endogenous metabolism of the system.

Metabolomic studies on physical exercise are scarce and most of them have been focused on the effects of intense exercise and training in professional sportsmen (10, 41, 42, 53, 64). There are numerous differences between persistently physically active and inactive individuals in the metabolome together indicating better metabolic health in the physically active than in inactive individuals (38).

The aim of this work was to study the adaptations in plasma metabolic profiles to lifelong spontaneous exercise in mice. We also aimed to correlate these changes with possible exercise-induced alterations in substrate utilization by the heart. The advantage of spontaneous running models is that animals are allowed to exercise at their own initiative, in terms of frequency, length, and intensity of training, which avoids some of the stressful factors related to forced training (32). To our knowledge no studies have been carried out to determine the metabolic responses during a long-term spontaneous exercise.

MATERIALS AND METHODS

Experimental Animals

Male C57Bl/6J mice, 3 mo of age, were randomly assigned to one of two groups: sedentary or spontaneous wheel running. The animals of the running group were housed in individual cages with running wheels allowing 24 h access (n = 72). On the same day the animals designated to be in the sedentary group (control) (n = 72) were

* D. Monleon and R. Garcia-Valles contributed equally to this work.

Address for reprint requests and other correspondence: J. Viña, Dept. of Physiology, Faculty of Medicine, Blasco Ibañez, 15, Valencia, Spain 46010 (e-mail: jose.vina@uv.es).
housed individually in standard rodent cages of the same dimensions. Thus each sedentary mouse was free to move around its cage but did not have access to running wheel. Of these 72 animals, 15 in each group were tested for performance, 10 were tested for food and water intake, 5 animals were used for the metabolomic study, and 5 animals were used for the PET study. The selection of the animals, in all the cases, was random. All animals were fed an ad libitum laboratory diet (Global diet 2014l; Harlan Teklad, Madison, WI) and were maintained at 23°C under a light/dark cycle of 12:12 h. The animals received water ad libitum. The animal weight and food intake were determined weekly during all the experimental period. Apparent food consumptions was determined by subtracting the amount eaten from the amount offered. No differences in weight and food intake were found between the experimental groups (data not shown).

Cage bottoms were cleaned once every 2 wk, and wheels were cleaned once every 4 wk. The experimental protocol was approved by the Committee on Ethics in Research of the Faculty of Medicine of the University of Valencia.

Wheel Running Activity

Activity of mice on the running wheels was monitored by a magnetic switch affixed to each wheel, which recorded the number of completed revolutions. Physical activity was recorded continuously and summed by week for analysis. Free open-field locomotor activity was completed revolutions. Physical activity was recorded continuously by a magnetic switch affixed to each wheel, which recorded the number of revolutions. Participants were selected on the bases of the animal (13, 26).

Performance Measurements

At the end of the exercise period we took the wheel running off to guarantee that the animals rested for 24 h before plasma sample collection. This resting period was chosen to negate any direct effects of exercise. This rest included a 4-h fasting period to negate the effects of postprandial food absorption. One milliliter of blood was drawn from the cava vein in heparinized tubes between 10:00 and 12:00 AM. Samples were then centrifuged for 10 min (3,000 rpm), and plasma was collected before storage at −80°C until NMR analysis.

Maximal running speed. When the mice were 18 mo old, they were acclimated to run on a treadmill Model 1050 LS Exer3/6 (Columbus Instruments, Columbus, OH). One day after acclimatization to treadmill, mice were given a graded intensity treadmill test to determine maximal running speed. After a warm-up of 6 min running at 6 m/min the treadmill speed was increased by 2 m/min every 120 s until exhaustion was reached. To encourage the mice to run, an electric shock grid at the base of the treadmill was activated to deliver a 0.2-mA pulse. This delivered an uncomfortable shock but did not injure or harm the mice. The maximal running speed was considered the maximal workload capacity of the animal (13, 26).

Grip strength test. The grip strength meter (Panlab, Harvard Apparatus) was employed to assess neuromuscular function by sensing the peak amount of force that the mice applied in grasping specially designed pull bar assemblies. Metering was performed with precision force gauges in such a manner as to retain the peak force applied on a digital display.

One week after performing the maximal running speed test the mice were weighed and tested for baseline grip strength. First, we allowed the animal to grasp the forelimb pull bar assembly. The animal was then drawn along a straight line leading away from the sensor. The animal released at some point and the maximum force attained was stored on the display. Peak force was automatically registered in grams force by the apparatus. Data were recorded, and four additional trials were immediately given (9). We report the average of the top three tests for each mouse.

Nuclear Magnetic Resonance Spectroscopy

Total preparation time for each sample before NMR detection was less than 15 min. This preparation consisted in the addition of 2 μl of D2O to 20 μl of mice plasma. An aliquot of 20 μl of this mixture was taken and placed in a 1-mm-high resolution NMR tube. All spectra were recorded in a Bruker Avance DRX 600 spectrometer (Valencia, Spain) operating at a 1H frequency of 600.13 MHz. The instrument was equipped with a triple resonance 1H/13C/31P probe. Lock homogeneity was achieved by extensive manual coil-shimming using the 1D water presaturation experiments in interactive mode. Visual line shape inspection on Alanine doublet signal at 1.475 ppm was used for lock homogeneity monitoring. Nominal temperature of the sample was kept at 310 K. A single-pulse presaturation experiment was acquired in all the samples. Number of transients was 256 collected into 65 k data points for all the experiments. Water presaturation was used during 1 s along the recycling delay for solvent signal suppression. Spectral width for all spectra was 8000 Hz for 1H. Before Fourier transformation, the free induction decay was multiplied with a 0.3-Hz exponential line broadening. Spectral chemical shift referencing on the alanine CH2 doublet signal at 1.475 ppm was performed in all spectra. All 11 spectra were processed using MestReNova 5.3 (Mestrelab Research S.L., Spain) and transferred to MATLAB (MatWorks, 2006) using in-house scripts for data analysis. The chemical shift reference including resonances between 0.50 and 4.70 ppm (the aliphatic region) and 5.20 to 10.00 ppm (the aromatic region) was investigated. Metabolite spin systems and resonances were identified by using literature data (49) and the commercial resonances database Chenomx (Chenomx). The spectra were normalized to total aliphatic spectral area to eliminate differences in metabolite total concentration. The spectra were binned into 0.01 ppm buckets for multivariate analysis. The binned data were subject to mean-centering before multivariate analysis. Signals belonging to selected metabolites were integrated and quantified using semiautomated in-house MATLAB peak-fitting routines. Final metabolite levels were calculated in arbitrary units as peak area normalized to total spectral area.

Determination of Heart Glucose Consumption In Vivo

Mice were deprived of food for 8 h before 18F-2- fluor-2-deoxyglucose (18F-FDG) injection. 18F-FDG (5.8 –11.1 MBq) was injected intraperitoneally after anesthesia with isoflurane (1.5–2% in 100% oxygen, IsoFlo; Abbott Laboratories). Positron emission tomography (PET) was started 60 min after 18F-FDG injection as described in Ref. 66. 18F-FDG was synthesized as previously described (67). The administered dose (FDG activity) was corrected for body weight. We acquired 20-min static images 60 min after injection of 18F-FDG. The biodistribution of 18F-FDG by the heart was compared between all the studied groups. The PET images were obtained with the Albira small animal PET (ONCOVISION; GEMImaging). Regions of interest were manually drawn over the heart with PMOD software. Tracer uptake by heart was quantified as SUV (standardized uptake value, Total Sum).

Western Blotting

Aliquots of heart lysate were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose membranes, which were incubated overnight at 4°C with appropriate primary antibodies: anti-PDK (H-300): sc-28783 (1:200, Santa Cruz Biotechnology), anti-PPARα (H-98): sc-9000 (1:200, Santa Cruz Biotechnology), and anti-α tubulin (TU-02): sc-8035 (1:1000, Santa Cruz Biotechnology). Thereafter, membranes were incubated with a secondary antibody for 1 h at room tempera-
ure. Specific proteins were visualized by using the enhanced chemiluminescence procedure as specified by the manufacturer (Amersham Biosciences, Piscataway, NJ). Autoradiographic signals were assessed by using a scanning densitometer (BioRad, Hercules, CA).

Data Analysis

The metabolomic statistical analysis was performed using in-house MATLAB scripts and the PLS_Toolbox (Eigenvector Research) statistical multivariate analysis library. Principal component analysis (PCA) was applied to NMR spectra data sets. PCA is able to find low dimensional embeddings of multivariate data in a way that optimally preserves the structure of the data (37). The main advantage of PCA models is that the main sources of variability in the data are modeled by the so-called principal components (PCs), and consequently, in their associated scores and loadings, allowing the visualization and understanding of different patterns and relations in the data. Principal components were chosen to explain at least 70% of the variance. The loading plots of the corresponding principal components were used to detect the positions of most discriminative variables in the NMR spectra.

For descriptive statistics, mean values and standard deviation were considered. Normality of distribution was checked with the Shapiro-Wilk test, and homogeneity of variance was tested by Levene's statistics. A two-way analysis of variance with repeated measures with groups (control and spontaneous wheel running) and time (3–18 mo) was used to test for interaction and main effects in body weight and food intake. Differences in maximal running speed and grip strength test between control and spontaneous animals were tested using a two-tailed Student's t-test for unpaired samples. Differences are considered significant at \( P < 0.05 \). Statistical calculations were performed using Sigma Stat statistical software (version 2.03; Sigma Stat, Chicago, IL).

RESULTS

Running Wheel Activity

Figure 1A shows the average spontaneous running distances of male C57Bl/6J mice for 18 mo. Mice ran an average of 4.6 ± 1.5 km/day at the beginning of the experiment. As in previous studies, there was a progressive decline in the distance run by the mice with advancing age (21, 31). The mice ran a total of 826.6 ± 35.6 km over the 18-mo period.

Exercise Performance Measurements

Grip strength of sedentary and wheel running mice is shown in Fig. 1B. An initial analysis was performed on the third month of the study (i.e., young mice), and no differences were found between the sedentary and the runners (data not shown). However, at 18 mo of age, wheel runners had significantly higher grip strength than sedentary ones. The difference in strength between groups, after correcting for body weight, was higher in magnitude in favor of the runners. We also found a significant increase in maximal running speed between runners and sedentary animals when we performed the test at the end of the study, i.e., when animals were old (Fig. 1C). Initial analysis for maximal running speed (month 3) indicated no differences between groups (data not shown).

---

Fig. 1. Physiological performance measurements in male C57Bl/6J mice. A: average running wheel distance for 18 mo. Data points represent the average distance per day (meters). B: maximal running speed (m/min) on a graded intensity treadmill test. C: grip strength test data in grams. Values are shown as mean ± SD. *\( P < 0.05 \).
Metabolic Profile Changes in Trained Animals at Rest

All our measurements were done in animals that were free to perform physical exercise but that were at rest when we obtained blood plasma to perform metabolomic analysis. The sedentary animals did not have the possibility of exercising at any time. Figure 2 shows the statistically significant metabolite levels according to Student’s \( t \)-test with a Bonferroni correction for multiple comparisons.

Long-term spontaneous exercise in mice induces a decrease in plasma alanine and lactate, i.e., two gluconeogenic substrates. Similarly, we found a decrease in glucose and insulin plasma levels. We also found an increase in the amount of total circulating and unsaturated fatty acids as well as of glycerol. These are all products of lipolysis. Thus adaptations to long-term spontaneous exercise include increase in lipolysis and modifications in glucose metabolism.

Total plasma creatine, which is critical in skeletal muscle energy metabolism, is lower in the wheel running animals, probably because of increased uptake by muscle. Plasma taurine concentrations also appeared to be lower in the long-term spontaneous wheel running mice.

The concentration of phosphocholine and choline (well known precursor of phospholipids) are lower in runners than in sedentary mice.

Two important aromatic amino acids like tyrosine and phenylalanine show higher relative concentrations in the runners group. Phenylalanine is a precursor of tyrosine and, therefore, the levels of these two amino acids are closely related. Ty-
Rosine is a precursor of catecholamines (DOPA, dopamine, noradrenaline, and adrenaline).

**Multivariate Analysis and NMR Spectra**

PCA on the mean-centered normalized plasma \(^1\)H-NMR spectra provides an overview of the global metabolic variations between runners and controls. The first two principal components expressed 79% of total variance. Figure 3A shows the PCA scores plot of mice plasma NMR spectra analysis. The metabolic changes induced by long-term spontaneous exercise are sufficient to achieve complete discrimination between groups in the PCA scores plot. The second principal component (PC2) loadings plot, which produces the best discrimination between controls and runners, reveals major metabolic contributions of total fatty acids, lactate, and total creatine. Other important contributors to PC1 in the aliphatic region include alanine, unsaturated fatty acids, glycerol, and phosphocholine. In the aromatic region, tyrosine and phenylalanine are the major contributions to PC1, although the contribution of the hump formed by resonances of protein amide protons (6.0 to 9.0 ppm) is also relevant. Spectral signal integration over these resonances provides relative metabolic quantification in the different samples.

Pulse-acquire NMR spectra are dominated by the presence of broad lipoprotein resonances (see Fig. 3B). The spectra cannot be separated between controls and runners by visual inspection, indicating that exercise is not associated with a major metabolic perturbation. All NMR spectra show narrow line widths and adequate signal-to-noise ratios with well-resolved spin-spin multiplicities. The 1D single-pulse presaturation experiment provides complete and unambiguous identification of the metabolic pattern of the examined samples. In all spectra, the aliphatic region has prominent signals of fatty acids in lipoproteins, choline-containing compounds, lactate, glycerol, and most amino acids.

**In Vivo Glucose Consumption by the Heart Measured by PET**

The marked metabolite changes found in animals that had run spontaneously, at rest, led us to study the in vivo glucose consumption by the major working muscle at rest, i.e., the heart. Figure 4A shows the effect of spontaneous exercise on glucose consumption in vivo in the heart of the mice by using the PET technology. We found that heart glucose consumption of the running group is significantly smaller than that of the sedentary animals (P < 0.05). We also determined the protein levels of PDK and PPARα, both involved in fatty acid oxidation, but no differences were found in their myocardial levels between the sedentary and the wheel running mice (Fig. 4B).

**DISCUSSION**

**Maximal Running Speed and Muscle Force in Exercised Animals**

Long-term physical inactivity causes many health problems (57). The exercise-induced adaptations are especially evident in the cardiorespiratory, musculoskeletal system, body composition, and metabolism (39, 60). The higher level of aerobic capacity (maximal running speed) and muscle force achieved after a training period has been related to the lower mortality rates in different studies (20, 47). However, no studies have been carried out to determine the whole metabolomic responses in a long-term spontaneous exercise intervention on controlled animals. We found a significant increase in the maximal running speed in mice after 18 mo of spontaneous running wheel, when compared with the sedentary ones (see Fig. 1C) (13). It has been shown that each 1-MET increase in exercise capacity confers an improvement in survival among healthy men (16, 47). Those results strengthen the relevance of the data on increased maximal running speed that we report here. The decrease in maximal aerobic capacity is usually accompanied by a decline in muscle strength and mass during aging (20). Many factors, including a sedentary lifestyle may contribute to muscle weakness during advanced age (3, 19). To determine if our protocol of long-term spontaneous exercise was accompanied by an increase in strength, we performed a grip strength test in our mice (Fig. 1B). The peak force for the spontaneous trained animals was 26.5% higher than those reported by the control ones. Our results show that spontaneous exercise not only leads to an increase in maximal running capacity but also to an increase in grip strength.
General Metabolic Effects of Exercise in Animals at Rest

The metabolic changes induced by long-term spontaneous exercise are sufficient to achieve complete discrimination between groups in the principal component analysis scores plot (see Fig. 3).

Our metabolic profiling by NMR reveals that spontaneous exercise induces sustained higher plasma levels of total and unsaturated fatty acids and glycerol in C57Bl/6J mice (see Fig. 2). Recently, it was found in a human study that physical activity induces a shift from saturated to a more polyunsaturated profile in serum fatty acid composition (38). Moreover, endurance training increases the capacities for FFA mobilization and oxidation during exercises of a given power output (36). As early as 1939, Christensen and Hansen (11) observed that endurance training reduced the respiratory exchange ratio during strenuous exercise, indicative of a decrease in carbohydrate oxidation and a corresponding increase in fatty acid oxidation (8). Basal whole body lipid kinetics has been evaluated in control and endurance-trained cyclists and it has been shown that the rate of appearance of glycerol (whole body lipolysis) and the rate of appearance of palmitate (index of fatty acid release) were two- to threefold higher in athletes than in untrained control subjects in resting conditions (55).

Exercise training alters substrate utilization by the heart (27). However, only a few studies have directly measured cardiac substrate utilization after exercise training and the results diverge (5, 6). Randle and coworkers (54) showed that the utilization of one nutrient inhibited the use of the other directly and without hormonal mediation. Because our trained animals had higher plasma fatty acid levels, we decided to study the in vivo heart glucose consumption by using the PET technology. Heart glucose consumption in the exercised group was significantly smaller than in the sedentary one (*P < 0.05) (see Fig. 4A). This may be explained by an increase in the myocardial oxidation rates of fatty acid, as previously reported by Burelle and coworkers (6). We determined the protein levels of PDK and PPARα, both involved in fatty acid oxidation, but no differences were found in their myocardial levels between the sedentary and the wheel running mice (See Fig. 4B). Although our data regarding inhibition of glucose utilization are in accordance with those reported by Randle et al. (54), we have not been able to demonstrate the increase in fatty acid oxidation.
utilization by the heart in our exercised mice. We should also take into account, to properly interpret our heart data, the possibility of an exercise training-induced resting bradycardia in our mice. It was previously described in animals but after higher intensity training protocols (12, 14, 18). The possibility that the cardiac metabolic rate would be reduced after our long-term spontaneous exercise due to resting bradycardia should be explored in future studies.

We found lower levels of circulating lactate in the wheel running group. This finding could be explained by a reduction in whole body rate of glycolysis. It could also be a result of enhanced lactate extraction into tissues from circulation or enhanced transport from the cytoplasm into mitochondria (28). However, we cannot exclude increased hepatic uptake of lactate to be used as a gluconeogenic precursor by the liver (33). Alanine is another relevant gluconeogenic precursor that is also significantly decreased in the group of mice with free access to the running wheel. Alanine plays an important role in exercise via the alanine-glucose cycle and a decrease in alanine serum levels was previously reported in metabolomic studies after an exercise training period (64). The reduction in plasma alanine could also theoretically be a result of a reduced need for nitrogen shuttling (reduced amino acid oxidation rate) or reduced glycolytic rate (less supply of pyruvate for transamination) after training.

We also found a decrease in glucose plasma levels in the wheel running group. These results are consistent with those reported previously in which lowered glucose levels were found in mice doing voluntary exercise for 6 wk (4). The paradoxical finding of a potential increase in gluconeogenesis, a decrease in glucose heart utilization and glycemia lead us to consider that there is an increase in skeletal muscle insulin sensitivity in the wheel running mice. We measured the insulin plasma levels. Because of lack of sufficient plasma to make an accurate determination, we pooled plasma from five sedentary and five exercised animals at rest and found that insulin levels were 100% higher in control (0.46 ng/ml) than in exercised animals (0.21 ng/ml). The lower insulin levels mirrored the lower plasma glucose levels, which may indicate higher insulin sensitivity in the exercised mice. However, to elucidate the significance of this difference, further experiments that include a glucose tolerance test measurements are required.

Tyrosine is a precursor of neurotransmitters and hormones. Mammals synthesize tyrosine from the essential amino acid phenylalanine. In the adrenal medulla, tyrosine is converted into the catecholamine hormones norepinephrine and epinephrine, both released by the adrenal glands in response to stress. We found that wheel runners exhibit increased levels of tyrosine and phenylalanine. This increment in polar amino acids in mice was previously reported after a short term (10 days) of moderate exercise training (17). This may indicate a lower rate of catecholamines synthesis by the adrenal glands after long-term spontaneous exercise. However, there is no evidence in the literature showing a significant training effect on adrenaline and noradrenaline resting values in animals (24, 25) and human studies (40, 44, 52, 62). There are several reports on the composition and content of amino acids in the blood of rodents undergoing forced exercise (35). However, to the best of our knowledge, this is the first report on blood amino acids in rodents under conditions of long-term voluntary running when blood was drawn at rest. More research is needed to determine the significance of the modifications in the tyrosine and phenylalanine levels induced by our exercise protocol.

Creatine, synthesized mostly in liver and kidney but not in muscle, is transported through the blood and taken up by tissues with high energy demands via an active transport.
system (22). Creatine phosphate is the fastest source for ATP resynthesis during exercise. In our study, long-term spontaneous exercise decreases total creatine in plasma. Lower circulating creatine may indicate either lower substrate availability or increased usage. Creatine exported from the liver and transported through the blood may be taken up by creatine requiring tissues. In skeletal muscle cells, a large pool of phosphocreatine is available for immediate regeneration of ATP hydrolyzed during short periods of intense work. The concentration of total creatine in muscle tissue seems to parallel the muscle glycolytic capacity and is one hundred times larger than that in blood (63). As a consequence, creatine transfer from blood to the muscle tissue works against a large concentration gradient from the blood. The lower levels of creatine found in the runners group may reflect an increased phosphocreatine pool built over time, an enhanced phosphocreatine storage capability, and better readiness for fast energy production in the skeletal muscle.

Taurine is a sulfur amino acid with high levels in skeletal muscle (34). As previously reported we find that taurine may be more readily used by skeletal muscle, decreasing its plasma concentration in the wheel running group (23). However, we cannot distinguish between increased taurine production or decreased utilization during exercise with our experimental design. The physiological implications of these modifications in taurine should be studied in future research.

Phosphocholine is an intermediate in the synthesis of phosphatidylcholine. Phosphatidylcholine is a major constituent of the phospholipid monolayer part of lipoproteins. Choline-containing lipids account for 75% of serum phospholipids. We detect a statistically significant decrease in phosphocholine and choline in lipids in plasma in the wheel running mice. However, this is not accompanied by a parallel variation in circulating cholesterol or in lipoprotein composition (data not shown). This suggests that long-term spontaneous exercise enhances phospholipid metabolism to a point equivalent to that of younger population (15). This effect seems unrelated to other cholesterol-mediated beneficial effects reported by other authors in exercise and metabolomic studies performed in humans (38).

The overall general overview that emerges from our study is in Fig. 5. It is important to emphasize that the findings reported here pertain to animals at rest (i.e., most of the time) but that perform exercise daily. The reported changes are thus dealing with adaptations and not with responses to exercise. However, we would like to point out that, in human studies, it has been reported that some of the metabolic changes that we have studied, for instance the exercise-induced FFA mobilization, can persist for 12–24 h after exercise (29, 43). Thus, although at the end of the 18-mo exercise period we took the wheel running off to guarantee that the animals rested for 24 h before plasma sample collection, this resting period may not have been sufficient to completely exclude some effects of the last exercise bout.

One month for rodents may be equivalent to 2 years for humans (56, 61). Thus the 18-mo duration of the exercise period for our mice may be equivalent to more than 36 years for humans, i.e., lifelong active people. However, one should be careful when extrapolating data on mice to humans because of fundamental differences between the different species and, of course, results in experimental animals must be validated in humans, when possible.

We identified plasma indicators of an increase in fatty acid oxidation (elevated unsaturated fatty acids and glycerol), a decrease in insulin plasma levels, a decrease in glucose plasma levels and in heart glucose consumption (PET), and altered glucose metabolism (decreased alanine and lactate) in the wheel running group. Collectively these data are compatible with an increase in skeletal muscle insulin sensitivity in the active mice.

We also found an increase in amino acids involved in catecholamine synthesis (tyrosine and phenylalanine), in the skeletal muscle pool of creatine phosphate and taurine, and changes in phospholipid metabolism (phosphocholine and choline in lipids) when we compared the sedentary and the active mice.

In conclusion, long-term spontaneous wheel running induces significant plasma and tissue (heart) metabolic responses that remain even when the trained animals are at rest, i.e., most of the time.

GRANTS

This work was supported by grants ISCIII/2006-RED13-027 and ISCIII/2012-RED-43-029 from the “Red Tematica de Investigacion Cooperativa en envejecimiento y fragilidad” (RETICFEC); SAF2013-44663-R; PROMETEOII/2014/056 from “Conselleria d’Educacio, Cultura i Esport de la Generalitat Valenciana”; 35NEURO GentsGent from “Fundacio Gent Per Gent de la Comunitat Valenciana”; RS2012-609 Intramural Grant from INCLIVA and EU Funded CM1001 and FRAILOMIC-HEALTH.2012.2.1.1-2. This study has been cofounded by FEDER funds from the European Union.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


J Appl Physiol • doi:10.1152/japplphysiol.00585.2014 • www.jappl.org


