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Amino Acids Changes and Muscle Damage During the 400 km Ultra Trail Gobi Race

Running title: Ultra endurance exercise and muscle proteolysis

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Abstract

Current researches demonstrated that completing the Ultra Trail Gobi (UTG) could lead to severe muscle damage. Our study was designed to analysis the muscle damage and amino acid changes reacted to a 400 km ultra-endurance race in experienced runners. Peripheral blood samples from 16 male athletes (mean age 40.3 ± 7.0 years, mean finish time 121.2 ± 21.8 hours), taken 48 h before and immediately after completing the Ultra Trail Gobi Race (UTG), were analyzed for 39 amino acids, 15 steroid hormones and 4 muscle damage factors.

In all participants, the 4 biomarkers for muscle damage, i.e., creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) increased significantly after the race, whose mean post-race values were 13.7-, 7.3-, 4.7- and 1.5-fold higher than the pre-race values, respectively. 5 amino acids, i.e., alanine, valine, proline, ornithine and citrulline showed significant decrease, whose mean values decreased by $40.4 \pm 18.7\%$, $38.9 \pm 9.3\%$, $48.1 \pm 15.2\%$, $44.8 \pm 15.1\%$ and $23.4 \pm 30.8\%$ after the race, respectively.

Our study revealed that progressive decline in amino acids contents may further contribute to the factors increasing the muscle damage during the UTG.

Key words: Ultra-marathon, Amino acid metabolism, Muscle damage, Energy supplement, Genetics

Introduction

Moderate physical exercise is regarded as a good way to improve health status. In particular it can prevent or lower the risk of chronic diseases such as cirrhosis, type 2 diabetes, hypertension and hyperlipidemia¹. Running is an easy and convenient sport for most people compared to other sports. Recently, participation in running such as marathons, Iron-man triathlons, and ultra-marathons is rising. However, excessive or long-time physical exercise could lead to metabolic changes within the body, and may bring healthy risks. Negative metabolic changes are independent of the ages of ultra-marathon runners and occur in both young and aged participants². Various researches have indicated that prolonged intense endurance

exercise can induce fluid and electrolyte imbalance, increase oxidative stress and inflammation, as well as muscle damage. Muscle damage induces rhabdomyolysis via structural damage to the myocytes and protein leakage during long distance running³.

Creatinine kinase (CK) and lactate dehydrogenase (LDH) are biochemical markers of acute or chronic muscular damage and cell necrosis. Activation of CK and LDH may increase symptoms such as pain, fatigue, and decline in muscular strength during high-intensity, long-distance exercise because of damaged skeletal muscles³. The level of LDH has been observed related to both biochemical adaptations to physical load and muscle states, while CK correlate with both intensity and duration of exercise⁴. Alanine transaminase (ALT) and aspartate aminotransferase (AST) serve as markers of liver disease, and increases in AST, ALT, and LDH after long-distance exercise such as an ultra-marathon induces chronic liver injury. CK, LDH, AST, and ALT are the most common biochemical markers indicating the runners' physical status and skeletal muscle damage caused by overtraining^{1,3}. Kim et al showed that injury-related parameters (CPK, LDH, ALT, AST, Hs-CRP and IL-6) increased and greater muscle damage occurred in the latter half (100-200 km) of ultra-endurance races⁵. Raised activities of CK, LDH, AST and ALT were significantly related to asymptomatic exertional rhabdomyolysis in a 246-km continuous running competition⁶. The mean values of CK, LDH, AST and ALT were $29,384 \pm 4,327$, 585 ± 89 , 5615 ± 902 , and $1606 \pm 331\%$, well above the corresponding pre-race values, respectively⁶.

There is some evidence suggesting efficacy of essential amino acids and other supplements in minimizing muscle damage induced by prolonged endurance exercise in running events⁷. Alanine is the key factor which suppresses the activity pyruvate kinase, consequently regulating gluconeogenesis and glycolysis to ensure glucose output in the event of energy shortage through "alanine-glucose cycle"⁸. The "alanine-glucose cycle" could achieve mutual conversion of glucose and alanine in muscle and liver⁹. Branched-chain amino acids (BCAAs) consisting of valine, leucine and isoleucine are the main matrix of muscle¹⁰. During long-time endurance exercises, BCAAs may be widely captured in skeletal muscles instead of the liver and oxidized for ATP re-synthesis to meet the energy demand, which induces muscle proteolysis¹¹. BCAAs supplements can effectively reduce the muscle soreness and fatigue through attenuating the muscle damage and inflammation by decreasing LDH, CK and granulocyte elastase levels during an intensive endurance training¹². Furthermore, we wish to investigate the correlation between changes in amino acids and muscle damage indicated by biochemical markers CK, LDH, AST, and ALT. Studies have shown that the decomposition of amino acids and nitrogen-containing compounds increases significantly during prolonged exercise, producing ammonia^{13,14}.

Genetics plays an indispensable role in sport performance and is increasingly recognised as a momentous method for predicting injury. Of late genetic susceptibility to injury has become a hot field of research directions¹⁵. To explore the relevance of genes to sport injury, we also sequenced a number of common SNPs associated with injury.

By now, the Ultra Trail Gobi Race (UTG) is China's longest and toughest ultra-distance running race, which takes place on September 2016. It was a 400-kilometers, non-stop, self-navigated and self-supported race. The cut-off time of completing the entire course was 150 hours. Runners were to run across one of the most arid regions in the world - The Great Gobi, China's protected desert ecosystem, where 80% of the place is uninhabited land. The temperature during the race was from a low of 7°C to a

high of 37°C. During the UTG, runners faced great challenges physically and psychologically, which may induce dramatic metabolic changes to the body. This was the first study researching the metabolic reactions to the most enduring and continuous running competition in China. Further more, it was designed to examine the relationship between amino acids metabolic changes and muscle damage induced by the UTG, thereby providing suggestions for runners to reduce muscle damage and improve physical performance.

Methods

Participants

Sixteen male runners (mean age 40.3 ± 7.0 years, training history 10 ± 7.0 years, mean finish time 121.2 ± 21.8 h) volunteered to provide buccal swabs before the race and blood samples before and after the race. Before the event began, the runners completed a questionnaire about their training and medical history, and signed their consents to participate in the study after being informed of all the experimental procedures and research purpose. All sample collections and research plans were approved by the Institutional Review Board on Bioethics and Biosafety of BGI in Shenzhen, China with approval number BGI-IRB16077.

Runners ran across the Gobi Desert and completed the 400 kilometers course in 146 hours, along with the temperature from 7°C to 37°C during the race. The subjects had consumed liquids (water, sports drinks, beverages and broth) and food (energy bars, breads, rice, meats and fruits) carried by self-choice and provided at each rest point (10 rest points in total: RP1 to RP10) throughout the race.

Blood sampling

Samples of peripheral blood collected 2 days before starting and immediately after completing the race, were measured for metabolic markers including amino acids and hormones. Samples of peripheral blood collected 2 days before, at midway (RP 6) and immediately after the race, were measured for hematology and biochemical markers including CK, AST, ALT, LDH, etc. The category of collected blood samples is shown as Table 1.

Blood analysis

2 ml blood sample (anti-coagulated with EDTA) was collected for blood hematology analysis on an Automated Hematology Analyzer MEK-8222K. 3.5 ml whole blood was collected to analysis the serum activities of biochemical markers including CK, ALT, AST and LDH which were measured using the clinical biochemical analyzer MS-880.

5 ml whole blood was collected and stored at 4°C for 30 minutes. Plasma was separated from whole blood by centrifugation at 1600 g for 10 min at 4°C. 150 µl of the plasma sample was used for amino acids analysis and 550 µl for hormone analysis. 39 amino acids and 15 hormones were detected by mass spectrometer. The mass spectrometer, a Sciex Q-trap 5500 (AB Sciex, Framingham, MA, USA/Concord, Ontario, Canada) equipped with APCI ion source, was operated in the positive ion mode. The curtain gas, collision gas, nebulizer current, source temperature and ion source gas 1 was set to 35.0, medium, 3.0, 500 and 60 respectively. Quantification was achieved by using the mass spectrometer in multiple reaction monitoring (MRM) mode. The quantitation was done by Multiquant (2011 Ab sciex, Version

1.1.1296.0) software. The peak area was integrated and quantified by the MQ4 method.

In order to prevent batch effects, blood samples were uploaded onto the mass spectrometer with a standard QC solution with a known concentration, quantitation of metabolite samples' concentrations were calibrated by the reference standard, to ensure the accuracy of detection for different batches.

Genotyping

Buccal swabs were collected and stored at room temperature until total DNA extraction. DNA was extracted using a sequenced extraction technique (MagPure Swab DNA Kit, Magen, Guangzhou, China) as per the manufacturer's recommendations. All subjects were genotyped for the 81 SNPs related to exercise and nutrition by MALDI-TOF (MassARRAY Analyzer 4, Agena, San Diego, CA, USA), including *MMP12* rs2276109¹⁶, *GDF5* rs143383¹⁷, *COL5A1* rs12722¹⁸ and *HBPI* rs4730250¹⁹. Molecular mass could be distinguished based on the position of peak by MassARRAY Analyzer. The result of SNP genotyping was obtained.

Statistical Analysis

Pre-race and post-race values were compared by Wilcoxon Signed-Rank Test. Spearman's (non-parametric) correlations were used to assess the relationships between selected variables. Significance was set at $P < 0.05$ for all statistical comparisons. All analyses were performed using the R software (R for windows, version 3.3.2).

Results

Changes of biochemical enzyme

Dramatic increases over the baseline values in muscle damage biochemical markers including CK, AST, ALT and LDH were observed after the race. The pre-race and post-race mean values of serum activities of CK, AST, ALT and LDH are presented in Table 2. Notably, post-race serum activities of CK, AST, ALT and LDH were 13.7-, 7.3-, 4.7- and 1.5-fold higher than the corresponding values before the race, respectively. Each runner's changes of CK, AST, ALT and LDH before, at midway (RP 6) and after the race are shown in Figure 1.

Changes of amino acids and hormones

Various metabolic markers also showed differences before and after the race. We detected the concentration of 39 amino acids and 15 steroid hormones in all runners, as shown in table3 and table 4, respectively. Amino acids alanine, valine and proline showed significant decrease after the race. Furthermore, the hormone testosterone also declined significantly. The pre-race and post-race values of alanine, valine, proline, ornithine and citrulline as well as testosterone, are presented in Figure 2.

Correlation between amino acids and biochemical enzymes

Through in-depth Spearman's analysis, we found valine and proline had negative association with these selected biochemical markers including CK, AST and LDH ($p < 0.05$). The results showed the decreased valine had the highest correlation with increased CK ($r = -0.671$, $p < 0.01$), as shown in Figure 3.

Genetics and amino acid changes

We correlated pre-race mean values and changes of amino acid and the four biomarkers of muscle damage with genotypes of injury-related genes by Wilcoxon Signed-Rank Test. It is found that the differences of the decreases of citrulline and ornithine after the race between *COL5A1* CC (n=6) and CT (n=10) genotype are significant. The p values are 0.026 and 0.034, respectively.

Discussion

Our project is the first one that collects samples and researches the influence of continuous, ultra-endurance running on the muscle under extreme conditions in China. Previous study demonstrated that muscular injury caused by the 308-km course (low-intensity, long-distance running) was higher than the marathon or 100-km ultra-marathon³. Our study confirmed that prolonged, continuous, ultra-marathon race will lead to severe muscle damage. Relationship between material-energy flow, genetics and muscle damage showed in Figure 5. We will separately discuss the changes in muscle damage-related enzymes, energy, amino acids and hormones, and the genetic effects on them below.

Many researches have reported the influence of extreme endurance exercises on biochemical and metabolic markers. Serum enzymes like CK, AST, ALT and LDH have been used as biochemical markers indicating the muscle damage. Ultra-endurance exercises lead to significant sarcolemma damage and leakage of intracellular proteins. Elevated AST, ALT and LDH after a 24-hour marathon implied skeletal muscle and hepatic cells damages²⁰. Skenderi et al correlated extremely elevated muscle and liver damage indicators with prolonged exercise⁶, and the similar observations occurred in our study where the muscle fatigue and damage biochemical indicators extremely increased after the race, i.e. the activities of CK, AST, ALT and LDH increased by 13.7-, 7.3-, 4.7- and 1.5-fold, respectively.

In ultra-endurance exercises, most participants will have a large energy deficit. This phenomenon is caused by the suppress appetite and gastrointestinal problems such as abdominal fullness, cramp and vomiting, which are common problems during ultra-endurance exercise²¹. Enqvist reported the average energy expenditure during 24-h kayaking, running, and cycling exercise was about $9,590 \pm 770$ kcal²². During a 477.3-km endurance race, the energy consumption and intake of athletes were $24,516 \pm 5,424$ kcal and $14,738 \pm 4,335$ kcal, respectively, which led to an energy deficit of $9,779 \pm 1,020$ kcal²³. The major sources of energy are carbohydrates and fat, while only 3–6% of total ATP supplied from oxidation of amino acids during prolonged endurance exercise^{24, 25, 26}. However, the energy contribution from amino acids may be up to 10% when taking exercise under the presence of low glycogen concentration or the exercise duration is prolonged^{27, 28}. In addition, gluconeogenesis can be activated by exercise²⁹. Our results demonstrated that running a 400-km ultra-endurance race resulted in significant decreases in plasma concentrations of alanine, valine and proline which were gluconeogenic amino acids produced in muscles by protein degradation and transamination. Substantial decreases in plasma activities of alanine, valine and proline are indications of energy shortage and significant amino acid loss, which could increase risks of muscle damage.

The important aspect of amino acids metabolic reaction to ultra-endurance events is the role of alanine involved in the “alanine-glucose cycle”. The concentration of alanine is significantly related to the intense and duration of exercise²⁷. Alanine is generated through protein breakdown and delivered to the

liver via the blood circulation, which accelerates the “alanine-glucose cycle”. This cycle ensures the glucose production during the early stage of ultra-endurance race along with a large amount of ammonia produced. Within the continuous prolonged exercise, the concentration of alanine in serum is decreasing due to the shortage of amino acid source and low rate of protein degradation³⁰. What’s more, most researches of the amino acids’ response to prolonged endurance efforts have assessed alanine alterations during the race. The alanine level gradually declines after 60-90 mins endurance exercise following an initial increase by 50-75% at the start of the exercise²⁸. These researches were consistent with our result that alanine concentration decreased $40.4 \pm 18.7\%$ after the UTG.

Shortage of alanine may weaken the endurance performance. BCAAs are preferentially taken up by muscle tissue and involved in the “alanine-glucose cycle”. Muscle damage induced by long time ultra-endurance exercise is followed by an increased uptake of skeletal muscle BCAAs from the serum. BCAAs were used as energy source and/or participated in translation initiation signaling pathway involved in muscle remodeling⁷. It had been reported that serum concentrations of free amino acids, including BCAAs were reduced to 35–85% of pre-race values during a 100-km ultra-marathon¹⁴. Thus, decreases in plasma valine, leucine and isoleucine levels supported the hypothesis that BCAAs’ supplement was insufficient and muscle damage happened to athletes in our study. Furthermore, our study demonstrates the correlation between amino acids loss and muscle damage biochemical markers increasement. The decreased valine had the highest correlation with increased CK ($r=-0.671$, $p<0.05$), which means those athletes experiencing the most valine deficiency in race may have a higher risk of muscle damage. Leucine and isoleucine were also decreased during the UTG, but they were not as significant as valine.

Proline is positively correlated with collagen production. Collagen is a major extracellular matrix protein as elongated fibrils in the endomysium of skeletal muscles. Prolonged exercise has a negative impact on protein synthesis and the autophagy of collagen³¹. In highly trained runners, it was found that prolonged endurance running resulted in decreases in total serum amino acids concentration along with increased free fatty acids, more significantly in alanine and proline²⁷. The mean concentration of proline decreased $48.1 \pm 15.2\%$ after the race in all participants in our study. Furthermore, proline had negative association with the muscle damage biochemical markers including CK ($r=-0.541$), AST ($r=-0.603$) and LDH($r=-0.535$). It indicates shortage of proline may cause muscle damage through decreasing the production of collagen and breaking the structure of skeletal muscles.

Studies have shown that amino acids and nitrogen-containing compounds decompose significantly during prolonged exercise^{13,14}. Ammonia, which is produced during the decomposition in the form of NH_4^+ and aspartate, is detoxified into urea by means of the urea cycle after transit to the liver by blood³². NH_4^+ is converted to carbamoyl phosphate and citrulline with ornithine, and the amino group of aspartate and the carbonyl group of citrulline form arginine, which is cleaved by arginase to product urea and ornithine in the last step³³. Accordingly, both ornithine and citrulline could reduce exercise-related accumulations of ammonia by increasing the urea cycle³⁴. It was reported that serum concentrations of ornithine and citrulline decreased after a 100-km ultra-marathon¹⁴, which is consistent with our study that the mean concentrations decreased $44.8 \pm 15.1\%$ and $23.4 \pm 30.8\%$ after the race in all participants, respectively.

Genetics research showed the *COL5A1* gene encodes the $\alpha 1$ chain of type V collagen, which plays a key

role in regulating collagen fibril assembly and cross growth (fibrillogenesis) even though its content is much less compared with other fibrillar collagen³⁵. The rs12722 in *COL5A1* gene 3'-UTR contains the C allele and the T allele identified prevalingly in the Achilles tendinopathic patients. What's more, the T functional form of rs12722 was associated with an overall increase in mRNA stability, which will increase type V collagen production and exert an influence on the amount incorporation into the collagen fibril^{36, 37}. Studies have shown that excessive type V collagen inhibits the self-aggregation of type I collagen³⁸, which facilitates collagen degradation along with producing more ammonia (NH₄⁺ and aspartate). NH₄⁺ is converted to carbamoyl phosphate and citrulline with ornithine in mitochondria, while aspartate and citrulline form arginine, which finally produce urea in cytoplasm. Therefore, citrulline of the participants carrying the T allele of rs12722 was significantly reduced, while ornithine was significantly increased compared with the C allele, which indicates that aspartate is produced more than NH₄⁺ to save energy. We speculate that carrying the T allele is beneficial to the degradation of ammonia in the form of aspartate, which may promote amino acid metabolism and muscle degradation. When energy is insufficient during exercise, the rs12722 with T allele is more likely to decompose muscles for energy by accumulating type V collagen, thereby causing muscle damage.

Testosterone is one of the major anabolic hormones and closely related to exercise capacity³⁹, by promoting skeletal muscle protein synthesis and enhancing muscle strength. What's more, it can also promote the production of erythropoietin and the hematopoiesis of bone marrow directly. It is well known that physical exercise can exert an influence on many hormones in the endocrine system, and testosterone is not an exception. A large number of studies have shown that exercise-induced changes in blood testosterone are mainly affected by the mode, frequency, load intensity and duration of exercise bout⁴⁰. It was demonstrated that participants in a 1700-km ultra-endurance cycling race showed a significant downward trend (4.2 ± 2.5 versus 3.9 ± 2.6 ng/L) in testosterone⁴¹. There was also a significant decreased level of testosterone in participants when completing a 161-km Western States Endurance Run (WSER). In addition, running the WSER continuously influences endocrine function until two days after the race and produces severe muscle damage⁴². During the UTG, the concentration of testosterone was decreased $62.8 \pm 54.4\%$ compared to pre-race, which was in accordance with previous research. Reductions in testosterone have a negative effect on the adaptation process associated with skeletal muscle and compromise the participants' health status during the prolonged ultra-endurance exercise³⁹. Some researches implied the low testosterone level may provide an enabling environment to mobilize amino acids away from protein synthesis and redirect them to gluconeogenesis⁴².

Conclusion and perspectives

The present study shows that completing the UTG could induce muscle damage in the runners, as seen by increased activities of corresponding biomarkers CK, LDH, ALT and AST. The significant loss of gluconeogenic amino acids alanine, valine and proline was an indication of energy shortage during the UTG. The data indicates runners experiencing the most valine defect may have a higher risk of muscle damage. Additionally, running the UTG decreased the levels of testosterone which may further increase the leakage of amino acids and produce severe muscle damage. Both ornithine and citrulline could reduce exercise-related accumulations of ammonia by increasing the urea cycle. The T allele of the rs12722 in *COL5A1* is associated with muscle proteolysis. Therefore, the level of alanine, valine, proline, ornithine, citrulline and testosterone in plasma as well as *COL5A1* genotype could be potential metabolic markers in monitoring physical status during the prolonged, ultra-endurance race. Furthermore, the findings in

the study can also provide nutritional supplements suggestions for endurance runners.

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Conflict of interest

The authors declare that they have no conflict of interests.

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Table 1. Samples collected by category

Category	Sample	Used Consumable
Hematology	2 ml anti-coagulated blood	5 ml EDTA tube
Biochemical	3.5 ml blood (serum)	5 ml Biochemical tube
Hormones/Amino acids	5 ml anti-coagulated blood (plasma)	5 ml EDTA tube

Table 2. Activities in serum biomarkers of muscle damage before and after the UTG

	Pre-race	Post-race
Creatinine kinase (IU•L⁻¹)	195.3 ± 123.0	2518.5 ± 2276.2***
Aspartate aminotransferase (IU•L⁻¹)	21.6 ± 4.6	183.4 ± 153.3***
Alanine transaminase (IU•L⁻¹)	19.6 ± 8.0	101.7 ± 69.2***
Lactate dehydrogenase (IU•L⁻¹)	203.2 ± 42.1	511.8 ± 249***

Mean ± SD for 16 runners completing the race. *Significance of the differences between pre- and post-race values (*p<0.05, **p<0.01, ***p<0.001), as analyzed by Wilcoxon Signed-Rank Test.

Table 3. Concentrations of 39 amino acids in plasma before and after the UTG

Amino acid (μmol•L ⁻¹)	Pre-race	Post-race	Amino acid (μ mol•L ⁻¹)	Pre-race	Post-race
Alanine	410.5 ± 93.3	233.3 ± 49.6***	Aspartic acid	2.2 ± 0.4	2.9 ± 3.5
Proline	210.7 ± 58.3	105.3 ± 29.5***	Glutamine	449.8 ± 141.4	499.5 ± 94.2
Valine	240.5 ± 30.0	147.2 ± 31.3***	Glutamic acid	36.2 ± 11.7	36.4 ± 10.6
Leucine	136.3 ± 16.1	103.4 ± 20.1***	Cystathionine	0.6 ± 0.3	1.0 ± 0.4**
Isoleucine	68.8 ± 11.7	51.5 ± 9.8***	Cystine	22.4 ± 3.8	37.6 ± 8.0***
Ornithine	105.2 ± 20.5	56.4 ± 14.4***	Homocysteine	0.01 ± 0.01	0.01 ± 0.01
Citrulline	36.1 ± 7.4	26.0 ± 7.0**	1-Methyl-L- histidine	7.4 ± 7.2	7.1 ± 10.3
Arginine	42.1 ± 16.0	52.5 ± 14.1	3-Methyl-L- histidine	4.2 ± 1.0	4.8 ± 1.8
Tryptophan	46.2 ± 9.1	40.7 ± 8.6	α-Amino-n- butyric acid	15.7 ± 4.7	13.9 ± 3.0
Histidine	75.3 ± 7.6	75.5 ± 6.8	Argininosuccini c acid	0.04 ± 0.01	0.06 ± 0.02*
Lysine	152.4 ± 12.2	133.1 ± 18.2**	β-Alanine	2.1 ± 0.6	2.5 ± 0.7
Methionine	24.2 ± 4.1	28.3 ± 5.9*	Carnosine	0.004 ± 0.005	0.01 ± 0.02
Phenylalanine	48.4 ± 5.3	60.4 ± 10.7***	Ethanol amine	8.7 ± 1.6	9.3 ± 1.6
Threonine	144.5 ± 34.6	106.5 ± 20.9**	Homocitrulline	0.4 ± 0.2	0.3 ± 0.1
Glycine	272.7 ± 50.2	206.3 ± 58.3**	Hydroxylysine	0.6 ± 0.4	0.4 ± 0.1
Serine	121.6 ± 19.9	106.1 ± 28.7	Hydroxyproline	20.0 ± 13.6	6.8 ± 3.0***
Taurine	69.6 ± 16.0	88.1 ± 22.2*	Phosphoryletha nolamine	3.2 ± 1.0	5.1 ± 1.3**

Tyrosine	50.4 ± 11.2	67.4 ± 7.9***	Phosphoserine	0.7 ± 0.3	0.4 ± 0.2**
α-aminoadipic acid	0.9 ± 0.4	1.0 ± 0.3	Sarcosine	2.5 ± 0.4	1.6 ± 0.3***
Asparaginate	61.9 ± 9.0	57.8 ± 7.4			

Mean ± SD for 16 runners completing the race. *Significance of the differences between pre- and post-race values (*p<0.05, **p<0.01, ***p<0.001), as analyzed by Wilcoxon Signed-Rank Test.

Table 4. Activities of 15 plasma steroid hormones before and after the UTG

Hormone (ng•mL ⁻¹)	Pre-race	Post-race	Hormone (ng•mL ⁻¹)	Pre-race	Post-race
Testosterone	4.7 ± 2.2	1.3 ± 1.4***	Aldosterone	0.10 ± 0.03	0.15 ± 0.04**
Progesterone	0.09 ± 0.06	0.05 ± 0.02**	Dehydroepian drosterone	2.2 ± 0.9	1.7 ± 0.8
17α-Hydroxyproge sterone	0.71 ± 0.24	0.32 ± 0.27**	Dehydroepian drosterone sulfate	1452.1 ± 658.0	2399.0 ± 1689.7*
11-Deoxycortisol	0.12 ± 0.07	0.08 ± 0.06	4-Androstenedio ne	0.94 ± 0.27	0.53 ± 0.24**
11-Deoxycorticost erone	0.02 ± 0.01	0.01 ± 0.01	Dihydrotestost erone	0.41 ± 0.57	0.28 ± 0.11
Cortisol	103.6 ± 42.1	188.5 ± 68.7**	Estrone	0.008 ± 0.004	0.02 ± 0.007**
Corticosterone	1.7 ± 1.3	3.4 ± 3.0	Estradiol	0.008 ± 0.006	0.010 ± 0.004
Cortisone	19.9 ± 4.6	19.4 ± 3.5			

Mean ± SD for 16 runners completing the race. *Significance of the differences between pre- and post-race values (*p<0.05, **p<0.01, ***p<0.001), as analyzed by Wilcoxon Signed-Rank Test.

Figure legends

Figure 1. Changes of CK, AST, ALT and LDH of each runner before (pre, grey), at midway (mid, red), and after the race (post, green).

Figure 2. Concentration values of alanine, valine, proline, ornithine, citrulline and testosterone before (pre, red) and after the race (post, green).

Figure 3. Spearman analysis for correlation between amino acids and biochemical markers.

Figure 4. Differences of changes of citrulline and ornithine after the race between *COL5A1* CC (n=6) and CT (n=10) genotype, as analyzed by Wilcoxon Signed-Rank Test ($p < 0.05$).

Figure 5. Relationship between material-energy flow, genetics and muscle damage. The upward and downward red arrows indicate that the concentration of the substance increases and decreases after the race, respectively, while dotted lines imply the changes of the marked compound were derived from existing studies. Green and yellow arrows represent regulating and driving factors, respectively, while the dashdotted lines mean the reasons come from our hypothesis.

Figures

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Amino Acids Changes and Muscle Damage During the 400 km Ultra Trail Gobi Race

Figures

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5