



## Exercise Biochemistry Review

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### An 8-week, low carbohydrate, high fat, ketogenic diet enhanced exercise capacity through improved ketolysis and lipolysis in mice

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**Objective** Carbohydrates, lipids and proteins are utilized both for energy production and structure of body. Among them, protein is the most important component of our body, carbohydrates and lipids are more flexible for energy supply system. Due to carbohydrate pitfall and lipid reserve abundance, coaches and elite athletes aspirate for an effective way to enhance fat utilization. Meanwhile, intramuscular triacylglycerol (IMTG) is a special way for skeletal muscle to store lipids. During exercise, IMTG may contribute up to 20% of total energy turnover, thus contribute significantly for ATP synthesis during exercise. However, abnormal or excessive fat deposition in skeletal muscle may induce insulin resistance as well. Intramuscular lipolysis regulation is crucial for energy supply system during exercise. It is reported by Amati and colleagues that well-trained athletes exhibit higher levels of IMTG and diacylglycerol (DAG) as well as well-preserved sensitivity to insulin, indicating lipolysis ability may be enhanced during exercise. In our previous study, we reported that an 8-week, a low carbohydrate, ketogenic diet increased running time till exhausted in male C57BL/6J mice, presuming the mechanism to be enhanced fat utilization. In the present study, we observed the alternation pattern of messenger RNAs related to lipid mobilization, fatty acid utilization and ketone body oxidation in muscle and adipose tissue immediately after exercise in both Type 1 and Type 2 muscle fibers.

#### 2. Materials and Methods

Male C57BL/6J mice (n = 35) were purchased from Takasugi Experimental Animals Supply (Kasukabe, Japan) at 7 weeks of age. All mice were randomly divided into four groups: chow diet (control: Con), including chow diet, sedentary (n = 8) and chow diet plus exercise (Con + Ex, n = 9), ketogenic diet (KD), including KD, sedentary, n = 9, and KD plus exercise (KD + Ex, n = 9) groups. A KD diet TP-201450 (consisting of 76.1% fat, 8.9% protein and 3.5% carbohydrate, 7.342 kcal/g) and a chow diet AIN93G (consisting of 7% fat, 17.8% protein and 64.3% carbohydrate, 3.601 kcal/g) wt/wt were obtained from Trophic (TROPIC Animal Feed High-tech Co., Ltd., Nantong, Jiangsu, China). Mice were maintained on *ad libitum* chow diet or KD.

Total RNA was extracted from the gastrocnemius muscle, soleus muscle and epididymal adipose tissue using the RNeasy Mini Kit or RNeasy Lipid Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. PCR was performed with the Fast 7500 real-time PCR system (Applied Biosystems) using the Fast SYBR® Green PCR Master Mix (Applied Biosystems).

Plasma IL-6 was measured using a R&D Mouse ELISA Duo set (R&D Systems, Minneapolis, USA) according to the manufacture's instructions. Plasma glycerol was measured using Glycerol Colorimetric Assay Kit (Cayman Chemical Co., Ann Arbor, MI, USA).

#### 3. Results and Discussion

##### 3.1 IL-6 concentration and exercise-induced myokine IL-6 mRNA alternation in both muscle fiber

IL-6 plays essential roles in immune responses. However, exercise induced IL-6 is reported to be able to stimulate lipolysis both in IMTG pool intramuscularly and adipocytes. Defined as exercise factor, or so-called myokines, muscle-derived IL-6 exhibits regulating function in various experiment

circumstances. Recombinant human IL-6 infusion showed an enhanced lipolysis and fat oxidation capacity in human subjects. Genetically IL-6 deficient mice presented a reduced ability on lipolysis and fatty acid oxidation. During KD administration, fat oxidation is no doubt the predominant, if not the only origin of energy, this make us to suspect IL-6 may be altered by acute exercise.

As shown in Figure 1, IL-6 mRNA increased rapidly, with a nearly 100-fold change in slow-twitch muscle fiber, and KD helped to this up-regulation. Transcription IL-6 level increased significantly in KD, compared to Con group subject, in soleus muscle, under the context of exhaustive exercise. This makes us to suspect that IL-6 may contribute to enhanced lipolysis and fatty acid mobilization. However, the effect is not observed in fast-twitch muscle. The result indicated that IL-6 mRNA expression exhibited a muscle fiber specification. Slow-twitch muscle fiber contributes more to endurance exercise, as fast-twitch muscle fiber mainly contributes to explosive strength and acceleration. The difference of fiber function leads to a different secretion mode of IL-6. The error bar is high in both exercise group. We observed an interesting phenomenon: the mice who quit at around 200 minutes has the highest IL-6 mRNA expression (gastrocnemius muscle) and plasma IL-6 concentration in both groups. One reason may be that, as the exercise taking on and gradually reach final fatigue, the call for fatty acid decreases with time.

As shown in Figure 2, both muscular IL-6 protein and plasma IL-6 were increased by exhaustive exercise. However, plasma IL-6 is significantly lower in the KD plus exercise group, though IL-6 rose nearly 5-fold in control feed group, it only rose to 2.5-fold in the KD group after exercise. For this phenomenon, the best explanation is that the well-adaption of lipid-centered metabolism, including metabolic flexibility and increased IMTG reservoir weakened the need to pull the trigger; this may also be the answer why KD mice had lower weight.

### *3.2 Fatty acid mobilation related RNA alternation after exhaustive exercise under endogenous ketosis in epididymal adipose tissue*

Adipose triglyceride lipase (ATGL) is also known as desnutrin in the first place, is a kind of lipase whose substance is patatin-like phospholipase domain-containing protein. Hormone-sensitive lipase (HSL) is also known as cholesteryl ester hydrolase (CEH), is another intracellular neutral lipase. ATGL and HSL cooperated to break apart fatty acids from TG, after which IMTG-origin fatty acid will be directly used for beta-oxidation, or lipid drop-origin fatty acids will be transported though lipoprotein shipping in the form of VLDL from adipose tissue into muscle fibers during exercise. As shown in Figure 3, mRNA expression levels of lipase were significantly enhanced by KD or exercise, indicating the up-regulated lipid mobilization and utilization ability is enhanced by exercise in adipocytes. However, KD plus exercise reversed this increase. One plausible explain for this phenomenon is the lack of plasma IL-6, thus the ability to mobilize fatty acid from adipose tissue is reduced. Adrenergic blocking agents are reported to harm fatty acid mobilization during fasting, and IL-6 is reported to function as adrenergic hormone.

Adipocyte-specific HSL deficiency mice present lowered submaximal exercise capacity. Our experiment design, the protocol for treadmill running is similar to a submaximal exercise. Under this circumstance, fat mobilization seems to be critical. Loss of this mobilizing ability, while exercise capacity is yet enhanced, makes us to suspect whether IMTG plays dominant role in this process.

### *3.3 Ketolytic RNA alternation after exhaustive exercise under conditions of endogenous ketosis in Type 1 and Type 2 muscle fiber*

Ketolysis is a complete oxidation of ketone bodies. Ketone bodies are utilized by mitochondria of extrahepatic tissues via a series of enzymatic reactions. Ketolysis is regulated by a rate-limiting enzyme 3-oxoacid CoA-transferase 1 (OXCT)-1 and hydroxybutyrate dehydrogenase (HBDH). Thus, we measured the transcriptional alternation of these enzymes in different muscle tissues. In our previous study, plasma ketone body (KB) increased rapidly in the sedentary KD group. However, after exhaustive exercise, blood KB of those KD mice dropped dramatically, while situation of blood KD in the Con mice showed a different figure. These results indicated that 1n 8-week KD administration has improved ketolysis, the ability for subjects to utilize KB. To investigate the mechanism of this enhancement, we assessed key enzymes in ketolysis in both fiber types.

As shown in Figure 4, gene expressions of these enzymes also present a fiber-specification. Since fast-twitch muscle fiber plays a second role in endurance exercise, exercise did not alter ketolytic enzymes in the transcriptional level, in gastrocnemius tissue. However, in the slow-twitch muscle fiber, it was changed. HBDH is up-regulated significantly in the case of KD plus exercise. Results here indicated that HBDH plays the key role in the improvement of exercise capacity by an 8-week KD.

### *3.4 Lipolysis- and fatty acid oxidation related RNA alternation after exhaustive exercise during endogenous ketosis in Type 1 and Type 2 muscle fiber*

After reaching working site, muscle lipoprotein lipase (LPL) hydrolyzes VLDL and harvests fatty acids at last, which will be finally utilized as primary fuel. Carnitine palmitoyl transferase (CPT)-1A, acyl-CoA oxidase (ACO), hydroxyacyl-coenzyme A dehydrogenase (HADH), medium chain acyl-CoA dehydrogenase (MCAD) and malonyl-CoA decarboxylase (MCD) are key regulating enzymes during fatty acid beta-oxidation. And for IMTG, the fatty acid is harvest by intramuscular lipase, ATGL and HSL. In an article published several years ago, the authors called adipose ATGL and HSL, “the mover and shaker of muscle lipolysis”. As shown in Figure 5, both ATGL and HSL mRNA expression are up-regulated by KD, but not by exhaustive exercise. Combined with results in the above part, enhanced mobilization of fatty acid intramuscularly is the main factor, but not the mobilization from adipocyte. LPL mRNA expression in gastrocnemius exhibited the same pattern of lipase mRNA synthesized by adipose tissue. Combined together, the reduced fatty mobilization from adipocyte, partly being the results of higher blood NEFA and TG, accompanied with enhanced fatty acid gain from IMTG pool, need for LPL was reduced.

As shown in Figure 6, in fast-twitch muscle fiber, CPT-1a, ACO and HADH mRNA expressions are enhanced by KD during exhaustive exercise. In slow-twitch muscle fiber, feed played as a main factor regulating fatty acid oxidation. CPT1a, MCAD and MCD mRNA expressions are enhanced. In summary, though tissue specific specificity were observed, overall ability of intramuscular fatty acid mobilization and fatty acid oxidation were enhanced by an 8-week KD feeding, thus contributed to exercise capacity. Compared to a glucose-centered metabolic system, a long-term KD feeding leads to establishment of a fatty acid oxidation-centered metabolic system. Metabolic flexibility is used as a term for the ability to adapt to conditional change in metabolic demand, and an 8-week KD helped established lipid-focused metabolic system through keto-adaption, thus increasing the metabolic flexibility. This is not a denial for the conception “glycogen loading” before competitions, while adequate KD meal may help our body to be more flexible during fuel choosing. Moderate training may enhance the ability to utilize ketone bodies as well as fatty acid, or to increase fatty acid mobilization from adipose tissue. Further investigation is urged to be carried out.

#### **4. Conclusions**

In the present study, we investigated how an 8-week KD remodeled adipose and muscle metabolic adaptation towards ketolysis, lipolysis and fatty acid oxidation under the circumstance of exhaustive exercise. Along with enhanced fatty acid oxidation capacity, KD also enhanced fatty acid mobilization capacity, ketolysis and lipolysis. These results revealed that an 8-week KD administration enhance exercise performance by up-regulated ketolytic and free fatty acid oxidation ability, indicating KD being a promising diet approach in athletes.