DOCTORAL THESIS

Metabolomics study of regulatory effects of exercise training on db/db type 2 diabetic mice

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Date of Award:
2018

ABSTRACT

Type 2 diabetes mellitus (T2DM) is mainly caused by genetic modifications and inappropriate life styles. The complexity of T2DM has brought us challenges for a comprehensive understanding of altered metabolic pathways that contributing to the development of T2DM. Therefore, a comprehensive metabolic analysis is needed. To date, taking regular exercise is a common and effective therapeutic way known to antagonize the metabolic disorders of T2DM. However, the regulatory effects of exercise on T2DM or T2DM-induced complications have not been clearly characterized.

We investigated the effect of physical activity on biochemical changes in diabetic *db/db* mice in plasma, urine, skeletal muscle, kidney and liver samples. Based on the techniques of liquid chromatography-high resolution Orbitrap mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), untargeted and targeted metabolomics studies were developed to delineate metabolic signatures in various kinds of biofluid and tissue samples. Targeted quantification methods on acylcarnitines and acyl-CoA were developed. Untargeted metabolomics analysis by GC-MS and LC-MS were also developed to draw a more comprehensive view of the metabolic changes in response to T2DM and exercise on *db/db* diabetic mice. The transcript expressions of mRNA in pathways of interest were measured to confirm the hypothesis.

Firstly, untargeted metabolomics study was carried on plasma samples to give a general view of exercise effect on *db/m+* control and *db/db* diabetic mice. A total of 24 differential metabolites were identified contributing to the metabolic differentiation of the 2 types of mice. The levels of three biomarkers, including creatine, uridine and lysine, were substantially reversed by exercise on *db/db* mice. Notably, the level of palmitoylcarnitine and pantothenic acid, which participate in FAO, were significantly

increased by exercise on *db/db* mice rather than on *db/m+* mice. The findings indicated that *db/db* diabetic mice may be more susceptible to exercise for energy expenditure. The results demonstrated that physical activity might have the ability to mitigate insulin resistance in T2DM mice through improving FAO and eliminating overloaded intermediate which contribute to insulin resistance.

Based on the findings from plasma sample, we noticed that acylcarnitines that play an important role in fatty acid β-oxidation (FAO) have been substantially changed between *db/m+* and *db/db* mice. In order to acquire more information of the disturbance of this pathway, a targeted quantification method of acylcarnitines was developed. Acylcarnitines exert a variety of biological functions depending on the differences in lengths, saturation levels and conjugation groups, which to a great extent contributes to the challenges of acylcarnitines quantifications due to the various kinds of isomers. Here, we describe a novel method by using high resolution parallel reaction monitoring (PRM) on LC-MS platform. Both reversed-phase and normal-phase columns were used in order to get accurate, reliable, widespread quantification of acylcarnitines, without tedious sample preparation procedure. The method provided the most comprehensive acylcarnitine profile with high resolution MS and MS/MS confirmation to date. A total of 117 acylcarnitines were detected from plasma and urine samples. The application of targeted profiling of acylcarnitines in *db/m+* control and *db/db* diabetic mice showed incomplete amino acid and fatty acid oxidation in diabetic mice. Interestingly, the reduction of medium odd-numbered chain acylcarnitines in urine samples was firstly observed between *db/m+* and *db/db* mice. The high resolution PRM method allowed monitoring the widespread metabolic changes of the acylcarnitines in response to stimuli. Besides, the accurate MS and MS/MS spectra data

of the 117 acylcarnitines could be used as mass spectrometric resources for the identification of acylcarnitines.

Skeletal muscle is the major tissue that responsible for insulin sensitivity. Any changes in skeletal muscle mass, responsiveness to hormones or metabolic rate could substantially influence the overall homeostasis of energy in the body. In Chapter 4, untargeted metabolomics analysis was performed to investigate the effect of regular exercise on *db/db* mice in skeletal muscle. Both LC-MS and GC-MS were carried out to monitor a wide range of regulated metabolites. Ninety-five metabolites were identified which contribute to the discrimination of *db/m+* control and *db/db* diabetic mice. The regulatory effects of exercise on these metabolites were mainly associated with attenuating the levels of long-chain fatty acids (C14 to C18) and medium- to long-chain acylcarnitines (C12 to C18), indicating that exercise might play a positive role in inhibiting the accumulation of excessive lipids, which contributed to insulin resistance. In addition, uric acid that is a risk factor for inflammation, cardiovascular complications, and fatty liver in diabetic patients, together with its intermediates (such as inosinic acid, hypoxanthine, *etc*.) were also substantially down regulated after exercise, indicating that exercise might also be protective against hyperuricemia related risks in T2DM. These findings revealed that regular exercise played a positive role in improving the efficiency of lipid metabolism and meanwhile enhancing uric acid clearance to prevent lipid accumulation in skeletal muscle, which might contribute to improved body fitness and body muscle composition.

In addition, the regulatory effect of exercise on diabetic nephropathy, a major complication of T2DM recognized to cause severe morbidity and mortality in diabetic patients, was also investigated in chapter 5. Untargeted and targeted metabolomics studies based on GC-MS and LC-MS were performed. The results demonstrated that

exercise exhibited beneficial effect in reducing hyperlipidemia, expression levels of inflammatory markers (TNFα, IL-6 and COX2) and fibrosis markers (Collagen 1), and alleviated DN induced mesangial expansion in kidneys of diabetic mice. The results of metabolic changes in kidney of DN mice revealed that the accumulation of acyl-CoA, phospholipids and hydroxylated acylcarnitines were substantially ameliorated by exercise, and the reduction of important enzymes *CTP1α* and *Acadl* in FAO were partially reversed. In addition, branched-chain amino acids (BCAA) metabolism that positively related to inflammation (TNFα) was down-regulated in diabetic mice. However, exercise up-regulated BCAA catabolism via reducing the level of leucine and increasing the expression of *Acad8*, *Bcat2*, *Hibch* and *Hmgcl* in this pathway. Furthermore, the accumulation of uric acid, which contributes to inflammation and tubulointerstitial fibrosis in kidney disease, together with its six precursors (guanosine,succinyladenosine, inosine, guanine and xanthine) were substantially reduced. The above results demonstrated that exercise might play beneficial effect in alleviating lipotoxicity through improving FAO efficiency in diabetic kidneys. In addition, exercise also ameliorated diabetic induced inflammation and fibrosis via promoting BCAA catabolism and accelerated the elimination of uric acid.

Together, mass spectrometry-based metabolomics study is a powerful tool to investigate the regulatory effect of exercise on complex metabolic diseases. The obtained results in this study may provide informative insights into the underlying the mechanism of exercise on T2DM and T2DM-induced complications.

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