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Polyol accumulation in muscle and liver in a mouse model of type 2 diabetes

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ABSTRACT

Aims: Type 2 diabetes (T2D) is a complex metabolic disease leading to complications in multiple organs. Diabetic myopathy and liver disease are common complications of T2D, but are incompletely understood. To gain insight into the pathogenesis of these conditions we performed metabolomic analysis of skeletal muscle and liver in a mouse model of T2D.

Methods: Tissue metabolomics were performed by GC/MS and LC/MS of the skeletal muscle and liver in the MKR mouse model of T2D, compared with control mice. MKR mice were treated with the β-3 adrenergic receptor agonist, CL-316,243 to determine metabolite changes after correcting hyperglycemia.

Results: Blood glucose was higher in MKR vs WT mice, and normalized with CL-316,243 treatment. Compared with WT mice, MKR mice had 2.5 fold higher concentrations of sorbitol and 1.7 fold lower concentrations of reduced glutathione in skeletal muscle. In liver, MKR mice had 2 fold higher concentrations of the pentitol ribitol. CL-316,243 treatment normalized sorbitol and ribitol concentrations in MKR skeletal muscle and liver, respectively to the levels of the WT mice.

Conclusions: These results demonstrate tissue-specific accumulation of polyols in a mouse model of T2D and provide novel insights into the pathogenesis of myopathy and liver disease in T2D.

1. Introduction

Type 2 diabetes (T2D) and its complications cause significant morbidity and premature mortality in patients, and major economic costs to the healthcare system. The molecular mechanisms that underlie T2D and many of its complications are incompletely understood. Therefore, developing a greater understanding of T2D and its complications would greatly benefit individual patients and the healthcare system. Metabolomic studies in humans with diabetes have largely focused on plasma and urine metabolites (Bain et al., 2009; Menni et al., 2013; Palmer et al., 2015). These studies have aimed to identify biomarkers of the disease or its complications, and have revealed novel changes in metabolites in those with diabetes (Bain et al., 2009; Palmer et al., 2015). However, as plasma metabolite levels frequently do not reflect concentrations in specific tissues (Schooneman, Achterkamp, Argmann, Soeters, & Houten, 2014), it is difficult to know how the circulating metabolite profile reflects the changes in tissues that may contribute to the pathogenesis or complications of diabetes.

Both type 1 (T1D) and T2D negatively impact skeletal muscle health and may lead to diabetic myopathy, characterized by reduced physical capacity, strength and muscle mass. Murine models of T1D and T2D display skeletal muscle dysfunction, impaired muscle growth, development and regeneration, reduced muscle mass and myofiber size and mitochondrial content, and metabolic inflexibility (D'Souza, Al-Sajee, & Hawke, 2013; Kim et al., 2006; Nguyen, Cheng, & Koh, 2011). The decrease in regeneration is associated with decreased stem cell proliferation and/or migration into the damaged muscle regions (D'Souza et al., 2013). Diabetic myopathy correlates with the other complications of diabetes, however the mechanisms underlying diabetic myopathy are incompletely understood (Aragno et al., 2004).

Individuals with T2D also have high rates of liver disease, which includes non-alcoholic fatty liver disease (NAFLD), hepatic cirrhosis and carcinoma (Leite, Villela-Nogueira, Cardoso, & Salles, 2014). Diabetes is a major contributor to end stage liver disease (Bugianesi, Vanni, & Marchesini, 2007). Developing a greater insight into the metabolite changes that occur in diabetic liver disease will advance the understanding of the development of liver disease in T2D. Additionally, a major challenge to the diagnosis of NAFLD is to identify non-invasive tests that may diagnose the disease prior to the

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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development of advanced fibrosis or cirrhosis, to allow for timely monitoring and therapeutic intervention to prevent or treat progressive fibrosis, cirrhosis and cancer.

The aim of our present study was to perform metabolomic analysis of skeletal muscle and liver tissues in a mouse model of T2D, to identify novel tissue specific metabolite changes that occur in the setting of T2D, which may contribute to diabetic myopathy and liver disease. In addition, we treated the mice with a β3-adrenergic receptor agonist, CL-316,243, that we have previously found to normalize the hyperglycemia in the diabetic mice in order to determine if the treatment of T2D would normalize the identified metabolite changes. We used a well-characterized mouse model of T2D, the MKR mouse (Fernández et al., 2001). Here, we discovered tissue specific differences in polyols in the MKR mice compared with wild-type (WT) mice, that were reversed upon treatment with CL-316,243, and provide novel insights into the pathogenesis of diabetic myopathy, and liver disease in T2D. The changes we have identified in skeletal muscle of our rodent model are consistent with limited cadaveric human studies that have previously been performed (Vander Jagt, Robinson, Taylor, & Hunsaker, 1990) and suggest that aldose reductase inhibitors may provide clinical benefit to individuals with diabetic myopathy. The identification of a specific polyol, ribitol, in the liver of the diabetic mice may provide a novel marker of hepatic disease in those with diabetes, or provide new insight into the mechanisms of hepatic dysfunction in T2D.

2. Materials and methods

2.1. Animal care and treatment

Animal studies were approved by the Icahn School of Medicine at Mount Sinai School of Institutional Animal Care and Use Committee (IACUC). Mice were housed in Icahn School of Medicine at Mount Sinai Center for Comparative Medicine and Surgery, Association for Assessment and Accreditation of Laboratory Animal care International (AALAC) and Office of Laboratory Animal Welfare (OLAW) accredited facility, where animal care and maintenance were provided. Mice were kept on a 12 h light/dark cycle, had free access to diet (Picolab Rodent Diet 20, 5053) and fresh water. All MKR and WT mice used in these studies were male, on the Friend Virus B/National Institutes of Health (FVB/N) background and were 10–16 weeks of age. The generation and characterization of MKR mice, including their skeletal muscle and hepatic phenotype has been previously described (Fernández et al., 2001). Male MKR mice have many other abnormalities associated with T2D, including hepatic steatosis and skeletal muscle dysfunction (Fernández et al., 2001; Kim et al., 2006). Skeletal muscle changes include a decreased cross-sectional area, the accumulation of myocellular lipids, decreased response to exercise training and impaired skeletal muscle regeneration after injury when compared with control mice (Heron-Milhavet, Mamaeva, LeRoith, Lamb, & Fernandez, 2010; Kawashima et al., 2009; Kim et al., 2006; Mallipattu et al., 2014). Non-fasted mice were euthanized at the end of each experiment. Liver and quadriceps (skeletal muscle) were collected and flash frozen in liquid nitrogen for subsequent metabolomic experiments.

Nine to ten week old male WT and MKR mice were injected intraperitoneally with CL-316,243 (1 mg/kg BW/day) or with an equivalent volume vehicle (sterile phosphate buffered saline) for three weeks. Body weight was measured before treatment and weekly during treatment. Body composition analysis was performed using the EchoMRI 3-in-1 NMR system (Echo Medical Systems, Houston, TX, USA) before treatment, and at the end of treatment. Non-fasting blood glucose measurements were performed on tail vein whole blood using a Bayer Contour Glucometer (Bayer Healthcare, Mishawaka, IN, USA), prior to commencing treatment and weekly thereafter. Serum or plasma collected from the retro-orbital sinus were used to measure circulating insulin (Mercodia, Uppsala, Sweden) and triglycerides (TG) (Pointe Scientific, Canton, MI).

2.2. Metabolomic study

Five biological replicates from MKR vehicle treated, WT vehicle treated, and CL-316,243 treated MKR mice from skeletal muscle and liver tissues were shipped overnight on dry ice to Metabolon (Durham, NC, USA). At the time of analysis samples were extracted and prepared for analysis using Metabolon's standard solvent extraction method. The extracted samples were split into equal parts for analysis on the gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) platforms. Also included were several technical replicate samples created from a homogeneous pool containing a small amount of all study samples ("Client Matrix"). The sample preparation process was carried out using the automated MicroLab STAR® system from Hamilton Company. Recovery standards were added prior to the first step in the extraction process for quality control (QC) purposes. Sample preparation was conducted using a proprietary series of organic and aqueous extractions to remove the protein fraction while allowing maximum recovery of small molecules. The resulting extract was divided into two fractions; one for analysis by LC and one for analysis by GC. Samples were placed briefly on a TurboVap® (Zymark, Hopkinton, MA) to remove the organic solvent. Each sample was then frozen and dried under vacuum. Samples were then prepared for the appropriate instrument, either LC/MS or GC/MS. For quality assurance (QA)/QC purposes, a number of additional samples were included with each day's analysis. Furthermore, a selection of QC compounds was added to every sample, including those under test. These compounds were carefully chosen so as not to interfere with the measurement of the endogenous compounds. The LC/MS portion of the platform was based on a Waters ACQUITY UPLC and a Thermo-Finnigan LTQ mass spectrometer, which consisted of an electrospray ionization (ESI) source and linear ion-trap (LIT) mass analyzer. The sample extract was split into two aliquots, dried, then reconstituted in acidic or basic LC-compatible solvents, each of which contained 11 or more injection standards at fixed concentrations. One aliquot was analyzed using acidic positive ion optimized conditions and the other using basic negative ion optimized conditions in two independent injections using separate dedicated columns. Extracts reconstituted in acidic conditions were gradient eluted using water and methanol both containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5 mM ammonium bicarbonate. The MS analysis alternated between MS and data-dependent MS^2 scans using dynamic exclusion. The samples destined for GC/MS analysis were re-dried under vacuum desiccation for a minimum of 24 h prior to being derivatized under dried nitrogen using bistrimethyl-silyl-triflouroacetamide (BSTFA). The GC column was 5% phenyl and the temperature ramp is from 40° to 300 ° C in a 16 minute period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. The instrument was tuned and calibrated for mass resolution and mass accuracy on a daily basis. The information output from the raw data files was automatically extracted as discussed below.

Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Identification of known chemical entities was based on comparison to metabolomic library entries of purified standards. As of this writing, more than 1000 commercially available purified standard compounds had been acquired and registered into LIMS for distribution to both the LC and GC platforms for determination of their analytical characteristics. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific compound or an isobaric entity. Additional entities could be identified by virtue of their recurrent

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