Original article

MOLECULAR METABOLISM

Cardiomyocyte-specific deficiency of ketone body metabolism promotes accelerated pathological remodeling

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ABSTRACT

Objective: Exploitation of protective metabolic pathways within injured myocardium still remains an unclarified therapeutic target in heart disease. Moreover, while the roles of altered fatty acid and glucose metabolism in the failing heart have been explored, the influence of highly dynamic and nutritionally modifiable ketone body metabolism in the regulation of myocardial substrate utilization, mitochondrial bioenergetics, reactive oxygen species (ROS) generation, and hemodynamic response to injury remains undefined.

Methods: Here we use mice that lack the enzyme required for terminal oxidation of ketone bodies, succinyl-CoA:3-oxoacid CoA transferase (SCOT) to determine the role of ketone body oxidation in the myocardial injury response. Tracer delivery in *ex vivo* perfused hearts coupled to NMR spectroscopy, *in vivo* high-resolution echocardiographic quantification of cardiac hemodynamics in nutritionally and surgically modified mice, and cellular and molecular measurements of energetic and oxidative stress responses are performed.

Results: While germline SCOT-knockout (KO) mice die in the early postnatal period, adult mice with cardiomyocyte-specific loss of SCOT (SCOT-Heart-KO) remarkably exhibit no overt metabolic abnormalities, and no differences in left ventricular mass or impairments of systolic function during periods of ketosis, including fasting and adherence to a ketogenic diet. Myocardial fatty acid oxidation is increased when ketones are delivered but cannot be oxidized. To determine the role of ketone body oxidation in the remodeling ventricle, we induced pressure overload injury by performing transverse aortic constriction (TAC) surgery in SCOT-Heart-KO and αMHC-Cre control mice. While TAC increased left ventricular mass equally in both groups, at four weeks post-TAC, myocardial ROS abundance was increased in myocardium of SCOT-Heart-KO mice, and mitochondria and myofilaments were ultrastructurally disordered. Eight weeks post-TAC, left ventricular volume was markedly increased and ejection fraction was decreased in SCOT-Heart-KO mice, while these parameters remained normal in hearts of control animals.

Conclusions: These studies demonstrate the ability of myocardial ketone metabolism to coordinate the myocardial response to pressure overload, and suggest that the oxidation of ketone bodies may be an important contributor to free radical homeostasis and hemodynamic preservation in the injured heart.

Keywords Myocardial ketone body metabolism; Nuclear magnetic resonance (NMR) measurement of substrate metabolism; Ventricular remodeling; Oxidative stress; Mitochondrial metabolism

1. INTRODUCTION

During evolution of adverse ventricular remodeling that culminates in cardiomyopathy and congestive heart failure, myocardial fuel utilization becomes inefficient and inflexible, and mechanistic studies in pre-clinical models indicate that altered substrate and energy metabolism can cause cardiomyopathy [1—5]. Therefore, deeper understanding and ultimately, judicious nutritional and pharmacological manipulation of myocardial metabolism are expected to improve morbidity and mortality attributable to heart failure [6,7]. While the roles of the primary myocardial fuels, fatty acids and glucose, in myocardial homeostasis and disease have been explored extensively, the influence of myocardial ketone bodies, considered a secondary substrate class due to their low circulating concentrations in fed states, remains ill-defined. However, ketone bodies are highly competitive substrates for myocardial oxidative metabolism, and ketone bodies

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circulate at increased concentrations in the setting of heart failure, at concentrations directly proportional to severity of the cardiomyopathy [8–10].

The liver is the source of the circulating ketone bodies D-β-hydroxybutyrate (D-βOHB) and acetoacetate (AcAc), whose myocardial oxidation requires the mitochondrial matrix enzyme succinyl-CoA:3-oxoacid-CoA transferase (SCOT, encoded by nuclear *Oxct1*), which as the only CoA transferase encoded by the mammalian genome, uniquely catalyzes a near-equilibrium reaction that transfers coenzyme A (CoA) between AcAc and succinate (see Ref. [11] for a recent review on integrated ketone body metabolism). Congenital and ubiquitous absence of SCOT causes significant morbidity in humans [12], and is incompatible with neonatal life in mice [13]. SCOT is absolutely required for terminal oxidation of ketone bodies [13], and through its ability to influence succinate metabolism, the reaction it catalyzes directly interfaces with the tricarboxylic acid (TCA) cycle and the electron transport chain [11]. Consequently its ability to influence myocardial bioenergetics may transcend physiological states considered 'ketotic'.

The heart is the highest ketone body consumer per unit mass, oxidizing ketone bodies in proportion to their delivery at the expense of fatty acid oxidation and glucose oxidation [14–19]. Compared with fatty acid oxidation, ketone bodies are more energetically efficient, yielding more energy available for ATP synthesis per molecule of oxygen invested [20–22]. Furthermore, the oxidation of ketone bodies may attenuate ROS production associated with the oxidation of fatty acids [21–23] suggesting that myocardial ketone body oxidation could protect against injury and adverse ventricular remodeling responses, which promote the development of cardiomyopathy and heart failure [24]. Indeed, clinical data suggest that myocardial ketone body metabolism may serve as an important therapeutic target. At least a subset of patients diagnosed with SCOT-deficiency exhibited dilated cardiomyopathy on presentation [25–28]. Moreover, extraction of delivered ketone bodies is maintained in failing hearts, but not skeletal muscle, of humans with advanced heart failure [29]. Similarly, the contribution of ketone bodies to cardiac energy metabolism may be elevated in patients with dilated and hypertrophic cardiomyopathies [30]. These results suggest that the diminution of myocardial fatty acid oxidation that typically occurs during the evolution of advanced cardiomyopathy may not be coupled to reduction of ketone body oxidation [1,2,5]. It is unclear whether maintained, or even elevated, utilization of ketone bodies preserves cardiac function, is a pathologic consequence, or is an innocent bystander in the adversely remodeling heart.

Therefore, mechanistic studies using tissue-selective genetic rodent models will begin to establish when and whether myocardial ketone body metabolism may be protective or deleterious to the heart. Here, we sought to investigate the role of ketone utilization in the heart during periods of myocardial stress by using mice with cardiomyocyte-specific loss of SCOT (SCOT-Heart-KO) to determine the physiological and pathological responses of myocardium lacking the ability to utilize ketones as an energy source.

2. MATERIALS AND METHODS

2.1. Animals and diets

The Animal Studies Committee at Washington University approved all experiments prior to their performance. SCOT-Heart-KO mice were generated by successive generations of breeding of *Oxct1*$^{flox/flox}$ mice to mice expressing Cre recombinase under control of the alpha myosin heavy chain promoter (αMHC-Cre, Jackson Laboratory, stock number 011038) [31]. SCOT-Heart-KO and αMHC-Cre control mice (male mice were studied) were maintained for at least ten generations on a C57BL/6N × C57BL/6J hybrid substrain background. A few experiments were performed using a tamoxifen inducible SCOT-Heart-KO strain that was generated by breeding *Oxct1*$^{flox/flox}$ mice to mice expressing tamoxifen inducible Cre recombinase under control of the αMHC promoter (MerCreMer, Jackson Laboratory, stock number 005657). To induce knockdown of SCOT in the cardiomyocytes of adult mice, tamoxifen (20 mg/kg) was administered intraperitoneally for 21 d. Mice were maintained on Lab Diet (5053) *ad libitum* and autoclaved water on cedar chip bedding at 22 °C. Lights were off between 1800 and 0600. Food was removed and fresh bedding was provided at the onset of fasting for mice fasted for 24 h; water was provided *ad libitum* to fasting animals. For some studies, mice were maintained on a rodent ketogenic diet (Harlan-Teklad TD.110633), which was described previously in Ref. [32] and is comprised of 94.1% fat, 4.6% protein, and 1.3% carbohydrate. The contributing fat sources are lard and milkfat; casein is the protein source, and sucrose is the minimal carbohydrate source; the diet is deficient in choline. A cohort of mice was also maintained on a 40% fat diet (Harlan-Teklad TD.110290), which is comprised of 40.7% fat, 40.3% carbohydrate, and 19% protein, in which lard and milkfat are the fat sources, casein is the protein source, and sucrose (18.3% by mass) and cornstarch are the carbohydrate sources.

2.2. Metabolite and insulin measurements

Serum samples were acquired from animals that had been fasted for 4 h on fresh cedar chip bedding. Serum glucose, D-βOHB, total ketone bodies (TKB, D-βOHB plus AcAc), triacylglycerols (TAG), and insulin concentrations were measured as previously described in Ref. [33].

2.3. Langendorff heart perfusions

Mouse heart perfusions were performed as previously described in Ref. [33]. Briefly, SCOT-Heart-KO and αMHC-Cre mice received 100 units of heparin by intraperitoneal injection and 10 min later were anesthetized with an intraperitoneal injection of 390 mg/kg sodium pentobarbital. Hearts were excised and placed in ice-cold Krebs-Henseleit (KH) bicarbonate solution (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 0.4 mM KH_2PO_4, 2.5 mM $CaCl_2$, pH 7.4) supplemented with 5 mM glucose. Hearts were cannulated *via* the aorta and then perfused in the Langendorff mode at a constant pressure of 60 mmHg, with continuous bubbling of a 95% O_2/5% CO_2 gas mixture into the KH-bicarbonate buffer with 3% fatty acid free bovine serum albumin (BSA). Experimental perfusion conditions are described in the legends for Figures 1 and 2. Insulin used for all perfusions was human insulin (rDNA origin; Lilly).

2.4. NMR spectroscopy of myocardial extracts

After 20 min of perfusion, freeze-clamped (liquid N_2-cooled tongs) hearts were homogenized in 3.6% perchloric acid, followed by removal of precipitated debris and pH neutralization of the supernatant with KOH. Neutralized perchloric acid tissue extracts were dissolved in 310 μL of D_2O (Cambridge Isotope Laboratories) spiked with 1 mM trimethylsilyl propionate (TSP), which provides a chemical shift reference and an internal concentration standard to quantify extract metabolite concentrations. Extracts were transferred into thin-walled 5 mm NMR tubes (Shigemi) and profiled using ^{13}C-edited proton nuclear magnetic resonance (NMR) measured at 11.75 T (Varian/Agilent Direct Drive-1) *via* first increment gradient heteronuclear single-quantum correlation (gHSQC) as previously described in Refs. [13,31,33,34], obtaining integrals for carbon #4 of glutamate, carbon #2 of taurine, carbon #3 of lactate, carbons #2/#3 of succinate, carbon

