Basic Science and Experimental Studies

Triheptanoin Alleviates Ventricular Hypertrophy and Improves Myocardial Glucose Oxidation in Rats With Pressure Overload

T. DUNG NGUYEN, MD,[1] YASUSHIGE SHINGU, MD,[2] PAULO A. AMORIM, MD,[1] MICHAEL SCHWARZER, PhD,[1] AND TORSTEN DOENST, MD[1]

Jena, Germany; and Sapporo, Hokkaido, Japan

ABSTRACT

Objective: Cardiac hypertrophy is characterized by changes in substrate utilization and activity of the Krebs cycle. We assessed the effects of triheptanoin, an odd-chain fat that might support the Krebs cycle, on cardiac metabolism and function in a model of cardiac hypertrophy.

Methods and Results: Rats were subjected to aortic banding (AoB) to induce pressure overload (PO). Starting at 1 week after AoB, rats were blindly fed a control diet or a special diet containing triheptanoin at 7% (T7 group) or 30% (T30 group) of total energy value. Six weeks after AoB, echocardiography revealed attenuated hypertrophy and improved diastolic function of the left ventricle. Isolated working heart perfusion showed similar cardiac power, fatty acid oxidation, substrate preference, and insulin response among groups. However, cardiac glucose oxidation (GO) was increased in the T30 group compared with the T7 and control groups. Blood levels of the odd-chain ketone body beta-hydroxypentanoate confirmed adequate bioavailability of triheptanoin. Importantly, they were directly proportional to cardiac GO.

Conclusions: Treatment with triheptanoin-enriched diet reduces ventricular hypertrophy and improves diastolic function in rats with PO, which is associated with enhanced cardiac GO. The results suggest targeting supplementation of the Krebs cycle to approach ventricular and metabolic remodeling in cardiac hypertrophy. (*J Cardiac Fail 2015;21:906–915*)

Key Words: Triheptanoin, anaplerosis, cardiac hypertrophy, cardiac metabolism.

Krebs cycle intermediates are dynamically channeled into numerous cellular processes and therefore need to be supplemented to maintain optimal activity of the cycle. The continuous replenishment of the Krebs cycle through various metabolic pathways is a critical process that is generally termed anaplerosis.[1] In the heart, impairment of anaplerosis may rapidly cause contractile dysfunction.[2,3] A number of anaplerotic pathways have been identified. These include the carboxylation of pyruvate to oxaloacetate or malate, the formation of α-ketoglutarate from glutamate, of succinyl–coenzyme A (CoA) from propionyl-CoA, and of fumarate through the purine nucleotide cycle.[4]

Altered anaplerosis has been identified in some models of heart disease. In cardiac hypertrophy induced by pressure overload, there is consistent evidence suggesting increased anaplerotic flux of pyruvate through nicotinamide adenine dinucleotide phosphate (NADP)–malic enzyme.[5–7] However, the role of this change for metabolism and function of the hypertrophied heart remains obscure, partly because of the high technical challenge in assessing specific anaplerotic pathways.[4] Although approaches modulating cardiac anaplerosis may also provide insight into the functional relevance of anaplerotic changes, studies focusing on short-term effects of anaplerotic treatments have delivered neutral results.[8,9]

Triheptanoin is a triglyceride containing 3 molecules of the 7-carbon fatty acid heptanoate (C7:0). In contrast to even-chain fatty acids, oxidation of heptanoate in the

From the [1]Department of Cardiothoracic Surgery, Jena University Hospital, Friedrich Schiller University Jena, Jena, Germany and [2]Department of Cardiovascular and Thoracic Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan.

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Reprint requests: Torsten Doenst, MD, Department of Cardiothoracic Surgery, Friedrich Schiller University Jena, Erlanger Allee 101, 07747 Jena, Germany. Tel: +49-3641-932 29 01; Fax: +49-3641-932 29 02. E-mail: doenst@med.uni-jena.de

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mitochondria delivers not only acetyl-CoA but also propionyl-CoA, with the latter exerting anaplerotic effect by entering the Krebs cycle as succinyl-CoA. In the liver, propionyl-CoA derived from oxidation of heptanoate can be converted to the C5-ketone body β-hydroxypentanoate (BHP) which reenters the circulation and provides other tissues with acetyl-CoA and propionyl-CoA.[10] Because of the hepatic passage and metabolism of heptanoate, enteral administration of triheptanoin exerts anaplerotic effect mostly through C5-ketone bodies.[11] The plasma levels of BHP may therefore be used to monitor dietary treatment with triheptanoin.

In view of the characteristic changes of anaplerosis in the hypertrophied heart and the anaplerotic potential of triheptanoin, we hypothesized that long-term treatment with a triheptanoin-enriched diet may affect cardiac metabolism and function in rats subjected to pressure overload (PO)–induced hypertrophy. We found that triheptanoin treatment reduced ventricular hypertrophy and improved diastolic function in rats with PO, which was associated with enhanced cardiac glucose oxidation (GO).

At 1st glance, the rationale of the study may appear to be counterintuitive by suggesting that increasing a process that is already elevated in a pathologic state would yield benefit. However, it is important to note that anaplerosis per se is neither good nor bad. Anaplerosis may occur through various pathways, each of which interacts with cellular processes differently. Therefore, the functional outcome of modulating anaplerosis always depends on the specific anaplerotic pathways that are changed.

Methods

Animals

Male Sprague-Dawley rats were obtained from Charles River (Sulzfeld, Germany) and housed under a light-dark cycle of 12 hours at 21°C with free access to food and water. All animals received humane care, and the experimental protocols were approved by the responsible Institutional Animal Care and Use Committee.

Experimental Design

Three-week-old rats were subjected to aortic banding (AoB) to induce PO. At 1 week after AoB, when full recovery was achieved, standard chow was removed and the rats were randomly assigned to a treatment group that received a control diet or a diet with either 7% or 30% of calories from triheptanoin. The diets were differently colored and their identities were unknown to all experimenters during the treatment and data acquisition. At 6 weeks after AoB, we performed experiments and collected blood samples. After finalizing data acquisition, blood concentrations of the C5-ketone body BHP were measured, leading to unblinding.

Surgical Procedures

Our surgical protocol for the induction of hypertrophy and heart failure in rats has been described in detail earlier.[12] Briefly, 3-week-old rats were anesthetized and ventilated with room air during the operation. After partial sternotomy and removal of the thymus, a titanium clip was placed around the aortic arch between the brachiocephalic trunk and the left carotid artery. The clip had a remaining opening of 0.35 mm, causing progressively increased afterload.

Diets

All diets were obtained from Ssniff (Soest, Germany). Triheptanoin oil for the production of the treatment diets was provided by Braun (Melsungen, Germany). Soybean oil represented the source of other fatty acids in the diets. The amounts of vitamins, minerals, protein, and essential amino acids matched those of the standard chow and were equal among all diets. See Table 1 for detailed compositions of the diets.

Echocardiographic Assessments

We previously described this method in detail.[13] Briefly, 2-dimensional short-axis views of the left ventricle at the level of the papillary muscle were obtained. M-Mode measurements included anterior wall thickness in diastole and systole (AWd and AWs), left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), and posterior wall thickness in diastole and systole (PWd and PWs).

Left ventricular mass index (LVMI) was used to assess ventricular hypertrophy and was calculated from M-mode measurements and body weight (BW) with the use of the following formula:

$$\text{LVMI (g/kg)} = \frac{\left(1.05\ \left[(\text{LVEDD} + \text{PWd} + \text{AWd})^3 - \text{LVEDD}^3\right] \times 0.8\right)}{\text{BW}}$$

Endocardial fractional shortening (eFS) was used to evaluate systolic function and was calculated as follows:

$$\text{eFS (\%)} = \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD} \times 100}$$

Stroke volume (SV) and cardiac output (CO) were calculated as follows:

$$\text{SV (mL)} = (\text{flow velocity integral}) \times (\text{cross} - \text{section area of LV outflow tract})$$

$$\text{CO (mL/min)} = \text{SV} \times \text{heart rate}$$

CO and SV indices were defined as the ratio to body weight.

Mitral inflow pattern was assessed from the apical 4-chamber view with the use of pulsed-wave Doppler and the sample volume placed at the mitral leaflet tips. Mitral inflow Doppler measurements included early (E) and late (A) diastolic peak velocities, deceleration time of E (DT), and isovolumic relaxation time (IVRT). Derived parameters of mitral inflow Doppler involved the ratios E/A and E/DT.

Tissue Doppler assessments were conducted from the apical 4-chamber view with the sample volume placed at the septal insertion site of the mitral leaflet. We measured the early (e') and late (a') diastolic peak velocities as well as the systolic (s') peak velocity of the septal mitral annulus. Derived parameters of tissue Doppler assessments included the ratios E/e' and e'/a'.

All values were averaged from 3 consecutive measurements.