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Mitochondrial Swelling Induced by Redox Imbalance Affects more Liver than Kidney in a Genetic Model of Obese Rat

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Abstract

The aim of this study was to evaluate the redox status and mitochondrial function in renal and hepatic tissues (homogenate and isolated mitochondria) of a genetically obese rat. Ten adult male rats were distributed into Zucker (fa-/fa-, n=5) and lean Zucker (fa+/fa-, n=5). Osmotic swelling, reactive oxygen species (ROS), oxidation of thiols and reduced glutathione (GSH) were measured in of renal and hepatic tissues of lean and Zucker rats. The kidney mitochondrial swelling was increased in the obese vs. lean in all concentrations, but it was significant only in Ca^{+2} (10, 25 or 50 μM); the same was observed in the liver mitochondrial swelling. ROS were significantly increased in liver mitochondria of obese vs. lean, but with no significance statistical in kidney mitochondria. Thiols levels in liver and kidney mitochondrial and homogenate of obese rats did not show statistical differences vs. lean, except in liver homogenate. Obese presented significant reduction of mitochondrial and homogenate GSH levels in kidney and liver vs. lean. These findings demonstrated that obese had higher thiols and ROS and, lower GSH levels, which contributed to mitochondrial dysfunction significant in kidney and liver vs. lean rats. Mitochondrial oxidative stress between obese and lean rats showed difference only in liver; however, the antioxidant system was lower in obese than lean in both, kidney and liver. The results showed that obesity promotes redox imbalance, contributing to mitochondrial dysfunction in both, kidney and liver, being the liver more susceptible than kidney to mitochondria permeabilization, which can be linked to cell death and injury in tissue-specific mitochondrial obesity.

1. Introduction

The obesity is a worldwide epidemic that affects both developed and developing

countries and promotes considerable risks to health [1].

Obesity is characterized as a chronic inflammatory disease associated to insulin resistance. Besides promoting cardiovascular, hepatic and renal alterations, the accumulation of adipose tissue generates an increased renin-angiotensin-aldosterone system activity, increased oxidative stress and mitochondrial dysfunction [2], [3].

In recent years, attention was drawn to the role of mitochondria as an efficient regulator of cytosolic calcium signals. Several studies with mitochondria targeted calcium probes indicate a dramatic and rapid increase in free intra mitochondrial calcium and it has been shown that an uptake by mitochondria has a big effect on the metabolic state of the cells as it can up regulate the activity of the enzymes in oxidative metabolism [4].

Altered calcium homeostasis and calcium signaling pathway activation are potential mechanisms that account for an interrelated toxic pathway for oxidative stress and mitochondrial dysfunction [4]. The latter plays an important role in the etiology of heart failure, myocardial infarction, type 2 diabetes, Alzheimer's, atherosclerosis, Parkinson's, endothelial dysfunction and nephropathy [5-10]. Changes in mitochondrial respiration, membrane permeability transition, ROS generation, lipid peroxidation and antioxidant defense characterize mitochondrial dysfunction [11]. These changes are widely studied in cardiac tissue, but little is known about the condition of tissue-specific mitochondria in obesity.

The kidney disease and diabetic kidney disease have great correlations with the development of cardiovascular dysfunction and mortality. Kidney proximal tubule cells and cardiomyocytes are mitochondrially rich which facilitates glucose recycling and muscle contractility, respectively. This results in susceptibility to mitochondrial dysfunction leading to increase in ROS generation [8].

Thus, the aim of this study was to evaluate the kidney and liver mitochondrial function of Zucker rats through cellular respiration, membrane potential, osmotic swelling, oxidative stress and antioxidant defense.

2. Material and Methods

2.1. Animals

Ten adult male rats with 10 weeks of age were maintained in the Animal Housing of Nephrology Division at a temperature of $22 \pm 1°C$ and at a light–dark cycle of 12/12 h, beginning at 6:00 am. The animals had free access to standard chow (Nuvilab, PR, Brazil) and water. These animals were distributed into obese (Zucker rats strain with a mutation in the gene fa^-/fa^- and obese phenotype, n=5) and lean rats (lean Zucker rats strain, heterozygous fa^+/fa^- phenotype and eutrophic, n=5). All protocols were approved by the Ethics Committee in Research of Universidade Federal de Sao Paulo under#1853/11 and all efforts were made to minimize animal suffering and reduce the number of animals used.

2.2. Isolation of Mitochondria

Kidney and liver mitochondria were isolated using conventional differential centrifugation [12]. The tissue was homogenized in 250 mM sucrose, 1 mM EGTA, and 10 mM HEPES-KOH buffer (pH 7.2). Homogenate was centrifuged at 770 g for 5 min and the resulting supernatant was further centrifuged at 13300 g for 10 min. Pellets were suspended in the same medium containing 0.3 mM EGTA and centrifuged at 4500 g for 15 min. The final pellet was suspended in 250 mM sucrose and 10 mM HEPES-KOH buffer (pH 7.2). The isolation procedure was performed at 4°C. During experiments, the mitochondrial suspension was kept on ice. The mitochondrial protein amount was determined using the Biuret reaction [13]. All studies using isolated mitochondria were performed within a 3 h period.

2.3. Mitochondrial Swelling

Kidney and liver mitochondria (0.27 mg protein/mL) were added to a buffer containing 125 mM sucrose, 65 mM KCL, 10 mM HEPES-KOH, pH 7.4, at 30°C plus 5 mM potassium succinate, 2.5 μM rotenone and different concentrations of $CaCl_2$ (10, 25, 50 μM) to assess the mitochondrial ability to capture calcium without mitochondrial swelling; $CaCl_2$ 50 μM plus 0.5% of bovine serum albumin (BSA) was utilized as antioxidant and, $CaCl_2$ 10μM plus *tert*-butylhydroperoxide (*t*-BOOH) was used as an oxidant agent. Mitochondrial swelling was estimated from the decrease in relative absorbance at 540 nm for 10 minutes using a Hitachi U-2000 spectrophotometer (Tokyo, Japan).

2.4. Generation of ROS

Mitochondrial ROS production was estimated in kidney and liver using the oxidative stress indicator 2'.7' dichlorodihydrofluorescein diacetate (DCFH-DA). The method is based on the intramitochondrial peroxide dependent oxidation of DCFH-DA to form the fluorescent product 2'.7'-dichlorofluorescein (DCF). Mitochondria (1 mg/mL) were incubated in a medium containing 130 mM KCl, 10 mM HEPES-KOH, pH 7.4, and 5 mM potassium succinate (plus 2.5 μM rotenone) for 10 min at 37°C in the presence of 4 μM DCFH-DA. The relative ROS production was measured in a Hitachi F-2500 spectrofluorometer (Tokyo, Japan), using as the wavelengths of excitation and emission 503 and 529 nm, respectively and the results were described as relative fluorescence units (RFU).

2.5. Determination of Protein-Thiols Content (SH)

Mitochondrial membrane and homogenate thiols groups of kidney and liver were measured using DTNB [5.5-dithiobis (2-nitrobenzoic) acid, Ellman's reagent]. After 15 min incubation under swelling conditions, the mitochondrial suspension was treated with trichloroacetic acid (5% final concentration) and centrifuged at 4500 g for 15 min. The
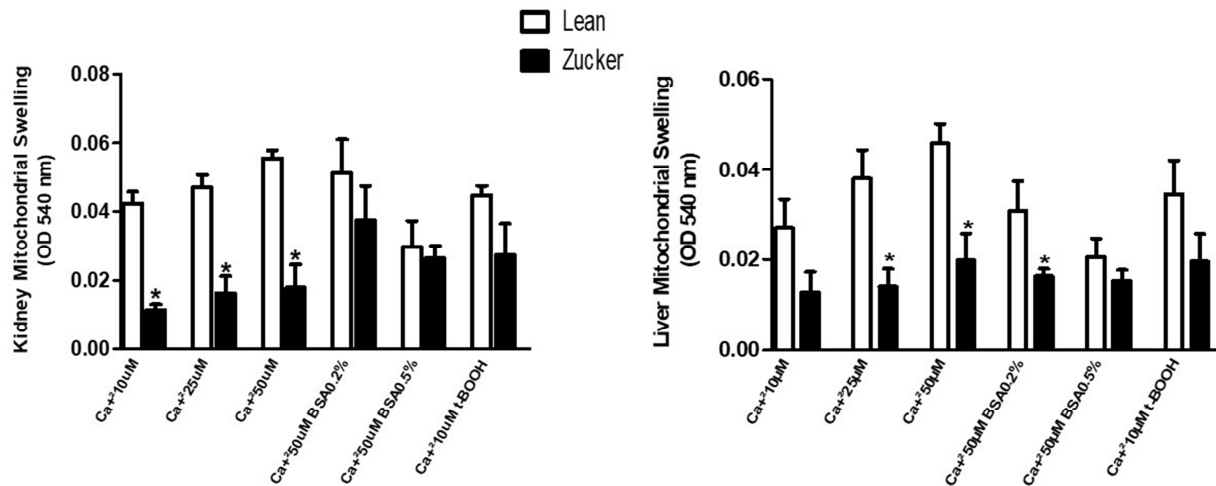
pellet was suspended with 1 mL of 0.5 M potassium phosphate buffer, pH 7.6. After addition of 0.1 mM DTNB, absorbance was determined at 412 nm. The amount of thiol groups was calculated from $\varepsilon = 13, 600$ M^{-1} [14].

2.6. Estimation of GSH Levels

After a 15 minutes incubation under swelling conditions, the mitochondrial suspension and homogenate of kidney and liver were treated with trichloroacetic acid (5% final concentration) and centrifuged at 4500 g for 15 min. Aliquots (100 µL) of supernatant were mixed with 2 mL of 100 mM NaH$_2$PO$_4$ buffer, pH 8.0, containing 5 mM EGTA. Ortho-phthalaldehyde (OPT, 1 mg/mL) was added, and the fluorescence was measured in a Hitachi F-2500 spectrofluorometer (Tokyo, Japan) using 350/420 nm excitation/emission wavelengths [15].
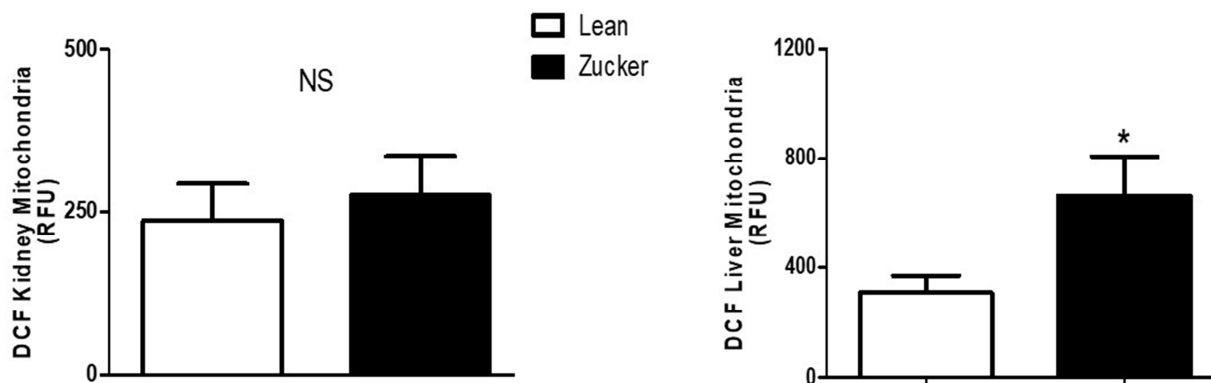
2.7. Statistical Analysis

Results were expressed as mean and standard error media (SEM). All data were analyzed by Unpaired T test (parametric) or Mann Whitney (nonparametric data) test when appropriate. Significance was defined as P<0.05.

3. Results

3.1. Mitochondrial Swelling

There was a reduced absorbance in obese (0.011 ± 0.002; 0.016 ± 0.005; 0.018 ± 0.007) vs. lean (0.042 ± 0.003; 0.047 ± 0.003; 0.056 ± 0.002) rats, demonstrating significant increase in kidney mitochondrial swelling of these animals, in all measurements at different Ca^{+2} concentrations respectively 10, 25 and 50µM, except when supplemented with BSA or t-BOOH. Similar result was observed in the absorbance of obese (0.014 ± 0.004; 0.020 ± 0.006; 0.016 ± 0.002) vs. lean (0.038 ± 0.006; 0.046 ± 0.004; 0.031 ± 0.007) rats in liver mitochondrial swelling, only in Ca^{+2} 25, 50µM concentrations and, when supplemented with BSA 0.2%, respectively, as seen in Figure 1.

Figure 1. Kidney and liver mitochondrial swelling in obese and lean rats. Data presented as mean ± SEM; n=5 per group. Unpaired T or Mann Whitney test; P<0.05: *vs. lean.

3.2. ROS Production in Mitochondria

ROS levels measured by DCF and expressed in relative fluorescence units (RFU) in kidney mitochondria did not show difference between obese and lean rats; however, in liver mitochondria, there was a significant increase in Zucker (664 ± 143) compared to lean (307 ± 62) group (Figure 2).

Figure 2. ROS levels in kidney and liver mitochondrial in obese and lean rats. Data presented as mean ± SEM; n=5 per group. Unpaired T or Mann Whitney test; P<0.05: *vs. lean.

3.3. Analysis of SH Levels

In relation to oxidation of SH in mitochondria of kidney and liver, lean and obese rats showed no significant difference (Figure 3). However, in the homogenate, there was a significant increase this parameter in liver of Zucker (0.57 ± 0.03) compared to lean (0.42 ± 0.01) rats, according to Figure 4.
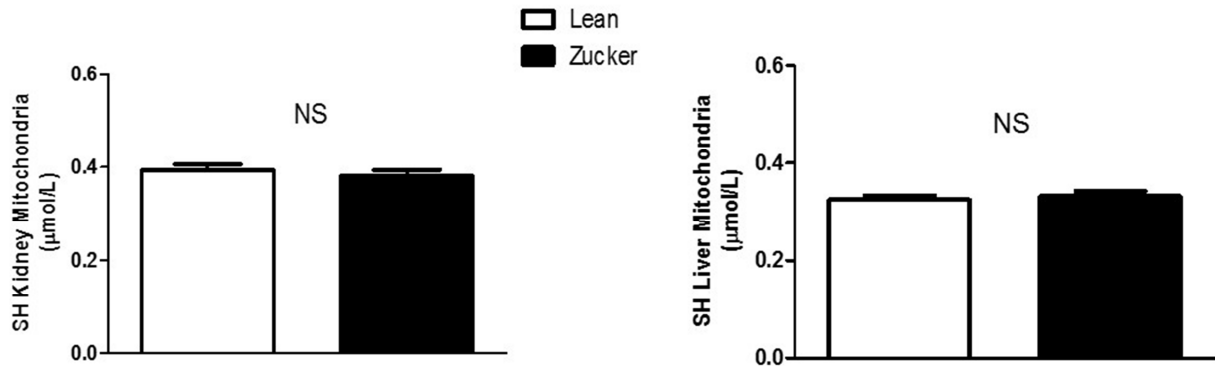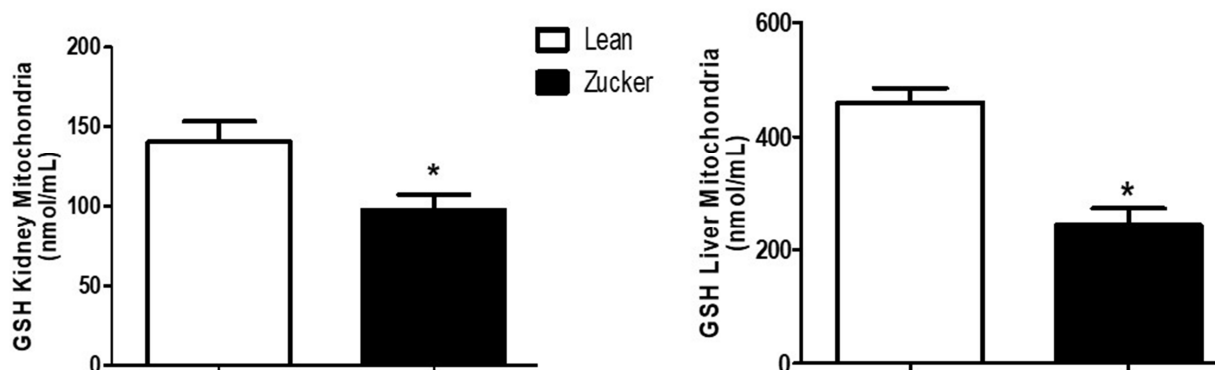

Figure 3. *SH of kidney and liver mitochondrial in obese and lean rats. SH: thiols-protein content. Data presented as mean ± SEM; n=5 per group. Unpaired T or Mann Whitney test; NS: not significant.*
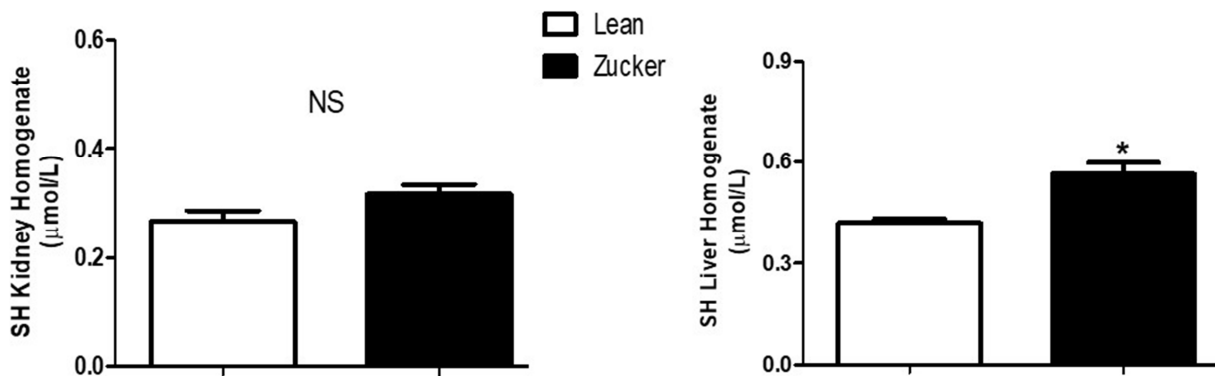
Figure 4. *SH of kidney and liver homogenate in obese and lean rats. SH: thiols-protein content. Data presented as mean ± SEM; n=5 per group. Unpaired T or Mann Whitney test; NS: not significant. P<0.05: *vs. lean.*

3.4. Analysis of the GSH Levels

The GSH level was significantly lower in obese (98 ± 10; 245 ± 29) than lean (141 ± 13; 460 ± 25) respectively in kidney and liver mitochondria (Figure 5). In relation to homogenate, the similar occurred; there was a significant decrease of this parameter in obese (123 ± 9; 1695 ± 102) compared to lean (157 ± 15; 2559 ± 152) rats, respectively in kidney and liver, according to Figure 6.

Figure 5. *GSH levels in kidney and liver mitochondrial of obese and lean rats. Data presented as mean ± SEM; n=5 per group. Unpaired T or Mann Whitney test; P<0.05: *vs. lean.*
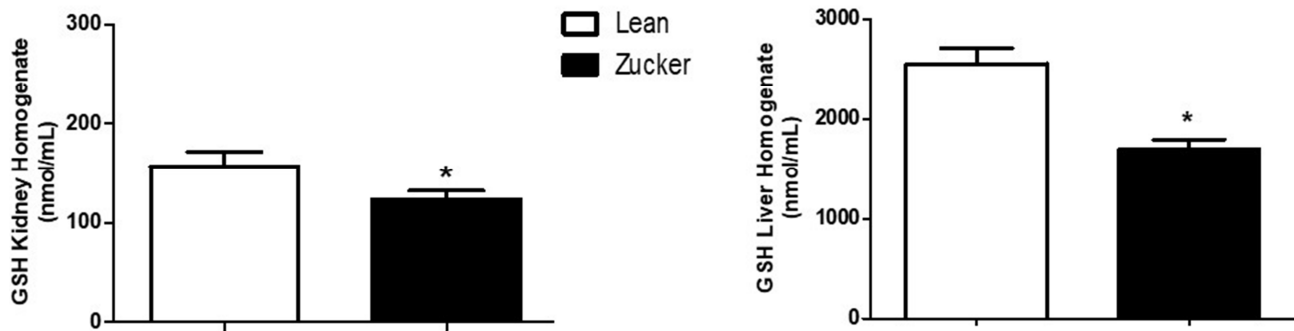
Figure 6. *GSH levels in kidney and liver homogenate of obese and lean rats. Data presented as mean ± SEM; n=5 per group. Unpaired T or Mann Whitney test; P<0.05: *vs. lean.*

4. Discussion

Measurements of mitochondrial function are also essential to understanding metabolic, aging, cancer, and neurodegenerative diseases. The mitochondria represent a strategic and important drug target in both health and disease. From the production of energy to the control of cell death pathways, intracellular signaling, calcium homeostasis, and intermediate metabolism, mitochondria are remarkable dynamic structures, with an important, yet undesirable, role as mediator of several disease processes [11].

When obese are compared to lean individuals, mitochondria have less clearly defined inner membranes, lower energy-generating capacities, and reduced fatty acid oxidation. These differences might promote the development and progression of obesity, with therapeutic implications [16], [17]. Furthermore, obesity can affect the integrity of mitochondrial membranes leading to the inefficient voltage dependent anion channel, mitochondrial permeability transition pore (MPTP) and other ion exchanger channels thus performing the mitochondria leaky and loss of integrity related to their membrane potentials [18]. In the present study, it was found that the mitochondrial swelling in obese was increased when compared to lean rats in different calcium concentrations, in kidney and liver; without showing any change after the use of t-BOOH, an inductor of ROS, did not change, which could be explained through loss of membrane potential.

A reduction of mitochondrial transcription factor A (TFAM) in obese can result in decreased mitochondrial DNA copy number and altered levels of proteins of the electron transport chain; it results in decreased complex I activity, greater oxygen consumption and increased uncoupled respiration. In consequence, the mitochondrial oxidative capacity of the adipose tissue is increased and outpaces metabolic flux through the tricarboxylic acid cycle, but this occurs without indication of oxidative stress or damage in mice on a normal chow diet [19]; this could explain the data of the present study, once that obese rats did not show differences in the parameters of mitochondrial swelling after oxidative stress induced by t-BOOH in kidney and liver.

Low mitochondrial respiration rate results in increased O_2 concentration, which in turn accelerates ROS formation.

High membrane potential inhibits forward electron transfer and stimulates reverse electron transfer in complexes I and III. The semiquinone form of CoQ is stabilized in complex III center (what is favorable for superoxide formation), and moreover ROS generation is somehow initiated in complex I [20], [21]. The ROS are a main source of cellular damage, mostly generated at complexes I and III of the cellular respiratory chain [22]. A review made by Skulachev described evidence that mitochondrial ROS are of great importance under many physiological and pathological conditions [23]. Dugan et al [24] demonstrated that there is a reduction in parameters of diabetic kidney disease and an increase in superoxide production due to stimulation of mitochondrial structure and function, via AMPK activation in type 1 diabetic mice.

In this study, there were no differences between obese and lean on the kidney mitochondria ROS generation, evaluated by DCF as well as SH; however, there was a significant difference of DCF and SH in liver mitochondria and homogenate, respectively, suggesting a condition of tissue-specific mitochondria in obesity. Its known that increased ROS decreases significantly the antioxidant activity [25] and that an imbalance between the generation and detoxification of ROS results in oxidative stress. ROS are unavoidable physiological by products which act as double edge in the biological system [26]. This is important because mitochondria can regulate apoptosis in response to cellular stress signals [27]; in this study, it was observed that kidney and liver mitochondria showed deregulated response to stress signal, demonstrating mitochondrial dysfunction in obese rats.

Lower levels of GSH are associated with cardiovascular diseases, neuro-degeneration and diabetes [28] and others diseases as cirrhosis; type 2 diabetes and alcoholic liver disease are associated with depletion of mitochondrial GSH [15], [29-31]. Benipal and Lash showed an increased susceptibility of proximal tubular cells of rat to nephrotoxicity and a significant degree of redox imbalance and basal oxidative stress in renal mitochondria [32]; these data corroborate the present study, once that the concentrations of the antioxidant GSH were reduced in obese compared to lean rats in kidney and liver mitochondria and homogenate, showing that the antioxidant system can be injured by the obesity.

5. Conclusion

In summary, this study demonstrated that obese rats had an increased mitochondrial swelling compared with leans in kidney and liver, demonstrating up regulation of oxidative stress and down regulation of antioxidant system in obese than lean rats, revealing increased liver sensitivity rather than kidney, which could be important to understand the mechanisms involved in tissue-specific mitochondrial obesity.

Conflict of Interest

The authors declare that they have no competing of interests.

Acknowledgements

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