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Mitochondrial energy metabolism and redox responses to hypertriglyceridemia

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Abstract In this work we review recent findings that explain how mitochondrial bioenergetic functions and redox state respond to a hyperlipidemic *in vivo* environment and may contribute to the maintenance of a normal metabolic phenotype. The experimental model utilized to evidence these adaptive mechanisms is especially useful for these studies since it exhibits genetic hypertriglyceridemia and avoids complications introduced by high fat diets. Liver from hypertrigliceridemic (HTG) mice have a greater content of glycerolipids together with increased mitochondrial free fatty acid oxidation. HTG liver mitochondria have a higher resting respiration rate but normal oxidative phosphorylation efficiency. This is achieved by higher activity of the mitochondrial potassium channel sensitive to ATP (mitoK$_{ATP}$). The mild uncoupling mediated by mitoK$_{ATP}$ accelerates respiration rates and reduces reactive oxygen species generation. Although this response is not sufficient to inhibit lipid induced extra-mitochondrial oxidative stress in whole liver cells it avoids amplification of this redox imbalance. Furthermore, higher mitoK$_{ATP}$ activity increases liver, brain and whole body metabolic rates. These mitochondrial adaptations may explain why these HTG mice do not develop insulin resistance and obesity even under a severe hyperlipidemic state. On the contrary, when long term high fat diets are employed, insulin resistance, fatty liver and obesity develop and mitochondrial adaptations are inefficient to counteract energy and redox imbalances.

Keywords hypertriglyceridemia · mitochondrial uncoupling · redox state · mitochondrial ATP-sensitive potassium channels

Hypertriglyceridemia is commonly associated with diabetes, obesity, metabolic syndrome and atherosclerosis (Grundy et al. 2004) and thus, contributes to the risk of morbidity and mortality in these conditions. The mechanism that link hyperlipidemia to the pathogenesis of these diseases is often ascribed to a lipid toxic action in different target tissues such as arteries, liver, pancreatic islet, heart and muscles. Lipid overflow cause ectopic fat deposition which impairs the function of these organs (Després and Lemieux 2006). Furthermore, chronic elevations in serum non-esterified fatty acids inhibit insulin's ability to promote peripheral glucose uptake into muscle and fat and to reduce hepatic glucose production, thus, disturbing glucose homeostasis and inducing insulin resistance (Rosen and Spiegelman 2006). Since lipid metabolism is under the tight control of insulin action, in the context of metabolic diseases where both, hyperlipidemia and insulin resistance coexist, it is difficult to discriminate the relative contribution of each of them as triggering or aggravating factors. Concerning intracellular lipotoxicity, multiple biochemical mechanisms have been proposed that indicate that the excess of lipids induces cell oxidative stress and damage (Schönfeld and Wojtczak 2008).

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In search of such mechanisms, we studied the effects of elevated plasma triglycerides (TG) and free fatty acids (FFA) levels on the mitochondrial bioenergetic functions and redox state using genetically modified mice. The hypertriglyceridemia in this model is a consequence of the overexpression of the apolipoprotein CIII gene (Ito et al. 1990). The overexpression of this protein impairs the recognition of the plasma TG-rich lipoproteins by their specific liver receptors (Aalto-Setala et al. 1992), resulting in extended lipoprotein half-life in the plasma compartment. Under high plasma concentration of TG, the intravascular lipolysis mediated by lipoprotein lipase increases the release and availability of FFA. Despite high plasma TG and FFA levels, under low fat diet, these mice exhibit normal glucose homeostasis (Reaven et al. 1994), normal pancreatic insulin secretion and peripheral insulin sensitivity (Amaral et al. 2002), as well as normal body weight gain (Alberici et al. 2006) and adiposity (Salerno et al. 2007). Therefore, this mouse model is particularly useful to study the effects of hyperlipidemia per se, independently of insulin resistance, obesity, and other confounders' factors such as inflammation caused by high fat diets.

Since there is a strong association between hyperlipidemia and disturbances in glucose homeostasis and body fat mass (Grundy et al. 2004; Després and Lemieux 2006), we hypothesized the existence of compensatory mechanisms in these hypertriglyceridemic (HTG) mice to explain their normal metabolic phenotype, at least under low fat diet. Changes in mitochondrial energy metabolism promoted by high cellular FFA levels are often related to the activity of uncoupling proteins (UCPs). UCPs are inner mitochondrial transmembrane proteins first identified in mitochondria from brown adipose tissue (Nicholls 1976). After identification of UCP occurrence in plants (Vercesi et al. 1995), other UCP family members were found widely distributed in many mammalian organs (Jesek and Garlid 1998). UCP acts as an anion carrier that mediates the transfer of fatty acid anions from the matrix to the intermembrane space. Because of the proton (H^+) gradient, fatty acids are found protonated in the intermembrane space. These protonated fatty acids spontaneously and rapidly cross the inner membrane into the matrix side, where deprotonation occurs and UCP transfer them back completing the cycle. As a result, UCP activity uncouples mitochondria, a process that includes decrease of electrochemical transmembrane potential, increase of respiration rates and decrease of oxidative phosphorylation efficiency (ADP/O ratio) (Garlid et al. 2000). Thus, increased mitochondrial uncoupling leads to energy transfer inefficiency and increased energy expenditure. Accordingly, it has been shown that UCPs activity induces alterations in body mass in experiments with genetically modified mice. UCP1-deficient mice exhibit increased susceptibility to diet-induced obesity (Kontani et al. 2005); mice expressing UCP1 in white fat display a lean phenotype (Kopecky et al. 1995); and mice overexpressing UCP-3 in skeletal muscle are hyperfagic and lean (Clapham et al. 2000).

In earlier experiments in HTG mice, we found elevated oxygen consumption during resting respiration of isolated liver mitochondria, which suggested that an uncoupling mechanism was activated in these mice. Furthermore, intracellular availability of FFA was associated to this process since *in vivo* treatment with the hypolipidemic drug ciprofibrate, which accelerates intracellular fatty acid beta-oxidation, restored the resting respiration in HTG liver mitochondria (Alberici et al. 2003). However, we found no changes in UCP content and oxidative phosphorylation efficiency (ADP/O ratio) in these mitochondria. In addition, the resting respiration in HTG liver mitochondria remained higher in the presence of bovine serum albumin and in the presence of UCPs inhibitor, GDP (Alberici et al. 2003), thus excluding an uncoupling mediated by UCPs. The activity of the adenine nucleotide carrier (ANT) may also cause some proton leak, and hence, a certain degree of uncoupling (Samartsev and Mokhova 1997); however, the use of the ANT inhibitor carboxyatractyloside showed no effect on the higher resting respiration in HTG mitochondria. On the other hand, the resting respiration was reestablished to control levels when all K^+ salts were changed by Li^+ salts in the reaction medium, i.e., when respiration rates were measured in absence of K^+ (Alberici et al. 2006). These results indicated that the uncoupling in HTG liver mitochondria is not related to the classical mechanism by UCPs but otherwise involves K^+ transport across the inner mitochondrial membrane.

The inner membrane of mitochondria is almost impermeable to K^+. However, K^+ uptake into the mitochondrial matrix occurs through mitochondrial ATP-sensitive K^+ channels (mitoK_{ATP}) and is accompanied by phosphate and water movement, resulting in mitochondrial swelling. The alteration in mitochondrial volume activates the K^+/H^+ antiporter (Garlid and Paucek 2003) generating a futile cycling of K^+ across the inner membrane. The net result of this process is the entrance of a H^+ for every K^+ exchanged, resulting in a slight decrease in membrane potential which is reestablished by an increase in the mitochondrial respiration rate (mild uncoupling). In fact, the higher resting respiration in HTG liver mitochondria was sensitive to mitoK_{ATP} antagonists (ATP, 5-hydroxydecanoate (5HD) and glyburide). In addition, mitochondrial swelling mediated by these channels in HTG liver mitochondria was also sensitive to antagonists of mitoK_{ATP} and inhibited in the absence of K^+ in the reaction medium. Together these results demonstrated that the increase in resting respiration in liver mitochondria isolated from HTG mice is mediated by a higher activity of the mitoK_{ATP} (Alberici et al. 2006).

MitoK$_{ATP}$ opening has previously been shown to be regulated *in vitro* by thiol oxidation (Zhang et al. 2001; Fornazari et al. 2008; Facundo et al. 2007). In fact, we found an oxidized status in HTG livers characterized by elevated levels of carbonylated proteins, malondialdehydes, and GSSG/GSH ratio. This oxidized profile as well as the higher mitoK$_{ATP}$ activity and resting respiration rate in HTG liver mitochondria were all reversed by *in vivo* treatment of HTG mice with the antioxidant N-acetylcysteine (NAC). These results demonstrated that the mitoK$_{ATP}$ activation is dependent on the oxidized state of HTG livers. The HTG cell redox imbalance was explained by an elevated cytosolic and microsomal reactive oxygen species (ROS) production promoted by higher activities of xanthine and NADPH oxidases, respectively (Alberici et al. 2009). These ROS-generating enzymes can be activated by intracellular FFA accumulation (Leclercq et al. 2000) or their oxidized products (Cighetti et al. 2001), which were found in the livers from HTG mice. On the other hand, mitochondrial compartment in HTG livers was found to be protected from oxidative stress, since malondialdehyde levels and GSSG/GSH ratio were similar in HTG and control liver mitochondria. Moreover, aconitase activity, a mitochondrial matrix enzyme that acts as a superoxide radical anion sensor, was enhanced in HTG liver mitochondria while fumarase activity, another mitochondrial matrix enzyme that is not a ROS target, was not altered (Alberici et al. 2009). The maintenance of a more reduced state inside mitochondria in an outside oxidized environment indicated an improved antioxidant system and/or reduced ROS generation in HTG liver mitochondria. Mitochondria continuously generate superoxide radical anions and other ROS owing to monoelectronic reduction of oxygen molecules at intermediary stages of the electron transport chain (mainly through complexes I and III) (Boveris 1977). Faster electron transport rates, as promoted by UCP or mitoK$_{ATP}$, may decrease ROS formation by keeping mitochondria in a low oxygen tension microenvironment and decreasing the lifetime of intermediates capable of donating electrons toward superoxide radical formation (Skulachev 1991). Indeed, isolated liver mitochondria from HTG mice showed lower ROS release in a manner sensitive to mitoK$_{ATP}$ antagonists or in the medium devoid of K^+ salts. In addition, the content and activity of Mn-superoxide dismutase (MnSOD) were also decreased in HTG liver mitochondria (Alberici et al. 2009). Because antioxidant enzymes are substrate-inducible (Meilhac et al. 2000), the downregulation of MnSOD was probably caused by a less oxidized microenvironment inside the mitochondria. Together, these results demonstrated that lower ROS release in HTG mitochondria can be attributable to enhanced respiration rates promoted by mitoK$_{ATP}$ activity, which acts a redox sensor allowing a cross talk between extra-and intramitochondrial compartments.

Our previous and present studies with tissue biopsies show that these findings are not restricted to isolated HTG liver mitochondria. HTG mice presented increased liver oxygen consumption sensitive to *in vivo* treatments with NAC or mitoK$_{ATP}$ antagonist 5-HD (Alberici et al. 2006; Alberici et al. 2009). Like liver, HTG brain also shows elevated oxygen consumption sensitive to 5-HD as shown in Fig. 1a. Together, liver and brain can achieve 35% of body basal metabolic rates (BMR) (Greenberg 1999; Greenberg and Boozer 2000). On the other hand, skeletal muscle, a tissue that have lower BMR (per unit of tissue mass) than liver and brain, shows oxygen consumption rates similar between HTG and wild type (WT) mice (Fig. 1b). Possibly, this HTG tissue have no FFA excess to induce mitoK$_{ATP}$ opening, since these hyperlipidemic mice have no insulin resistance (Amaral et al. 2002). Nevertheless, HTG mice have increased

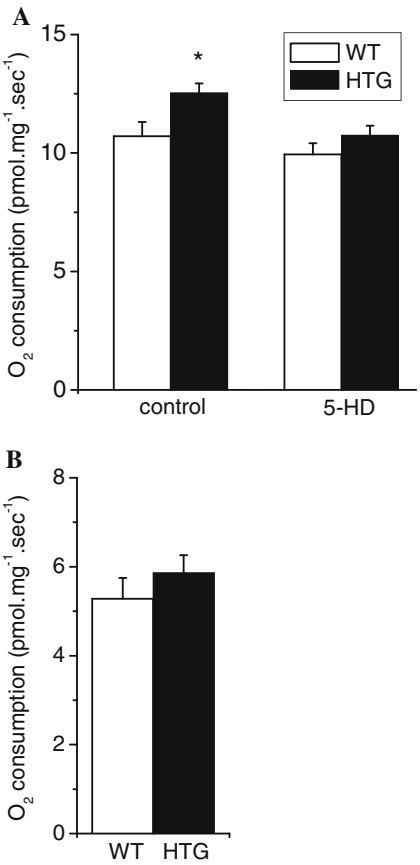

Fig. 1 Higher resting respiration rate in brain tissue of HTG mice. Animal model was described in details elsewhere (Alberici et al. 2006). Fresh brain and soleus skeletal muscle were stored in ice cold "high energy storage solution", as described by Sperl et al. (1997). **a** Biopsies of brain (5 mg) were incubated in a 37°C DMEM (plus 25 mM Hepes) in the absence (control) or presence of 0.1 mM 5-HD; **b** Biopsies of skeletal muscle (3 mg) were incubated in a 37°C incubation medium described by Sperl et al. (1997). Measurements of oxygen consumption were performed in an OROBOROS-Oxygraph (Paar, Graz, Austria). Respiration rates given in pmol oxygen mg^{-1} sec^{-1}. Mean ± sem (n=4–6). *P<0.05 vs WT by Student t test

overall body metabolic rates, as evidenced by 0.7°C elevation in body temperature and higher CO_2 production rates sensitive to *in vivo* NAC treatment (Alberici et al. 2009). These results point to a new biological role of the mitoK$_{ATP}$ in the regulation of body energy homeostasis.

Although a study has shown that the mitoK$_{ATP}$ activity in heart and liver mitochondria decline with age (Krylova et al. 2006), we show here that this mitochondrial adaptation remains in aged HTG mice. Figure 2 shows enhanced O_2 consumption rates of liver mitochondria isolated from elderly HTG mice when compared to liver mitochondria isolated from elderly wild type (WT) mice. These higher respiratory rates were reversed when mitoK$_{ATP}$ was inhibited by ATP or in the absence of K^+, whereas the channel agonist diazoxide restored elevated respiration in a manner sensitive to the antagonist 5-HD. No significant differences in respiratory rates in the presence of mitoK$_{ATP}$ agonist and antagonists could be observed in elderly WT mitochondria, indicating low levels of mitoK$_{ATP}$ activity, exactly as found in mitochondria from young adult WT (Alberici et al. 2006). Accordingly, elderly HTG liver mitochondria still maintained low levels of ROS production, sensitive to ATP (data not shown). The increased mitoK$_{ATP}$ activity persists with aging probably because the provocative stimuli of high intracellular lipid and oxidized derivatives also persist in elderly HTG mice.

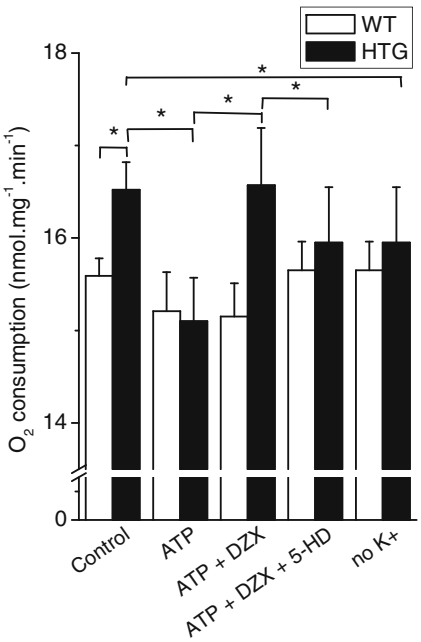

Fig. 2 Higher oxygen consumption promoted by mitoK$_{ATP}$ activation in isolated liver mitochondria from aging HTG mice. 22 month old WT and HTG mice liver mitochondria were prepared as described elsewhere (Alberici et al. 2006). Liver mitochondria (0.5 mg/ml) were incubated in a reaction medium (Alberici et al. 2006) in the presence of 0.1 mM ATP, 30 μM diazoxide (DZX) and/or 60 μM 5-HD. Mean ± sem (n=8). Respiration rates given in nmol oxygen · mg protein^{-1} · min^{-1}. *P<0.05 by one-way analysis of variance

The obese phenotype induced by long term use of high fat diets generally displays inflammation, insulin resistance, and non-alcoholic fatty liver disease, in addition increased adiposity. It has been shown that elevated mitochondrial ROS production can disturb insulin signaling and result in insulin resistance (Nishikawa et al. 2007). Apparently mitochondria cannot compensate for the high fat diet induced oxidative stress. Salerno et al (2007) have shown that five months of a high saturated fat diet increases the liver mitochondrial resting respiration rate in wild type C57Bl6 mice but does not further increase the already high resting respiration in HTG mitochondria. Cardoso et al (2010) showed that 2 month of high polyunsaturated fat diet increased mitoK$_{ATP}$ activity but had no effect on mitochondrial respiration rates and increased ROS generation in the liver of Swiss mice. After 10 months on this diet, mitoK$_{ATP}$ was no longer active and phosphorylation efficiency (ADP/O ratio) was markedly decreased, suggesting UCPs action; however, ROS production remained elevated. In fact, UCP3 is upregulated in skeletal muscle during a high fat diet as well as in food deprivation (Zhang et al 2010; Hesselink et al. 2003). These observations raise the possibility that UCP3 might be of importance in protecting mitochondria against fatty acid accumulation and may help to maintain muscular fat oxidative capacity (Hesselink et al. 2003; Schrauwen and Hesselink 2004). However, this seems not to be the case in liver. In fact, a recent study showed that a high fat diet modulates UCPs expression in a tissue-specific manner (Zhang et al. 2010).

In conclusion, the results reported indicated that the hypertriglyceridemic state followed by intracellular lipid accumulation increases the activity of extramitochondrial ROS-producing systems promoting oxidative damage in liver. As a redox sensor, mitoK$_{ATP}$ channels open, increase resting respiration rates and FFA catabolism, and decrease mitochondrial ROS generation. This cross talk between extra-and intramitochondrial compartments conserves the organelle in a more reduced state which can avoid amplification of overall cell oxidative stress. This mitochondrial adaptation in response to hypertriglyceridemia increases liver and brain energy expenditure, and perhaps occurs also in other lipid metabolizing tissues, resulting in an elevated overall body energy metabolism and preventing lipotoxicity. On the contrary, when long term high fat diets are employed, insulin resistance, fatty liver and obesity develop and mitochondrial adaptations are inefficient to counteract energy and redox imbalances.

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References

Aalto-Setala K, Fisher EA, Chen X, Chajek-Shaul T, Hayek T, Zechner R, Walsh A, Ramakrishnan R, Ginsberg HN, Breslow JL (1992) J Clin Invest 90:1889–1900

Alberici LC, Oliveira HC, Bighetti EJ, de Faria EC, Degaspari GR, Souza CT, Vercesi AE (2003) J Bioenerg Biomembr 35:451–457

Alberici LC, Oliveira HC, Patrício PR, Kowaltowski AJ, Vercesi AE (2006) Gastroenterology 131:1228–1234

Alberici LC, Oliveira HC, Paim BA, Mantello CC, Augusto AC, Zecchin KG, Gurgueira SA, Kowaltowski AJ, Vercesi AE (2009) Free Radic Biol Med 47:1432–1439

Amaral ME, Oliveira HC, Carneiro EM, Delghingaro-Augusto V, Vieira EC, Berti JA, Boschero AC (2002) Horm Metab Res 34:21–26

Boveris A (1977) Adv Exp Med Biol 78:67–82

Cardoso AR, Cabral-Costa JV, Kowaltowski AJ (2010) J Bioenerg Biomembr 42:245–253

Cighetti G, Bortone L, Sala S, Allevi P (2001) Arch Biochem Biophys 389:195–200

Clapham JC, Arch JR, Chapman H, Haynes A, Lister C, Moore GB, Piercy V, Carter SA, Lehner I, Smith SA, Beeley LJ, Godden RJ, Herrity N, Skehel M, Changani KK, Hockings PD, Reid DG, Squires SM, Hatcher J, Trail B, Latcham J, Rastan S, Harper AJ, Cadenas S, Buckingham JA, Brand MD, Abuin A (2000) Nature 406:415–418

Després JP, Lemieux I (2006) Nature 444:881–887

Facundo HT, de Paula JG, Kowaltowski AJ (2007) Free Radic Biol Med 42:1039–1048

Fornazari M, de Paula JG, Castilho RF, Kowaltowski AJ (2008) J Neurosci Res 86:1548–1556

Garlid KD, Paucek P (2003) Biochim Biophys Acta 1606:23–41

Garlid KD, Jaburek M, Jezek P, Varecha M (2000) Biochim Biophys Acta 1459:383–389

Greenberg JA (1999) Med Hypotheses 52:15–22

Greenberg JA, Boozer CN (2000) Mech Ageing Dev 113:37–48

Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C, American Heart Association, National Heart, Lung, and Blood Institute (2004) Circulation 109:433–438

Hesselink MK, Mensink M, Schrauwen P (2003) Obes Res 11:1429–1443

Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL (1990) Science 249:790–793

Jezek P, Garlid KD (1998) Int J Biochem Cell Biol 30:1163–1168

Kontani Y, Wang Y, Kimura K, Inokuma KI, Saito M, Suzuki-Miura T, Wang Z, Sato Y, Mori N, Yamashita H (2005) Aging Cell 4:147–155

Kopecky J, Clarke G, Enerback S, Spiegelman B, Kozak LP (1995) J Clin Invest 96:2914–2923

Krylova IB, Kachaeva EV, Rodionova OM, Negoda AE, Evdokimova NR, Balina MI, Sapronov NS, Mironova GD (2006) Exp Gerontol 41:697–703

Leclercq IA, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR (2000) J Clin Invest 105:1067–1075

Meilhac O, Zhou M, Santanam N, Parthasarathy S (2000) J Lipid Res 41:1205–1213

Nicholls DG (1976) FEBS Lett 61:103–110

Nishikawa T, Kukidome D, Sonoda K, Fujisawa K, Matsuhisa T, Motoshima H, Matsumura T, Araki E (2007) Diabetes Res Clin Pract 77:161–164

Reaven GM, Mondon CE, Chen YD, Breslow JL (1994) J Lipid Res 820–824.

Rosen ED, Spiegelman BM (2006) Nature 444:847–853

Salerno AG, Silva TR, Amaral ME, Alberici LC, Bonfleur ML, Patrício PR, Francesconi EP, Grassi-Kassisse DM, Vercesi AE, Boschero AC, Oliveira HC (2007) Int J Obes (Lond) 31:1586–1595

Samartsev VN, Mokhova EN (1997) Biochem Mol Biol Int 42:29–34

Schönfeld P, Wojtczak L (2008) Free Radic Biol Med 45:231–241

Schrauwen P, Hesselink MK (2004) Proc Nutr Soc 63:287–292

Skulachev VP (1991) FEBS Lett 294:158–162

Sperl W, Skladal D, Gnaiger E, Wyss M, Mayr U, Hager J, Gellerich FN (1997) Mol Cell Biochem 174:71–78

Vercesi AE, Martins IS, Silva MAP, Leite HMF, Cuccovia IM, Chaimovich H (1995) Nature 375:24

Zhang DX, Chen YF, Campbell WB, Zou AP, Gross GJ, Li PL (2001) Circ Res 89:1177–1183

Zhang CM, Gu Y, Qing DN, Zhu JG, Zhu C, Zhang M, Guo XR (2010) Mol Biol Rep 37:3177–3182