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Research paper

Fatty liver and insulin resistance in obese Zucker rats: No role for mitochondrial dysfunction

Mélissa Flamment [a,*], Matthieu Arvier [a], Yves Gallois [b,c], Gilles Simard [a,b,c], Yves Malthièry [a,b,c], Patrick Ritz [a,b,d], Pierre-Henri Ducluzeau [a,b,d]

[a] *INSERM, U694, CHU Angers, 4 rue Larrey, F-49033 Angers, France*
[b] *Univ Angers, F-49035 Angers, France*
[c] *CHU Angers, Département de Biochimie et Génétique, F-49033 Angers, France*
[d] *CHU Angers, Département d'Endocrinologie-Diabète-Nutrition, F-49033 Angers, France*

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Abstract

The relationship between insulin resistance and mitochondrial function is of increasing interest. Studies looking for such interactions are usually made in muscle and only a few studies have been done in liver, which is known to be a crucial partner in whole body insulin action. Recent studies have revealed a similar mechanism to that of muscle for fat-induced insulin resistance in liver. However, the exact mechanism of lipid metabolites accumulation in liver leading to insulin resistance is far from being elucidated. One of the hypothetical mechanisms for liver steatosis development is an impairment of mitochondrial function. We examined mitochondrial function in fatty liver and insulin resistance state using isolated mitochondria from obese Zucker rats. We determined the relationship between ATP synthesis and oxygen consumption as well as the relationship between mitochondrial membrane potential and oxygen consumption. In order to evaluate the quantity of mitochondria and the oxidative capacity we measured citrate synthase and cytochrome *c* oxidase activities. Results showed that despite significant fatty liver and hyperinsulinemia, isolated liver mitochondria from obese Zucker rats display no difference in oxygen consumption, ATP synthesis, and membrane potential compared with lean Zucker rats. There was no difference in citrate synthase and cytochrome *c* oxidase activities between obese and lean Zucker rats in isolated mitochondria as well as in liver homogenate, indicating a similar relative amount of hepatic mitochondria and a similar oxidative capacity. Adiponectin, which is involved in bioenergetic homeostasis, was increased two-fold in obese Zucker rats despite insulin resistance. In conclusion, isolated liver mitochondria from lean and obese insulin-resistant Zucker rats showed strictly the same mitochondrial function. It remains to be elucidated whether adiponectin increase is involved in these results.
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Keywords: Mitochondria; Fatty liver; Insulin resistance; Oxidative phosphorylation; Zucker rat

1. Introduction

Mechanisms leading to insulin resistance, involving fat accumulation and metabolic abnormalities are not clearly understood. The relationship between insulin resistance and mitochondrial function is increasingly of interest [1]. Recent evidences indicate that insulin resistance in skeletal muscle could be due to reduced mitochondrial oxidative capacity [2–7]. In fact, insulin resistance is also closely associated with alterations of hepatic metabolism, an organ which plays a central role in whole body insulin sensitivity. The cellular mechanisms responsible for causing hepatic insulin resistance remain controversial, with several hypotheses attributing causality to visceral fat [8], adipokines [9,10], endoplasmic reticulum stress [11], and inflammation [12]. An alternative hypothesis is that the development of hepatic steatosis per se leads to hepatic insulin resistance. Indeed, recent studies

Abbreviations: COX, cytochrome *c* oxidase; CS, citrate synthase; ANT, adenine nucleotide translocator; TG, triglyceride.

* Corresponding author. Tel: +33 241 73 59 65; fax: +33 241 35 40 17.
E-mail address: mflamment@yahoo.fr (M. Flamment).

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have revealed a similar mechanism to that of muscle for fat-induced insulin resistance in liver, where accumulation of intracellular lipid metabolites activates a serine kinase cascade involving PKCε, leading to a decrease of insulin receptor kinase activity resulting in impaired insulin action [13,14]. However, the exact mechanism of lipid metabolites accumulation in liver leading to insulin resistance is far from being elucidated. One of the hypothetical mechanisms for liver steatosis development is an impairment of mitochondrial function.

ATP is synthesized in mitochondria through oxidative phosphorylation, which couples substrate oxidation by the respiratory chain to ADP phosphorylation by F1-F0 ATP synthase [15]. However, it has long been known that oxidative phosphorylation coupling is not a perfect chemical reaction and a fraction of the proton gradient is dissipated by proton leak across the inner membrane [16]. Alterations in mitochondrial oxidative capacities might contribute to a diminished ability to oxidize fatty acid, and so might promote intracellular fat accumulation. This ectopic fat accumulation could be due to: (i) an increase of oxidative phosphorylation efficiency, and so in this case for the same quantity of synthesized ATP there are less consumed energetic substrates; (ii) a decrease of oxidative capacities without variations in efficiency; (iii) a decrease in the quantity of mitochondria.

In this study we decided to look at hepatic mitochondrial function, in particular oxidative phosphorylation efficiency, in fatty liver and insulin resistance state with using isolated mitochondria from the livers of lean and obese Zucker rats. These rats, which lack functional leptin receptor exhibit hyperphagia, hyperinsulinemia, and hyperlipidemia [17] and are commonly used in studies of insulin resistance [18]. Studies relating to proton leak in Zucker rats lead to divergent results. Ramsey et al. [19] showed that liver mitochondria from obese Zucker rats exhibit less proton leak than their lean littermates. Brookes et al. [20] carried out a similar study in which there was no difference in proton leak in isolated liver mitochondria between obese and lean Zucker rats. However, as previously discussed by Brookes et al. [20], in the study of Ramsey et al. [19] there is no bovine serum albumin in the experimental medium and the proton leak curve of obese Zucker rats appears aberrant in shape. Recently a decrease has been shown in state 3 (phosphorylating state) respiration in liver mitochondria from obese Zucker rats which is associated with a decrease in the content of adenine nucleotide translocator (ANT) [21]. Nevertheless in these studies we have no information about ATP synthesis nor, therefore, about oxidative phosphorylation efficiency.

The aim of this study was to examine whether obese Zucker rats, used as a fatty liver and insulin resistance model, exhibit differences in liver mitochondria function. We determined the relationship between membrane potential and oxygen consumption in non-phosphorylating (state 4) and phosphorylating (state 3) isolated mitochondria. In order to actually evaluate oxidative phosphorylation efficiency we determined the kinetic of the relationship between ATP synthesis and oxygen consumption in isolated liver mitochondria which really corresponds to the physiological variations in oxidative phosphorylation. To investigate mitochondrial content and oxidative capacity we determined citrate synthase and cytochrome *c* oxidase activities.

2. Materials and methods

2.1. Animals

The present investigation was performed in accordance with the guiding principles in the care and use of animals. Ten male obese (*fa*/*fa*) Zucker rats and ten male lean (*fa*/+) Zucker rats were purchased from Charles River (L'Arbresle, France). Animals were caged individually in a temperature-controlled room (22 °C) with a dark/light cycle of 12:12 h. They were provided with water ad libitum and a standard diet (U-A-R A04; SAFE, Epinay-sur-Orge, France) composed of (% total weight) 16% protein, 3% fat, 60% carbohydrate and 21% water, fiber, vitamins and minerals. All rats were fasted during the night before being killed. Eleven-week-old rats were anesthetized by inhalation of isoflurane and blood was collected from the heart. Immediately after blood collection, the liver was rapidly removed and weighed.

2.2. Liver mitochondria preparation

Liver mitochondria were isolated using a modification of the differential centrifugation procedure of Krahenbuhl et al. [22] with all steps carried out at 4 °C. Liver was homogenized in an isolation medium containing 100 mM sucrose, 50 mM KCl, 5 mM EGTA and 50 mM Tris–base, pH 7.4 (10 ml/g tissue) followed by centrifugation at 600 × *g* for 10 min. The supernatant was filtered through cheesecloth and centrifuged at 7000 × *g* for 10 min. The pellet was resuspended in the isolation medium (10 ml/g tissue) and centrifuged at 3500 × *g* for 10 min. The resulting pellet was resuspended in a minute volume of isolation medium. Protein concentration was determined using the bicinchoninic acid assay kit (Interchim, Montluçon, France) with bovine serum albumin as standard.

2.3. Mitochondrial respiration and membrane potential

Oxygen consumption and membrane potential were measured simultaneously using electrodes sensitive to oxygen and to the potential-dependent probe triphenylmethylphosphonium ($TPMP^+$). Mitochondria (0.5 mg protein/ml) were incubated in a respiratory reaction medium containing 120 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 2 mM $MgCl_2$, 3 mM HEPES and 0.3% bovine serum albumin (w/v), pH 7.4, and supplemented with 5 μM rotenone and 80 ng/ml nigericin. The TPMP electrode was calibrated by sequential 0.5 μM additions up to 2 μM TPMP, then 5 mM succinate was added to start the reaction. After each run, 2 μM of carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) was added to dissipate the membrane potential and release all TPMP back into the medium for baseline correction. Membrane potentials were calculated as previously described by Brand [23], assuming a TPMP binding correction of 0.42 (μl/mg of protein)$^{-1}$ [24].

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