Research Article

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Effects of a high-fat diet on energy metabolism and ROS production in rat liver

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Background & Aims: A high-fat diet affects liver metabolism, leading to steatosis, a complex disorder related to insulin resistance and mitochondrial alterations. Steatosis is still poorly understood since diverse effects have been reported, depending on the different experimental models used.

Methods: We hereby report the effects of an 8 week high-fat diet on liver energy metabolism in a rat model, investigated in both isolated mitochondria and hepatocytes.

Results: Liver mass was unchanged but lipid content and composition were markedly affected. State-3 mitochondrial oxidative phosphorylation was inhibited, contrasting with unaffected cytochrome content. Oxidative phosphorylation stoichiometry was unaffected, as were ATPase and adenine nucleotide translocator proteins and mRNAs. Mitochondrial acylcarnitine-related H_2O_2 production was substantially higher and the mitochondrial quinone pool was smaller and more reduced. Cellular consequences of these mitochondrial alterations were investigated in perifused, freshly isolated hepatocytes. Ketogenesis and fatty acid-dependent respiration were lower, indicating a lower β-oxidation rate contrasting with higher RNA contents of CD36, FABP, CPT-1, and AcylCoA dehydrogenases. Concomitantly, the cellular redox state was more reduced in the mitochondrial matrix but more oxidized in the cytosol: these opposing changes are in agreement with a significantly higher *in situ* mitochondrial proton motive force.

Conclusions: A high-fat diet results in both a decrease in mitochondrial quinone pool and a profound modification in mitochondrial lipid composition. These changes appear to play a key role in the resulting inhibition of fatty acid oxidation and of mitochondrial oxidative-phosphorylation associated with an increased mitochondrial ROS production. Mitochondrial quinone pool could have prospects as a crucial event, potentially leading to interesting therapeutic perspectives.

Keywords: Steatosis; β-Oxidation; Redox-state; Oxidative-phosphorylation; Ubiquinone.

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Abbreviations: NASH, non-alcoholic steatohepatitis; FA, fatty acids; C, control group; HF, high-fat diet; CS, citrate synthase; TMPD/Asc/DNP, tetramethyl-1,4-phenylenediamine/ascorbate/2,4-dinitrophenol; *J*, flux; 3-OHbut, beta-hydroxybutyrate; AcAc, acetoacetate.

Introduction

The non-alcoholic fatty liver disease is one of the most common etiologies of chronic liver disease starting from steatosis to non-alcoholic steatohepatitis (NASH) and liver cirrhosis. Insulin resistance, fatty acid metabolism alteration, and mitochondrial dysfunction may be the main contributing factors [11,27]. However, the exact contribution and the relationships between these factors have yet to be investigated since conflicting results have been reported on the metabolism of hepatocytes chronically exposed to high concentrations of free fatty acids (FFA) *in vivo*. Using perfused liver, isolated cells or tracers *in vivo*, both an increase [12,19,20] and decrease [7] in mitochondrial β-oxidation have been reported. Similarly, CPT1-L expression has been reported to increase [3,26], decrease [22] or remain unchanged [27]. Alterations in the oxidative phosphorylation pathway are also frequently reported; however, the resulting picture is not clear. The respiratory chain activity measured on isolated mitochondria is reported to be either decreased [21,28], unchanged [31] or even increased [3,4,28]. The presence of conflicting results also concerns respiratory chain complex activities [27]. Intrinsic activity of the complex V is unchanged [31], while hepatic ATP is depleted [4]. Production of reactive oxygen species (ROS) is reported to be frequently increased. However, the exact mechanism of production and the deleterious consequences of ROS require further clarification. ROS can be generated either at the mitochondrial respiratory chain level and/or at cytochrome P450 (CYP2E1) level [18,22]. The mitochondrial production is linked, at least in part, to the hyperpolarization of the inner mitochondrial membrane (Δp_m), which prolongs the half-life of mobile electron carriers. Some studies suggest a mitochondrial uncoupling in steatosis and NASH involving UCPs, leading to a lower efficiency of ATP synthesis [4], despite the controversy about the presence of UCP in hepatocytes [23]. Assessment of oxidative phosphorylation efficiency (ATP/O) under conditions

close to intracellular condition (i.e. with a control strength of the pathway exerted by matricial phosphate potential, see below) is still lacking. Interestingly, it has recently been suggested that a lower oxygen supply to liver cells could be an important factor for the initiation and progression of this complex disease, emphasizing the role of mitochondrial dysfunction [18]. Therefore, in a rodent model, we conducted a comprehensive study on the effect of long-term high-fat diet exposure on liver energy metabolism by coupling an approach on isolated mitochondria with measurements on intact hepatocytes. We then compared, in the same rat model, the effects of a high-fat diet on mitochondrial function, metabolic fluxes, as well as cellular adaptations including gene expression and membrane composition alterations. In this study, we report that a high-fat diet clearly affects hepatocyte energy metabolism leading to depressed β-oxidation and respiration, despite a more reduced mitochondrial matrix and an increased mitochondrial ROS production. These alterations relate to both a lower mitochondrial quinone content and a profound modification in the lipid composition of the mitochondrial membranes.

Materials and methods

Animals and experimental design

Four month-old male Wistar rats (321 g ± 11 g) were housed individually at 22 °C, in 12 h/12 h light/dark conditions and with a 50% relative humidity. Rats were randomly assigned to be fed over an eight-week period with either a standard carbohydrate diet (C: 16.1% proteins, 3.1% lipids, 60% cellulose; A04, Safe, France), or a high-fat diet (HF: 31.5% proteins, 54% lipids (50% lard, 4% soya-bean oil w/w), 7% cellulose) (Supplementary Table 1). Because of the lower food intake (g/day) in HF, due to the higher energy density, protein content in the HF diet was 2-fold higher; allowing a similar daily protein intake in both groups. Body weight and food intake were recorded twice weekly. The experiments followed the European Union recommendations concerning the care and use of laboratory animals for experimental and scientific purposes. All animal work has been approved by the Comité Régional d'Ethique pour l'Expérimentation Animale Rhone-Alpes (regional board of ethics) for advice and was notified to the research animal facility of the Department of Biology, Université Joseph Fourier Grenoble.

Details of Materials and methods are provided as Supplementary materials/methods.

Statistical procedures

All data are presented as mean ± SE. One-way analysis of variance (ANOVA) was used to determine the global effects of treatment. When appropriate, differences between groups were tested with a PLSD Fisher post hoc test. Statistical significance was accepted at $p < 0.05$ (∗, significantly different between C and HF). Mann and Whitney tests were applied when values were not normally distributed.

Results

Effects of a HF diet on growth, energy expenditure and body composition

As shown in Fig. 1, an 8-week HF diet did not affect animal growth. Caloric intake was higher in the HF group during the first 4 weeks and was identical during the last 4 weeks. Energy expenditure was significantly lower in the HF group during the entire study period. Interestingly, HF rats failed to increase oxygen consumption during the nocturnal (active and feeding) period, conversely to the control group. No difference in the respiratory quotient was noticed between the groups during the diurnal period. However, the large increase observed during the nocturnal period in the control group, was absent in the HF group.

Effects of a HF diet on the lipid composition of plasma, liver tissue, and liver mitochondria

The high lipid content of the diet was responsible for a marked change in body lipid content and composition. Mesenteric, retroperitoneal, and epididymal fat deposits together with liver lipid content were significantly higher in HF. However, liver mass and liver protein content were similar in both groups, while liver glycogen was lower in HF (Supplementary Table 2). Total plasma fatty acids were moderately increased and fatty acid composition was markedly altered in HF (Table 1). There was no difference in the amount of saturated fatty acids (SFA) between the groups but the composition was different: stearic acid (18:0) was 2-fold higher, while 14:0, 16:0, and 20:0 were significantly lower. Total unsaturated fatty acids (UFA) were lower in HF, although 18:1 *n*-9; 20:3 *n*-6, and 20:4 *n*-6 were higher. Total *n*-3 FAs and *n*-3-to-*n*-6 ratios were also lower in HF. The FA liver content was nearly 3-fold higher in HF, with a marked increase in mono-unsaturated fatty acids (MUFA), largely due to oleic acid increase. As seen above in the plasma, *n*-3 FAs, and *n*-3-to-*n*-6 ratios were lower in livers from the HF group. The changes found in liver mitochondrion fractions were quite similar to those found in the plasma: there was no difference between the two groups regarding total FAs, *n*-6 FAs, MUFAs, and SFAs, but *n*-3FAs and *n*-3-to-*n*-6 ratios were lower in HF. The lack of change in total SFAs contrasted with a significant modification of their proportions: 14:0, 16:0 were lower, while 18:0 was substantially higher. Finally, there was an increase in 18:1 *n*-9; 20:3 *n*-6, and 20:4 *n*-6 in HF, as displayed in the plasma.

Inhibition of mitochondrial oxidative phosphorylation and increase of ROS production in HF

Mitochondrion state-3 (phosphorylating) oxygen consumption was lower in the HF group independent of the substrate (Table 2). By contrast, state-4 respiration was higher only with glutamate/malate. Also, fully uncoupled respiration with TMPD/ascorbate/DNP was significantly lower in the HF group. The decrease in state-3 respiration was not accompanied by any change in phosphorylation efficiency (see Supplementary Fig. 1). Hence, the lower state-3 respiration rate exhibited by the HF group also corresponded to a lower ATP synthesis rate.

In the presence of glutamate/malate, basal mitochondrial production of H_2O_2 was increased after rotenone addition and further still after adding antimycin (Fig. 2). In the presence of succinate and regardless of the diet, high basal production of H_2O_2 was inhibited by rotenone, indicating a production related to a reverse electron flux at the complex-1 [1,30]. Antimycin addition induced an increase in production, which was higher in the HF group as compared to controls. The addition of glutamate/malate and succinate resulted in an intermediary situation between the image depicting glutamate/malate or succinate alone. Mitochondrial H_2O_2 production with palmitoyl-carnitine was very low in controls and was substantially higher in HF.

Effects on the respiratory chain

The HF diet was responsible for a significant decrease in the amount of reduced and oxidized Q9; the resulting ratio of