



HAL
open science

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

Melissa Flamment, Matthieu Arvier, Yves Gallois, Gilles Simard, Yves Malthièry, Patrick Ritz, Pierre-Henri Ducluzeau-Fieloux

► **To cite this version:**

Melissa Flamment, Matthieu Arvier, Yves Gallois, Gilles Simard, Yves Malthièry, et al.. Fatty liver and insulin resistance in obese Zucker rats: No role for mitochondrial dysfunction. *Biochimie*, 2008, 90 (9), pp.1407-1413. 10.1016/j.biochi.2008.05.003 . hal-01389572

HAL Id: hal-01389572

<https://hal.science/hal-01389572>

Submitted on 10 Aug 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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 Malthiery ^{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

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.

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).

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 PKC ϵ , 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 (*falfa*) Zucker rats and ten male lean (*fal+*) 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 \times g for 10 min. The supernatant was filtered through cheesecloth and centrifuged at 7000 \times g for 10 min. The pellet was resuspended in the isolation medium (10 ml/g tissue) and centrifuged at 3500 \times 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 KH₂PO₄, 1 mM EGTA, 2 mM MgCl₂, 3 mM HEPES and 0.3% bovine serum albumin (w/v), pH 7.4, and supplemented with 5 μ M rotenone and 80 ng/ml nigericin. The TPMP electrode was calibrated by sequential 0.5 μ M additions up to 2 μ M TPMP, then 5 mM succinate was added to start the reaction. After each run, 2 μ M 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 (μ l/mg of protein)⁻¹ [24].

The relationships between oxygen consumption and membrane potential were established by carrying out titrations in non-phosphorylating (state 4) and phosphorylating (state 3) states. In the non-phosphorylating state, respiration and membrane potential were progressively inhibited through successive steady states induced by additions of malonate (up to 5 mM) in the presence of oligomycin (1 µg/ml). In the phosphorylating state, respiration and membrane potential were recorded in the respiratory reaction medium supplemented with 20 mM glucose and 125 µM ATP with increasing concentrations of hexokinase ranging from 10 to 175 mU/ml, in the absence of oligomycin.

2.4. Mitochondrial ATP synthesis

Mitochondria (0.5 mg/ml) were incubated in the respiratory reaction medium supplemented with 5 µM rotenone, 5 mM succinate, 20 mM glucose and 125 µM ATP. The rate of oxidative phosphorylation flux was modulated by different concentrations of hexokinase (10–175 mU/ml) and ATP production was monitored by glucose-6-phosphate formation, as previously described [25]. For each steady state, oxygen consumption was recorded for 2 min, thereafter aliquots were taken from the respiratory reaction medium and immediately put in perchloric acid (6%), centrifuged (8000 × *g*, 3 min) and the supernatant was removed and neutralized to pH 7 using KOH (5 M). NADP⁺ (0.5 mM) and glucose 6-phosphate dehydrogenase (0.25 U) were then added and ATP production was monitored from NADPH content by spectrophotometry at 340 nm.

2.5. Mitochondrial enzyme activities

The activities of citrate synthase (CS) and cytochrome *c* oxidase (COX) were measured spectrophotometrically at 37 °C in liver homogenate and in isolated mitochondria via an adaptation of the method described by Malgat et al. [26]. The activity of CS was measured in a reaction medium consisting of 0.1 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (regenerated with Tris buffer, 1 M, pH 8.1), 1 mM oxaloacetic acid, 0.3 mM acetyl CoA and Triton X-100 (4%). After 3 min of incubation, the reaction was initiated by adding the homogenate (30 µg protein) or isolated mitochondria (10 µg protein) and the change in optical density at 412 nm was recorded for 3 min. The activity of COX was measured by monitoring the oxidation of reduced cytochrome *c* at 550 nm. An 80 µM solution of reduced cytochrome *c* (92–97% reduced using dithionite) in 50 mM KH₂PO₄, was preincubated for 5 min. The reaction was initiated by adding the homogenate (30 µg protein) or isolated mitochondria (5 µg protein) and the change in optical density at 550 nm was recorded for 1.5 min.

2.6. Liver triglyceride content

Liver triglyceride (TG) content was determined using the method described by Xu et al. [27]. Briefly, 100 mg of liver tissue was homogenized in 1 ml of ethanol followed by

centrifugation at 15,000 × *g* for 10 min. TG were measured with a Sigma kit.

2.7. Serum parameters

Glucose concentrations were measured with a glucometer. Serum insulin and leptin concentrations were measured using enzyme-linked immunosorbent assay kits (Linco Research, St Charles, MO). Serum adiponectin concentrations were measured using a radioimmunoassay kit (Linco Research, St Charles, MO). Serum β-hydroxybutyrate concentrations were measured by an enzymatic spectrophotometric method adapted on a Cobas Mira automatic analyzer (Roche) using 3-hydroxybutyrate dehydrogenase to catalyze the conversion of 3-hydroxybutyrate to acetoacetate, in presence of NAD⁺ and at pH 9.5. The concentration of β-hydroxybutyrate was calculated from the NADH produced.

2.8. Statistical analysis

Results are expressed as mean ± SEM. The statistical significance of observed variations was assessed using unpaired Student's *t*-test. A *P* value < 0.05 was considered significant in all cases. All analyses were performed using Statview 5.0 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Obesity, fatty liver and insulin resistance state

Results concerning body weight, liver mass, triglyceride content and serum parameters are shown in Table 1. Body weight of obese Zucker rats was significantly higher (+14%, *P* < 0.05) than that of lean Zucker rats. Liver relative weight of obese Zucker rats was significantly higher (+17%, *P* < 0.05) than that of lean Zucker rats. Liver triglyceride content was significantly higher (+43%, *P* < 0.0001). Fasting serum glucose, insulin, leptin and adiponectin concentrations of obese Zucker rats were significantly higher than those of lean Zucker rats. Serum β-hydroxybutyrate concentration was not significantly different between lean and obese Zucker rats.

Table 1
Body weight, liver mass, liver triglyceride content and serum parameters

	Lean	Obese
Body weight (g)	273 ± 8	316 ± 13*
Liver mass (% weight)	3.25 ± 0.17	3.93 ± 0.08*
Liver TG (mg/g liver)	45.3 ± 4.2	105.9 ± 9.9 [†]
Glucose (g/l)	1.1 ± 0.09	2.05 ± 0.3*
Insulin (µU/ml)	12.05 ± 1.7	186.6 ± 24.2 [†]
Leptin (ng/ml)	1.33 ± 0.18	42.1 ± 2.9 [†]
Adiponectin (µg/ml)	1.76 ± 0.07	2.92 ± 0.17 [†]
β-Hydroxybutyrate (µmol/l)	905.5 ± 124.8	722.56 ± 175.8

Results are expressed as mean ± SEM. **P* < 0.05; [†]*P* < 0.0001 compared with lean Zucker rats.

3.2. Relationship between membrane potential and oxygen consumption

To evaluate proton leak, membrane potential and oxygen consumption in state 4 respiration was determined by additions of malonate, an inhibitor of complex II, in the presence of oligomycin (Fig. 1). By inhibiting complex V (ATP synthase), oligomycin blocks phosphorylation of ADP in ATP (non-phosphorylating conditions). Under these conditions, the proton motive force is only consumed by the proton leak. There was strictly no difference in oxygen consumption and membrane potential in state 4 between obese and lean Zucker rats.

To evaluate the capacity of the respiratory chain to generate and maintain a potential, in state 3 respiration, we measured oxygen consumption and membrane potential with increasing concentration of hexokinase, in the absence of oligomycin (Fig. 2). There was no difference in oxygen consumption and membrane potential in state 3 between obese and lean Zucker rats.

3.3. Oxidative phosphorylation efficiency

To quantify the oxidative phosphorylation rate and so directly evaluate the mitochondrial energy efficiency, we measured oxygen consumption and ATP synthesis with increasing concentration of hexokinase (Fig. 3). There was no difference in oxygen consumption and ATP synthesis between obese and lean Zucker rats.

3.4. Mitochondrial enzyme activities

Measurement of the enzymatic activities of citrate synthase (CS) and cytochrome *c* oxidase (COX) in isolated mitochondria and liver homogenate allows us to determine the quantity of

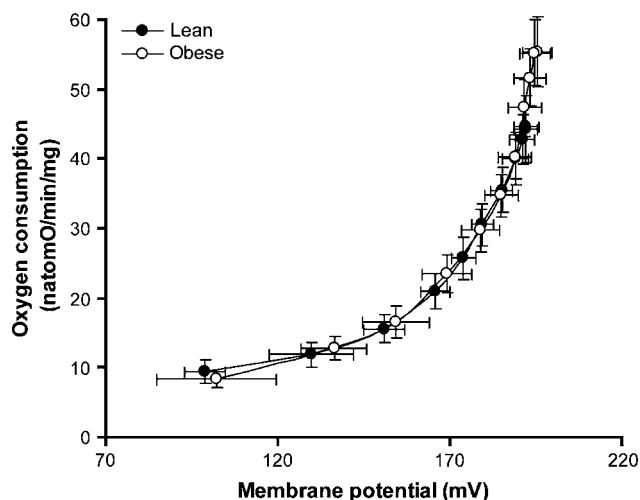


Fig. 1. Relationship between membrane potential and oxygen consumption in state 4 respiration. Oxygen consumption and membrane potential were progressively inhibited through successive steady states induced by additions of malonate (up to 5 mM) in the presence of oligomycin (1 μ g/ml) in liver mitochondria of lean (\bullet) and obese (\circ) Zucker rats. Results are expressed as mean \pm SEM.

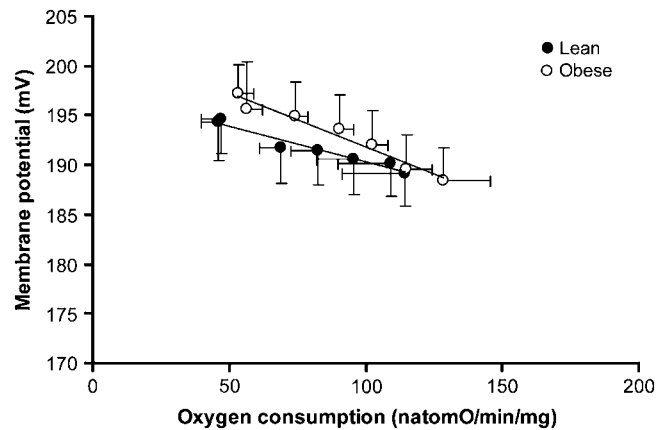


Fig. 2. Relationship between membrane potential and oxygen consumption in state 3 respiration. Oxygen consumption was modulated by subsaturating ADP concentrations generated by hexokinase (10–175 mU/ml) in liver mitochondria of lean (\bullet) and obese (\circ) Zucker rats. Results are expressed as mean \pm SEM.

mitochondria in liver and to evaluate the oxidative capacity of mitochondria. As shown in Table 2, there was no difference in CS and COX activities between obese and lean Zucker rats in isolated mitochondria and in liver homogenate.

4. Discussion

Factors underlying the development of insulin resistance have not been fully elucidated, however the relationship between insulin resistance and mitochondrial function is increasingly of interest [1]. Most studies are performed on skeletal muscle and show that insulin resistance in skeletal muscle could be due to reduced mitochondrial oxidative capacity [2–7]. Only a few studies have been done on 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 [13,14]. However, the exact mechanism of lipid metabolites accumulation

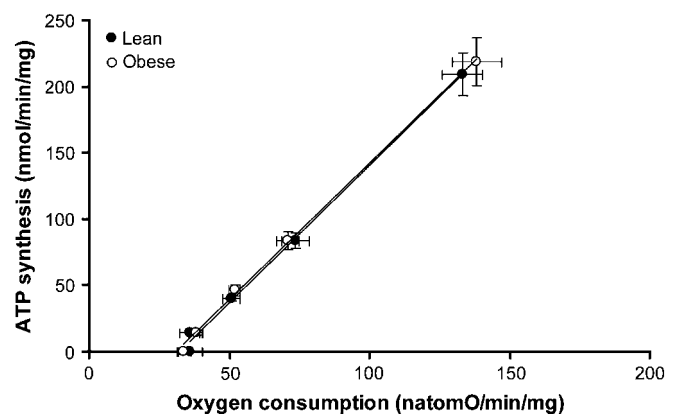


Fig. 3. Relationship between ATP synthesis and oxygen consumption. Oxygen consumption was modulated by subsaturating ADP concentrations generated by hexokinase (10–175 mU/ml) in liver mitochondria of lean (\bullet) and obese (\circ) Zucker rats. Results are expressed as mean \pm SEM. Linear regression equations are $y = 2.07x - 67.12$ ($R^2 = 0.996$) for lean and $y = 2.06x - 64.28$ ($R^2 = 0.998$) for obese Zucker rats.

Table 2
Mitochondrial enzyme activities

		Lean	Obese
Citrate synthase	Homogenate	192.2 ± 8.5	177 ± 4.4
	Mitochondria	331.4 ± 25.4	362.7 ± 21.3
Cytochrome <i>c</i> oxidase	Homogenate	336.1 ± 30.2	358.2 ± 18.2
	Mitochondria	2044.6 ± 111.9	2139.2 ± 129.6

CS and COX activities in liver homogenate and isolated mitochondria are expressed in nmol/min per mg protein. Results are expressed as mean ± SEM.

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. In the current study we studied oxidative phosphorylation efficiency using isolated mitochondria from the livers of Zucker rats, a fatty liver and insulin resistance model. We determined relationships between membrane potential and oxygen consumption in non-phosphorylating (state 4) and phosphorylating (state 3) isolated mitochondria and the relationship between ATP synthesis and oxygen consumption in isolated liver mitochondria. To investigate mitochondrial content and oxidative capacity we determined citrate synthase and cytochrome *c* oxidase activities.

In order to evaluate basal proton leak within mitochondria we determined the relationship between membrane potential and oxygen consumption in non-phosphorylating (state 4 oligomycin) conditions (Fig. 1). Oligomycin is an inhibitor of complex V and so blocks the phosphorylation of ADP in ATP. Under these conditions, the protonmotive force is only consumed by the proton leak. Oxygen uptake is thus proportional to the intensity of the proton leak and to the capacity of the respiratory chain to generate and maintain membrane potential [28]. There was no difference in oxygen consumption and membrane potential in state 4 between obese and lean Zucker rats. These results are in agreement with those of Brookes et al. [20], who observed a similar proton leak in obese and lean Zucker rats. To evaluate the capacity of the respiratory chain to generate and maintain a potential, in state 3 respiration, we measured oxygen consumption and membrane potential with increasing concentrations of hexokinase, in the absence of oligomycin (Fig. 2). There was no difference in oxygen consumption and membrane potential in state 3 between obese and lean Zucker rats. To quantify the oxidative phosphorylation rate and so directly evaluate the mitochondrial energy efficiency, we measured oxygen consumption and ATP synthesis with increasing concentrations of hexokinase in order to obtain a kinetic which really corresponds to the physiological variations in oxidative phosphorylation (Fig. 3). There was no difference in oxygen consumption and ATP synthesis between obese and lean Zucker rats, indicating an identical mitochondrial energy efficiency. The slopes of the curves represent the ATP/O ratio, which in our study are 2.07 for lean rats and 2.06 for obese rats. These values are close to those found in the literature [29]. This result is also in agreement with our earlier finding that there was no difference in mitochondrial state 3 oxygen consumption between our two rats groups (Fig. 2). Our results are not in accordance with the recent study which showed that in liver mitochondria from obese Zucker rats there is

a decrease in state 3 respiration which is associated with a decrease in ANT content and an increase in state 4 oligomycin respiration with succinate as the respiratory substrate [21]. It appears difficult to compare their results with ours, because we did not use the same experimental design. In particular, they measured oxygen consumption and membrane potential at 25 °C compared to 37 °C for our measurements. However mitochondrial energetics at sub-physiological temperatures are different than at normal body temperature [30]. For example, the contribution of slip redox to resting respiration could be dependent on the experimentation temperature [31]. This last mechanism could participate in the increase in state 4 oligomycin respiration in obese Zucker rats obtained by Teodoro et al. [21] which is not observed in our study. To explain the discrepancy in results concerning state 3, we can speculate that this is due to the difference in experimental procedure used to obtain state 3 respiration. In their study, state 3 respiration rate was stimulated by saturating ADP concentration. In this case, newly synthesized ATP accumulated in the medium, with a subsequent increase in the ATP/ADP ratio, which is well known to slow down oxidative phosphorylation, in part via ANT inhibition [32], due to competition between ATP and ADP for ANT binding and subsequent translocation across the mitochondrial membrane [33,34]. Given the fact that in their study, obese Zucker rats exhibited a decrease in ANT content, ATP accumulation could be responsible for a stronger inhibition of oxidative phosphorylation in obese than in lean Zucker rats. Indeed, it has been shown that ANT exerts a high flux control on respiration in liver mitochondria, and that control strength of ANT on respiration is more pronounced when the extramitochondrial ATP/ADP ratio increases [35]. In our study we used the “hexokinase system” which allows a regeneration of ADP from ATP and so prevents ATP accumulation and maintains ADP concentration and the corresponding respiration at a constant physiological rate.

The measure of the enzymatic activities of citrate synthase (CS) and cytochrome *c* oxidase (COX) on isolated mitochondria and homogenate from liver allows us to determine the quantity of mitochondria in liver and to evaluate the oxidative capacity of mitochondria. It has been shown that in the muscle of insulin-resistant offspring of type 2 diabetic parents there is a decrease in mitochondrial density [2,7] which could explain the reduced muscle mitochondrial ATP production. In the current study there was no difference in CS and COX 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. The absence of variation of CS and COX activities is in agreement with the result obtained by Wardlaw and Kaplan [36] carried out on Zucker rats. The absence of mitochondrial dysfunction especially for the β -oxidation pathway was confirmed by the fact that serum β -hydroxybutyrate which is an indicator of liver β -oxidation capacity, was not altered in obese compared to lean Zucker rats.

In our study, liver mitochondrial function seemed to be unaffected in obese Zucker rats despite fatty liver and insulin resistance. Effectively, rats in this study displayed liver triglyceride accumulation and hyperglycemia associated with strong

hyperinsulinemia arguing for increased hepatic glucose production and liver insulin resistance. Serum leptin concentrations showed a dramatic increase in obese Zucker rats compared to their lean littermates, which could not be fully explained by the increased fat mass noted in obese rats. Indeed, strong hyperleptinemia observed in obese Zucker rats is, at least in part, a consequence of the homozygote mutation of their leptin receptor gene. Indeed, the white adipose tissue of *falfa* rats overexpresses the *ob* gene, thus inducing massive synthesis of leptin [37]. Only the increase in serum adiponectin concentration in obese Zucker rats seems to be inconsistent with the generally accepted association of high adiponectin with insulin sensitivity. Adiponectin is an adipokine that plays a central role in glucose and lipid metabolism [38]. Low adiponectin levels are found in obesity and type 2 diabetes [39–41], and reduced adiponectin levels have also been correlated to increased risk of developing obesity and type 2 diabetes [42,43]. Surprisingly, we showed that in obese Zucker rats adiponectin levels are increased. This result is in accordance with many studies on Zucker rats that have observed a higher adiponectin level in obese than in lean Zucker rats [44–46]. This increased adiponectin level could be an adaptative phenomenon that exists in obese Zucker rats, which lack leptin signal. It has been reported that adiponectin regulates mitochondrial bioenergetics in muscle by increasing mitochondrial biogenesis as well as fatty acid oxidation [47]. In hepatocytes adiponectin, via AMP-activated kinase (AMPK), could increase the entry of fatty acid into mitochondria and so increase their oxidation [48]. We could speculate that this paradoxical increase of adiponectin in obese Zucker rats could counteract the mitochondrial abnormalities expected in these animals, and that fatty liver and insulin resistance appear, in this particular model, via an independent pathway of mitochondrial dysfunction.

5. Conclusion

In conclusion we showed that there was no difference in oxygen consumption, ATP synthesis, and membrane potential (state 4 and 3 respiration) between lean and obese Zucker rats, used as a fatty liver and insulin resistance model. Taken together, these results showed that isolated mitochondria of both rats were equally efficient for coupling respiration to phosphorylation. We showed also that there was no difference in CS and COX activities between our two groups of rats indicating a similar quantity of mitochondria and a similar oxidative capacity. So it appears that in the livers of obese Zucker rats, isolated mitochondria do not exhibit any mitochondrial dysfunction despite fatty liver and insulin resistance. The question of the impact of the adapted increased adiponectin level on mitochondrial function remains to be elucidated.

Acknowledgements

Mélissa Flamment was supported by a grant from “Conseil Régional Pays de la Loire/Inserm”. The authors thank Jennifer Deschamps and Maud Poirier for animal care.

References

- [1] B.B. Lowell, G.I. Shulman, Mitochondrial dysfunction and type 2 diabetes, *Science* 307 (2005) 384–387.
- [2] D.E. Kelley, J. He, E.V. Menshikova, V.B. Ritov, Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes, *Diabetes* 51 (2002) 2944–2950.
- [3] K.F. Petersen, D. Befroy, S. Dufour, J. Dziura, C. Ariyan, D.L. Rothman, L. DiPietro, G.W. Cline, G.I. Shulman, Mitochondrial dysfunction in the elderly: possible role in insulin resistance, *Science* 300 (2003) 1140–1142.
- [4] K.F. Petersen, S. Dufour, D. Befroy, R. Garcia, G.I. Shulman, Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes, *N. Engl. J. Med.* 350 (2004) 664–671.
- [5] M.E. Patti, A.J. Butte, S. Crunkhorn, K. Cusi, R. Berria, S. Kashyap, Y. Miyazaki, I. Kohane, M. Costello, R. Saccone, E.J. Landaker, A.B. Goldfine, E. Mun, R. DeFronzo, J. Finlayson, C.R. Kahn, L.J. Mandarino, Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 8466–8471.
- [6] V.K. Mootha, C.M. Lindgren, K.F. Eriksson, A. Subramanian, S. Sihag, J. Lehar, P. Puigserver, E. Carlsson, M. Ridderstrale, E. Laurila, N. Houstis, M.J. Daly, N. Patterson, J.P. Mesirov, T.R. Golub, P. Tamayo, B. Spiegelman, E.S. Lander, J.N. Hirschhorn, D. Altshuler, L.C. Groop, PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes, *Nat. Genet.* 34 (2003) 267–273.
- [7] K. Morino, K.F. Petersen, S. Dufour, D. Befroy, J. Frattini, N. Shatzkes, S. Neschen, M.F. White, S. Bilz, S. Sono, M. Pypaert, G.I. Shulman, Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents, *J. Clin. Invest.* 115 (2005) 3587–3593.
- [8] M. Kabir, K.J. Catalano, S. Ananthnarayan, S.P. Kim, G.W. Van Citters, M.K. Dea, R.N. Bergman, Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance, *Am. J. Physiol. Endocrinol. Metab.* 288 (2005) E454–E461.
- [9] T. Kadowaki, T. Yamauchi, N. Kubota, K. Hara, K. Ueki, K. Tobe, Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome, *J. Clin. Invest.* 116 (2006) 1784–1792.
- [10] R.R. Banerjee, S.M. Rangwala, J.S. Shapiro, A.S. Rich, B. Rhoades, Y. Qi, J. Wang, M.W. Rajala, A. Poci, P.E. Scherer, C.M. Steppan, R.S. Ahima, S. Obici, L. Rossetti, M.A. Lazar, Regulation of fasted blood glucose by resistin, *Science* 303 (2004) 1195–1198.
- [11] U. Ozcan, E. Yilmaz, L. Ozcan, M. Furuhashi, E. Vaillancourt, R.O. Smith, C.Z. Görgün, G.S. Hotamisligil, Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes, *Science* 313 (2006) 1137–1140.
- [12] S.E. Shoelson, J. Lee, A.B. Goldfine, Inflammation and insulin resistance, *J. Clin. Invest.* 116 (2006) 1793–1801.
- [13] V.T. Samuel, Z.X. Liu, X. Qu, B.D. Elder, S. Bilz, D. Befroy, A.J. Romanelli, G.I. Shulman, Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease, *J. Biol. Chem.* 279 (2004) 32345–32353.
- [14] V.T. Samuel, Z.X. Liu, A. Wang, S.A. Beddow, J.G. Geisler, M. Kahn, X.M. Zhang, B.P. Monia, S. Bhanot, G.I. Shulman, Inhibition of protein kinase C ϵ prevents hepatic insulin resistance in nonalcoholic fatty liver disease, *J. Clin. Invest.* 117 (2007) 739–745.
- [15] P. Mitchell, J. Moyle, Proton translocation coupled to ATP hydrolysis in rat liver mitochondria, *Eur. J. Biochem.* 4 (1968) 530–539.
- [16] J.A. Stuart, S. Cadenas, M.B. Jekabsons, D. Rousset, M.D. Brand, Mitochondrial proton leak and the uncoupling protein 1 homologues, *Biochim. Biophys. Acta.* 1504 (2001) 144–158.
- [17] L.M. Zucker, T.F. Zucker, Fatty, a new mutation in the rat, *J. Hered.* 52 (1961) 275–278.
- [18] J. Terretaz, B. Jeanrenaud, In vivo hepatic and peripheral insulin resistance in genetically obese (*fa/fa*) rats, *Endocrinology* 112 (1983) 1346–1351.

- [19] J.J. Ramsey, D.E. Johnson, K.L. Hossner, K.A. Johnson, Metabolic rate, organ mass, and mitochondrial proton leak variations in lean and obese rats, *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.* 113 (1996) 461–466.
- [20] P.S. Brookes, J.A. Buckingham, A.M. Tenreiro, A.J. Hulbert, M.D. Brand, The proton permeability of the inner membrane of liver mitochondria from ectothermic and endothermic vertebrates and from obese rats: correlations with standard metabolic rate and phospholipid fatty acid composition, *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 119 (1998) 325–334.
- [21] J. Teodoro, A.P. Rolo, P.J. Oliveira, C.M. Palmeira, Decreased ANT content in Zucker fatty rats: relevance for altered hepatic mitochondrial bioenergetics in steatosis, *FEBS Lett.* 580 (2006) 2153–2157.
- [22] S. Krahenbuhl, C. Talos, S. Fischer, J. Reichen, Toxicity of bile acids on the electron transport chain of isolated rat liver mitochondria, *Hepatology* 19 (1994) 471–479.
- [23] M.D. Brand, Measurement of mitochondrial protonmotive force, in: G.C. Brown, C.E. Cooper (Eds.), *Bioenergetics, A Practical Approach*, IRL Press, Oxford, 1995, pp. 39–62.
- [24] D.F. Rolfe, A.J. Hulbert, M.D. Brand, Characteristics of mitochondrial proton leak and control of oxidative phosphorylation in the major oxygen-consuming tissues of the rat, *Biochim. Biophys. Acta.* 1188 (1994) 405–416.
- [25] V. Nogueira, M.A. Piquet, A. Devin, C. Fiore, E. Fontaine, G. Brandolin, M. Rigoulet, X.M. Leverve, Mitochondrial adaptation to in vivo polyunsaturated fatty acid deficiency: increase in phosphorylation efficiency, *J. Bioenerg. Biomembr.* 33 (2001) 53–61.
- [26] M. Malgat, G. Durrieu, J.P. Mazat, Enzymatic and polarographic measurements of the respiratory chain complexes, in: P. Lestienne (Ed.), *Mitochondrial Diseases*, Springer Verlag, Paris, 1999, pp. 357–377.
- [27] H. Xu, D. Wilcox, P. Nguyen, M. Voorbach, T. Suhar, S.J. Morgan, W.F. An, L. Ge, J. Green, Z. Wu, R.E. Gimeno, R. Reilly, P.B. Jacobson, C.A. Collins, K. Landschulz, T. Surowy, Hepatic knock-down of mitochondrial GPAT1 in ob/ob mice improves metabolic profile, *Biochem. Biophys. Res. Commun.* 349 (2006) 439–448.
- [28] R.P. Hafner, M.D. Brand, Effect of protonmotive force on the relative proton stoichiometries of the mitochondrial proton pumps, *Biochem. J.* 275 (1991) 75–80.
- [29] P.C. Hinkle, P/O ratios of mitochondrial oxidative phosphorylation, *Biochim. Biophys. Acta.* 1706 (2005) 1–11.
- [30] S. Dufour, N. Rousse, P. Canioni, P. Diolez, Top-down control analysis of temperature effect on oxidative phosphorylation, *Biochem. J.* 314 (1996) 743–751.
- [31] M. Canton, S. Luvisetto, I. Schmehl, G.F. Azzone, The nature of mitochondrial respiration and discrimination between membrane and pump properties, *Biochem. J.* 310 (1995) 477–481.
- [32] W.E. Jacobus, R.W. Moreadith, K.M. Vandegaer, Mitochondrial respiratory control. Evidence against the regulation of respiration by extramitochondrial phosphorylation potentials or by [ATP]/[ADP] ratios, *J. Biol. Chem.* 257 (1982) 2397–2402.
- [33] R. Bohnsack, Control of energy transformation of mitochondria. Analysis by a quantitative model, *Biochim. Biophys. Acta.* 634 (1981) 203–218.
- [34] R.S. Balaban, Regulation of oxidative phosphorylation in the mammalian cell, *Am. J. Physiol.* 258 (1990) C377–C389.
- [35] L.I. Ivanovene, V.I. Borutaite, V. Iu. Zhilinskene, A.I. Toleikis, A.K. Prashkiavichius, Participation of the adenine nucleotide translocator in the regulation of pyruvate oxidation in heart mitochondria, *Biull. Eksp. Biol. Med.* 105 (1988) 540–542.
- [36] G.M. Wardlaw, M.L. Kaplan, Oxygen consumption and oxidative capacity of hepatocytes from young male obese and nonobese Zucker rats, *Proc. Soc. Exp. Biol. Med.* 183 (1986) 199–206.
- [37] S.C. Chua Jr., W.K. Chung, X.S. Wu-Peng, Y. Zhang, S.M. Liu, L. Tartaglia, R.L. Leibel, Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor, *Science* 271 (1996) 994–996.
- [38] A.H. Berg, T.P. Combs, P.E. Scherer, ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism, *Trends Endocrinol. Metab.* 13 (2002) 84–89.
- [39] E. Hu, P. Liang, B.M. Spiegelman, AdipoQ is a novel adipose-specific gene dysregulated in obesity, *J. Biol. Chem.* 271 (1996) 10697–10703.
- [40] Y. Arita, S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi, Y. Matsuzawa, Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity, *Biochem. Biophys. Res. Commun.* 257 (1999) 79–83.
- [41] K. Hotta, T. Funahashi, Y. Arita, M. Takahashi, M. Matsuda, Y. Okamoto, H. Iwahashi, H. Kuriyama, N. Ouchi, K. Maeda, M. Nishida, S. Kihara, N. Sakai, T. Nakajima, K. Hasegawa, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Hanafusa, Y. Matsuzawa, Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients, *Arterioscler. Thromb. Vasc. Biol.* 20 (2000) 1595–1599.
- [42] R.S. Lindsay, T. Funahashi, R.L. Hanson, Y. Matsuzawa, S. Tanaka, P.A. Tataranni, W.C. Knowler, J. Krakoff, Adiponectin and development of type 2 diabetes in the Pima Indian population, *Lancet* 360 (2002) 57–58.
- [43] J. Spranger, A. Kroke, M. Möhlig, M.M. Bergmann, M. Ristow, H. Boeing, A.F. Pfeiffer, Adiponectin and protection against type 2 diabetes mellitus, *Lancet* 361 (2003) 226–228.
- [44] F. Oana, H. Takeda, K. Hayakawa, A. Matsuzawa, S. Akahane, M. Isaji, M. Akahane, Physiological difference between obese (fa/fa) Zucker rats and lean Zucker rats concerning adiponectin, *Metabolism* 54 (2005) 995–1001.
- [45] S. de Assis, M. Wang, S. Goel, A. Foxworth, W. Helferich, L. Hilakivi-Clarke, Excessive weight gain during pregnancy increases carcinogen-induced mammary tumorigenesis in Sprague–Dawley and lean and obese Zucker rats, *J. Nutr.* 136 (2006) 998–1004.
- [46] C. Doyon, R.G. Denis, E.D. Baraboi, P. Samson, J. Lalonde, Y. Deshaies, D. Richard, Effects of rimonabant (SR141716) on fasting-induced hypothalamic–pituitary–adrenal axis and neuronal activation in lean and obese Zucker rats, *Diabetes* 55 (2006) 3403–3410.
- [47] A.E. Civitarese, B. Ukropcova, S. Carling, M. Hulver, R.A. DeFronzo, L. Mandarino, E. Ravussin, S.R. Smith, Role of adiponectin in human skeletal muscle bioenergetics, *Cell Metab.* 4 (2006) 75–87.
- [48] T. Yamauchi, J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B.B. Kahn, T. Kadowaki, Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase, *Nat. Med.* 8 (2002) 128812–128895.