Journal of Physiology and Biochemistry (2019) 75:65–72
https://doi.org/10.1007/s13105-018-0653-x

ORIGINAL ARTICLE

Cardiac mitochondrial respiration following a low-carbohydrate, high-fat diet in apolipoprotein E-deficient mice

Cynthia Rocha[1] · Olivia H. Koury[1] · Celena Scheede-Bergdahl[2] · Andreas Bergdahl[1]

Received: 17 May 2017 / Accepted: 10 October 2018 / Published online: 25 October 2018

Abstract

Low-carbohydrate diets are considered to be an effective approach to weight loss and have, subsequently, grown in popularity. Despite the apparent health benefits that these diets may provide for insulin resistance, hypertension, and dyslipidemia, their implications on cardiomyocyte oxidative capacity have yet to be investigated. To evaluate the adaptations induced by a 6-week low-carbohydrate, high-fat (LCHF) diet on mitochondrial respiration, two groups of male mice were investigated: Apolipoprotein E-deficient mice on a LCHF diet (L-DIET) and apolipoprotein E-deficient mice on a regular rodent diet (CON). Heart tissue was extracted and used for high-resolution respirometry (HRR), while immunoblotting was performed to quantify mitochondrial density and complexes. The results demonstrate increased expression of all five mitochondrial subunits in the L-DIET group compared to control condition. Furthermore, HRR revealed increased efficiency of substrate consumption, implying augmented oxidative capacity in the L-DIET group. These findings further support the notion that cardiomyocytes prefer lipids as a primary fuel source, by demonstrating that the shift in metabolism caused by a LCHF diet facilitates such an environment. This provides important information regarding the effects of a LCHF on cardiomyocytes, especially when considering free radical production and heart dysfunction.

Keywords Apolipoprotein E · Cardiomyocytes · Mitochondria · OXPHOS · Lipid metabolism · Low-carbohydrate, high-fat diet

Introduction

Obesity is associated with a plethora of negative health effects including heart disease, certain types of cancer, type 2 diabetes mellitus, and respiratory complications [14]. To reduce the risk of developing related comorbidities, overweight and obese patients are advised to strive for a healthy BMI. One of the most efficient means of weight loss is achieved by dieting, and more specifically, through carbohydrate restriction. In recent years, low-carbohydrate diets have gained widespread popularity with the public [6] as advocates claim they "burn more fat" and lead to quicker weight loss with an apparent absence of adverse long-term effects [2]. The altered macronutrient composition in these diets drastically affect cellular metabolism as the majority of energy is derived from fatty acids (FAs) and ketones due to the extreme carbohydrate reduction. In the case of low-carbohydrate, high-fat (LCHF) diets, 70% of caloric requirements comes from fatty acids via dietary fat or lipolysis, 20% from ketone bodies (acetoacetate and β-hydroxybutyrate), and 10% from glucose via gluconeogenesis or glycogenolysis [34]. This induces a shift from "glucocentric" metabolism to one which is more "adipocentric" in nature [33]. While both glucose and FAs are oxidized to ATP in the mitochondria, for cardiomyocytes, lipids are the preferred and more efficient substrate (generating 70% of total ATP), thus highlighting the importance of FA uptake from the plasma for cardiac viability [3, 29–31, 35]. Lipids are also necessary for the maintenance of the pacemaker activity of the cardiomyocytes, further demonstrating their significance [8]. Since the heart has limited substrate storage capacity, the uptake of nutrients needs to be finely balanced as the mitochondria have to respond appropriately and competently to the continuous flux in energy demand and subsequent substrate need [11]. Considering the need and production of ATP drives all cellular processes, the phosphorylation of ADP is imperative for a high

Cynthia Rocha and Olivia H. Koury contributed equally to this work.

✉ Andreas Bergdahl
andreas.bergdahl@concordia.ca

[1] Department of Health, Kinesiology & Applied Physiology, Concordia University, 7141 Sherbrooke West, Montreal, QC H4B 1R6, Canada

[2] Department of Kinesiology and Physical Education, McGill Research Centre for Physical Activity & Health, McGill University, 475 Pine Avenue West, Montreal H2W 1S4, QC, Canada

energy demand, high workload organ like the heart [11]. The capacity of cardiac mitochondria relies heavily on availability of lipids and carbohydrates which contribute to energy metabolism based on their utilization through β-oxidation and the Krebs cycle. β-Oxidation produces acetyl-CoA from FAs that subsequently enters the Krebs cycle, generating electrons that are successively transported to the electron transport system by NADH and $FADH_2$ to amplify ATP production [16]. Acetyl-CoA oxidation by Krebs cycle is responsible for two thirds of the ATP production and oxygen consumption making it the focal aerobic metabolic pathway for carbohydrates, lipids, and proteins [1]. Furthermore, the mitochondria are implicated in the pathophysiology of heart failure and overall cardiovascular health since uptake of cholesterol and lipids modifies the process of oxidative phosphorylation [28]. Elevated FA levels also increase mitochondrial uncoupling as fatty acids function as uncoupling protein activators [24]. Studies that associate high cardiac uncoupling levels with elevated FA have used the obese *ob/ob* and diabetic *db/db* mouse models, which have mutations in the leptin and leptin receptor respectively [5]. It is unknown whether cardiac mitochondrial uncoupling is elevated in the pre-diabetic form of elevated plasma lipids that we induce with our diet model. Under normal conditions, the mitochondria maintain a fine equilibrium between glucose and FA use; however, during physiological stress, there is a shift towards an increased carbohydrate metabolism. This change adds stress as it shunts available lipids into non-oxidative pathways creating more reactive oxygen species (ROS) than energy [13]. The same phenomenon occurs during reduced oxygen supply as a result of coronary vessel occlusion, a major cause of cardiovascular distress [10]. Low-carbohydrate diets have been studied extensively in the past decades regarding weight loss, lipid markers, insulin sensitivity, hypertension, and endothelial dysfunction [4, 22]. However, little has been done to investigate the effects on cardiac oxidative capacity, especially not in the elevated lipid plasma environment which is symptomatic for obese individuals.

This study examined whether apolipoprotein E-deficient (ApoE−/−) mice, with their characteristic high lipid levels [12, 23], exhibit altered cardiac mitochondrial capacity when exposed to a LCHF environment. For this, we compared cardiomyocyte oxygen consumption in 6-week-old ApoE−/− mice randomly assigned either a control or a LCHF diet. We hypothesized that the mitochondria from the LCHF cardiac tissue would demonstrate increased respiratory function in addition to altered energy metabolism when compared to controls.

Methods

Animal care

ApoE−/− mice were obtained from Jackson Laboratories (Bar Harbor, Maine, USA) and used for breeding. The resulting litters were separated based on gender at time of weaning (21–28 days). For the purpose of this study, the male mice were housed individually in a thermo-neutral environment (22 °C), on a 12:12 h photoperiod, and randomly assigned to either a control (CON) or a low-carbohydrate, high-fat diet (L-DIET) for 6 weeks. Male mice were used to avoid affects by the female estrous phase. Both groups had access to water and their respective food pellets ad libitum. All procedures were approved by the Animal Ethics Committee of Concordia University (protocol ID: #30000259) and were conducted in accordance with guidelines of the Canadian Council on Animal Care. This animal model was chosen as it provides a more human-comparable HDL: LDL ratio [21].

Diet specifications

Both control and LCHF diets were isocaloric; the diet specifications are given in Table 1. The control diet, 5075 Charles River autoclavable Rodent Diet, reflects a healthy, standard macronutrient distribution; the LCHF diet, obtained from Harlan Laboratories (TD.04524), simulates an Atkin's diet used for weight loss. This LCHF diet is a modification of TD.88137 (Harlan Laboratories), used for studies on atherosclerosis with a cholesterol content of 1.5 g/kg.

Experimental protocol

Immediately after euthanasia with CO_2, and according to the approved animal protocol, the heart was removed and split into two different portions. The upper region of the heart was snap frozen in liquid nitrogen, and stored at − 80 °C for biochemical analysis, while the apex was placed in an ice cold relaxing buffer (BIOPS) and used immediately to measure mitochondrial respiration. The BIOPS contains (in mM) CaK_2EGTA 2.77, K_2EGTA 7.23, Na_2ATP 5.77, $MgCl_2 \cdot 6H_2O$ 6.56, taurine 20, Na_2Phosphocreatine 15, imidazole 20, dithiothreitol 0.5, MES 50, and pH 7.1.

Table 1 Diet specifications

Product name	Agribrands 5075 Charles River Rodent Diet Control	Harlan Teklad TD.04524 LCHF
Calories from carbohydrate (%)	63	11
Calories from protein (%)	24	46
Calories from fat (%)	14	43
Caloric density (kcal/g)	4.1	4.4

Preparation of permeabilized cardiac fibers

The fiber bundles from the apex were separated using sharp forceps and subsequently incubated for 30 min on ice in BIOPS containing 50 μg/ml saponin followed by washing in ice-cold buffer (MiR05) for 2 × 10 min. MiR05 contains (in mM) EGTA 0.5, $MgCl_2 \cdot 6H_2O$ 3.0, K-lactobionate 60, taurine 20, KH_2PO_4 10, HEPES 20, sucrose 110, BSA 1 g/l, and pH 7.1.

Mitochondrial respiratory measurements

Measurements of oxygen consumption were performed in MiR05 at 37 °C using a polarographic oxygen sensor (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) as previously described [26]. Approximately 2.0 to 2.5 mg of muscle tissue (wet weight) was placed in either chamber in a cross-sectional design. O_2 flux was resolved by DatLab and all experiments were carried out in hyperoxygenated levels to avoid O_2 diffusion limitations. A sequential substrate addition protocol was used to allow functional dissection of the electron transport system: state 2 respiration (absence of adenylates) was assessed by addition of malate (2 mM) and octanoyl carnitine (1.5 mM), by adding ADP (5 mM), state 3 respiration for complex I was reached. This was followed by addition of glutamate (10 mM) and succinate (10 mM) to achieve maximal coupled state 3 respiration with parallel electron input to complex I and II. To measure state 4 respiration, oligomycin (2 μg/ml) was added to block complex V. This was followed by antimycin A (2.5 μM) to inhibit complex III. Finally, ascorbate (2 mM) and TMPD (500 μM) were added to evaluate complex IV respiration.

Mitochondrial uncoupling

Six different protocols were used to test for mitochondrial uncoupling: Protocol 1 began with the addition of oligomycin (2 μg/ml) followed by succinate (10 mM), then FCCP step titrations (1 μl/step) and finally antimycin A (2.5 μM). Protocol 2 was identical to protocol 1 except GDP (10 mM) was added between succinate and FCCP. Protocol 3 consisted of sequential addition of succinate (10 mM), ADP (5 mM) followed by a simultaneous addition of glutamate (10 mM) and malate (2 mM). Protocol 4 required addition of malate (2 mM), octanoyl carnitine (1.5 mM), ADP (5 mM), glutamate (10 mM), oligomycin (2 μg/ml) followed by multiple additions of GDP (10 mM). Protocol 5 began with the addition of malate (2 mM) then octanoyl carnitine (1.5 mM), glutamate (10 mM), oligomycin (2 μg/ml), and again multiple additions of GDP (10 mM). Protocol 6 consisted of adding malate (2 mM) then octanoyl carnitine (1.5 mM), oligomycin (2 μg/ml), and then multiple additions of GDP (10 mM). Uncoupling protein 2 and 3 (UCP2 and UCP3)-mediated uncoupling could hence be investigated through the titrations of oligomycin, GDP, and antimycin A.

Protein extraction and immunoblotting

A portion of the snap frozen tissue excised from the region above the apex was homogenized in liquid nitrogen and mixed with 150 μl lysis buffer containing (in mM) NaCl 250, HEPES 50, $MgCl_2$ 1.5, EGTA 1, $Na_4P_2O_7$ 10, NaF 1, glycerol 10%, triton X-100 1%, Na_3VO_4 800 μM, and pH 7.5. Following 1 h on ice, the cell slurry was centrifuged at 13,000 rpm for 10 min, after which the supernatant was collected. Ten microliters of lysate was mixed with 2 μl DTT and 2 μl sample buffer and loaded on a 12.5% acrylamide-SDS gel. This was followed by transfer onto a 0.45-μm nitrocellulose membrane (162-0115 Bio-Rad, Mississauga, ON, Canada) in 10 mM sodium tetraborate buffer. Even protein loading and transfer were confirmed by Ponceau S staining. Staining of total protein on the blotting membrane was also used to normalize loading conditions according to Moritz [18]. The membranes were then blocked in 3% bovine serum albumin in 0.1% Tween in Tris-buffered saline (10 mM Tris-HCl, 150 mM NaCl, pH 7.5) for 1 h at room temperature, followed by overnight incubation at 4 °C with total OXPHOS rodent antibody cocktail (1:2000, MS604 MitoSciences, Eugene, OR, USA). The membranes were washed and incubated with secondary antibody (1:15000, ab6728 Abcam, Toronto, ON, Canada). Membranes were exposed with ECL chemiluminescence (Immun-Star Chemiluminescent; 1705070; Bio-Rad, Mississauga, ON, Canada) and developed bands were analyzed with ImageJ Software.

Statistical analysis

Summarized data are presented as means ± standard error of the mean (SEM) for immunoblotting, and standard estimate (SE) for the respirometry data. Statistical comparisons were done using a two-tailed Student's *t* test. *n* represents the sample number. For all statistical evaluations, the figures presented $*p < 0.05$ and $** p < 0.01$.

Results

All mice consumed a similar weight of food each day (see Table 1 for dietary composition); however, the LCHF diet has a higher caloric density than the standard laboratory chow therefore these mice consumed approximately 10% more calories. Despite this, the animals in both groups were comparable in weight at 6 weeks (Fig. 1a).

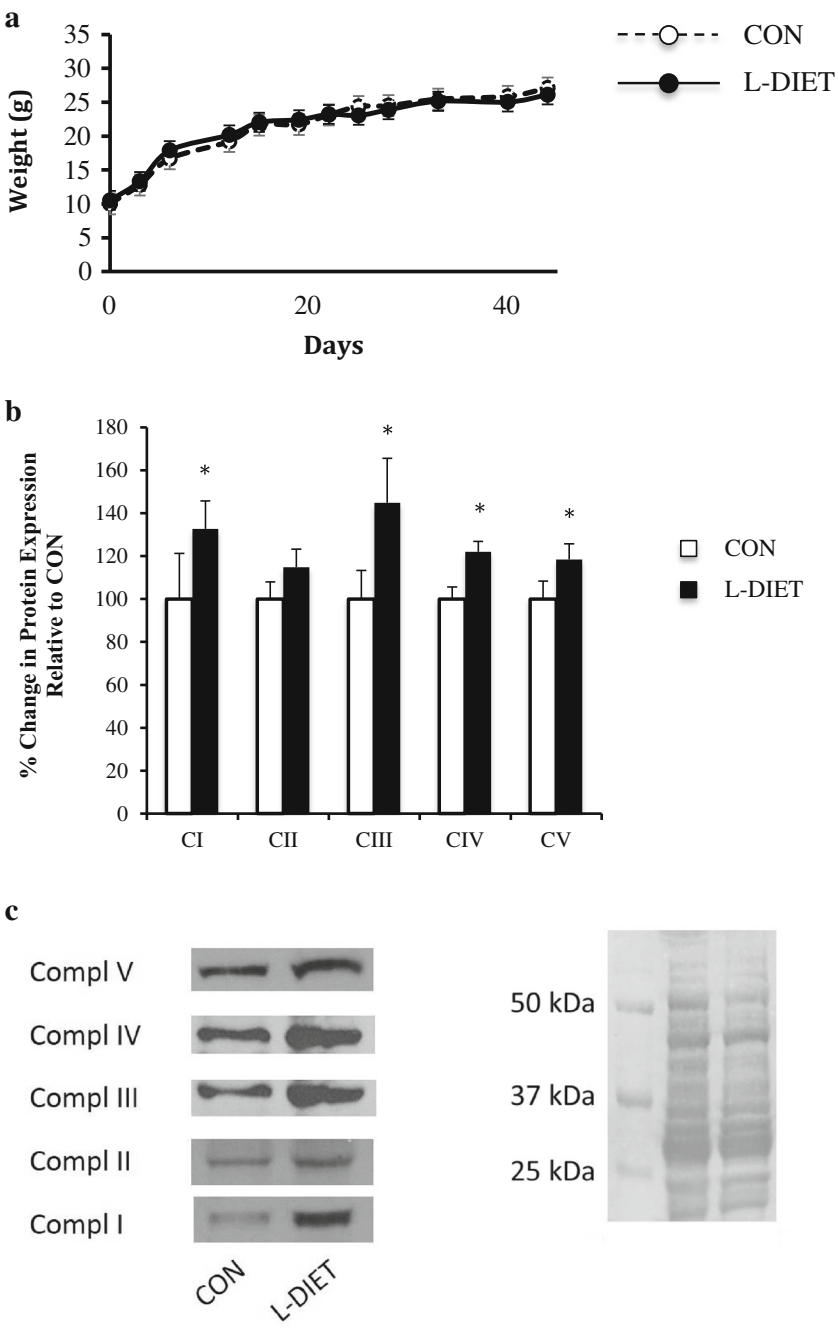

Fig. 1 **a** Summarized increase in body weight over 6 weeks ($n = 7$). **b** Immunoblotting of complex I to V using total OXPHOS rodent antibody cocktail from MitoSciences (MS604). Trends indicate an increase in all five mitochondrial subunits in the L-DIET group with significance for all but complex II relative to CON. Data were normalized to the staining of total protein on the blotting membrane (Ponceau S) ($n = 7$). **c** Representative blots for complex I–V as well as Ponceau S

Relative expression of complexes I–V

Immunoblotting revealed an increasing trend for all five mitochondrial complexes in the L-DIET mice, when normalized to CON (CI 100% ± 19.8 and 132.7% ± 11.1, CII 100% ± 8 and 114.8% ± 8.5, CIII 100% ± 13.3 and 144.9 ± 20.8, CIV 100% ± 5.7 and 122.1% ± 5.7, CV 100% ± 8.4 and 128.5% ± 7.3, respectively, $n = 7$). As seen in Fig. 1b, Complexes I, III, IV, and V demonstrated a statistically significant increase in expression ($p < 0.05$).

Mitochondrial respiration

There was a substantial difference in substrate utilization, as seen in Fig. 2. Data show a significant increase in the respiration (in pmol/s/mg) following addition of octanoyl carnitine (28.3 ± 2.8 and 38.4 ± 2.9, $p < 0.03$), ADP (75.0 ± 5.6 and 140.0 ± 25.1, $p < 0.03$), glutamate (87.2 ± 5.1 and 161.8 ± 25.9, $p < 0.02$), succinate (155.5 ± 27.0 and 362.8 ± 47.9, $p < 0.01$), oligomycin (74.8 ± 14.8 and 122.1 ± 13.5, $p < 0.04$), and ascorbate+TMPD (212.5 ± 24.8 and 292.9 ±

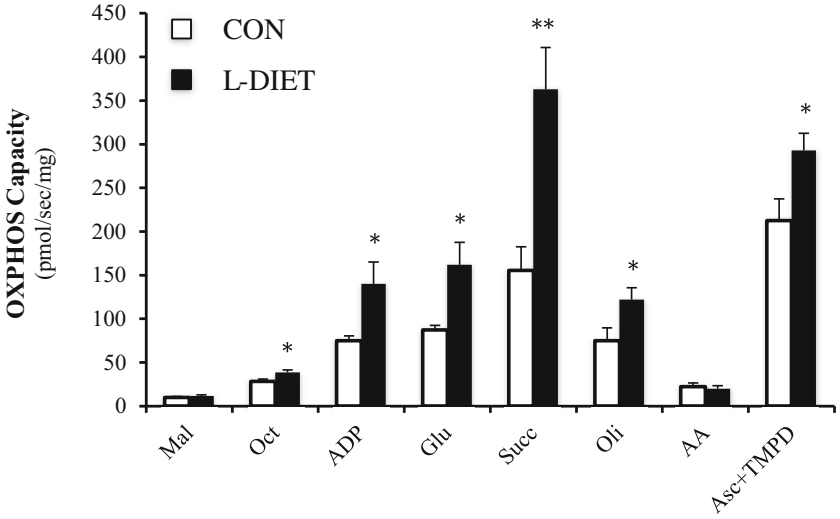

Fig. 2 Substrate utilization by the mitochondria of permeabilized cardiac myofibers assessed through high-resolution respirometry

19.5, $p < 0.03$) by the L-DIET group compared to CON ($n = 7$). Consumption of malate and inhibition with antimycin A were not significant (9.7 ± 1.2 and 11.6 pmol/s/mg ± 1.34; 22.4 ± 4.3 and 20.0 pmol/s/mg ± 3.5, respectively). Residual oxygen consumption (ROX) was similar in both groups (data not shown). Mitochondrial LEAK, estimated as antimycin A flux rates subtracted from oligomycin flux rates, was significantly increased in the L-DIET group when compared to CON (Fig. 3) (52.4 ± 16.1 and 102.2 ± 12.1 pmol/s/mg, respectively, $p < 0.03$). There was also a significant difference between the two groups in terms of substrate control ratio for succinate (GM3/GMS3) (0.62 ± 0.08 and 0.44 ± 0.02, respectively, $p < 0.05$) as illustrated in Fig. 4a. The lipid (L/P) coupling control ratio indicated no significant difference between the L-DIET and CON as seen in Fig. 4b. Neither the respiratory control ratio (RCR, state 3 over state 4 respiration) (2.2 ± 0.8 and 3.0 ± 0.6) nor the acceptor control ratio (ACR, maximal, ADP stimulated respiration divided by basal, ADP restricted respiration) (8.5 ± 1.4 and 12.0 ± 1.4), representing the degree of coupling between oxidation and phosphorylation, demonstrated any significant differences as seen in Fig. 5a, b. None of the protocols used with the uncoupling inhibitor GDP significantly changed the respiration rates for states 3 or 4 respectively (data not shown).

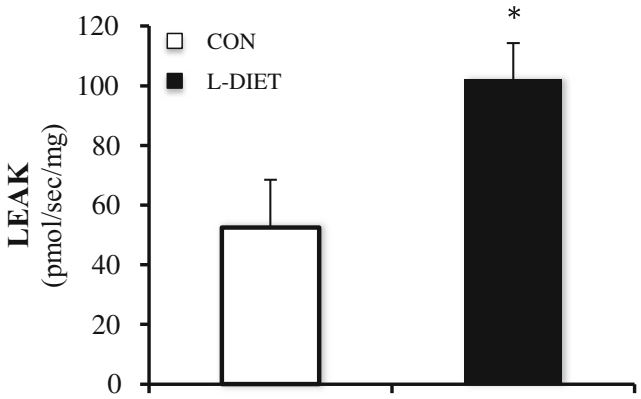

Fig. 3 Mitochondrial LEAK assessed by the difference between oligomycin and antimycin A demonstrates a significant increase in the L-DIET group compared to CON, $n = 7$

Discussion

The novel finding in this study is that mitochondrial respiration is higher in the cardiomyocytes from mice who consumed a LCHF diet when evaluating lipid and ADP-stimulated complex I respiration. Additionally, an increase in ADP

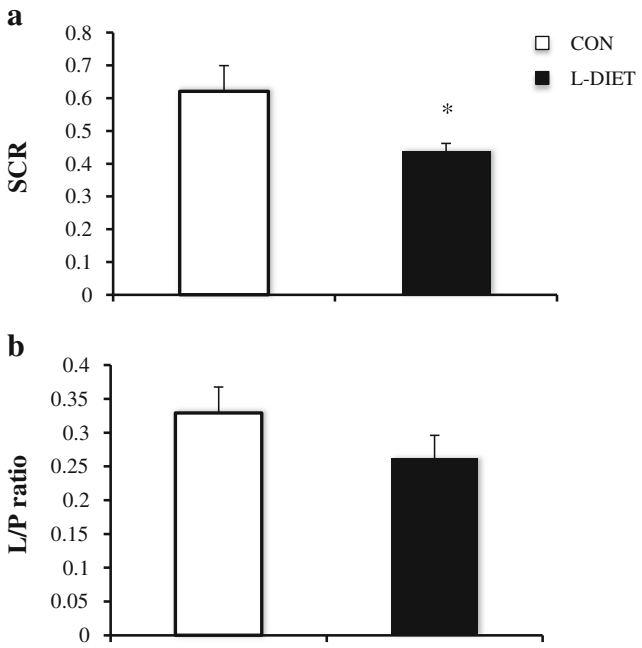

Fig. 4 **a** Substrate control ratio (SCR) show a significant decrease from CON to the L-DIET group, $n = 6$. **b** Lipid coupling control ratio (L/P) reflecting the efficiency to utilize lipids under similar respiratory levels show a decreasing trend when comparing CON to L-DIET ($n = 7$)

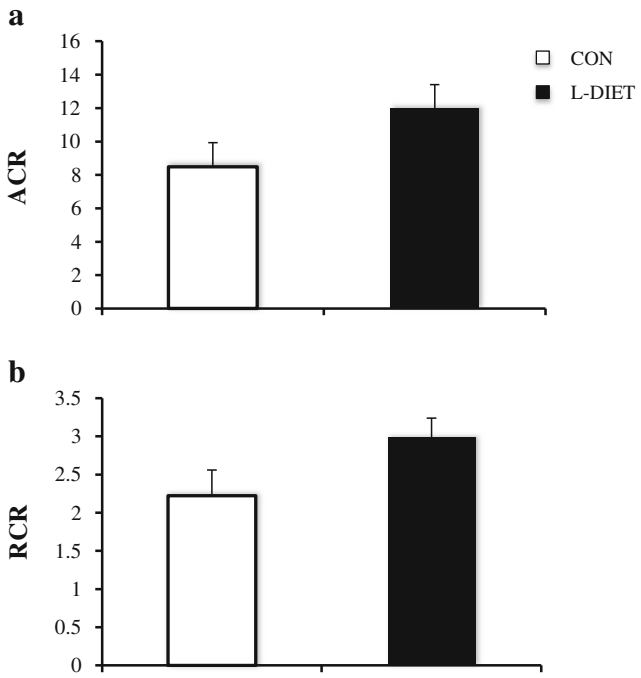

Fig. 5 **a** The acceptor control ratio (ACR), the relative quality of phosphorylation determined by dividing ADP by malate average rates of respiration. The data illustrate a non-significant increase in the ACR of the L-DIET group compared to CON, $n = 7$. **b** The respiratory control ratio (RCR), state 3 over state 4 respiration demonstrate a non-significant increase in the RCR of CON in comparison to L-DIET, $n = 7$

accelerated complex I- and II-linked OXPHOS capacity was also detected in the L-DIET mice when compared to CON. This suggests that, at least in the short term, a LCHF diet, presumably through elevated serum fatty acid levels, enhances oxidative metabolism in cardiomyocytes.

Despite that LCHF diets are conventionally used to achieve weight loss [2, 6], we did not find any difference in weight between the two groups at 6 weeks of LCHF consumption. This is consistent with a previous study that investigated a similar isocaloric LCHF in an ApoE −/− model where no significant difference in weight was detected until after 12 weeks of dietary intervention [7]. Mourmoura and colleagues also detected no significant differences in body weight between a group of rats fed a LCHF diet versus another that were fed standard chow, although those fed the intervention diet for a period of 8 months had significantly greater epididymal, visceral retroperitoneal, and abdominal adipose tissue weight [19].

Although weight loss was not evident in our study, after 6 weeks of dietary intervention, there was a significant effect of LCHF on mitochondrial function in cardiomyocytes of L-DIET mice as we detected an enhanced oxidative respiration, thus indicating a more efficient energy production. The significant decrease in substrate control ratio ($CI+II_P/CI_P$), the flux control ratio at constant mitochondrial coupling, and increase in substrate utilization, specifically for ADP, indicate an increase in mitochondrial efficiency to metabolize various substrates [15]. Similar results have been previously reported: Wistar rats that were fed a LCHF diet for 8 months demonstrated improved mitochondrial phosphorylation when compared to rodents fed a standard chow diet [19]. Although, on the surface, this observation seems to suggest that high-fat diets are ultimately beneficial for mitochondrial-mediated protection against ischemia/reperfusion injury in the heart, it must also be emphasized that this enhanced oxidative phosphorylation was accompanied by an increased mitochondrial hydrogen peroxide release in cardiomyocytes that remained undetected in plasma measurements. The authors hypothesized that the augmentation in lipid-related oxidative stress, in addition to observed decreases in left ventricle developed pressure, is a contributing factor to the early phases of cardiomyopathy [19]. This hypothesis is further supported by observations that the myocardium, even in moderately overweight adult rats (approximately 10% greater than control) who were overfed in the post-natal period, was found to have both metabolic and oxidative disturbances and a higher susceptibility to cardiac damage, presumably in part via ventricular remodeling [9].

In addition to enhanced lipid-mediated oxidative respiration, we also report an increased protein expression of complexes I–V following the LCHF diet. The mechanism in normal cardiomyocytes responsible for the increased oxygen consumption is elusive, but may be associated to direct action of the FAs on mitochondrial uncoupling. However, we found that the state 3 and state 4 respiration were not affected by GPD which indicates that uncoupling proteins were not responsible for the increased respiration in the L-DIET mice. Furthermore, this does not discount the possibility that the direct action of FAs and other lipids may cause uncoupling in the absence of changes in mitochondrial proteins. In addition, the increased oxygen uptake, related to the FA substrate, is not necessarily specific for generating ATP since it may also play a role in fatty acid esterification and reactive oxygen species production [32]. In our experiments, we noticed a reduction in potency of oligomycin, a well-known inhibitor of mitochondrial ATPase/ATP synthase, following the LCHF diet. The lack of inhibitory effects for oligomycin suggests that the oxygen that is still being used is shunted into ROS and is consistent with the increased levels of oxidative stress reported in other studies [9, 19].

The elevated mitochondrial function, seen in the L-DIET mice, could result in a higher membrane potential, subsequently leading to elevated ROS which often accompanies diminished mitochondrial function. This is likely for our experimental model as mitochondrial ROS production has been shown to be favored by incomplete, branched amino acid chains and FA oxidation [20]. It would thus be logical to assume that the ApoE−/− state has negative effects on the mitochondria, which in turn, over time, induces cardiac damage. In prolonged obesity, and subsequent elevation of FAs,

there is a decrease in the efficiency of myocardial energy transduction to contractile work [36]. These alterations are similar to observations made in experimental animal models of obesity and may be responsible for the impairments in cardiac contractility often seen in obese individuals. We therefore demonstrate results consistent with findings that myocardial FA metabolism increases before ventricular contractile dysfunction [17]. Further studies are needed to elucidate the relationship between ROS production in combination with the evaluation of mitochondrial OXPHOS capacity. As ApoE−/− is a model of atherosclerosis and elevated plasma lipid levels, our findings imply that FA action in cardiomyocytes couples directly to mitochondrial energetics and substrate selection. Elevated intrinsic mitochondrial function following LCHF diet may be due to a compensatory effect, although this remains speculative.

As wild-type mice have low plasma LDL and, therefore, do not develop a pro-atherogenic environment, the murine ApoE −/− model is an appropriate model to study cardiovascular disease [21, 23] and allows for the accumulation of blood lipids when challenged through LCHF administration. Foo and colleagues, using the identical mouse strain and diet composition, found that after 6 weeks, serum total cholesterol and non-esterified fatty acids were significantly higher than a regular chow diet [7]. The LCHF content therefore induces a shift in the mitochondria that promotes a greater preference for fatty acids as the source of energy and consequently alters the metabolism in the heart towards its preferred fuel, lipids [25].

One limitation of this study is that the functional properties of the heart were not directly measured (i.e., heart perfusion and coronary reactivity [as in 9 and 19] or myocardial collagen content and matrix metalloprotease activity [19]. Consequently, it cannot be established in this study whether the LCHF diet in the ApoE-deficient mice alters, in addition to mitochondrial function, mechanical or structural aspects of the heart. We also did not directly measure plasma FA levels, although there is evidence provided by Foo and colleagues that serum total cholesterol and non-esterified fatty acids are significantly elevated in the ApoE−/− model at 6 weeks of LCHF feeding [7]. Other limitations include the lack of direct measurements of insulin resistance or oxidative stress markers, although this data has been previously published elsewhere [9].

In summary, we have demonstrated that a LCHF diet induces an increased capacity to oxidize glucose and FA substrates in a rodent model that is comparable to a human pro-atherogenic environment. We propose that the higher intrinsic respiratory capacity may be linked to an increased production of reactive oxygen species, which could result in cell damage. These changes may underlie the abnormalities in cardiac energetics observed in obese individuals prior to the development of functional impairments. Similar studies in humans with dietary-induced elevations in plasma lipid levels are warranted as the development and progression of associated complications have been linked to mitochondrial abnormalities. Additionally, more information is required to establish the mechanistic underpinnings of this condition and how it ultimately contributes to heart failure and other pathological mechanisms. Given recent and timely data that raises concerns about the increased mortality associated with diets that are both high in carbohydrates or low in carbohydrates/high in fat and protein content [27], the careful interpretation of data such as ours must be emphasized as well as highlighting the need for further studies in the area.

Acknowledgements We are very grateful to the Concordia University Animal Care Facility Manager Aileen Murray and her staff for the housekeeping and caregiving they provided throughout this project.

Funding information Concordia University provided funding for this project. CR was funded by a CIHR Master student scholarship.

Compliance with ethical standards

All procedures were approved by the Animal Ethics Committee of Concordia University (protocol ID: #30000259) and were conducted in accordance with guidelines of the Canadian Council on Animal Care.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Akram M (2014) Citric acid cycle and role of its intermediates in metabolism. Cell Biochem Biophys 68(3):475–8
2. Atkins RC (1998) Dr. Atkins' new diet revolution. Avon Books, New York
3. Bing RJ, Siegel A, Ungar I, Gilbert M (1954) Metabolism of the human heart II: studies on fat, ketone and amino acid metabolism. Am J Med 16:504–515
4. Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP (2005) Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type-2 diabetes. Ann Intern Med 142(6):403–411
5. Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, Aziz S, Johnsson JI, Bugger H, Zaha VG, Abel ED (2007) Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. Diabetes 56:2457–2466
6. Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD, Bravata DM (2003) Efficacy and safety of low-carbohydrate diets. A systematic review. JAMA 289(14):1837–1850
7. Foo SY, Heller ER, Wykrzykowska J, Sullivan CJ, Manning-Tobin JJ, Moore KJ, Gerszten RE, Rosenzweig A (2009) Vascular effects of a low-carbohydrate high-protein diet. Proc Natl Acad Sci U S A 106(36):15418–15423
8. Fujimoto A, Harary I (1965) The effects of lipids on enzyme levels in beating rat heart cells. Biochem Biophys Res Commun 20(4): 456–462
9. Habbout A, Delemasure S, Goirand F, Guilland JC, Chabod F, Sediki M, Rochette L, Vergely C (2012) Postnatal overfeeding in

rats leads to moderate overweight and to cardiometabolic and oxidative alterations in adulthood. Biochimie 94(1):117–124
10. Hansson A, Hance N, Dufour E, Rantanen A, Hultenby K, Clayton DA, Wibom R, Larsson NG (2004) A switch in metabolism precedes increased mitochondrial biogenesis in respiratory chain-deficient mouse hearts. Proc Natl Acad Sci U S A 101(9):3136–3141
11. Huss JM, Kelly DP (2005) Mitochondrial energy metabolism in heart failure: a question of balance. J Clin Invest 115:547–555
12. Jackson Laboratory. Blood chemistry survey of 11 inbred strains of mice. MPD:Jaxpheno3. Mouse Phenome Database web site, the Jackson Laboratory, Bar Harbor, Maine USA. http://phenome.jax.org [Cited 13 Aug, 2014]
13. Kolwicz SC, Tian R (2011) Glucose metabolism and cardiac hypertrophy. Cardiovasc Res 90:194–201
14. Kopelman PG (2000) Obesity as a medical problem. Nature 6(4):635–643
15. Larsen S, Scheede-Bergdahl C, Whitesell T, Boushel R, Bergdahl A (2015) Increased intrinsic mitochondrial respiratory capacity in skeletal muscle from rats with streptozotocin-induced hyperglycemia. Physiol Rep 3(7):e12467
16. Lopaschuk GD, Belke DD, Gamble J, Toshiyuki I, Schönekess BO (1994) Regulation of fatty acid oxidation in the mammalian heart in health and disease. Biochim Biophys Acta 1213:263–276
17. Lopaschuk GD, Ussher JR, Folmes CDL, Jaswal JS, Stanley WC (2010) Myocardial fatty acid metabolism in health and disease. Physiol Rev 90:207–258
18. Moritz CP (2017) Tubulin or not tubulin: heading toward total protein staining as loading control in Western blots. Proteomics 17(20):1600189–1600201
19. Mourmoura E, Rigaudière JP, Couturier K, Hininger I, Laillet B, Malpuech-Brugère C, Azarnoush K, Demaison L (2016) Long-term abdominal adiposity activates several parameters of cardiac energy function. J Physiol Biochem 72(3):525–537
20. Newgard CB (2012) Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab 15:606–614
21. Pendse AA, Arbones-Mainar JM, Johnson LA, Altenburg MK, Maeda N (2009) Apolipoprotein E knock-out and knock-in mice: atherosclerosis, metabolic syndrome and beyond. J Lipid Res 50:178–182
22. Phillips SA, Jurva JW, Syed AQ, Syed AQ, Kulinksi JP, Pleuss J et al (2008) Benefit of low-fat over low-carbohydrate diet on endothelial healthy in obesity. Hypertension 51:376–382
23. Piedrahita JA et al (2000) Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci U S A 89:4471–4475
24. Ray J, Noll F, Daut J, Hanley PJ (2002) Long chain fatty acids increase basal metabolism and depolarize mitochondria in cardiac muscle cells. Am J Physiol Heart Circ Physiol 282:H1495–H1501
25. Rocha C, Scheede-Bergdahl C, Whitesell T, Bergdahl A (2014) Implications of apolipoprotein E deficiency on cardiac mitochondrial oxygen consumption in a young mouse model. Eur J Cardiovasc Med 3(1):394–400
26. Scheede-Bergdahl C, Bergdahl A (2017) Adaptation of mitochondrial expression and ATP production in dedifferentiating vascular smooth muscle cells. Can J Physiol Pharmacol 95(12):1473–1479
27. Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, Folsom AR, Rimm EB, Willett WC, Solomon SD (2018) Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. Lancet Public Health 3(9):e419–e428
28. Sheeran FL, Pepe S (2006) Energy deficiency in the failing heart: linking increased reactive oxygen species and disruption of oxidative phosphorylation rate. Biochim Biophys Acta 1757:543–552
29. Shipp JC, Opie LH, Challoner D (1961) Fatty acid and glucose metabolism in the perfused heart. Nature 189:1018–1019
30. Stanley WC, Chandler MP (2002) Energy metabolism in the normal and failing heart: potential for therapeutic interventions. Heart Fail Rev 7:115–130
31. Taegtmeyer H (1994) Energy metabolism of the heart: from basic concepts to clinical applications. Curr Probl Cardiol 19:59–113
32. Vik-Mo H, Mjøs OD (1981) Influence of free fatty acids on myocardial oxygen consumption and ischemic injury. Am J Cardiol 48:361–365
33. Westman EC, Mavropoulos J, Yancy WS, Volek JS (2003) A review of low-carbohydrate ketogenic diets. Curr Atheroscler Rep 5:476–483
34. Westman EC, Feinman RD, Mavropoulos JC, Vernon MC, Volek JS, Wortman JA, Yancy WS, Phinney SD (2007) Low-carbohydrate nutrition and metabolism. Am J Clin Nutr 86:276–284
35. Wisnecki JA, Gertz EQ, Neese RA, Mayr M (1987) Myocardial metabolism of free fatty acids: studies with ^{14}C labelled substrates in humans. J Clin Invest 79:359–366
36. Zhou Y-T, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH (2000) Lipotoxic heart disease in obese rats: implications for human obesity. Proc Natl Acad Sci U S A 97:1784–1789