Molecular and Cellular Biochemistry **233:** 119–125, 2002.

Effects of chronic caloric restriction on mitochondrial respiration in the ischemic reperfused rat heart

Tom L. Broderick,[1] Terry Belke,[2] William R. Driedzic[3]

[1]*Department of Physiology, Midwestern University, Glendale, AZ, USA;* [2]*Department of Psychology, Mount Allison University, Sackville, New Brunswick;* [3]*Ocean Sciences Centre, Memorial University of Newfoundland, St-John's, Newfoundland, Canada*

Received 4 October 2001; accepted 18 January 2002

Abstract

Dietary restriction increases life span and delays the development of age-related diseases in rodents. We have recently demonstrated that chronic dietary restriction is beneficial on recovery of heart function following ischemia. We studied whether the metabolic basis of this benefit is associated with alterations in mitochondrial respiration. Male Wistar rats were assigned to an *ad libitum*-fed (AL) group and a food restricted (FR) group, in which food intake was reduced to 55% of the amount consumed by the AL group. Following an 8-month period of restricted caloric intake, isolated working hearts perfused with glucose and high levels of fatty acids were subjected to global ischemia followed by reperfusion. At the end of reperfusion, total heart mitochondria respiration was assessed in the presence of pyruvate, tricarboxylic acid intermediates, and palmitoylcarnitine. Recovery of heart function following ischemia was greater in FR hearts compared to AL hearts. Paralleling these changes in heart function was an increase in state 3 respiration with pyruvate. The respiratory control ratios in the presence of pyruvate and tricarboxylic acid intermediates were higher in FR hearts compared to AL hearts, indicating well-coupled mitochondria. Overall energy production, expressed as the ADP:O ratio and the oxidative phosphorylation rate, was also improved in FR hearts. Our results indicate that the beneficial effect of FR on recovery of heart function following ischemia is associated with changes in mitochondrial respiration. (Mol Cell Biochem **233**: 119–125, 2002)

Key words: food restriction, cardiac function, mitochondria, reperfusion

Introduction

Food restriction (FR) has elicited a great research interest because it not only increases the longevity of rodents, but also reduces age-related pathologies by delaying the aging process [1–3]. In fact, chronic FR can prevent the onset of certain cardiomyopathies and attenuate the decline in myocardial performance normally associated with aging [4, 5]. We have recently reported that hearts from chronically FR animals are more resistant to severe ischemia [6].

Several changes induced by chronic FR can account for these reported benefits on cardiac function, including improved neurotransmitter content and favorable changes of both the inotropic and chronotropic state of the myocardium [5, 7]. From a metabolic point of view, there is evidence suggesting that FR can maintain insulin sensitivity of tissues by preventing the loss of glucose transporter proteins in tissues, including heart [8, 9]. Considering this, it is an intriguing possibility that changes in glucose metabolism may be an adaptation of hearts in response to FR, particularly during reperfusion when the extent of ventricular recovery is correlated with mitochondrial use of this substrate [10]. Therefore, we thought it important to determine whether the metabolic basis for the improved heart function with FR, if

Address for offprints: T.L. Broderick, Department of Physiology, Midwestern University, 19555 North 59th Avenue, Glendale, AZ 85308, USA (E-mail: tbrode@arizona.midwestern.edu)

present, can be attributed to changes in mitochondrial respiration.

Materials and methods

Experimental groups and food restriction protocol

Male Wistar rats were obtained from Charles River Laboratories at 2 months of age. Animals were caged singly and had free access to food (standard rat chow) and water until their body weight reached 400 g, corresponding to an age of 3 months. Thereafter, one-half of the animals were assigned to an *ad libitum* (AL) group, and the other half to a food restricted (FR) group. In those assigned to the FR group, the amount of food provided on a daily basis was reduced to 55% of the amount consumed by the AL-fed animals. AL animals were provided with food and water *ad libitum*, whereas FR rats had free access to water. Restricted caloric intake was maintained for a period of 8 months. This 8-month period of FR was selected because hearts from caloric-restricted animals are more resistant to ischemia following this period [6]. All animals used in this study were cared for according to the recommendations in the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals.

Ischemia-reperfusion protocols

Experiments were performed on animals in a 3–4 h postprandial state. Hearts from Na^+ pentobarbital-anesthetized (65 mg/kg) rats were perfused and cannulated as working hearts. Briefly, hearts were quickly excised, placed in ice-cold buffer, and immediately perfused retrogradely via the aorta with Krebs-Henseleit buffer containing in (mM): 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 0.5 EDTA, 25 $NaHCO_3$, 11 glucose (pH 7.4, gassed with 95% O_2-5% CO_2). During this perfusion, the hearts were trimmed of excess tissue and the openings of the left atria were cannulated. Hearts were then switched to the working mode and perfused at a 11.5 mmHg left atrial filling pressure and 80 mmHg aortic afterload in a recirculating buffer system containing 11 mM glucose and 1.2 mM palmitate prebound to 3% bovine serum albumin. We chose to perfuse hearts with 11 mM glucose since glucose uptake is saturated at this concentration and tissue glycogen levels remain stable under aerobic conditions [11]. Low glycogen content in the heart prior to ischemia may affect the ability of the heart to withstand the ischemic episode. In addition, hearts were perfused with an elevated concentration of fatty acid since it mimics that observed in the clinical setting of ischemia and reperfusion associated with cardiac surgery [12]. After 20 min of aerobic perfusion, hearts were subjected to 25 min of global ischemia by clamping off both the left atrial filling and aortic afterload line. Following ischemia, left atrial and aortic flow were restored and hearts reperfused for 15 min. At the end of reperfusion, hearts were removed from the cannulae for the immediate isolation of mitochondria, as described below. Rapid recovery of heart function following global ischemia occurs in hearts from caloric restricted animals [6], and for this reason we chose to isolate mitochondrial from these hearts early in the reperfusion period.

Throughout the entire perfusion, heart rate and pressure development by the heart were recorded using a Harvard pressure transducer inserted in the aortic line. The aortic pulse signal generated by the heart was recorded by a standard strip chart system. Heart function was expressed as rate-pressure product (RPP), which is the product of heart rate and systolic pressure. Aortic flow, measured by timed collection, was also used as index of function. Throughout the entire perfusion protocol, water-jacketed chambers kept the temperature of the perfusate at 37°C.

Isolation of heart mitochondria

Mitochondria were prepared by differential centrifugation, as described previously [13]. Hearts were immersed in ice-cold isolation media containing (in mM): 250 sucrose, 1 EGTA, 4 Tris base, 0.2% fatty acid free bovine serum albumin, pH 7.4, then minced with scissors, and homogenized in a glass Potter-Elvehjem using a motor-driven Teflon pestle. The homogenate was centrifuged for 10 min at 500 g at 4°C after which the supernatant was collected and further centrifuged for 10 min at 10,000 g. The resulting supernatant was decanted and the pellet containing the mitochondria was gently resuspended in chilled isolation media. A portion of this suspension was used for the determination of protein content by the Bradford method using bovine serum albumin.

Mitochondrial respiration

Mitochondrial oxygen consumption was measured using a Clark-type electrode mounted in a water-jacketed closed reaction chamber with magnetic stirring, interfaced to a YSI oxygen meter and strip chart recorder. The respiratory buffer consisted of (in mM): 230 mannitol, 70 sucrose, 20 Tris, 0.02 EDTA, 20 potassium phosphate buffer, pH 7.2. Rates of oxygen utilization were measured in the presence of 1 µmole of malate with (in µmoles) either 5 pyruvate, 5 glutamate, 5 α-ketoglutarate, or 120 nanomoles of palmitoylcarnitine. State 3 respiration was stimulated following the addition of 0.5 µmole of adenosine 5′ diphosphate (ADP) and 150 µl of mitochondrial pellet into 1.2 ml of respiratory buffer. Rates of mitochondrial respiration were expressed in nanoatoms

oxygen/mg mitochondrial protein/min. The respiratory control ratio (RCR), calculated as the state 3 to state 4 ratio, was assessed to determine the ratio of the rate of oxygen consumption in the presence of ADP to that in the absence of ADP. This is a measure of the tightness of the respiratory coupling. The efficiency of adenosine triphosphate (ATP) production was determined by the ADP:O ratio, which is the ratio of ADP phosphorylated to the total amount oxygen consumed by the respiring mitochondria. The ADP:O ratio was determined as the product of the total deflection in state 3 respiration normalized for mitochondrial protein and the concentration of oxygen, in natoms, in the respiratory buffer. The oxidative phosphorylation rate (OPR), also an index of energy production in the form of ATP, was obtained from the product of state 3 respiration and ADP:O ratio [14]. The ADP:O ratio was expressed as nmol ADP/nanoatom O, whereas the OPR was expressed as nmol ATP/mg mitochondrial protein/min.

Statistical analysis

For statistical comparison of group means, the paired and unpaired Student *t*-tests were used. A value of $p < 0.05$ was considered significant. All data are reported as mean ± S.E.M.

Results

The physical characteristics of AL and FR animals are shown in Table 1. As expected, following 8 months of restricted caloric restriction, both body weight and heart weight were lower in the FR group compared to the AL group. However, the heart weight-to-body weight ratio was elevated in the FR group, suggesting cardiac mass sparing during chronic food restriction. This higher ratio in FR hearts could reflect differences in glycogen and water content [15]. Blood levels of glucose also reflect restricted food intake. Compared to AL rats, levels of postprandial blood glucose were significantly lower in the FR rats.

Table 1. Physical characteristics of control and caloric restricted animals

Parameter	AL	FR
Food intake (g/day)	25.0 ± 0.2	13.1 ± 0.1*
Body weight (g)	589 ± 28	339 ± 3*
Heart weight (g)	2.01 ± 0.12	1.20 ± 0.04*
HW/BW ratio (g/g × 10^{-3})	3.3 ± 0.1	3.8 ± 0.1*
Glucose (mmol/L)	8.6 ± 0.5	6.3 ± 0.9*

Values are reported as mean ± S.E. for 6 animals for each group. AL – *ad libitum*-fed; FR – food restricted. *significant compared to the AL group, $p < 0.05$.

The effects of chronic FR on heart function during the aerobic perfusion and reperfusion following global ischemia are shown in Table 2. Under aerobic conditions, mechanical function was similar between hearts from AL and FR groups. During reperfusion, differences in the extent of recovery between AL and FR hearts were seen. Heart rate, aortic flow, and RPP remained depressed in AL hearts compared to preischemic function. In FR hearts, only aortic flow was lower compared to preischemic function. However, aortic flow remained significantly higher in these hearts compared to AL hearts. Additionally, RPP was higher in FR hearts, as a result of increased systolic pressure.

Figure 1 shows rates of isolated mitochondrial respiration in reperfused hearts. There were no differences in the rates of state 3 respiration with glutamate, α-ketoglutarate, and palmitoylcarnitine between AL and FR hearts. In the presence of pyruvate, however, state 3 was significantly higher in FR hearts (Fig. 1A). Rates of state 4 respiration with glutamate and a-ketoglutarate were lower in the FR group. With pyruvate, although not significant, state 4 was also lower. As a result, the RCR in the presence of these substrates in FR hearts was significantly higher, indicating well-coupled mitochondrial function (Fig. 1C).

High myocardial ADP:O ratios and increased OPR during reperfusion translate into greater mitochondrial efficiency in converting ADP into ATP. The ADP:O ratios and OPR of AL and FR hearts are illustrated in Fig. 2. In the presence of all substrates, both the ADP:O ratios (Fig. 2A) and OPR (Fig. 2B) were significantly higher in the FR group compared to the AL group.

Table 2. Effects of chronic food restriction on heart function

Group	HR (beats/min)	SP (mmHg)	DP (mmHg)	AF (ml/min)	RPP (10^{-3})
Aerobic					
AL	222 ± 8	124 ± 4	58 ± 2	21 ± 3	27.6 ± 1.3
FR	203 ± 8	125 ± 3	59 ± 2	28 ± 3	26.9 ± 2.0
Reperfusion					
AL	176 ± 6†	87 ± 14	49 ± 5	6 ± 3†	16.1 ± 3.4†
FR	202 ± 4	120 ± 3*	58 ± 2	18 ± 2*‡	24.1 ± 0.7*
Percent recovery of function					
AL	79 ± 6	69 ± 11	86 ± 10	25 ± 14	57 ± 12
FR	101 ± 5*	96 ± 3*	99 ± 5	67 ± 9*	96 ± 3*

Values are reported as mean ± S.E. for 6 hearts for each group. HR – heart rate; SP and DP – systolic and diastolic pressure, respectively, determined by aortic pressure development and relaxation; AF – aortic flow; RPP – heart rate-pressure product in mmHg × 10^{-3}/min. AL – *ad libitum*-fed; FR – food restriction. *significant compared to AL hearts, $p < 0.05$. †significant compared to AL preischemic function, $p < 0.05$. ‡significant compared to FR preischemic function, $p < 0.05$.

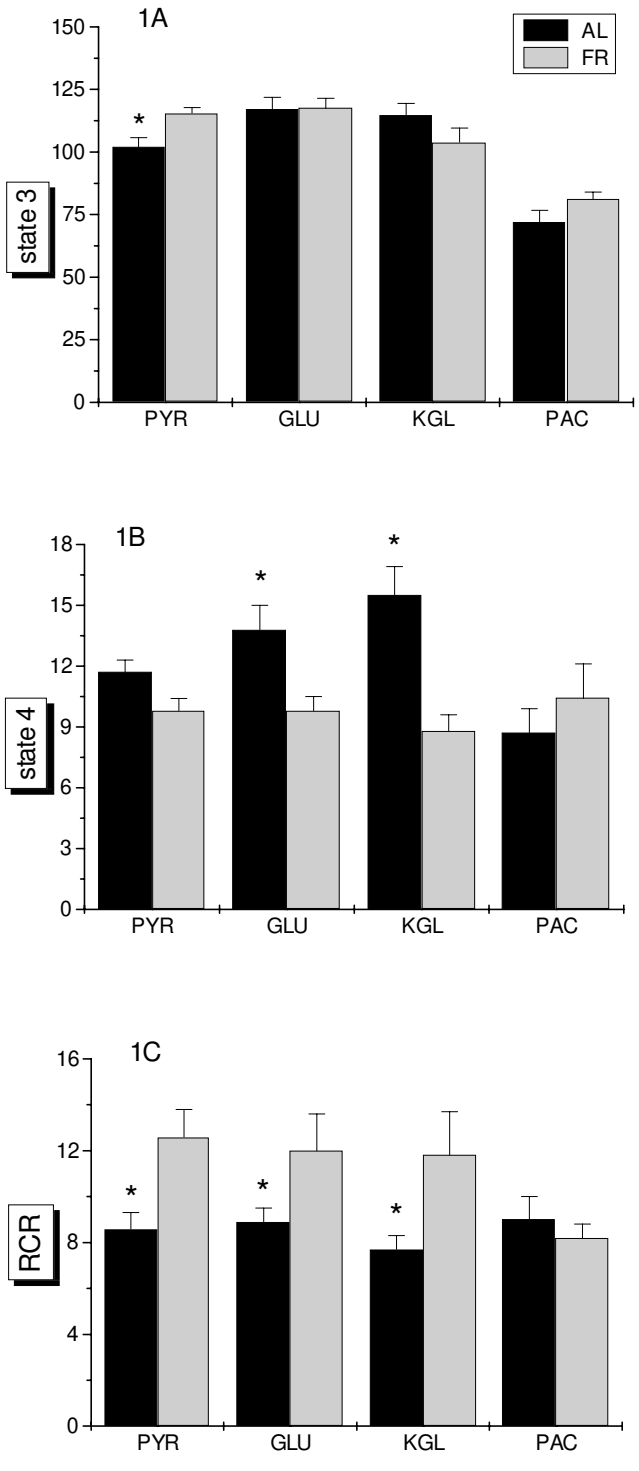

Fig. 1. Rates of isolated mitochondrial respiration shown as state 3 (A), state 4 (B), and RCR (C) in reperfused hearts. Respiration rates are expressed as nanoatoms of oxygen/mg protein/min. PYR – pyruvate; GLU – glutamate; KGL – α-ketoglutarate; PAC – palmitoylcarnitine. Values are shown as mean ± S.E. for 6 hearts in each group. AL – *ad libitum*-fed; FR – food restricted. *significant compared to AL, $p < 0.05$.

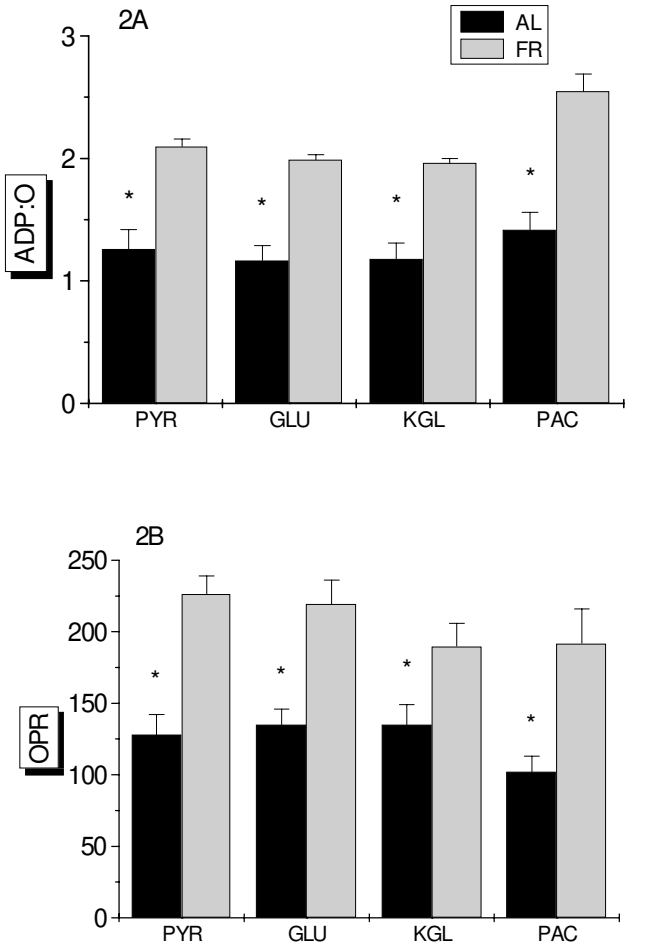

Fig. 2. The ADP:O (A) in nmol ADP/nanoatom O and OPR (B) expressed in nmol ATP/mg mitochondrial protein/minute. The ADP:O and OPR were determined in hearts reperfused following ischemia. PYR – pyruvate; GLU – glutamate; KGL – α-ketoglutarate; PAC – palmitoylcarnitine. Values are shown as mean ± S.E. for 6 hearts in each group. AL – *ad libitum*-fed; FR – food restricted. *significant compared to AL, $p < 0.05$.

Discussion

This study was carried out to examine whether the beneficial effect of chronic diet restriction on recovery of heart function following ischemia was accompanied by an improvement in the mitochondrial respiration of fuels. Following an eight-month period of restricted caloric intake, isolated working rat heart function and mitochondrial respiration were measured during reperfusion shortly following global ischemia. Our results show that FR was clearly of benefit and enhanced overall contractile pump function, confirming our recent study [6]. Hearts from FR animals demonstrated a rapid recovery of rate-pressure product and aortic flow. Paralleling these improvements in mechanical function, as a possible mechanism explaining a beneficial effect of FR, were

the following changes in mitochondrial respiration: (1) increased state 3 respiration with pyruvate, (2) improved mitochondrial coupling exemplified by the RCR with pyruvate, glutamate and α-ketoglutarate, and (3) greater efficiency of energy production expressed as higher ADP:O ratios and OPR with pyruvate, glutamate, α-ketoglutarate, and palmitoylcarnitine.

It is tempting to speculate as to what could be responsible for the ischemic tolerance of FR hearts. One possibility is that enhanced pyruvate metabolism seen during reperfusion may be of benefit. Defective mitochondrial pyruvate metabolism during reperfusion is an important factor determining the extent of recovery of mechanical function following ischemia [10, 16, 17]. In our study, hearts were perfused in the presence of elevated levels of fatty acids, which not only inhibits pyruvate utilization but also accelerates ischemic failure [18]. Stimulating pyruvate metabolism during reperfusion is of benefit and facilitates recovery of heart function [10, 16, 17]. Our results indicate that compared to AL hearts, FR hearts subjected to ischemia are more resistant to injury and that state 3 respiration with pyruvate was increased. This observation would be consistent with metabolic strategies intended to favor pyruvate metabolism, which exert anti-ischemic efficiency.

Studies in the intact rat heart suggest that glycogen content prior to ischemia may affect the ability of the heart to withstand the ischemic episode. High glycogen stores would maintain glycolysis, providing a critical source of ATP during ischemia, resulting in an increased tolerance of the heart to ischemia [19]. Increases in cardiac glycogen content have been reported in response to food restriction [15]. Therefore, it is not surprising that in hearts from FR animals higher rates of glycogenolysis occur during ischemia, thus reducing the extent of postischemic dysfunction in these hearts.

It was of interest to observe that the oxidative phosphorylation rates in the presence of glutamate, α-ketoglutarate, and palmitoylcarnitine were increased in reperfused FR hearts. These results suggest that FR may actually benefit the myocardium by facilitating overall oxidative use of fuels. While improved mitochondrial oxidation of fuels may be effective in preserving cardiac function, our results are not consistent with Kirsch and Savabi's study demonstrating that FR is associated with defects in mitochondrial respiration, particularly that of α-ketoglutarate [20]. Defects at this level have the potential to impair the removal of reducing equivalents from α-ketoglutarate and interfere with the transfer of these equivalents in the respiratory chain. Daneshrad *et al*. have also shown a depression in mitochondrial respiration following caloric restriction [21]. Using cardiac myofibrillar mitochondria, the stimulatory effects of creatine were greatly attenuated with diet restriction, suggesting a depression in ADP production under the effect of mitochondrial creatine kinase. This would, in turn, reduce the availability of ADP for exchange with ATP from the adenine nucleotide translocase, limiting energy production. Depressed cardiac function is a typical response under these conditions [22], since this may be an adaptive mechanism allowing the heart to contract under conditions of reduced energy availability. Additionally, the reasons for these contrasting findings are not known, but may relate to differences in the rate of oxidative metabolism between hearts. In the present study, isolated mitochondrial respiration was assessed during early reperfusion, a period characterized by rapid return of oxidative metabolism, and often exceeding preischemic levels [18]. Mitochondrial metabolism of fuels under these conditions is likely to be different from those obtained from *in situ* hearts [20].

The mechanisms explaining the benefits of chronic caloric restriction in the ischemic heart remain to be determined. The potential involvement of mitochondrial antioxidant defenses to enhance the heart's ability to withstand ischemia should be considered. An increase in heart free radical production and oxidative damage associated with aging can be attenuated or prevented with dietary restriction [23–25]. Increases in the activities of cardiac superoxide dismutase and catalase occur following chronic caloric intake, and as a result, lipid peroxidation is reduced in these hearts [23–25]. Antioxidant defense is of clinical concern since a rapid accumulation of oxygen free radicals immediately upon reperfusion can impair postischemic recovery of ventricular function [26].

Limitations of the study

In summary, the results of our study indicate that FR exerts a protective effect against ischemia and reperfusion in hearts perfused with elevated concentrations of fatty acids. This beneficial effect of FR on reperfusion recovery is associated with changes in mitochondrial respiration of energy substrates. Despite these findings, a cause and effect relationship between enhanced mitochondrial metabolism of fuels and improved recovery of heart function following ischemia could not be determined. Consequently, it is not possible to distinguish whether FR is stimulating energy metabolism, owing to the improved ischemic tolerance or whether it is the noted tolerance that accounts for the changes in mitochondrial respiration. The possibility exists that changes in mitochondrial metabolism of fuels, favoring both glucose and fatty acid utilization may be present in non-ischemic hearts. Under these conditions, we would expect an improvement in cardiac function [27], which was not the case in non-ischemic hearts of this study. However, metabolic strategies that increase only glucose utilization in the heart can do so with greater efficiency without altering cardiac function [28]. Clearly, further studies are warranted to quantify the effects of long term FR on fuel oxidation in normoxic and ischemic hearts.

Furthermore, we measured respiratory function from a total mitochondrial population isolated from hearts of FR rats. Distinct mitochondrial populations, however, do exist in the myocardium [29]. These include the subsarcolemmal mitochondrial population, residing beneath the plasma membrane, and interfibrillar mitochondrial population, located between the myofibrils. Previous studies have shown that aging, heart failure, and ischemia affect these functionally distinct populations [30–32]. Although our results can provide a basis for the understanding of biochemical changes that occur with chronic FR, these should be interpreted with caution, since it is not known how respiration is selectively altered in the distinct mitochondrial subpopulations.

Acknowledgements

This study was supported by grants from the New Brunswick Heart and Stroke Foundation of Canada (TLB), Medical Research Fund of New Brunswick (TLB, WRD), and the Natural Sciences and Engineering Research Council of Canada, grant #OGP0017002 (TB).

References

1. Yu BP, Masoro EJ, Murata I, Bertrand HA, Lynd FT: Life span study of the SPF Fischer 344 male rats fed *ad libitum* or restricted diets: Longevity, growth, lean body mass and disease. J Gerontol 37: 130–141, 1982
2. Masoro E: Antiaging action of caloric restriction: Endocrine and metabolic aspects. Obes Res 3(suppl 1): 241–247, 1995
3. Sohal RS, Weindruch R: Oxidative stress, caloric restriction, and aging. Science 273: 59–63, 1996
4. Klebanov S, Herlihy JT, Freeman GR: Effect of long-term food restriction on cardiac mechanics. Am J Physiol 273: H2333–H2342, 1997
5. Kelley GR, Herlihy JT: Food restriction alters the age-related decline in cardiac beta-adrenergic responsiveness. Mech Ageing Dev 103: 1–12, 1988
6. Broderick TL, Driedzic WR, Gillis M, Jacob J, Belke T: Effects of chronic food restriction and exercise training on recovery of cardiac function following ischemia. J Gerontol Biol Sci 56: B1–B5, 2001
7. Kim SW, Yu BP, Sanderford M, Herlihy JT: Dietary restriction modulates the norepinephrine content and uptake of the heart. Proc Soc Exp Biol Med 207: 43–47, 1994
8. Wetter TJ, Gazdag AC, Dean DJ, Cartee GD: Effect of calorie restriction on *in vivo* metabolism by individual tissues in rats. Am J Physiol 276: E728–E738, 1999
9. Papa PC, Seraphim PR, Machado UF: Loss of weight restores GLUT 4 content in insulin-sensitive tissues of monosodium glutamate-treated obese mice. Int J Obes Relat Metab Disord 21: 1065–1070, 1994
10. Bunger R, Mallet RT, Hartman DA: Pyruvate-enhanced phosphorylation potential and inotropism in normoxic and postischemic isolated working heart. Eur J Biochem 180: 231–233, 1989
11. Neely JR, Whitmer M, Mochizuki S: Effects of mechanical activity and hormones on myocardial glucose and fatty acid utilization. Circ Res 38: I22–I30, 1976
12. Svensson S, Svedjeholm R, Ekroth R, Milocco I, Nilsson F, Dabel KG, William-Olsson G: Trauma metabolism in the heart: Uptake of substrates and the effects of insulin early after cardiac operations. J Thorac Cardiovasc Surg 99: 1063–1073, 1990
13. Paulson DJ, Shug AL: Inhibition of the adenine nucleotide translocator by matrix-localized palmitoyl-CoA in rat heart mitochondria. Biochem Biophys Acta 766: 70–76, 1984
14. Edoute Y, Kotze JCN, Lochne A: Oxidative phosphorylation rate: An index for evaluation of mitochondrial function in myocardial ischemia. J Mol Cell Cardiol 11: 831–833, 1979
15. Arnall DA, Palmer WK, Miller WC, Oscai LB: Effect of fasting on myocardial substrates in male and female rats. Am J Physiol 254: C560–C563, 1988
16. Lewandowski ED, White LT: Pyruvate dehydrogenase influences on postischemic heart function. Circulation 91: 2071–2079, 1985
17. McVeigh JJ, Lopaschuk GD: Dichloroacetate stimulation of glucose oxidation improves recovery of ischemic rat hearts. Am J Physiol 259: H1070–H1085, 1990
18. Lopaschuk GD: Alterations in fatty acid oxidation during reperfusion of the heart after myocardial ischemia. Am J Cardiol 80: 11A–16A, 1997
19. Vanoverschelde JLJ, Janier MF, Bakke JE, Marshall DR, Bergmann SR: Rate of glycolysis during ischemia determines the extent of ischemic injury and functional recovery after reperfusion. Am J Physiol 267: H1785–H1794, 1994
20. Kirsch A, Savabi F: Effect of food restriction in the phosphocreatine energy shuttle components in rat heart. J Mol Cell Cardiol 24: 821–830, 1992
21. Daneshrad Z, Novel-Chate V, Birot O, Serrurier B, Sanchez H, Bigard AX, Rossi A: Diet restriction plays an important role in the alterations of heart mitochondria following exposure of young rats to chronic exposure. Eur J Physiol 442: 12–18, 2001
22. Savabi F, Kirsch A: Diabetic type of cardiomyopathy in food-restricted rats. Can J Physiol Pharmacol 70: 1040–1047, 1992
23. Xia E, Rao G, Van Remmen H, Heydari AR, Richardson A: Activities of antioxidant enzymes in various tissues of male Fischer 344 rat are altered by food restriction. J Nutr 125: 195–201, 1995
24. Sohal RS, Ku HH, Agarwal S, Forster MJ, Lal H: Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. Mech Ageing Dev 74: 121–133, 1994
25. Lass A, Sohal BH, Weindruch R, Forster MJ, Sohal RS: Caloric restriction prevents age-associated accrual of oxidative damage to mouse skeletal muscle mitochondria. Free Radic Biol Med 25: 1089–1097, 1988
26. Bolli R, Patel BS, Jeroudi MO, Lai EK, McCay PB: Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap alpha-phenyl N-tert-butyl nitrone. J Clin Invest 82: 476–485, 1988
27. Broderick TL, Driedzic WR, Paulson DJ: Propionyl-L-carnitine effects on postischemic recovery of mechanical function and substrate oxidation in the diabetic heart. Mol Cell Biochem 206: 151–157, 2000
28. McCormack JG, Barr RL, Wolff AA, Lopaschuk GD: Ranolazine stimulates glucose oxidation in normoxic, ischemic, and reperfused ischemic hearts. Circulation 93: 135–142, 1996
29. Palmer JW, Tandler B, Hoppel CL: Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. J Biol Chem 252: 8731–8739, 1977
30. Hoppel CL, Tandler B, Parland W, Turkaly JS, Albers LD: Hamster

cardiomyopathy: A defect in oxidative phosphorylation in cardiac interfibrillar mitochondria. J Biol Chem 257: 1540–1548, 1982
31. Lesnefsky EJ, Ramani K, Fannin S, Slabe TJ, Hoppel CL: Aging selectively decreases oxidative capacity in interfibrillar rat mitochondria. Circulation 90: I209, 1994
32. Ueta H, Ogura M, Sugiyama A, Kagiyama A, Shin G: Spin trapping of cardiac submitochondrial particles isolated from ischemic and non-ischemic myocardium. J Mol Cell Cardiol 22: 893–899, 1990