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The heart is better protected against myocardial infarction in the fed state compared to the fasted state

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ABSTRACT

Objective. A variety of calorie restriction diets and fasting regimens are popular among overweight people. However, starvation could result in unexpected cardiovascular effects. Therefore, it is necessary to evaluate the short-term effects of diets on cardiovascular function, energy metabolism and potential risk of heart damage in case of myocardial infarction. The objective of the present study was to investigate whether the increased level of glucose oxidation or reduction of fatty acid (FA) load in the fed state provides the basis for protection against myocardial infarction in an experimental rat model of ischemia–reperfusion.

Materials/Methods. We tested the effects of the availability of energy substrates and their metabolites on the heart functionality and energy metabolism under normoxic and ischemia–reperfusion conditions.

Results. In a fasted state, the heart draws energy exclusively from FAs, whereas in a fed state, higher concentration of circulating insulin ensures a partial switch to glucose oxidation, while the load of FA on heart and mitochondria is reduced. Herein, we demonstrate that ischemic damage in hearts isolated from Wistar rats and diabetic Goto–Kakizaki rats is significantly lower in the fed state compared to the fasted state.

Conclusions. Present findings indicate that postprandial or fed-state physiology, which is characterised by insulin-activated glucose and lactate utilisation, is protective against myocardial infarction. Energy metabolism pattern in the heart is determined by insulin signalling and the availability of FAs. Overall, our study suggests that even overnight fasting could provoke and aggravate cardiovascular events and high-risk cardiovascular patients should avoid prolonged fasting periods.

Abbreviations: AN, area of necrosis; AR, area at risk; ATP, adenosine triphosphate; BSA, bovine serum albumin; CPT1A, CPT1B, carnitine palmitoyltransferase 1A and 1B; ETC, electron transfer chain; FA, fatty acid; FATP1, fatty acid transport protein 1; GIK, glucose–insulin–potassium; GK, Goto–Kakizaki; Glut1, Glut4, glucose transporter 1 and 4; HK2, hexokinase 2; HR, heart rate; HSL, hormone-sensitive lipase; IS, infarct size; KH, Krebs–Henseleit; LAD, left anterior descending coronary artery; LVDP, left ventricular developed pressure; P-Akt, phosphorylated Akt; PDHx, pyruvate dehydrogenase complex, component X; PDK4, pyruvate dehydrogenase lipoamide kinase isozyme 4; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator; PPAR-α, peroxisome proliferator-activated receptor alpha; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; ROS, reactive oxygen species; SEM, standard error of the mean; TG, triglycerides; UCP1, UCP3, uncoupling proteins 1 and 3.

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1. Introduction

Obesity and sedentary lifestyles are prevalent risk factors for heart disease [1]. Dietary and other lifestyle interventions resulting in greater than 15% loss in body weight can reduce cardiovascular risk by up to 45% [2], and various calorie restriction diets and fasting regimens are commonly used to reduce weight [3,4]. However, hypoglycaemia is potentially harmful and has been related to cardiovascular events and death [5]. It is important to lower the risk for cardiovascular events in the population through lifestyle, environmental and social changes while attempting to minimise hypoglycaemia-related cardiovascular side effects. Therefore, it is necessary to evaluate the short-term effects of diets on cardiovascular function, energy metabolism and potential risk of heart damage in cases of myocardial infarction.

Heart function is strongly dependent on energy derived from ATP generated during oxidative phosphorylation in mitochondria [6]. The most important energy substrates in the heart are fatty acids (FAs) and glucose as well as their intermediates, such as lactate, pyruvate and ketone bodies [7]. Hearts of healthy subjects are able to switch appropriately between the available energy substrates, and glucose and FAs compete with each other to enter the oxidative metabolism in mitochondria [8]. Because the heart has a rapid energy turnover and low energy storage capacity, myocardial functioning relies heavily on energy substrate levels in plasma [9,10]. In the fasted state, glucose and insulin levels are significantly lower, and energy metabolism switches predominantly to the oxidation of FAs [11]. In contrast, in the postprandial or fed state, glucose and insulin concentrations are higher, and the heart partially switches to glucose oxidation [12].

An improved recovery of ischemic myocardium can be achieved by stimulating the utilisation of glucose as an energy substrate, either directly or indirectly, via partially inhibiting FA oxidation [13]. The beneficial effects of switching from FAs to glucose oxidation in the myocardium are based on the increased efficiency of ATP production and a greater amount of mechanical energy obtained per mole of oxygen spent in glucose oxidation [14,15]. We hypothesise that ischemic damage is lower in the fed state compared to the fasted state due to physiologically facilitated glucose metabolism and reduced FA load during the fed state. Although a few previous studies have demonstrated certain beneficial effects of fasting on cardiac recovery after non-flow ischemia [16,17], other studies have demonstrated an increased severity of complications in cases of preoperative fasting [18–20]. The objective of the present study was to investigate whether the increased level of glucose oxidation or reduction of FA metabolism in the fed state provides the basis for protection against myocardial infarction in an experimental rat model of ischemia–reperfusion. We tested the effects of the availability of energy substrates and their metabolites on the heart contractile function and energy metabolism under normoxic and ischemia–reperfusion conditions.

2. Methods

All experimental procedures were performed in accordance with the guidelines of the European Community as well as local laws and policies and were approved by the Latvian Animal Protection Ethical Committee of the Food and Veterinary Service, Riga, Latvia. All experiments were performed in a blinded manner.

2.1. *Animals and treatments*

For energy metabolism and *ex vivo* myocardial infarction experiments, 78 male Wistar rats weighing 200–240 g were obtained from the Laboratory of Experimental Animals, Riga Stradins University (Riga, Latvia), and adapted for two weeks prior to the experiments. All rats were housed under standard conditions (21–23 °C, 12 h light/dark cycle, relative humidity 45%–65%) with unlimited access to food (R70 diet, Lactamin, Kimstad, Sweden) and water.

Rats were randomly separated into two experimental groups, fed (n = 34) and fasted (n = 44). Rats in the fed group

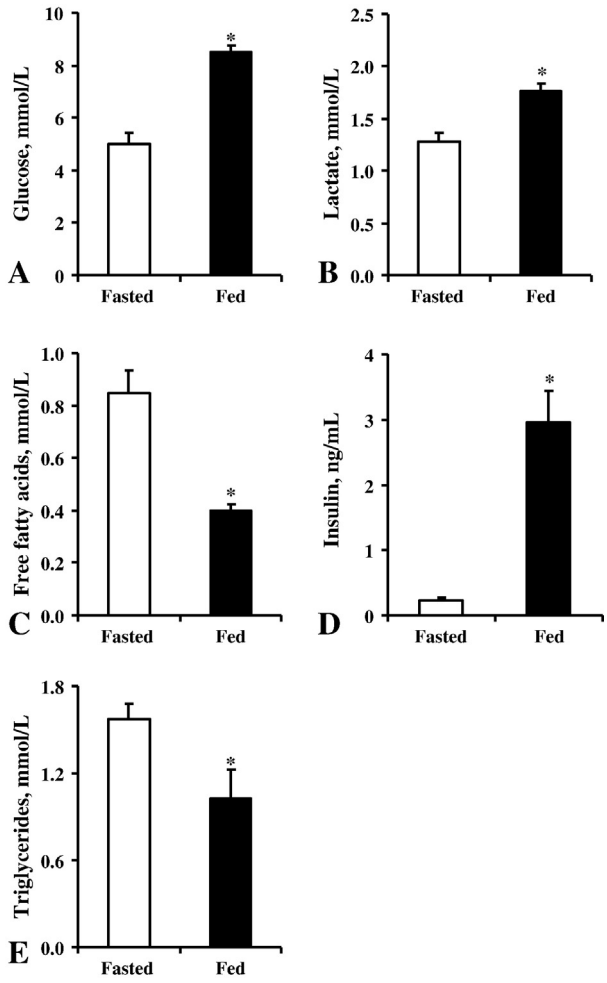

Fig. 1 – The concentrations of biochemical components in blood plasma samples from fed and fasted rats. Compared to fasted rats, blood plasma in fed rats contains higher concentrations of glucose (A), lactate (B) and insulin (D) but lower concentrations of FAs (C) and triglycerides (E). The presented results are average values ± SEM of at least 8 rats. *Significantly different from the fasted group (Student's t-test, P < 0.05).

had unlimited access to food, whereas those in the fasted group were deprived of food for 18 h prior to the start of the experiment. Fasting was started at the end of the light phase. In each group, 24 rats were randomly assigned for metabolic analysis, whereas the remaining rats (fed, n = 10; fasted, n = 20) were used for myocardial infarction studies. Fasted rat hearts used for infarction study were further separated into two subgroups: Fasted (n = 10) and Fasted + 'fed' buffer group (n = 10), in which the hearts from fasted rats were perfused with 'fed' Krebs–Henseleit buffer solution containing higher concentrations of glucose, insulin and lactate.

An additional 12 male Goto–Kakizaki (GK) rats and 12 Wistar rats (as non-diabetic GK control) were randomly separated into two experimental groups: fasted (Wistar, n = 6; GK, n = 6) and fed (Wistar, n = 6; GK, n = 6). The Fed group rats had unlimited access to food, whereas the fasted rats were deprived of food for 18 h before the beginning of the experiment.

2.2. *Isolated rat heart infarction study and hemodynamic measurements*

The effects of fasted and fed states on infarct size were investigated in an isolated rat heart infarction model. The infarction study was performed according to the Langendorff constant pressure recirculation technique using Krebs–Henseleit (KH) buffer solution (118 mmol/L NaCl, 2.52 mmol/L $CaCl_2$, 1.64 mmol/L $MgCl_2$, 24.88 mmol/L $NaHCO_3$, 1.18 mmol/L KH_2PO_4 and 0.05 mmol/L EDTA, pH 7.4 at 37 °C) supplemented to mimic the blood plasma parameters found in the fed and fasted states (Fig. 1; Supplementary Table 1). Briefly, hearts from fed rats were perfused with KH buffer solution 'fed' supplemented with 10 mmol/L glucose, 0.3 mmol/L sodium palmitate bound to 2% BSA, 2 mmol/L lactate, 0.2 mmol/L pyruvate and 3 ng/mL insulin, hearts from fasted rats were perfused with KH buffer solution 'fasted' supplemented with 5 mmol/L glucose, 1.2 mmol/L sodium palmitate bound to 2% BSA, 1 mmol/L lactate, 0.1 mmol/L pyruvate and 0.3 ng/ml insulin. Similar modified KH solutions have been used also in previous studies to mimic fed and fasted states [21–24]. Rats were anaesthetised using sodium pentobarbital (60 mg/kg ip) with the concomitant administration of heparin (1000 IU/kg). Hearts were excised and retrogradely perfused with the respective oxygenated (95% O_2–5% CO_2) perfusion solutions via the aorta at a constant pressure of 70 mm Hg. The heart rate, left-ventricle developed pressure, contractility and relaxation were continuously recorded using a PowerLab 8/30 system from ADInstruments. The coronary flow was measured using an ultrasound flow detection system connected to the PowerLab 8/30 instrument. Hearts were adapted for 20 min, and the occlusion of left anterior descending coronary artery (LAD) was performed for 20 min. Successful occlusion was confirmed by a 40% decrease in the coronary flow [25,26]. At the end of the 120 min reperfusion, the LAD was re-occluded, and the non-risk area was stained with 0.1% methylene blue solution in KH buffer perfused via the aortic root. Hearts were sectioned transversely from the apex to the base in 6 slices of 2 mm thickness and incubated in 1% triphenyl-tetrazolium chloride in phosphate buffer (pH 7.4, 37 °C) for 10 min to stain viable tissue red and necrotic tissue white. The planemetric analysis of left-ventricle cross-sectional images was performed using Image-Pro Plus v6.3 software to determine the area at risk (AR) and area of necrosis (AN), each expressed as a percentage of the left ventricle area [27]. The obtained values were then used to calculate the infarct size (IS) as a percentage of the risk area according to the formula IS = AN/AR × 100%.

In a similar manner, the infarction study was performed in Goto–Kakizaki and Wistar rats. Fed rat hearts were perfused with KH buffer solution 'fed', and fasted rat hearts were perfused with KH buffer solution 'fasted'. If compared to first experiment (Fig. 2A), the only difference was that the occlusion was performed for 30 min.

2.3. *Measurement of substrate oxidation in the isolated rat heart*

The rates of radiolabelled glucose, lactate and palmitate oxidation were measured in different sets of Wistar rat hearts as previously described [28] with certain modifications indicated below. The energy metabolism measurements were performed according to the Langendorff constant flow non-recirculation technique. Briefly, the hearts from fasted or fed Wistar rats were retrogradely perfused with the respective non-labelled oxygenated (95% O_2, 5% CO_2) KH buffer solution 'fed' or 'fasted' via the aorta at a constant flow of 10 ml/min for 10 min. The perfusate was then switched to the respective ('fed' or 'fasted') oxygenated radiolabelled KH buffer solution for 10 min. After 10 min, the hearts were switched back to the respective non-labelled KH perfusion solution and perfused for 10 min.

Glucose and lactate oxidation rates were determined by measuring the $^{14}CO_2$ released from the metabolism of [U-^{14}C] glucose (specific activity, 300 mCi/mmol) or [1-^{14}C]lactate (specific activity, 55 mCi/mmol), respectively. Palmitate oxidation was determined by measuring ^3H_2O released from [9,10-^{3}H]palmitate (specific activity, 60 Ci/mmol). Palmitate uptake in the heart was calculated from the amount of radiolabelled palmitate oxidised during the perfusion and the amount found in the cardiac tissues at the end of the perfusion.

2.4. *Determination of biochemical parameters in heart, liver and blood plasma*

To determine lipid profile and glycogen concentrations, frozen heart and liver tissues were prepared as previously described [25,29]. For biochemical measurements, the blood samples were collected from tail vein in heparin-containing tubes. To obtain plasma, samples were centrifuged at 1000 g for 10 min at 4 °C). All samples were stored at −80 °C until analysis.

The concentrations of free fatty acids and triglycerides were measured using commercially available enzymatic kits from Wako (Neuss, Germany) and Instrumentation Laboratory (Lexington, Massachusetts). The plasma glucose and insulin concentrations were determined using kit from Instrumentation Laboratory and Sensitive Rat Insulin RIA kit (Millipore, Billerica, USA), respectively. Lactate level was measured in samples using enzymatic kit from Roche Diagnostics (Mannheim, Germany). The glucose concentration was determined

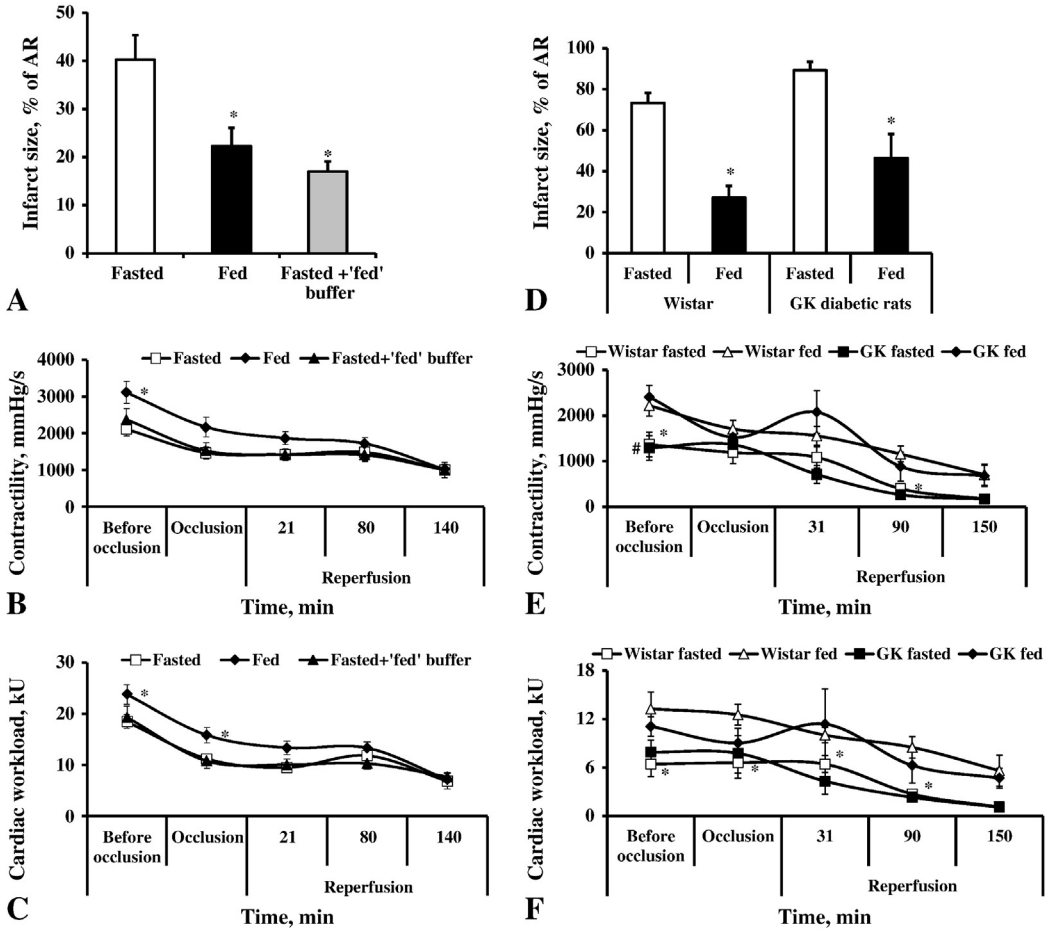

Fig. 2 – Differences in the myocardial infarct size and hemodynamic parameters in hearts from fasted and fed rats. The infarct size is approximately 2-fold smaller in hearts of rats in the fed state compared to the fasted state (A). Perfusion of hearts from fasted rats with 'fed' buffer reduces deleterious effects of fasting. In both Wistar and diabetic Goto–Kakizaki (GK) rat hearts, a 2-fold difference in the infarct size was observed between fed and fasted rats (D). As a result of insulin resistance, the infarct size in both fed and fasted GK rat hearts was increased by 20%–50% compared to Wistar rat hearts (D). The cardiac function, calculated as a product of the heart rate and left ventricle developed pressure (C and F), and contractility of the left ventricle (B and E) were lower in fasted rats. The presented results are the average values ± SEM of at least 5 rats. *Significantly different from the respective fasted group (Tukey's test, $P < 0.05$). #Significantly different from the respective Wistar group (Tukey's test, $P < 0.05$).

using a glucose determination kit from Instrumentation Laboratory. Glycogen concentration in heart and liver tissues was determined as previously described [25,29].

2.5. *Intraperitoneal glucose tolerance test*

To perform the glucose tolerance test, the diabetic and control rats were fasted overnight. Then, the glucose solution (1 g/kg of body weight) was administered intraperitoneally, and blood samples were then drawn from the tail vein at 0 (fasting), 5, 15, 30, 45, 60, 120 and 240 min.

2.6. *mRNA isolation and quantitative RT-PCR*

Total RNA from heart tissue was isolated using the TRI Reagent (Sigma, St. Louis, USA) according to the manufacturer's protocol. The quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis was performed as described previously [30]. The transcript levels for the constitutive housekeeping gene product β-actin were quantitatively measured for each sample, and PCR data were reported as the number of transcripts per number of β-actin mRNA molecules. To avoid genomic DNA contamination, the primers were designed to span an intron. The primer sequences used for the quantitative RT-PCR analysis are available upon request.

2.7. *Western blot analysis of tissue lysates and nuclear extracts*

Heart tissue were homogenized by an Ultra-Turrax® homogenizer (IKA, Germany) at a ratio of 1:10 (w/v) at 4 °C in a buffer containing 100 mmol/L Tris–HCl, pH 7.4, 10 mmol/L EDTA, 5 mmol/L $MgCl_2$, 1 mmol/L glycerol 3-phosphate, 1 mmol/L

NaF and protease inhibitors (10 μmol/L leupeptin, 1 μmol/L pepstatin, 1 μmol/L aprotinin, and 100 μmol/L PMSF). The nuclear extracts for western blot analysis were isolated and purified as described previously [31]. PAGE and western blot analysis of tissue lysates and nuclear extracts were performed as described by Liepinsh et al. [31]. The blots were developed using chemiluminescence reagents (Millipore). Western blot images were scanned and then analyzed using Gel-Pro Analyzer 6.0 software.

2.8. Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). To compare 2 groups Student's or Mann–Whitney t-tests were used. One-way analysis of variance (ANOVA) with Tukey's post-test was used to compare 3 and more groups. P values less than 0.05 were considered to be statistically significant. Statistical calculations were performed using Prism 3.0 software (GraphPad, San Diego, California).

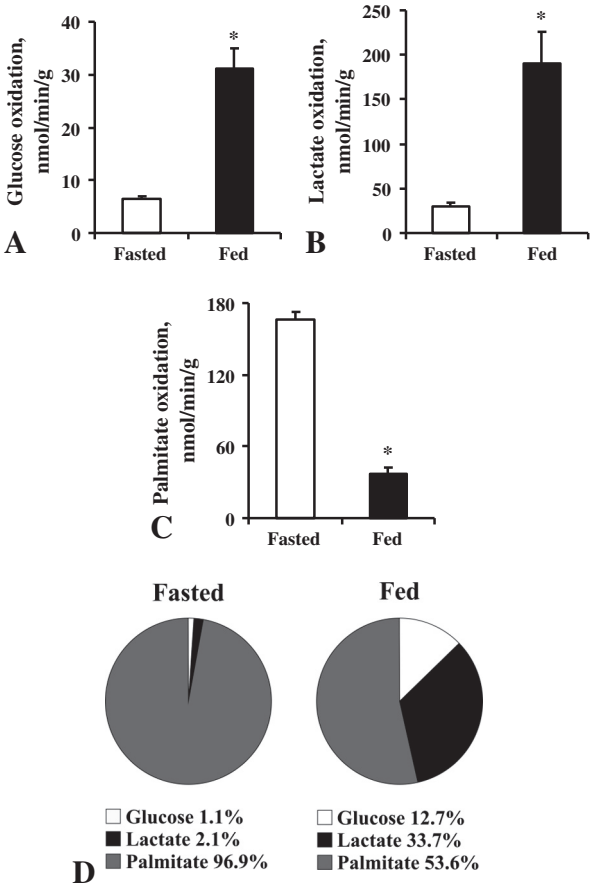

Fig. 3 – Energy substrate oxidation rates in isolated hearts from fasted and fed Wistar rats. In the fed state, the heart tissues oxidise more glucose (A) and lactate (B) but less palmitate (C). The calculations of energy substrate input in the energy production show that FA oxidation overrides glucose and lactate oxidation and prevails as the only energy source in the fasted state (D). The presented results are the average values ± SEM of at least 8 rats. *Significantly different from the fasted group (Student's t-test P < 0.05).

3. Results

3.1. The fed state is protective against myocardial infarction

The effects of fasted and fed states on infarct size were studied in an isolated rat heart infarction model. To mimic both states *ex vivo* in the isolated heart model, hearts from fasted and fed rats were perfused with 2 different solutions consisting of blood plasma components in concentrations found in the fed and fasted states (Fig. 1). 'Fed' buffer solution contained higher concentrations of glucose, lactate and insulin, but lower concentration of palmitate, compared to 'fasted' buffer solution. The cardiac area at risk was approximately 50% of the area of the left ventricle and was similar in all groups. The infarct size in fed Wistar rat hearts was almost 2-fold (46%) lower than that observed in fasted rat hearts (Fig. 2A). A similar effect was achieved when hearts from fasted rats were perfused with 'fed' buffer containing higher concentrations of glucose, insulin and lactate (Fasted + 'fed' buffer group). The basal contractility was significantly lower in fasted rat hearts compared to the fed group (Fig. 2B), although no significant differences were observed regarding the basal contractility and cardiac workload between the Fasted and Fasted + 'fed' buffer groups. During occlusion the cardiac workload (HR*LVDP) was significantly lower in fasted rat hearts compared to fed rat hearts (Fig. 2C). To characterize ischemia–reperfusion tolerance changes in hemodynamic parameters were normalized to the baseline function. All the parameters were found to be similar in fed and fasted states. In summary, although the changes in hemodynamic parameters relative to the baseline values were similar, the cardiac function in total was worse and the infarct size was significantly larger in hearts from fasted rats compared to those from fed rats. In addition, perfusion with 'fed' buffer was able to protect fasted hearts against infarction-induced damage, but could not improve cardiac function.

The effects of fasted and fed states on infarct size were studied also in the model of type 2 diabetes, GK rats. The GK rats had markedly reduced insulin sensitivity and impaired glucose tolerance (Supplementary Figure 1). Similar to Wistar rats, there was almost a 2-fold difference in the infarct size in GK rats between hearts from fed and fasted animals. As a result of insulin resistance, the infarct size in hearts from fed and fasted GK rats was 20%–50% larger than in Wistar rat hearts (Fig. 2D). Similar to Wistar rats, in GK rats the basal contractility was significantly lower (Fig. 2E) in hearts from fasted animals, although no significant difference was observed in cardiac workload (Fig. 2F).

3.2. Insulin signalling and reduced FA load determine cardioprotective effect in the fed state

Increased rate of FA flux could lead to reduced cardiac efficiency [15,32], while increased glucose oxidation is protective against myocardial infarction-induced cell damage. In order to determine changes in substrate utilization pattern behind the cardioprotection in the fed state, we measured glucose, lactate and FA oxidation rates in hearts isolated from

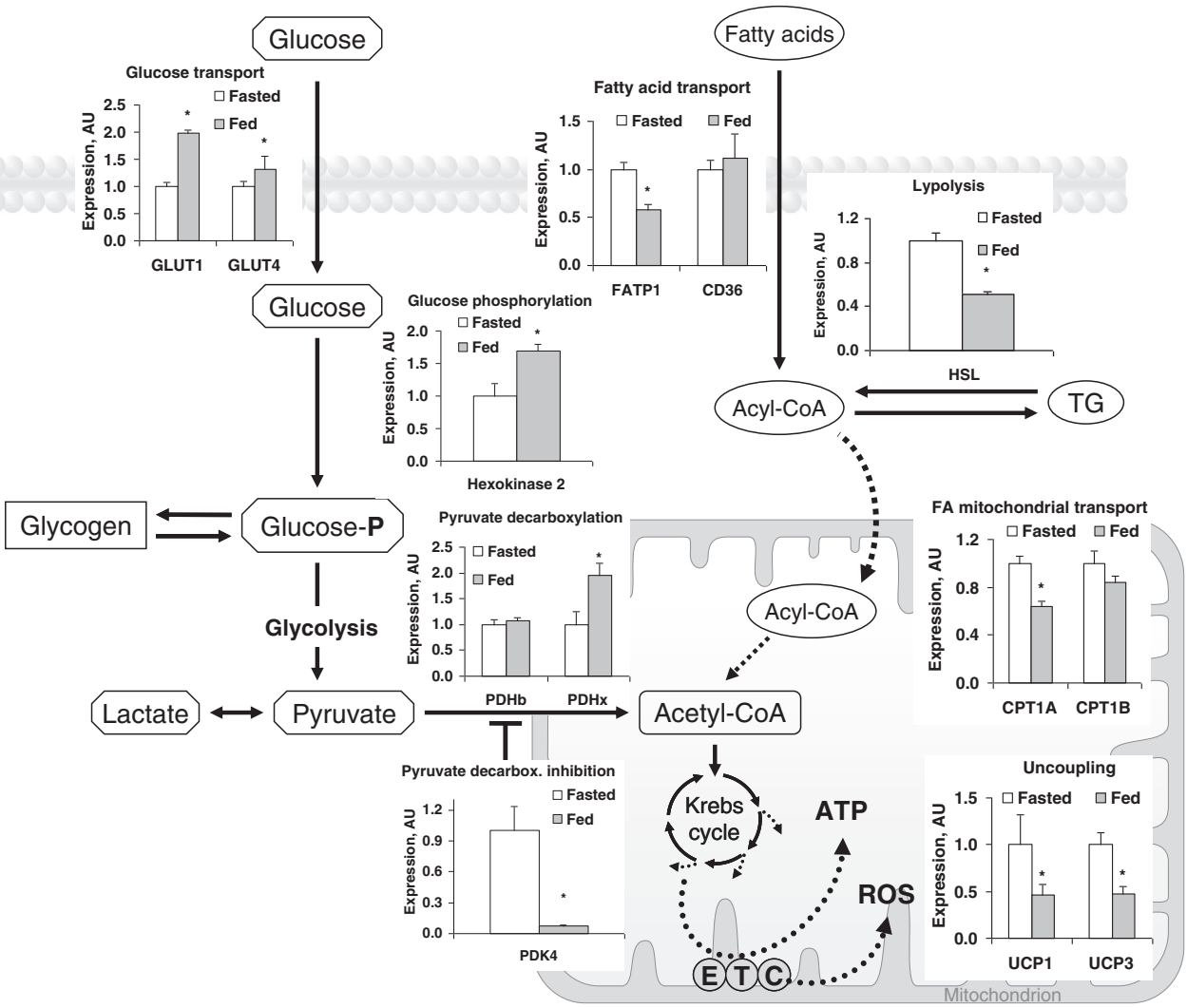

Fig. 4 – Differences in the gene expression profile in the hearts from fasted and fed Wistar rats. Genes involved in glucose uptake (Glut1 and Glut4), glucose phosphorylation (HK2) and pyruvate decarboxylation (PDHx) were upregulated in the fed state. Genes involved in lypolysis (HSL), FA transport (FATP1 and CPT1A), the inhibition of pyruvate decarboxylation (PDK4) and mitochondrial uncoupling (UCP1, UCP3) were upregulated in the fasted state. The presented results are the average values ± SEM of at least 8 rats. *Significantly different from the fasted group (Student's t-test $P < 0.05$).

fasted and fed rats. In the fasted state, the oxidation of lactate and pyruvate was very limited (Fig. 3A and B), and glucose was stored in the form of glycogen (Supplementary Figure 2B) or metabolised to lactate. The lactate content in the heart was similar in the fasted and fed states (Supplementary Figure 2A). In contrast, glucose and lactate oxidation rates were increased by 5- and 4-fold (Fig. 3A and B), respectively, although the circulating concentrations of glucose and lactate were only 2-fold higher (Fig. 1A and B) in the fed state relative to the fasted state. Thus, the increase in glucose, lactate and pyruvate oxidation rates directly depends on insulin signalling and indirectly on circulating glucose and lactate concentrations (Fig. 1A, B and D).

The heart has a relatively low free FA and triglyceride storage capacity (Supplementary Figure 2C and 2D), and FA oxidation depends on the levels of circulating FAs and triglycerides (Fig. 1C and E). We observed 3-fold reductions in the labelled palmitate uptake and oxidation rate in the fed state compared to the fasted state (Fig. 3C). In the fasted state, FA oxidation overrides glucose and lactate oxidation and prevails as the only energy source (Fig. 3D). An increased circulating concentration of insulin and consequential stimulation of glucose metabolism together with lower FA load determine the protection against ischemia–reperfusion injury in the fed state.

3.3. *Insulin and PPAR-α/PGC-1α mediate changes in the gene expression*

Insulin and PPAR-α/PGC-1α mediate the main signalling pathways involved in glucose and FA metabolism [33,34]. Insulin stimulates glucose uptake and oxidation, whereas the PPAR-α/PGC-1α pathway stimulates FA metabolism. In the fed state, the higher concentration of circulating insulin (Fig. 1D) together with activated insulin pathway (Fig. 5A) induced a significant 2-fold increase in the expression of genes involved

in glucose metabolism (uptake (Glut1, Glut4), phosphorylation (HK2) and pyruvate decarboxylation (PDHx)) (Fig. 4). Changes in gene expression in the fed state are associated with the increased oxidation of glucose and lactate in the heart (Fig. 3A and B).

In contrast, in the fasted state the PPAR-α/PGC-1α pathway and genes involved in lypolysis (HSL) and FA transport (FATP1, CPT1A) were upregulated (Fig. 5B). These gene expression results are consistent with the differences in FA metabolism between the fasted and fed states (Fig. 3C) in the heart. The expression of PDK4 (an inhibitor of pyruvate decarboxylation) was 10-fold higher in the fasted state compared to the fed state (Fig. 4). Thus, an increased expression of PDK4 provides a mechanism for the arrest of lactate oxidation in the fasted state (Fig. 3B). Moreover, because FAs are intrinsic activators of the uncoupling protein (UCP), high FA content results in 2-fold increase in cardiac UCP1 and UCP3 expression (Fig. 4).

4. Discussion

We have shown that in the hearts of Wistar rats, the infarct size is approximately 2-fold smaller in the fed state compared to the fasted state. In the fed state, the heart tissues oxidise more glucose and less palmitate than in the fasted state as a result of activated insulin signalling pathway-induced expression of glucose metabolism genes. In contrast, in the fasted state, the PPAR-α/PGC-1α pathway increases PDK4 expression, which switches off glucose and lactate oxidation. FA overloading in the fasted state leads to an uncoupling of mitochondrial oxidative phosphorylation and energy dissipation. Our results demonstrate that cardiac recovery from ischemia–reperfusion injury is improved in the fed state due to enhanced glucose and lactate oxidation and lower load of FAs.

There is abundant evidence that increased glucose oxidation is protective against myocardial infarction-induced cell damage [13,35,36]. In the fasted state, the oxidation of glucose, lactate and pyruvate contributes to less than 5% of produced ATP, which leads to marked heart tissue damage in cases of myocardial infarction. In the fed state, significantly increased glucose oxidation ensures better survival in ischemic conditions and improved recovery in reperfusion. The increases in glucose, lactate and pyruvate oxidation rates depend on insulin signalling rather than on circulating glucose and lactate concentrations. Insulin signalling influences glucose transport and metabolism by altering gene expression and enzyme activities. Similarly, FA oxidation is partially downregulated in hearts isolated from fed rats. Insulin-activated pathways induce inhibition of FA metabolism, resulting in significantly lower concentrations of long-chain FAs in the heart and mitochondria. As a result, in the fed state, there is a lower risk of mitochondrial damage by FAs in cases of myocardial infarction. Thus, insulin is the most important switch in energy metabolism in a myocardial fed–fasted cycle.

In line with previous studies [15,32], we also observed that high FA flux in the fasted state leads to reduced cardiac efficiency. Inefficiency of FA oxidation is related to energy dissipation by intrinsic activation of the uncoupling proteins, which leads to reduced ATP yield [37–39], that as observed in this study is associated with decreased cardiac function in the fasted state. Increased oxidation of FAs in the fasted state results in higher oxygen requirement, which increases the risk of heart damage under hypoxia-related conditions. Overall, these results provide additional evidence that FA is a less-effective energy substrate and that high FA load increases the

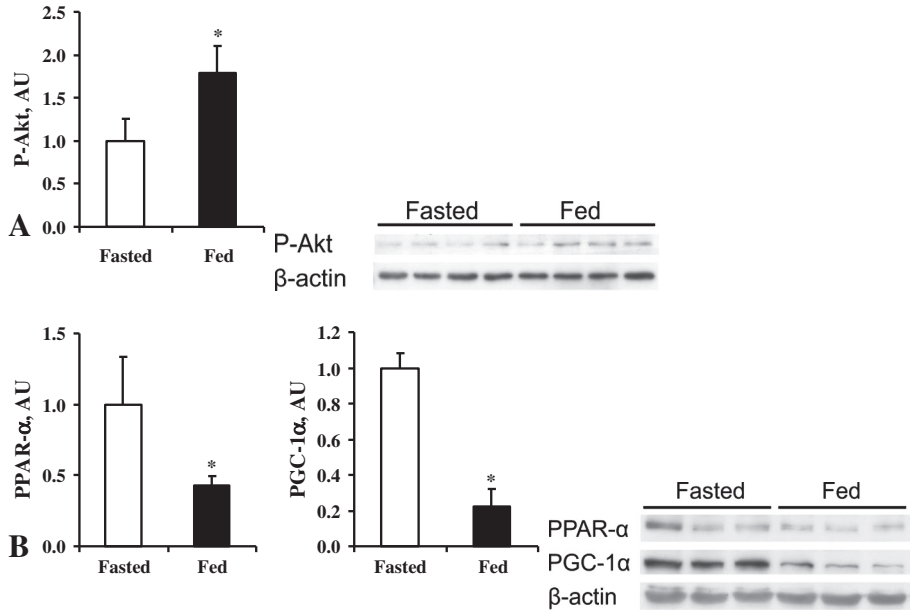

Fig. 5 – Nuclear contents of PPAR-α, PGC-1α and phosphorylated Akt (P-Akt) in the hearts from fed and fasted Wistar rats. In the fed state compared to fasted state, content of phosphorylated Akt (P-Akt) is higher (A) and nuclear contents of PPAR-α and PGC-1α are lower (B). The presented results are average values ± SEM of at least 4 rats. *Significantly different from the fasted group (Mann–Whitney test, P < 0.05).

risk for permanent heart damage and the possibility of lethal cardiovascular events.

Several previous studies have used *ex vivo* isolated perfused hearts to examine the differences between fed and fasted states in a global ischemia–reperfusion model [16,17,40–42]. These studies concluded that fasted rat hearts are better protected from mechanical dysfunction induced by ischemia–reperfusion. However, all previous studies used only one buffer solution that does not mimic both states in the *ex vivo* isolated heart model. Two studies performed by Montessuit and colleagues used a buffer that contained glucose, palmitate and insulin in concentrations similar to fed conditions [16,17], while other studies used glucose as the only energy substrate [41,42]. Previous results indicate the beneficial effects of acutely stimulated glucose metabolism rather than the effects of changes in energy metabolism pattern. Meanwhile, the present results highlight the importance of insulin-signalling and FA metabolism intensity to the outcome of ischemia–reperfusion injury.

Although preoperative fasting is mandatory for adults to prevent perioperative complications, this fasting could also cause hypoglycaemia-related effects [18–20]. A growing body of data on the interplay between glucose and FA metabolism during acute myocardial infarction supports the need for heart-specific glucose metabolism-stimulating agents [20,43–45]. GIK infusion has long been suggested for myocardial protection [46], although the results of clinical trials are controversial [47,48]. The present results suggest that, in subjects in the postprandial state, concentrations of glucose and insulin are already relatively high, and the possibilities for inducing additional increases in glucose oxidation by insulin and glucose infusion are very limited. Thus, protective effects could be expected only if the GIK is administered in the fasted state. In addition, the improvement of the clinical outcome can be achieved when GIK administration is started within the first hours after the onset of symptoms of acute coronary syndrome [47]. Thus, during fasting in case of symptoms of cardiovascular event the intake of foods or beverages rich in carbohydrates could be potentially life-saving.

Obesity-induced insulin resistance is an important risk factor for cardiovascular diseases [2,49]. Therefore, long-term low calorie diets are beneficial for preventing diabetes and cardiovascular complications. However, acute extreme fasting or starving could result in low plasma glucose concentrations, which can trigger cardiovascular events. Moreover, an increased FA availability in the fasted state enhances the severity of cardiovascular events. In low-carbohydrate diets, low plasma glucose levels or even hypoglycaemia is observed in the postprandial state. As a translational aspect our results demonstrate that it is important to suggest for high-risk patients to avoid very low-carbohydrate diets and prolonged fasting periods.

This research work provides an integrative view of effects of common dietary components and their metabolites on the heart function under normoxic and ischemia–reperfusion conditions and their translational potential in clinic. There are some limitations which should be mentioned. First, the current physiological results are obtained in rodents and future studies in humans to confirm the effects should be performed. Second, *ex vivo* perfusion model only partially reflects the systemic factors.

In conclusion, our study suggests that glucose and lactate oxidation is important for the survival of ischemic hearts, and even overnight fasting-induced hypoglycaemia could trigger cardiovascular events, such as angina pectoris and arrhythmias.

Author contributions

E.L. and M.D. designed the research. M.M., J.K., R.V., H.C., E.M., O.P., S.G. and E.S. performed experiments. E.L., M.D., M.M., J.K., R.V. and E.M. analysed and interpreted the data. These authors also provided input on the manuscript. E.L. wrote the manuscript. The study was supervised by E.L. and M.D. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no potential conflicts of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2013.09.014.

REFERENCES

[1] Hassan M, Latif N, Yacoub M. Adipose tissue: friend or foe? Nat Rev Cardiol 2012;9(12):689–702.
[2] Lavie CJ, Milani RV, Ventura HO. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. J Am Coll Cardiol 2009;53(21):1925–32.
[3] Klempel MC, Kroeger CM, Varady KA. Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet. Metabolism 2013;62(1):137–43.
[4] Numao S, Kawano H, Endo N, et al. Effects of a single bout of aerobic exercise on short-term low-carbohydrate/high-fat intake-induced postprandial glucose metabolism during an oral glucose tolerance test. Metabolism 2013;62(10):1406–15.
[5] Desouza CV, Bolli GB, Fonseca V. Hypoglycemia, diabetes, and cardiovascular events. Diabetes Care 2010;33(6):1389–94.
[6] Dzeja PP, Redfield MM, Burnett JC, et al. Failing energetics in failing hearts. Curr Cardiol Rep 2000;2(3):212–7.
[7] Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev 2005;85(3):1093–129.

[8] Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev 1998;14(4):263–83.
[9] Ardehali H, Sabbah HN, Burke MA, et al. Targeting myocardial substrate metabolism in heart failure: potential for new therapies. Eur J Heart Fail 2012;14(2):120–9.
[10] Opie LH. Cardiac metabolism—emergence, decline, and resurgence. Part I Cardiovasc Res 1992;26(8):721–33.
[11] Ramos-Roman MA, Sweetman L, Valdez MJ, et al. Postprandial changes in plasma acylcarnitine concentrations as markers of fatty acid flux in overweight and obesity. Metabolism 2012;61(2):202–12.
[12] Viscarra JA, Ortiz RM. Cellular mechanisms regulating fuel metabolism in mammals: role of adipose tissue and lipids during prolonged food deprivation. Metabolism 2013;62(7): 889–97.
[13] Ussher JR, Wang W, Gandhi M, et al. Stimulation of glucose oxidation protects against acute myocardial infarction and reperfusion injury. Cardiovasc Res 2012;94(2):359–69.
[14] Korvald C, Elvenes OP, Myrmel T. Myocardial substrate metabolism influences left ventricular energetics in vivo. Am J Physiol Heart Circ Physiol 2000;278(4):H1345–51.
[15] Simonsen S, Kjekshus JK. The effect of free fatty acids on myocardial oxygen consumption during atrial pacing and catecholamine infusion in man. Circulation 1978;58(3 Pt 1): 484–91.
[16] Montessuit C, Papageorgiou I, Tardy I, et al. Effect of nutritional state on substrate metabolism and contractile function in postischemic rat myocardium. Am J Physiol 1996;271(5 Pt 2):H2060–70.
[17] Montessuit C, Papageorgiou I, Tardy-Cantalupi I, et al. Post-ischemic recovery of heart metabolism and function: role of mitochondrial fatty acid transfer. J Appl Physiol 2000;89(1): 111–9.
[18] Awad S, Stephens F, Shannon C, et al. Perioperative perturbations in carnitine metabolism are attenuated by preoperative carbohydrate treatment: another mechanism by which preoperative feeding may attenuate development of postoperative insulin resistance. Clin Nutr 2012;31(5): 717–20.
[19] Ljungqvist O. Modulating postoperative insulin resistance by preoperative carbohydrate loading. Best Pract Res Clin Anaesthesiol 2009;23(4):401–9.
[20] van Hoorn DE, Boelens PG, van Middelaar-Voskuilen MC, et al. Preoperative feeding preserves heart function and decreases oxidative injury in rats. Nutrition 2005;21(7–8): 859–66.
[21] Crawford PA, Crowley JR, Sambandam N, et al. Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. Proc Natl Acad Sci U S A 2009;106(27):11276–81.
[22] Desrois M, Clarke K, Lan C, et al. Upregulation of eNOS and unchanged energy metabolism in increased susceptibility of the aging type 2 diabetic GK rat heart to ischemic injury. Am J Physiol Heart Circ Physiol 2010;299(5):H1679–86.
[23] Moreno KX, Sabelhaus SM, Merritt ME, et al. Competition of pyruvate with physiological substrates for oxidation by the heart: implications for studies with hyperpolarized [1-13C] pyruvate. Am J Physiol Heart Circ Physiol 2010;298(5): H1556–64.
[24] Vincent G, Bouchard B, Khairallah M, et al. Differential modulation of citrate synthesis and release by fatty acids in perfused working rat hearts. Am J Physiol Heart Circ Physiol 2004;286(1):H257–66.
[25] Kuka J, Vilskersts R, Cirule H, et al. The cardioprotective effect of mildronate is diminished after co-treatment with L-carnitine. J Cardiovasc Pharmacol Ther 2012;17(2):215–22.
[26] Liepinsh E, Vilskersts R, Loca D, et al. Mildronate, an inhibitor of carnitine biosynthesis, induces an increase in gamma-butyrobetaine contents and cardioprotection in isolated rat heart infarction. J Cardiovasc Pharmacol 2006;48(6):314–9.
[27] Liepinsh E, Kuka J, Dambrova M. Troubleshooting digital macro photography for image acquisition and the analysis of biological samples. J Pharmacol Toxicol Methods 2013;67(2): 98–106.
[28] Lopaschuk GD, Barr RL. Measurements of fatty acid and carbohydrate metabolism in the isolated working rat heart. Mol Cell Biochem 1997;172(1–2):137–47.
[29] Liepinsh E, Kuka J, Svalbe B, et al. Effects of long-term mildronate treatment on cardiac and liver functions in rats. Basic Clin Pharmacol Toxicol 2009;105(6):387–94.
[30] Liepinsh E, Vilskersts R, Skapare E, et al. Mildronate decreases carnitine availability and up-regulates glucose uptake and related gene expression in the mouse heart. Life Sci 2008;83(17–18):613–9.
[31] Liepinsh E, Skapare E, Svalbe B, et al. Anti-diabetic effects of mildronate alone or in combination with metformin in obese Zucker rats. Eur J Pharmacol 2011;658(2–3):277–83.
[32] Mjos OD. Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. J Clin Invest 1971;50(7): 1386–9.
[33] Finck BN. The PPAR regulatory system in cardiac physiology and disease. Cardiovasc Res 2007;73(2):269–77.
[34] Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001;414(6865): 799–806.
[35] Hafstad AD, Khalid AM, How OJ, et al. Glucose and insulin improve cardiac efficiency and postischemic functional recovery in perfused hearts from type 2 diabetic (db/db) mice. Am J Physiol Endocrinol Metab 2007;292(5):E1288–94.
[36] Liu B, Clanachan AS, Schulz R, et al. Cardiac efficiency is improved after ischemia by altering both the source and fate of protons. Circ Res 1996;79(5):940–8.
[37] Cole MA, Murray AJ, Cochlin LE, et al. A high fat diet increases mitochondrial fatty acid oxidation and uncoupling to decrease efficiency in rat heart. Basic Res Cardiol 2011;106(3): 447–57.
[38] Ray J, Noll F, Daut J, et al. Long-chain fatty acids increase basal metabolism and depolarize mitochondria in cardiac muscle cells. Am J Physiol Heart Circ Physiol 2002;282(4): H1495–501.
[39] Rider OJ, Cox P, Tyler D, et al. Myocardial substrate metabolism in obesity. Int J Obes (Lond) 2013;37(7):972–9.
[40] Doenst T, Guthrie PH, Chemnitius JM, et al. Fasting, lactate, and insulin improve ischemia tolerance in rat heart: a comparison with ischemic preconditioning. Am J Physiol 1996;270(5 Pt 2):H1607–15.
[41] Schaefer S, Ramasamy R. Glycogen utilization and ischemic injury in the isolated rat heart. Cardiovasc Res 1997;35(1): 90–8.
[42] Schneider CA, Taegtmeyer H. Fasting in vivo delays myocardial cell damage after brief periods of ischemia in the isolated working rat heart. Circ Res 1991;68(4): 1045–50.
[43] Diks J, van Hoorn DE, Nijveldt RJ, et al. Preoperative fasting: an outdated concept? JPEN J Parenter Enteral Nutr 2005;29(4): 298–304.
[44] Luttikhold J, Oosting A, van den Braak CC, et al. Preservation of the gut by preoperative carbohydrate loading improves postoperative food intake. Clin Nutr 2013;32(4):556–61.
[45] van den Brom CE, Bulte CS, Loer SA, et al. Diabetes, perioperative ischaemia and volatile anaesthetics: consequences of derangements in myocardial substrate metabolism. Cardiovasc Diabetol 2013;12(1):42.
[46] Sievers J, Lindh J, Johansson BW, et al. Acute myocardial infarction treated by glucose–insulin–potassium (GIK) infusion. Cardiologia 1966;49(4):239–47.

[47] Grossman AN, Opie LH, Beshansky JR, et al. Glucose–insulin–potassium revived: current status in acute coronary syndromes and the energy-depleted heart. Circulation 2013;127(9):1040–8.
[48] Zhao YT, Weng CL, Chen ML, et al. Comparison of glucose–insulin–potassium and insulin–glucose as adjunctive therapy in acute myocardial infarction: a contemporary meta-analysis of randomised controlled trials. Heart 2010;96(20):1622–6.
[49] Bhat SL, Abbasi FA, Blasey C, et al. Beyond fasting plasma glucose: the association between coronary heart disease risk and postprandial glucose, postprandial insulin and insulin resistance in healthy, nondiabetic adults. Metabolism 2013;62(9):1223–6.