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RESEARCH ARTICLE | *Energetics and Metabolism*

Diet-induced obese mouse hearts tolerate an acute high-fatty acid exposure that also increases ischemic tolerance

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Boardman NT, Pedersen TM, Rossvoll L, Hafstad AD, Aasum E. Diet-induced obese mouse hearts tolerate an acute high-fatty acid exposure that also increases ischemic tolerance. *Am J Physiol Heart Circ Physiol* 319: H682–H693, 2020. First published August 14, 2020; doi:10.1152/ajpheart.00284.2020.—An ischemic insult is accompanied by an acute increase in circulating fatty acid (FA) levels, which can induce adverse changes related to cardiac metabolism/energetics. Although chronic hyperlipidemia contributes to the pathogenesis of obesity-/diabetes-related cardiomyopathy, it is unclear how these hearts are affected by an acute high FA-load. We hypothesize that adaptation to chronic FA exposure enhances the obese hearts' ability to handle an acute high FA-load. Diet-induced obese (DIO) and age-matched control (CON) mouse hearts were perfused in the presence of low- or high FA-load (0.4 and 1.8 mM, respectively). Left ventricular (LV) function, FA oxidation rate, myocardial oxygen consumption, and mechanical efficiency were assessed, followed by analysis of myocardial oxidative stress, mitochondrial respiration, protein acetylation, and gene expression. Finally, ischemic tolerance was determined by examining LV functional recovery and infarct size. Under low-FA conditions, DIO hearts showed mild LV dysfunction, oxygen wasting, mechanical inefficiency, and reduced mitochondrial OxPhos. High FA-load increased FA oxidation rates in both groups, but this did not alter any of the above parameters in DIO hearts. In contrast, CON hearts showed FA-induced mechanical inefficiency, oxidative stress, and reduced OxPhos, as well as enhanced acetylation and activation of PPARα-dependent gene expression. While high FA-load did not alter functional recovery and infarct size in CON hearts, it increased ischemic tolerance in DIO hearts. Thus, this study demonstrates that acute FA-load affects normal and obese hearts differently and that chronically elevated circulating FA levels render the DIO heart less vulnerable to the disadvantageous effects of an acute FA-load.

NEW & NOTEWORTHY An acute myocardial fat-load leads to oxidative stress, oxygen wasting, mechanical inefficiency, hyperacetylation, and impaired mitochondrial function, which can contribute to reduced ischemic tolerance. Following obesity/insulin resistance, hearts were less affected by a high fat-load, which subsequently also improved ischemic tolerance. This study highlights that an acute fat-load affects normal and obese hearts differently and that obesity renders hearts less vulnerable to the disadvantageous effects of an acute fat-load.

acetylation; cardiac efficiency; heart perfusion; mitochondrial respiration; oxygen consumption; pressure volume

INTRODUCTION

An acute myocardial infarction is accompanied by increased circulating FAs due to adrenergic-driven lipolysis in adipose tissue (36, 42, 43). An ischemic heart will therefore not only be challenged by hypoxia but also by an acute high FA-load. In normal hearts, a high FA-load will increase mitochondrial reactive oxygen species (ROS) production and lead to impaired mitochondrial energetics (31, 41), intracellular acidosis (35), as well as Ca^{2+} dysregulation (17, 50), and oxygen wasting with subsequent mechanical inefficiency (9, 27, 28, 40). These FA-mediated changes could all potentially aggravate ischemia-reperfusion injury, and accordingly, high-FA levels have been reported to reduce ischemic tolerance in isolated perfused hearts (12, 14, 19, 35).

In obesity and diabetes, there is an increased risk of developing heart failure, independent of coronary artery disease and hypertension. This specific cardiomyopathy has been linked to hormonal and metabolic derangements. Accordingly, high circulating levels of insulin, glucose, and FAs may contribute in the pathogenesis of diabetic cardiomyopathy. Although preclinical studies generally report reduced ischemic tolerance in hearts from models of obesity/diabetes, it is less clear how high glucose, insulin, and FAs affect these hearts and their tolerance to ischemia. We have previously shown that high glucose and insulin had oxygen-sparing effects accompanied by improved postischemic functional recovery in hearts from obese diabetic mice (24). Thus, the aim of the present study was to elucidate the effects of an acute high FA-load under similar conditions.

It is well known that obese/diabetic hearts undergo metabolic alterations, making them more reliant on FAs as energy substrate (9, 25, 38, 39). This metabolic shift includes both allosteric control and translational and posttranslational modifications of metabolic proteins. The enhanced cellular FA processing is due to increased protein and gene expression of FA transporters (20), enzymes related to β-oxidation (25, 29, 38), and higher myocardial FA storage (4). Although the consensus is that the elevated supply of FAs in obesity/diabetes contributes to the development of cardiac dysfunction, we hypothesized that metabolic adaptation to elevated FA levels in the heart enhance the overall capacity of the heart for handling FAs and, consequently, their tolerance to an acute high FA-load.

MATERIALS AND METHODS

Animals. C57BL/6J male mice (5 to 6 wk) were purchased from Charles River Laboratories (Germany). Obesity and insulin resistance

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were induced by feeding mice an obesogenic diet for 20 wk (diet-induced obese, DIO) as previously described (25, 39). Age-matched mice were fed a regular chow diet served as controls (CON). All mice were housed in a room with a constant temperature of 23°C and 55% humidity, with a 12-h:12-h reversed light-dark cycle, and were given ad libitum access to water and their respective diets. To evaluate insulin resistance, plasma glucose (glucometer, FreeStyle Lite, Alameda, CA) and insulin (commercial kits from DRG Diagnostics, Marburg, Germany) were determined in blood samples obtained from the saphenous vein from fasted (4 h) mice. Homeostatic model assessment (HOMA) was calculated from fasting blood glucose and insulin levels. Plasma free fatty acids were determined in blood samples taken from the saphenous vein of fed animals before euthanasia, using commercial kits from Wako Chemicals (Neuss, Germany). Animal experiments were approved by the Norwegian National Animal Research Authority, which conforms to the National Institute of Health guidelines (NIH Publication No. 85-23, Revised 1996) and European Directive 2010/63/EU.

Assessment of left ventricular function, fatty acid oxidation, and oxygen consumption. Left ventricular (LV) function, fatty acid (FA) oxidation rate, and myocardial oxygen consumption ($M\dot{V}O_2$) were assessed in isolated perfused hearts. All hearts were perfused in a recirculating mode, using Krebs-Henseleit bicarbonate buffer that contained 5 mM glucose and either low (0.4 mM)- or high (1.8 mM)-FA concentration (palmitate prebound to 3% BSA) throughout the entire experiment. High-FA levels and high FA-load are used interchangeably throughout the manuscript.

LV pressure and volume were assessed in working hearts, perfused with either low- or high-FA concentration, using a conductance catheter (1 Fr) inserted through the apex (27). FA oxidation rates were also measured in working hearts using 9,10-[^{3}H] palmitate prebound to BSA (1). $M\dot{V}O_2$ was assessed using fiber-optic oxygen probes (FOXY-AL300; Ocean Optics, Duiven, The Netherlands), inserted into the perfusion line just above the aortic cannula and into the pulmonary artery to obtain the arterial-venous difference in P_{O_2}.

Cardiac power was calculated as the product of left ventricular developed pressure and cardiac output. Mechanical efficiency was calculated by dividing cardiac power by $M\dot{V}O_2$. This was expressed as a percentage as both parameters were converted to the same unit. Work-independent $M\dot{V}O_2$ was assessed by switching to Langendorff perfusion mode and inserting a vent through the apex. Under these conditions, the hearts were paced (7 Hz) and $M\dot{V}O_2$ was measured before and after KCl-induced cardiac arrest, representing O_2 cost in unloaded hearts ($M\dot{V}O_{2unloaded}$) and for basal metabolism ($M\dot{V}O_{2BM}$), as previously described (7). Oxygen cost for processes associated with excitation-contraction coupling ($M\dot{V}O_{2ECC}$) were calculated as the difference between $M\dot{V}O_{2unloaded}$ and $M\dot{V}O_{2BM}$.

RNA isolation and quantification RT-PCR. LV samples were immersed in RNAlater (Qiagen, Hilden, Germany), and total RNA was extracted using RNeasy Fibrous Tissue protocol (Qiagen). cDNA was obtained from 500 ng RNA, and real-time PCR was performed in a LightCycler96 System using a 1:6 dilution of the cDNA and FastStart Essential DNA Green Master (Roche). mRNA expression of genes of interest were normalized to the GeNorm value from the three housekeeping genes cyclophilin (*Cyclo*), hypoxanthine guanine phosphoribosyl transferase (*Hprt*), and hydroxymethylbilane synthase (*Hmbs*). Primer sequences for the gene expression of the housekeeping genes, pyruvate dehydrogenase kinase 4 (*Pdk4*), fatty acid tranlocase (*FAT/Cd36*), uncoupling protein 2 and 3 (*Ucp2* and *3*), carnitine palmitoyl transferase 1 (*mCpt1*), acyl-CoA thioesterases 2 (*Acot2*), hexokinase 2 (*Hk2*), glutathione peroxidase 3 (*Gpx3*), catalase (*Cat*), superoxide dismutase (*MnSod*), and lactate dehydrogenase (*Ldh*) are as previously reported (22, 23). Additional forward and reverse primer (5′-3′) sequences: acyl-CoA thioesterases 1 (*Acot1*): forward, AAC-ATC-ACC-TTT-GGA-GGG-GAG, and reverse, TCC-CCA-ACC-TCC-AAA-CCA-TCA; acyl-CoA oxidase (*Aox*): forward, GCG-CCA-GTC-TGA-AAT-CAA-G, and reverse, ACT-GCT-GCG-TCT-GAA-AAT-CC; glucose transporter 4 (*Glut4*): forward, GAC-GGA-CAC-TCC-ATC-TGT-TG, and reverse, GCC-ACG-ATG-GAG-ACA-TAG-C; and sirtuin 3 (*Sirt3*): forward, GGC-TCT-ATA-CAC-AGA-ACA-TCG-AC, and reverse, GAA-GGA-CCT-TCG-ACA-GAC-CGT; amino acid synthesis 5 like-1 (*GCN5L1/BLOS1*): forward, TCC-CGC-CTG-CTC-AAA-GAA-C, and reverse, GAG-GTG-ATC-CAC-CAA-CGC-TT.

Mitochondrial respiration. Mitochondrial respiration was assessed in isolated cardiac mitochondria from Langendorff-perfused hearts subjected to a low or high FA-load for 30 min. Respiration was determined by high-resolution respirometry using an oxygraph (O2k, Oroboros Instruments, Austria). Pyruvate (5 mM) and malate (2 mM) or palmitoyl-CoA (25 μM), L-carnitine (5 mM), and malate (2 mM) served as substrates. Uncoupled respiration (V_0) was assessed in the presence of substrates but without ADP. Coupled respiration (V_{max}, OxPhos) was defined as the peak respiration after adding 100 μmol/L ADP. Respiration rates were adjusted to total protein, and respiratory control ratio (RCR) was calculated as the ratio between V_{max} and V_0. A rate-independent coupling parameter, based on the ratio of molecules of ADP phosphorylated to each oxygen molecule consumed (ADP/O), was also calculated.

Mitochondrial protein acetylation. Mitochondrial proteins were lysed in buffer containing 75 mM Tris·HCL, 3.8% SDS, 4 M urea and Complete Protease Inhibitor Cocktail (Sigma-Aldrich). Laemmli buffer was added, and samples were boiled (95°C, 5 min). Protein (20 μg) was loaded onto a 15% criterion gel (Bio Rad) and transferred onto a nitrocellulose membrane (GE Healthcare) following electrophoresis. Membranes were blocked (5% milk, 1 h), followed by incubation with acetylated lysine antibody or VDAC antibody (Cell Signaling Technology) overnight at 4°C and thereafter washed and incubated with anti-rabbit antibody for 1 h. Immunopositive bands were developed in chemoluminescent peroxidase substrate 3 (Sigma) for acetylated lysine and LumiGlo (Cell Signaling Technology) for VDAC and visualized using a GE ImageQuant LAS 4000 (GE healthcare). Densitometry of bands was evaluated using Image Studio Protein Lite (LI COR Biosciences), and load was normalized using VDAC as a loading control.

Myocardial ROS. Following 30 min of Langendorff perfusion, LV tissue was embedded in OCT compound, frozen in cooled isopentane, and stored at −70°C. The samples were cryosectioned and stained with dihydroethidium (DHE) for evaluation of superoxide generation using epifluorescence microscope. From each heart, 10–15 images were obtained for quantification using ImageJ software.

Susceptibility to ischemic injury. Ischemic tolerance was examined in Langendorff-perfused hearts. LV function was recorded using an intraventricular fluid-filled balloon connected to a pressure transducer (39). The volume of the balloon was adjusted to give an end-diastolic pressure of 5–10 mmHg. After 20 min of stabilization, the hearts were subjected to 25 min global, no-flow ischemia, followed by 90 min reperfusion. Cardiac temperature was continuously monitored and kept at 37 ± 0.5°C throughout the perfusion protocol. At the end of reperfusion, hearts were frozen at −20°C, before slicing and staining using a 1% 2,3,5- triphenyl-2H-tetrazolium chloride solution. Infarct size was determined using ImageJ software (National Institutes of Health, Bethesda, MD).

Statistical analysis. Data are presented as means ± SE. Differences between two groups were analyzed using an unpaired Student's *t* test. Multiple comparisons were performed by a one-way or two-way ANOVA as indicated in the table or figure legends. When the ANOVA revealed differences, the data sets were compared using Holm-Sidak method as the post hoc test. Differences of $P < 0.05$ were considered significant.

RESULTS

Diet-induced obese (DIO) mice exhibited higher body weight and increased perirenal fat deposits (Table 1), elevated

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Table 1. *Animal characteristics of CON and DIO mice*

	Control	DIO
n	71	73
Body wt, g	31.7 ± 0.3	47.0 ± 0.5#
Tibia length, mm	18.3 ± 0.1	18.1 ± 0.1
Perirenal fat mass, g	0.34 ± 0.03	1.46 ± 0.06#
Liver wt, g	1.17 ± 0.02	2.05 ± 0.10#
Blood $glucose_{fasted}$, mmol/L	5.7 ± 0.2	6.9 ± 0.2#
Plasma $insulin_{fasted}$, μg/L	0.9 ± 0.1	3.2 ± 0.3#
HOMA IR	5.5 ± 0.5	25.2 ± 2.3#
Plasma free FA, μmol/L	371 ± 32	571 ± 37#
Heart wt, mg	155 ± 2	157 ± 2
Heart wt/tibia length	8.5 ± 0.1	8.7 ± 0.1

Values are means ± SE; *n*, number of animals used for assessment of blood glucose, plasma insulin and calculation of the homeostatic model assessment (HOMA IR) was 49 and 43 in control (CON) and diet-induced obese (DIO), respectively. Number of animals used for assessment of free fatty acids (FA) in nonfasted animals was 31 and 25 in CON and DIO, respectively. Data were analyzed using unpaired Student's *t*-test. #$P < 0.05$ vs. CON.

plasma free fatty acid (FA) levels, and a moderate increase in fasted blood glucose. These mice also showed marked insulin resistance, as indicated by increased HOMA-IR, which was primarily due to higher insulin levels (Table 1).

Left ventricular function. Left ventricular (LV) function was assessed in isolated perfused working hearts (Table 2). When perfused with a low-FA concentration, hearts from DIO mice are characterized by diastolic dysfunction, as indicated by elevated LV end-diastolic pressure, reduced LV relaxation (τ), and augmented end-diastolic pressure volume relationship (EDPVR) compared with control hearts perfused under the same conditions. These hearts developed a mild systolic dysfunction, with a small reduction in cardiac output and cardiac power, but without change in preload recruitable stroke work index (PRSWi) (Table 2). Subjecting CON and DIO to a high FA-load (a 4.5-fold increase in FA concentration) did not alter cardiac function in either CON or DIO hearts (Table 2).

Myocardial FA oxidation rate and metabolic gene expression. During perfusion with a low-FA concentration, myocardial FA oxidation rates were significantly elevated in DIO compared with CON hearts (Fig. 1*A*). This was accompanied by increased expression of FA uptake proteins (FAT/CD36), as well as mitochondrial transport (mCPT1) and oxidation of FAs (ACOX1). We also found the gene expression of PDK4 to be increased, supporting a shift toward enhanced utilization of FAs as energy substrate. In addition, DIO hearts showed increased expression of ACOT1 and -2, as well as UCP2 and 3 (Fig. 1*B*), while gene expression of the proteins GLUT4, HK, and LDH was unaltered (Fig. 1*C*).

As expected, elevation of the FA concentration in the perfusate increased FA oxidation rates in both CON and DIO hearts. Under these conditions, the rate of FA oxidation was no longer different between the two groups. Interestingly, we found that 30 min of high-FA perfusion resulted in a marked increase in the expression of PPARα target genes (PDK4, mCPT1, ACOX1, ACOT1 and -2, as well as UCP 2 and -3) in CON hearts, while genes of proteins not under the control of PPARα (HK, LDH, and GLUT4) remained unaltered. In DIO hearts, on the other hand, high-FA perfusion did not alter the mRNA expression of any of the genes examined, nor did we find PPARα gene expression to be affected by either obesity or by the acute high FA-load (data not shown).

Myocardial oxygen consumption and mechanical efficiency. Mechanical efficiency was calculated from cardiac power and $M\dot{V}O_2$ in isolated perfused working hearts (Table 2). Under low-FA conditions, DIO hearts showed mechanical inefficiency when compared with CON hearts under the same conditions (Fig. 2*A*). Increased $M\dot{V}O_2$ in the DIO hearts was also observed when hearts were subjected to unloaded conditions (Fig. 2*B*), which demonstrates a higher O_2 cost for nonmechanical processes in DIO as compared with control hearts, when perfused under low-FA conditions. Accordingly, DIO hearts also showed increased O_2 cost for processes related

Table 2. *LV function assessed in isolated perfused working hearts from control and DIO mice, perfused with low- and high-FA levels*

	Control Low FA	Control High FA	DIO Low FA	DIO High FA
n	9	8	11	11
Heart rate, beats/min	432 ± 9	461 ± 9*	423 ± 9	412 ± 9
Cardiac output, mL/min	16.7 ± 0.8	15.5 ± 0.8	14.1 ± 0.7#	14.1 ± 0.7
Coronary flow, mL/min	3.8 ± 0.2	4.5 ± 0.2	3.9 ± 0.2	3.7 ± 0.2#
LVEDP, mmHg	6.7 ± 0.5	5.6 ± 0.5	9.0 ± 0.5#	10.1 ± 0.5#
LVDP, mmHg	66.8 ± 1.4	67.2 ± 1.5	65.0 ± 1.4	62.9 ± 1.4
dP/dt_{max}, mmHg/s	4,923 ± 226	4,657 ± 119	4,457 ± 252	4,642 ± 126
dP/dt_{min}, mmHg/s	4,069 ± 241	3,828 ± 96	3,718 ± 243	3,531 ± 99
τ_{Glanz}, ms	16.9 ± 0.6	15.7 ± 0.4	19.0 ± 0.7#	20.0 ± 0.6#
EDPVR, mmHg/μL	0.15 ± 0.03	0.15 ± 0.03	0.30 ± 0.03#	0.33 ± 0.04#
PRSWi	66.8 ± 1.4	67.2 ± 1.5	65.0 ± 1.5	62.8 ± 1.4
Cardiac power, $J \cdot min^{-1} \cdot g^{-1}$	0.97 ± 0.04	0.87 ± 0.02	0.76 ± 0.06#	0.78 ± 0.05
$M\dot{V}O_2$, $J \cdot min^{-1} \cdot g^{-1}$	6.17 ± 0.39	7.82 ± 0.39*	6.49 ± 0.28	6.64 ± 0.30#

Values are means ± SE. Steady-state parameters were obtained in hearts perfused with a preload and afterload of 10 and 50 mmHg, respectively. All hearts were paced at 10% higher than their intrinsic heart rate, and intraventricular pressure was assessed using a conductance catheter inserted through the apex. dP/dt_{max} and dP/dt_{min}, maximum positive and negative first time derivative of left ventricular (LV) pressure; LVED, LV end-diastolic pressure; LVDP, LV developed pressure; τ, LV relaxation time constant; cardiac power, product of cardiac output and LVDP; $M\dot{V}O_2$, myocarardial oxygen consumption. Load-independent parameters were assessed from a family of pressure-volume loops created by a temporary preload occlusion in unpaced hearts from 7 to 10 hearts in each group. EDPVR, end-diastolic pressure-volume relationship; PRSWi, preload recruitable stroke work index. Data were analyzed with a two-way ANOVA with Holm-Sidak method as the post hoc test. *$P < 0.05$ vs. low FA. #$P < 0.05$ vs. CON at the same FA concentration.

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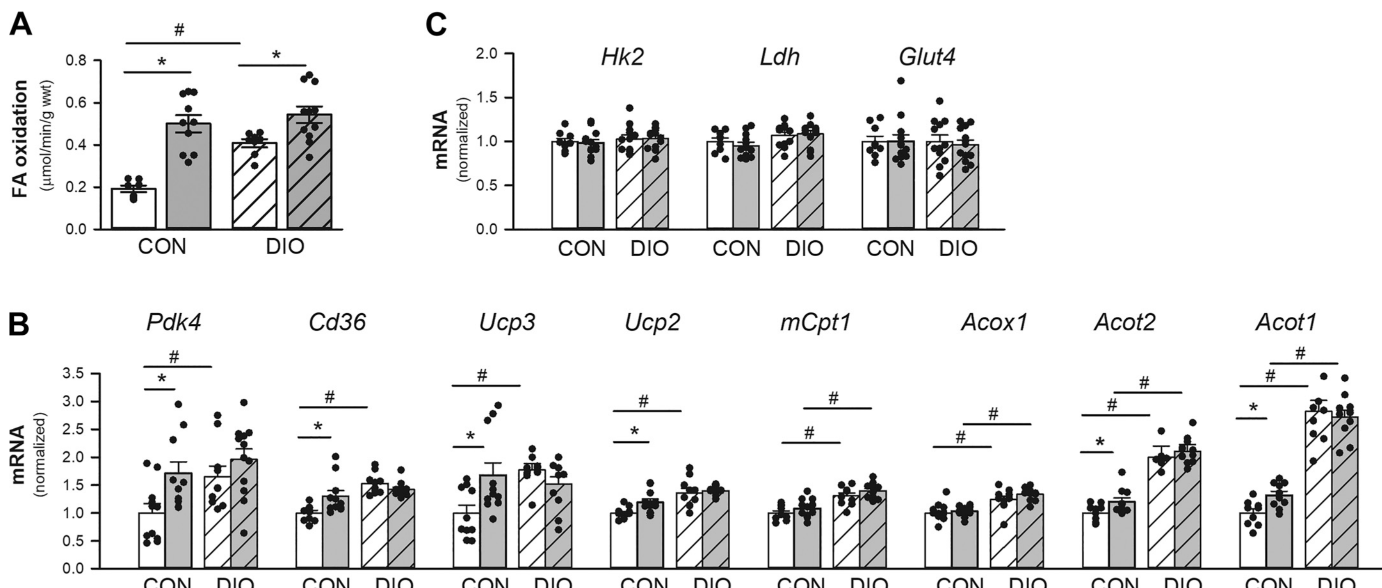

Fig. 1. FA oxidation rate (*A*) and mRNA expression (*B* and *C*) in hearts from control (CON) and diet-induced obese (DIO) mice, perfused with low (white bars)- or high (gray bars)-FA concentration. *A*: myocardial FA oxidation rate ($n = 7$–11) measured in working hearts. *B* and *C*: expression of cardiac fuel metabolism genes ($n = 10$–13) in hearts pre-perfused for 30 min with low- or high-FA concentration. This includes gene expression of pyruvate dehydrogenase kinase 4 (*Pdk4*), fatty acid translocase (*FAT/Cd36*), uncoupling protein 2 and 3 (*Ucp2* and *-3*), carnitine palmitoyl transferase 1 (*mCpt1*), acyl-CoA oxidase 1 (*Acox1*), acyl-CoA thioesterases 1 and 2 (*Acot1* and *-2*), hexokinase 2 (*Hk2*), lactate dehydrogenase (*Ldh*), and glucose transporter 4 (*Glut4*). Gene expression is normalized to low FA-perfused CON hearts. Data are means ± SE. Data were analyzed with a two-way ANOVA with Holm-Sidak method as post hoc test. *$P < 0.05$ vs. low FA; #$P < 0.05$ vs. CON at the same FA concentration.

to both basal metabolism ($M\dot{V}O_{2BM}$, Fig. 2*C*) as well as excitation-contraction (EC) coupling ($M\dot{V}O_{2ECC}$) (Fig. 2*D*).

The high FA-load markedly decreased mechanical efficiency in CON hearts (Fig. 2*A*). High FAs also increased $M\dot{V}O_2$ in unloaded CON hearts (Fig. 2*B*), and we confirmed that high FA-load increased the O_2 cost for both BM (Fig. 2*C*) and ECC (Fig. 2*C*). In contrast to CON hearts, the high FA-load neither decreased mechanical efficiency nor increased unloaded $M\dot{V}O_2$ nor oxygen cost of BM and ECC in DIO hearts (Fig. 2, *A–D*, respectively).

Mitochondrial respiration. Mitochondrial respiration was assessed using pyruvate and malate (Fig. 3, *A–C*) or palmitoyl-CoA, L-carnitine, and malate (Fig. 3, *D–E*) as substrates for respiration. Mitochondria from DIO hearts perfused with a low-FA concentration showed reduced coupled respiration (V_{max}) compared with mitochondria from controls perfused with low FA, both when pyruvate and palmitate served as substrate. Under the same conditions, uncoupled respiration (V_0) was also significantly reduced, such that the respiratory control ratio (RCR) was not different between CON and DIO,

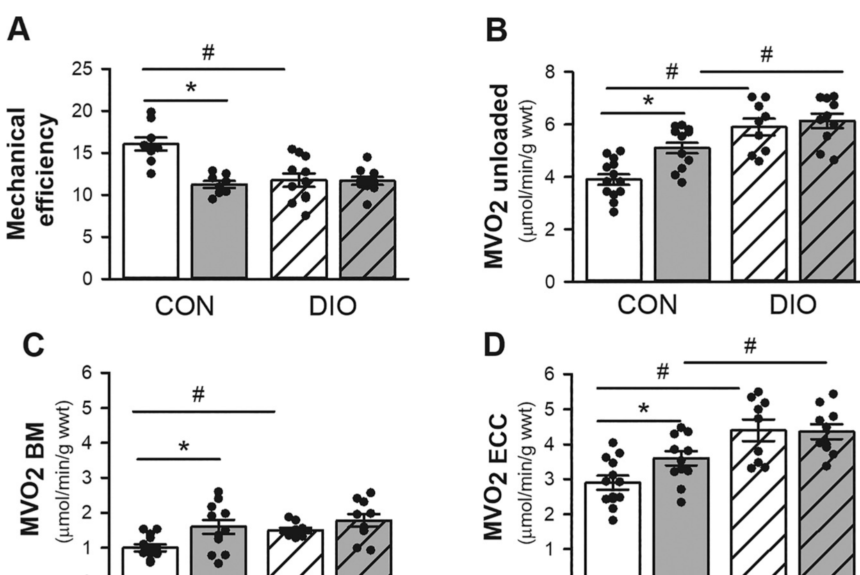

Fig. 2. Mechanical efficiency (*A*) was determined by measuring cardiac power and myocardial oxygen consumption ($M\dot{V}O_2$, $n = 8$–11) in isolated perfused working hearts from control (CON) and diet-induced obese (DIO) mice perfused with a low (white bars)- or high (gray bars)-FA concentration. $M\dot{V}O_2$ was also measured in electrically paced (7 Hz), unloaded Langendorff-perfused hearts ($n = 9$–13) before and after KCl-induced cardiac arrest, representing the oxygen cost of the heart in an unloaded condition (*B*; $M\dot{V}O_{2unloaded}$) and for basal metabolism (*C*; $M\dot{V}O_{2BM}$), respectively. The oxygen cost for excitation-contraction coupling (*D*; $M\dot{V}O_{2ECC}$) was calculated from the difference between $M\dot{V}O_{2unloaded}$ and $M\dot{V}O_{2BM}$. Data were analyzed with a two-way ANOVA with Holm-Sidak method as the post hoc test. Data are means ± SE. *$P < 0.05$ vs. low FA. #$P < 0.05$ vs. CON at the same FA concentration.

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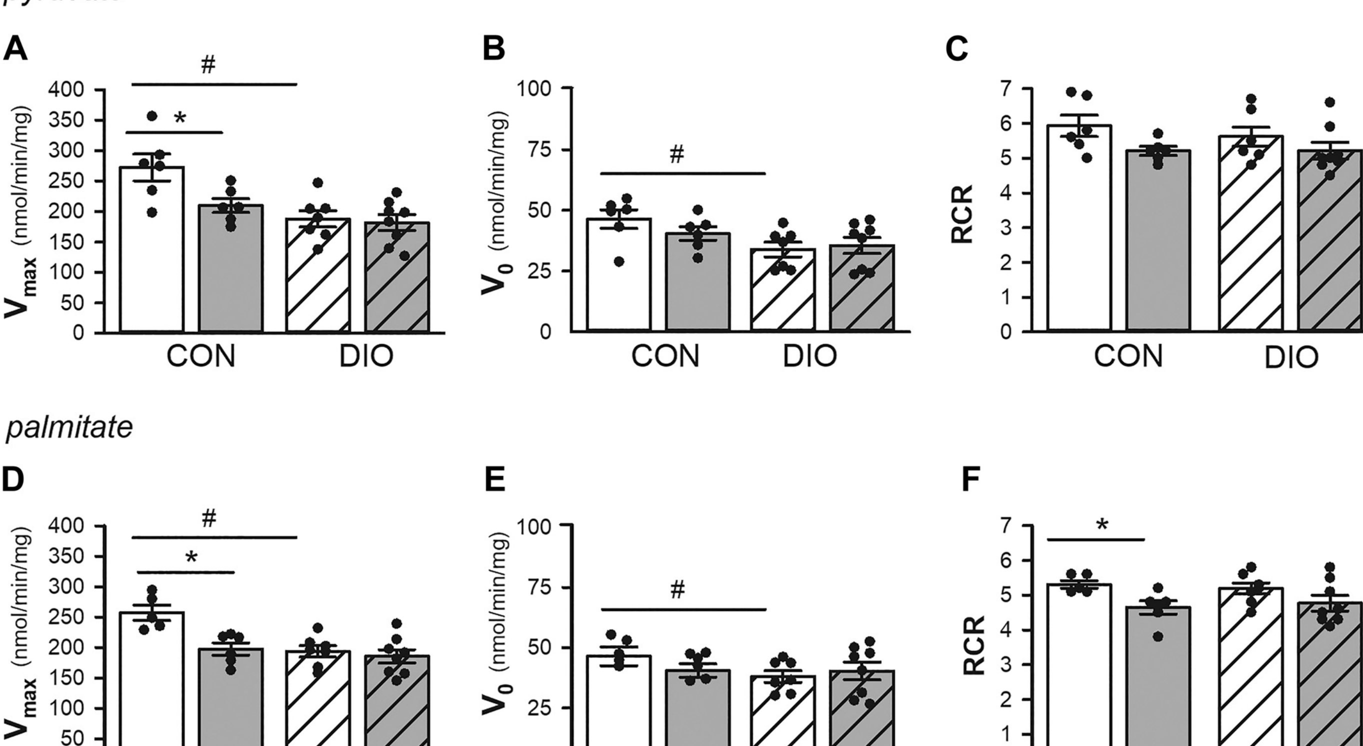

Fig. 3. Mitochondrial respiration measured in cardiac mitochondria isolated from control (CON, n = 5 to 6) and diet-induced obese (DIO, n = 7 to 8) hearts perfused with low (white bars)- or high (gray bars)-FA concentration before the isolation procedure. The respiratory medium contained 5 mM pyruvate and 2 mM malate (*A–C*) or 25 μM palmitoyl CoA, 5 mM L-carnitine, and 2 mM malate (*D–F*). V_0 respiration is defined as the respiratory state before ADP is added, and V_{max} is defined as the respiration peak after adding 100 μmol/L ADP. Respiratory control ratio (RCR) was calculated as the ratio between V_{max} and V_0. Mitochondrial respiration rates were normalized to protein. Data were analyzed with a two-way ANOVA with Holm Sidak method as the post hoc test. Data are means ± SE. *P < 0.05 vs. low FA. #P < 0.05 vs. CON at the same FA concentration.

regardless of substrate. Correspondingly, the ADP/O ratio was not different between CON and DIO (pyruvate; 2.23 ± 0.06 vs. 2.37 ± 0.06; palmitate; 2.08 ± 0.06 vs. 2.09 ± 0.04, in CON and DIO, respectively).

When CON hearts were exposed to a high FA-load before mitochondrial isolation, V_{max} was significantly reduced, accompanied by a small but not significant reduction in V_0. As the reduction in V_{max} was more pronounced than V_0, RCR was lower in mitochondria from hearts perfused with high as compared with low FAs (palmitate, $P < 0.05$; pyruvate, $P = 0.067$) (Fig. 3). The ADP/O was not altered by subjecting the CON hearts to a high FA-load (2.32 ± 0.03 and 2.16 ± 0.02, pyruvate and palmitate, respectively). In contrast to CON, high FA-load did not attenuate coupled (V_{max}) or uncoupled (V_0) respiration in mitochondria from DIO hearts, regardless of substrate conditions. Thus, both RCR (Fig. 3) and ADP/O were unaltered by high FAs in DIO (2.32 ± 0.06 and 2.11 ± 0.04, pyruvate and palmitate, respectively).

Myocardial ROS content and mitochondrial protein acetylation. ROS levels in LV tissue from DIO hearts were higher than in CON hearts that had been perfused with low-FA concentration (Fig. 4*A*). In these hearts, we found increased gene expression of key enzymes in the first-line antioxidant defense, such as catalase and glutathione peroxidase (*gpx3*), but not mn-superoxide dismutase (*mn-sod*). Subjecting CON hearts to the high FA-load significantly increased ROS levels, and the 30-min high-FA perfusion also increased mRNA expression of catalase and GPX3 in these hearts (Fig. 4*B*). DIO hearts, however, showed a resistance to these FA-induced changes. Interestingly, we did not find ROS content to be different between CON and DIO hearts exposed to a high FA-load.

The effect of altered FA concentrations on the lysine acetylation of mitochondrial proteins was also examined. It has been previously shown that diet-induced obesity increases protein acetylation in the obese/diabetic heart (18). As we did not observe this difference in mitochondria from low FA-perfused hearts, we also isolated mitochondria from hearts perfused without BSA-bound FAs (NoF). Under these conditions, there was a near twofold increase in acetylated mitochondrial proteins in DIO hearts compared with CON hearts (Fig. 5*A*).

When comparing the acetylation status with different concentrations of FAs, we found a marked dose-dependent increase in protein acetylation in CON hearts (Fig. 5*B*). Interestingly, a similar effect was not observed in DIO hearts (Fig. 5*C*). Gene expression analysis of tissue from these hearts revealed that perfusion with high FA-load did not alter the mRNA expression of sirtuin 3 or GCN5L1 (data not shown).

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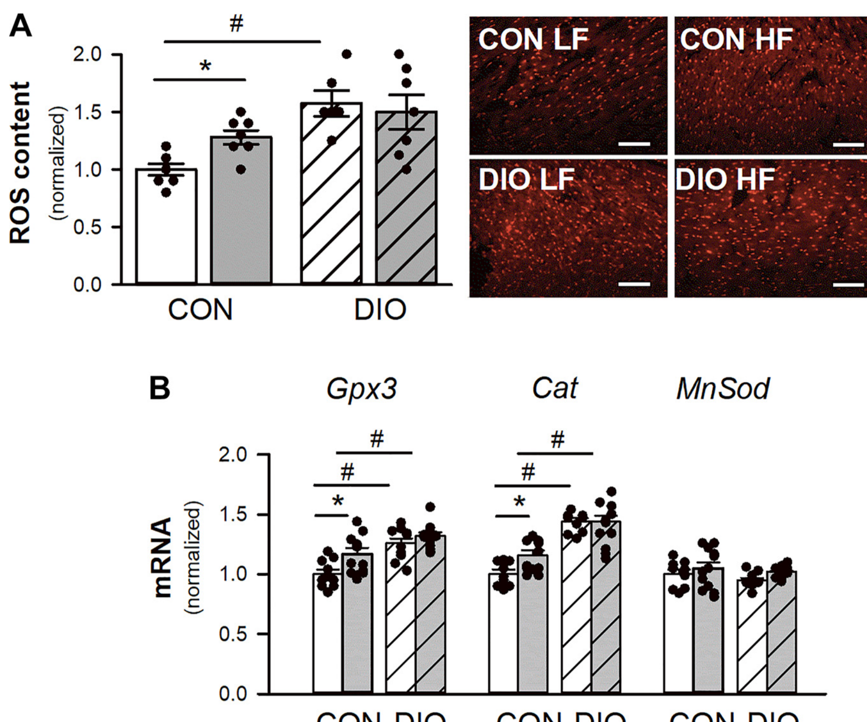

Fig. 4. Myocardial ROS content and mRNA expression control (CON) and diet-induced obese (DIO) mouse hearts. *A*: myocardial reactive oxygen synthase content (n = 6 to 7) was quantified as fluorescence intensity in left ventricular cryosections using dihydroethidium staining (scale bar, 100 μm). Data were analyzed with a two-way ANOVA with Holm-Sidak method as the post hoc test. *B*: gene expression of enzymes in antioxidant systems (n = 10–13) in LV tissue pre-perfused for 30 min with low- or high-FA concentration. This includes gene expression of catalase (*Cat*), glutathione peroxidase (*GPx3*) and Mn-superoxide dismutase (*MnSod*). Data are means ± SE. *P < 0.05 vs. low FA. #P < 0.05 vs. CON at the same FA concentration.

Susceptibility to ischemic injury. Ischemic tolerance was examined in Langendorff-perfused hearts, as this perfusion mode is a robust technique for examining myocardial ischemic tolerance. It should be noted, however, that this perfusion mode is less sensitive for picking up LV functional changes (45). Accordingly, we did not find differences in preischemic function between Langendorff-perfused CON and DIO hearts when perfused under low-FA conditions (Table 3), even though dysfunction was evident in DIO hearts in the working mode. A significantly lower heart rate was observed in high FA-perfused DIO hearts when compared with CON hearts. However, as the lower heart rate was accompanied by higher

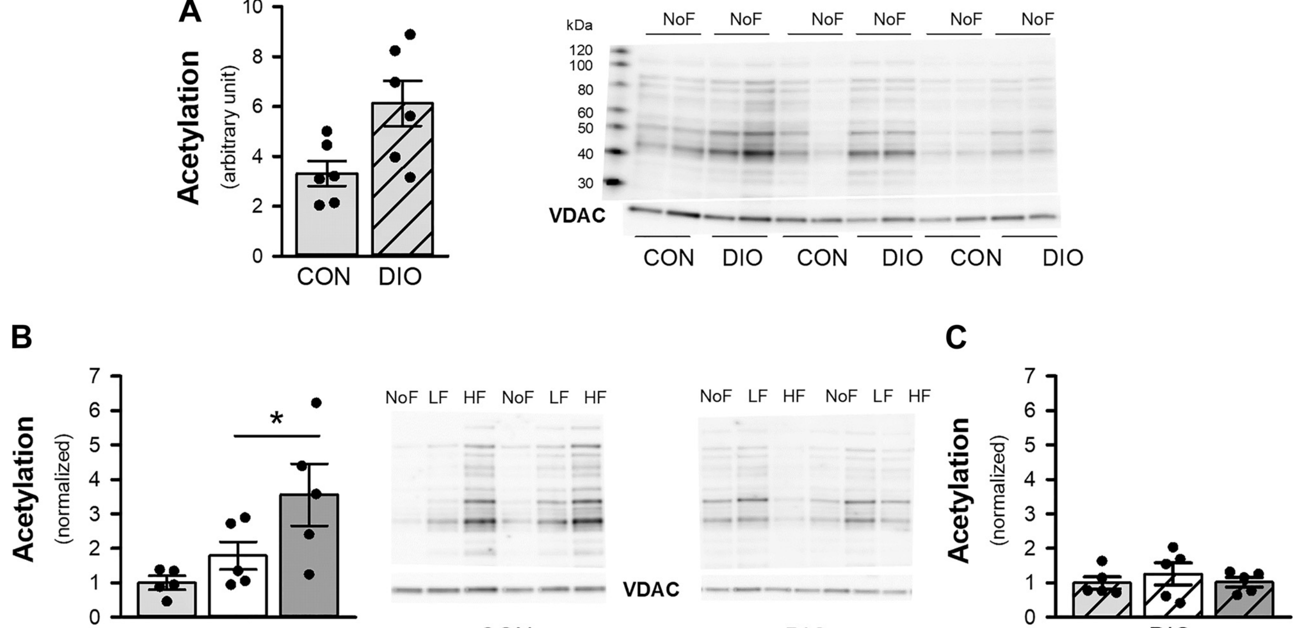

Fig. 5. Overall mitochondrial protein lysine acetylation in control (CON) and diet-induced obese (DIO) mouse hearts. *A*: protein acetylation in mitochondria isolated from CON and DIO hearts following perfusion without fatty acids (NoF, light gray bars, n = 6). *B*: protein acetylation in mitochondria isolated from CON hearts and DIO hearts following perfusion without FAs (NoF, light gray bars, n = 6), low FAs (LF, white bars, n = 6), or high FAs (HF, dark gray bars, n = 6). Data in *B* and *C* were normalized to NoF within each group (same data as in *A*) and analyzed with a one-way ANOVA with Holm-Sidak method as the post hoc test. Data are means ± SE. *P < 0.05 vs. low FA.

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Table 3. *Pre- and postischemic LV function in isolated Langendorff-perfused hearts from control and DIO mice, perfused with low- and high-FA levels*

	Low Fatty Acid			High Fatty Acid		
	Preischemia	Postischemia	%Recovery	Preischemia	Postischemia	%Recovery
Control						
n		14			14	
Heart rate, beats/min	296 ± 8	284 ± 9	96 ± 3	320 ± 10	256 ± 17	89 ± 4
CF, mL/min	3.0 ± 0.2	2.0 ± 0.2	65 ± 4	3.1 ± 0.2	2.0 ± 0.2	63 ± 4
LVDP, mmHg	134 ± 7	76 ± 5	58 ± 4	126 ± 7	61 ± 8	49 ± 6
dP/dt_{max}, mmHg/s	4847 ± 340	2764 ± 283	58 ± 4	4416 ± 333	2301 ± 367	53 ± 7
dP/dt_{min}, mmHg/s	3,377 ± 160	1,969 ± 159	59 ± 4	3,132 ± 133	1,617 ± 217	51 ± 6
RPP, mmHg·beats/min	39,702 ± 2486	20,968 ± 1638	55 ± 1	40,123 ± 2,409	17,979 ± 2,192	45 ± 5
Diet-induced obese						
n		15			12	
Heart rate, beats/min	293 ± 10	261 ± 15	89 ± 5	279 ± 12#	292 ± 11*,#	106 ± 4*,#
CF, mL/min	3.5 ± 0.2	2.6 ± 0.2#	74 ± 5	3.5 ± 0.2	2.4 ± 0.2	70 ± 4
LVDP, mmHg	147 ± 11	64 ± 10	42 ± 6	152 ± 7#	80 ± 6	53 ± 6
dP/dt_{max}, mmHg/s	5,413 ± 483	2,259 ± 388	41 ± 6	5,514 ± 420	2,899 ± 161	55 ± 7
dP/dt_{min}, mmHg/s	3,712 ± 237	1,641 ± 225	42 ± 5	3,878 ± 251#	2,019 ± 116	54 ± 6
RPP, mmHg·beats/min	42,034 ± 3,465	16,528 ± 2,661	38 ± 6#	42,042 ± 2,414	23,069 ± 1,226	56 ± 3*

Values are means ± SE. Left ventricular (LV) function was assessed using a fluid-filled balloon where the perfusion pressure was 60 mmHg and the end-diastolic pressure was adjusted to be between 5 and 10 mmHg. Postischemic functional recovery is calculated as percentage of preischemic values. CF, coronary flow; DIO, diet-induced obese; LVDP, LV developed pressure; dP/dt_{max} and dP/dt_{min}, maximum positive and negative first time derivative of LV pressure, respectively; RPP, rate pressure product (LVDP × heart rate). Data were analyzed with a two-way ANOVA with Holm-Sidak method as the post hoc test. *$P < 0.05$ vs. low fatty acid (FA). #$P < 0.05$ vs. control at the same FA concentration.

LV developed pressure (LVDP), the resulting preischemic rate pressure product (RPP) was similar to the other experimental groups (Table 3).

All hearts were subjected to 25 min of no-flow ischemia followed by reperfusion, with postischemic LV function assessed until maximum recovery. Functional recovery (expressed as percentage of the preischemic values) showed that under low-FA conditions, DIO hearts exhibited impaired recovery of RPP (Fig. 6*C*, Table 3) when compared with CON hearts under the same conditions. The impaired recovery was mainly due to a lower recovery of LVDP (Table 3). Corroborating this, infarct size was also greater in DIO hearts under these conditions.

Exposing CON hearts to high-FA levels did not significantly impair functional recovery, nor was infarct size increased (Fig. 6*C*, Table 3). In contrast, and to our surprise, FA-load was found to significantly improve postischemic recovery in DIO hearts compared with DIO hearts perfused under low-FA conditions (Fig. 6*C*). In line with improved postischemic functional recovery, infarct size was also reduced following the high FA-load in DIO hearts (Fig. 6*D*). Notably, although DIO hearts showed lower ischemic tolerance when compared with CON hearts when perfused under low-FA conditions, there were no significant differences with regards to postischemic functional recovery or infarct size between DIO and CON under high-FA perfusion (Fig. 6).

DISCUSSION

Both physiological and pathophysiological stresses can lead to acute high circulating levels of fatty acids (FAs). Although

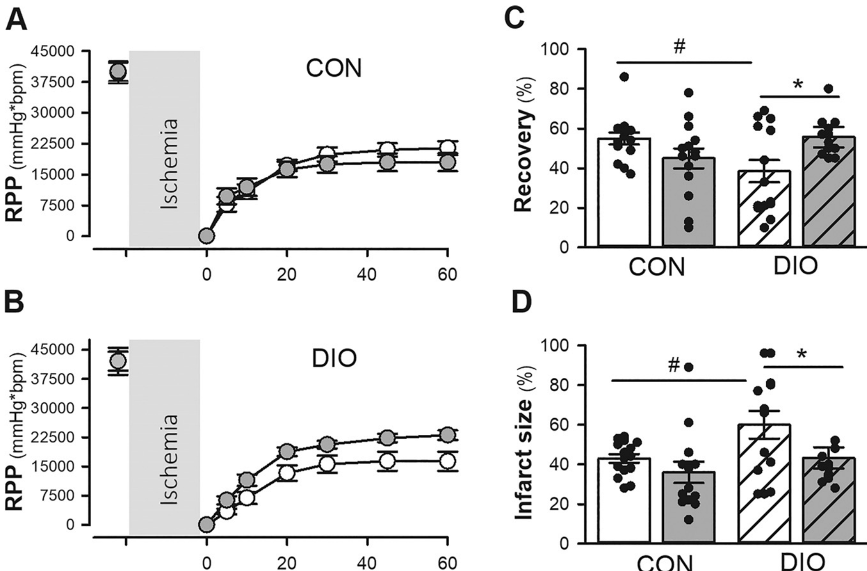

Fig. 6. Rate pressure product (RPP; *A* and *B*), the calculated postischemic recovery (*C*, in percentage of preischemic RPP values, and infarct size (*D*) in isolated perfused hearts from control (CON, n = 13–15) and diet-induced obese (DIO, n = 12–14) mice. Hearts were subjected to perfusion with a low (white circles and bars)- or high (gray circles and bars)-FA concentration. Data were analyzed with a two-way ANOVA with Holm-Sidak method as the post hoc test. Data are means ± SE. *$P < 0.05$ vs. low FA. #$P < 0.05$ vs. CON at the same FA concentration.

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it has been shown that an acute high FA-load can have adverse effects in the normal heart, to what extent this also occurs in the obese/diabetic heart is less clear. Thus, in the present study we have examined the effects of high FA-load in hearts from diet-induced obese (DIO) mice by comparing this to a low-FA condition. DIO hearts perfused under low-FA condition displayed the expected metabolic phenotype of diabetic cardiomyopathy, including a high reliance on FA oxidation, increased ROS, and mitochondrial dysfuncion. When exposed to a high-FA concentration, FA oxidation rate was increased in hearts from both control and DIO mice. This was associated with increased ROS, impaired mitochondrial OxPhos, augmented protein acetylation, and altered gene expression only in CON hearts. In addition, FA-mediated oxygen wasting and mechanical inefficiency only occurred in hearts from normal mice, suggesting that high FA-load results in disadvantageous changes in controls but did not further aggravate these parameters in the obese heart. Finally, high-FA levels did not affect ischemic tolerance in normal hearts, and we observed increased ischemic tolerance when DIO hearts were exposed to high FAs. These findings clearly show that a high FA-load affects normal and obese hearts differently, and may suggest that chronic hyperlipidemia renders the DIO hearts less vulnerable to the disadvantageous effects of an acute FA-load.

High FA-load in the normal heart. In normal hearts, elevation of FAs can have a range of cardiac effects. In the present study, acute high FA-load increased myocardial FA oxidation rate and likely also intracellular levels of FA and FA intermediates. Accordingly, we found mitochondrial protein acetylation to be increased, suggesting that the acute high FA-load led to a mismatch between the FA supply and oxidation and subsequent acetyl-CoA accumulation. Interestingly, our data also show that high FA-load markedly increased the expression of PPARα target genes, which supports a FA-mediated activation of PPARα due to increased intracellular FAs levels.

The present study confirms a FA-mediated decrease in mechanical efficiency in control hearts, due to an increase in $M\dot{V}O_2$ (8, 9, 27). $M\dot{V}O_2$ is determined by both work-dependent factors (e.g., pre- and afterload, heart rate, and wall stress) and work-independent factors (including myocardial Ca^{2+} control, mitochondrial membrane potential, protein synthesis, and transmembrane ionic balance), and we confirmed that the FA-mediated oxygen wasting was primarily linked to work-independent processes (8, 27). Although this is commonly attributed to the obligatory increase in myocardial FA oxidation, as the oxidation of FAs requires more oxygen for the same amount of ATP produced compared with glucose, there is evidence that the increased O_2 cost for FA oxidation per se is negligible, as an inhibition of myocardial FA oxidation does not abolish the increase in $M\dot{V}O_2$ in normal hearts perfused with high FAs (8, 28).

In the present study, the FA-mediated increase in O_2 wasting was accompanied by impaired mitochondrial energetics, as we found reduced OxPhos in mitochondria from high FA-perfused hearts. This suggests that O_2 wasting is not solely explained by excessive mitochondrial respiration but that high FA-load affects extramitochondrial processes which contribute to increase $M\dot{V}O_2$ as well. Accordingly, we found increased O_2 cost for excitation-contraction (EC) coupling (8), which could be the result of less efficient myocardial Ca^{2+} handling, due to SR Ca^{2+} leak and/or higher energy cost related to sarcolemmal ionic transport (5, 48). In addition, high FA has been shown to prolong the decay phase of the Ca^{2+} transient in the heart (17). Although the exact underlying mechanisms linking FA to altered O_2 cost for EC coupling remain to be determined, FA-mediated changes in redox balance (17, 50) may play a role as Ca^{2+}-handling proteins are known to be sensitive to redox-linked modifications (32). In accordance with this, FA-mediated oxidative stress was found to be accompanied by increased expression of catalase and glutathione peroxidase, key enzymes in the first-line antioxidant defense.

High FA-load in the obese heart. The diet-induced obese (DIO) mice used in the present study resemble a prediabetic state that is typically characterized by obesity, elevated circulating free FA levels, and insulin resistance. In accordance with our previous studies, left ventricular (LV) pressure-volume recordings revealed that these hearts primarily develop diastolic dysfunction (25, 39, 45) and display mechanical inefficiency due to an increased $M\dot{V}O_2$ (25, 39). This study also confirms that these hearts show higher FA oxidation rates, as well as increased and elevated expression of metabolic genes that are transcriptionally regulated by PPARα in DIO hearts. These include genes encoding for FA transport (CD36, mCPT1) and the regulation of FA and glucose oxidation (ACOX, PDK4), UCP2 and -3, and acyl-CoA thioesterases (ACOT) 1 and 2. The latter is suggested to have a regulatory role in controlling rates of FA oxidation and in subcellular trafficking of FAs. ACOT1 (the cytosolic isoform) catalyzes the cleave of long-chain acyl-CoAs into FA and CoA and thus contributes in regulating the ligand (FA) availability for the transcription factor PPARα.

The obese heart also showed impaired mitochondrial respiration (OxPhos rate) (2, 25), increased oxidative stress, and expression of antioxidant enzymes (2, 25). The obesity-induced increase in $M\dot{V}O_2$ may be related to altered substrate utilization, altered mitochondrial energetics, and increased oxidative stress, in addition to structural remodeling and inefficient Ca^{2+} transport, as reviewed in detail by Hafstad et al. (21). In support of the latter, we found augmented O_2 cost for the work-independent processes in the heart (21, 39). Although some of the changes induced by chronic FA exposure resemble the effects caused by the acute high FA-load in normal hearts, they are not comparable, since long-term exposure to high-circulating lipid levels seems to make these hearts uniquely adapted to handle high FAs, as discussed in the following subsection.

Despite high-FA supply, we did not observe a FA-mediated increase in mitochondrial protein lysine acetylation, nor did we find changes in the expression of PPARα target genes. Together, this suggests that obese hearts have a higher capacity to handle FAs, which may be related to an attenuated FA-mediated accumulation of acetyl-CoA (and thus acetylation) and free FA (and thus PPARα activation). There is also reason to believe that DIO hearts have enhanced capacity for a temporary lipid storage, which will also contribute to limiting the buildup of intracellular FAs and lipid intermediates (4).

Although high FA-load did not markedly alter LV function in DIO hearts when they were compared with DIO hearts perfused under low-FA conditions, FAs have been reported to improve cell shortening and Ca^{2+} transients in cardiomyocytes from obese and type 2 diabetic mice when compared with conditions where FAs are completely absent (17, 50). Like-

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wise, the addition of FAs during perfusion of isolated hearts from experimental models of diet-induced obesity (47) and type 2 diabetes (50) has been reported to increase LV function. This effect was reported to be particularly evident in hearts and cardiomyocytes subjected to metabolic (hyperglycemia) and/or adrenergic stress, where the improved function was linked to a FA-mediated increase in the content of reduced glutathione (GSH) and augmented mitochondrial ROS scavenging capacity (10, 50). These findings suggest that FAs are crucial to ensure a sufficient supply of reducing equivalents to prevent unfavorable ROS production and impaired energetics (50) and that metabolic inflexibility in obese/diabetic hearts may render them energy starved when FAs are omitted in experimental settings of increased cardiac work (6).

In accordance with a study by Cole et al. (9) using hearts from DIO rats, high FAs, compared with low FAs, did not alter LV mechanical efficiency in DIO mice, nor did high-FA levels alter unloaded $M\dot{V}O_2$ or the O_2 cost for EC coupling and BM, which is in accordance with a previous study using type 2 diabetic *db/db* mice (7). We did not find high-FA perfusion to alter mitochondrial respiration or coupling efficiency in DIO hearts, nor did it exacerbate myocardial ROS accumulation, corroborating findings in cardiomyocytes from *ob/ob* mice (17) that demonstrated a resistance to a FA-mediated increase in mitochondrial ROS emission (17). Taken together, the findings in this study suggest that the obese heart is somewhat protected from the potential harmful effects following exposure to an acute high FA-load. Future studies should address myocardial cellular FA processing in the diabetic heart to elucidate potential mechanisms that may enhance the capacity for processing FA.

The effect of acute high FA-load on ischemic tolerance in normal and obese hearts. Acute myocardial infarction and cardiac surgery are reported to be accompanied by a marked increase in circulating FA levels (36, 42, 43), which are due to a hyperadrenergic state, leading to increased lipolysis in white adipose tissue. Thus, the exposure to an acute high FA-load poses an additional challenge in the hypoxic state, where FA-mediated O_2 wasting could be particularly detrimental. Accordingly, high FA-perfused rat hearts have been reported to have impaired postischemic functional recovery when compared with hearts perfused without FAs (12, 14, 19, 35, 37), and inhibition of FA uptake and utilization during ischemia-reperfusion has been associated with improved postischemic recovery (31, 35, 41). Similarly, Dalgas et al. (12) recently showed that increasing the FA concentration from 0.4 to 1.2 mM reduced postischemic function and increased infarct size in normal rat hearts. In contrast, we did not find that high FAs exacerbated ischemic injury in normal hearts in the present study despite the disadvantageous effects that occurred in these hearts following FA-load. Although the reason for these discrepancies is not clear, it may be related to the severity of the ischemic insult. As the aim of this study was primarily to examine the effects of high FA-load in obese hearts, the duration of ischemia may have been too short to unmask the disadvantageous effects of high-FA levels in the control hearts.

In clinical studies, obesity and diabetes are known to worsen the long-term outcome of ischemic heart disease. Although, this can be due to decreased myocardial ischemic tolerance, it can also be due to impaired myocardial reperfusion, due to vascular dysfunction, and/or reduced coronary reserve (49). Futhermore, clinical studies have shown paradoxical and favorable effects of obesity on the outcome of acute coronary syndrome (3, 11), and studies using animal models of obesity and diabetes have reported both decreased (16, 34), unchanged (26, 33, 53), and even increased (13, 15, 29, 30, 51) ischemic tolerance. The discrepancies in preclinical studies could partly be due to differences in the severity of the metabolic disease in these models (44, 46, 51, 53); however, it does not seem to be the sole explanation (15, 26, 52), and differences in perfusion conditions may therefore also play a role. Accordingly, du Toit et al. (14) found that high-FA levels decreased ischemic tolerance when this was compared with a condition of no FAs in hearts from an obese rat model. In contrast, the present study demonstrated that in hearts from obese mice, high-FA levels did not worsen but rather improved the ischemic outcome

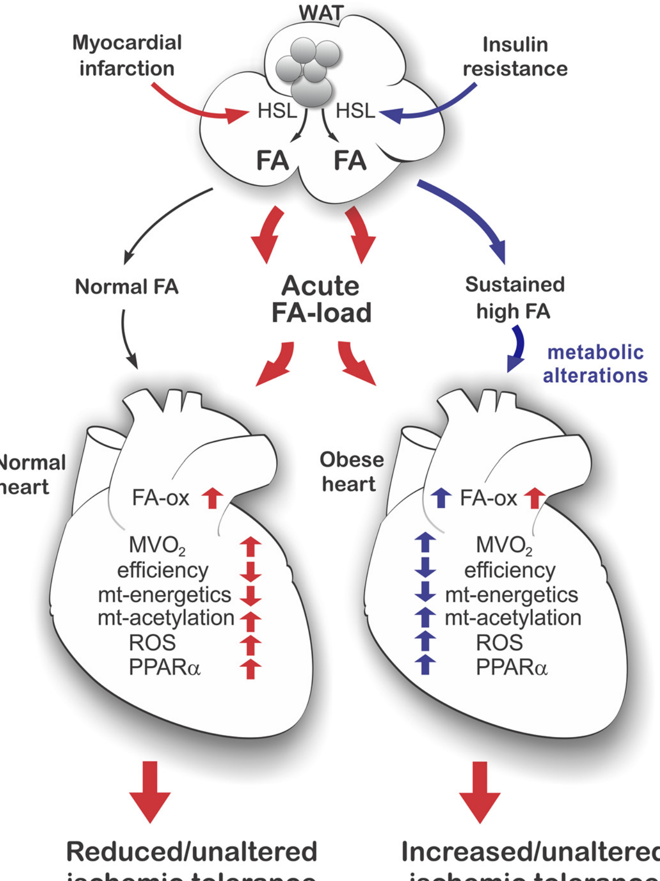

Fig. 7. While a normal heart is exposed to normal circulating fatty acid (FA) levels, obesity-related insulin resistance and lack of insulin-induced inhibition of the hormone-sensitive lipase (HSL) in white adipose tissue (WAT), leaves the obese heart exposed to sustained high-FA levels (blue arrows). This dyslipidemia alters the metabolic phenotype in obese hearts and contributes to the development of obesity-/diabetes-related cardiomyopathy. Under acute pathophysiological stress, such as a myocardial infarction, an adrenergic activation of HSL will lead to an acute high FA-load (red arrows). This high FA-load is considered unfavorable in normal hearts, due to FA-mediated changes in myocardial oxygen consumption ($M\dot{V}O_2$), mechanical efficiency, ROS production, and mitochondrial (mt) energetics. Following obesity and insulin resistance, however, the metabolic alterations induced by the sustained high-FA exposure will enhance these hearts' ability to handle an acute high FA-load, so that is does not futher alter the phenotype of these hearts. Thus, while an acute high FA-load can reduce the ischemic tolerance in normal hearts, it does not represent an additive stress in obese hearts.

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when compared with a physiologically more relevant FA condition. It should be noted, however, that the improved tolerance to ischemia occurred despite an unaltered phenotype. Although the exact mechanism underlying the protective effects remains to be elucidated, this study highlights a need for more focus on the role and effect of FAs under ischemic conditions which may also contribute to elucidate paradoxical favorable effects of obesity on the outcome of acute coronary syndrome in patients (3, 11).

Limitations. Although FAs and glucose are regarded as the main energy substrate to the heart, it should be acknowledged that additional substrates in the perfusion buffer such as lactate, ketone bodies, and branched chain amino acids could potentially have influenced both the ischemic tolerance and the effects of high FAs. In addition, the high-FA concentration used in this study (1.8 mM) during perfusion is at the upper range of the reported levels in patients with an acute myocardial ischemia (43). However, it should be noted that the high-FA concentration used here did not have any adverse effects on cardiac function. Finally, we recognize that this study has not identified the exact underlying mechanism related to the improved tolerance to an acute high FA-load in obese hearts. However, given the important role of FAs in cardiomyocytes (both in physiological and pathophysiological processes), we believe that this is clearly multifactorial and complex (as implied in this study) and will need to be addressed in future studies.

Concluding remarks. This study confirms that exposure of normal hearts to high- as compared with low-FA levels induces oxygen wasting, mechanical inefficiency, increased ROS content, hyperacetylation, and reduced OxPhos (as summarized in Fig. 7). Apart from transcriptional regulation of the metabolic machinery, other FA-mediated processes, such as increased ROS and protein acetylation, may also play a signaling role in the adaptation to match FA oxidation to the increased supply. These factors may lead to changes that increase $M\dot{V}_{O_2}$; however, this does not need to have any negative functional consequences, unless the heart is also challenged by pathophysiological stress. Thus, an acute FA-load may contribute to a reduced ischemic sensitivity in the normal heart. In obesity and insulin resistance, sustained high-circulating FA levels are likely to induce similar cardiac changes (Fig. 7). Although the chronic dyslipidemia and FA-mediated changes eventually may contribute in the pathogenesis of diabetic cardiomyopathy, emerging data also suggest that FAs are crucial for maintaining redox status, providing reducing equivalents and, hence, mitochondrial energetics, in these hearts. The results from the present study suggest that these hearts have enhanced capacity to handle an acute high FA-load, so that upon myocardial infarction, the accompanying high FA-load does not represent an additive stress. Accordingly, high FA-load did not aggravate mechanical efficiency, $M\dot{V}_{O_2}$, ROS, acetylation, or OxPhos in hearts from DIO mice. The fact that high FA-load improved ischemic tolerance despite unchanged phenotype implies that there must be other factors contributing to a worsened outcome following obesity. The therapeutic potential of targeting FA metabolism remains to be determined; however, this study highlights that future studies should focus on the importance of FAs beyond simply serving as an energy substrate for the heart.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.T.B., T.M.P., A.D.H., and E.A. conceived and designed research; N.T.B., T.M.P., L.R., and A.D.H. performed experiments; N.T.B., T.M.P., L.R., and A.D.H. analyzed data; N.T.B., T.M.P., L.R., and A.D.H. interpreted results of experiments; N.T.B., T.M.P., and E.A. prepared figures; N.T.B., T.M.P., and E.A. drafted manuscript; N.T.B., T.M.P., and E.A. edited and revised manuscript; N.T.B., T.M.P., L.R., A.D.H., and E.A. approved final version of manuscript.

REFERENCES

1. **Aasum E, Hafstad AD, Severson DL, Larsen TS.** Age-dependent changes in metabolism, contractile function, and ischemic sensitivity in hearts from db/db mice. *Diabetes* 52: 434–441, 2003. doi:10.2337/diabetes.52.2.434.
2. **Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD.** Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J Am Coll Cardiol* 54: 1891–1898, 2009. doi:10.1016/j.jacc.2009.07.031.
3. **Angerås O, Albertsson P, Karason K, Råmunddal T, Matejka G, James S, Lagerqvist B, Rosengren A, Omerovic E.** Evidence for obesity paradox in patients with acute coronary syndromes: a report from the Swedish Coronary Angiography and Angioplasty Registry. *Eur Heart J* 34: 345–353, 2013. doi:10.1093/eurheartj/ehs217.
4. **Aon MA, Bhatt N, Cortassa SC.** Mitochondrial and cellular mechanisms for managing lipid excess. *Front Physiol* 5: 282, 2014. doi:10.3389/fphys.2014.00282.
5. **Belke DD, Swanson EA, Dillmann WH.** Decreased sarcoplasmic reticulum activity and contractility in diabetic db/db mouse heart. *Diabetes* 53: 3201–3208, 2004. doi:10.2337/diabetes.53.12.3201.
6. **Bertero E, Sequeira V, Maack C.** Let's face the fats: palmitate restores cellular redox state in the diabetic heart. *J Physiol* 598: 1283–1284, 2020. doi:10.1113/JP277473.
7. **Boardman N, Hafstad AD, Larsen TS, Severson DL, Aasum E.** Increased O2 cost of basal metabolism and excitation-contraction coupling in hearts from type 2 diabetic mice. *Am J Physiol Heart Circ Physiol* 296: H1373–H1379, 2009. doi:10.1152/ajpheart.01264.2008.
8. **Boardman NT, Larsen TS, Severson DL, Essop MF, Aasum E.** Chronic and acute exposure of mouse hearts to fatty acids increases oxygen cost of excitation-contraction coupling. *Am J Physiol Heart Circ Physiol* 300: H1631–H1636, 2011. doi:10.1152/ajpheart.01190.2010.
9. **Cole MA, Murray AJ, Cochlin LE, Heather LC, McAleese S, Knight NS, Sutton E, Jamil AA, Parassol N, Clarke K.** A high fat diet increases mitochondrial fatty acid oxidation and uncoupling to decrease efficiency in rat heart. *Basic Res Cardiol* 106: 447–457, 2011. doi:10.1007/s00395-011-0156-1.
10. **Cortassa S, Caceres V, Tocchetti CG, Bernier M, de Cabo R, Paolocci N, Sollott SJ, Aon MA.** Metabolic remodelling of glucose, fatty acid and redox pathways in the heart of type 2 diabetic mice. *J Physiol* 598: 1393–1415, 2020. doi:10.1113/JP276824.
11. **Curtis JP, Selter JG, Wang Y, Rathore SS, Jovin IS, Jadbabaie F, Kosiborod M, Portnay EL, Sokol SI, Bader F, Krumholz HM.** The obesity paradox: body mass index and outcomes in patients with heart failure. *Arch Intern Med* 165: 55–61, 2005. doi:10.1001/archinte.165.1.55.
12. **Dalgas C, Povlsen JA, Løfgren B, Erichsen SB, Bøtker HE.** Effects of fatty acids on cardioprotection by pre-ischaemic inhibition of the malate-aspartate shuttle. *Clin Exp Pharmacol Physiol* 39: 878–885, 2012. doi:10.1111/j.1440-1681.2012.05749.x.

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13. **Donner D, Headrick JP, Peart JN, du Toit EF.** Obesity improves myocardial ischaemic tolerance and RISK signalling in insulin-insensitive rats. *Dis Model Mech* 6: 457–466, 2013. doi:10.1242/dmm.010959.
14. **du Toit EF, Smith W, Muller C, Strijdom H, Stouthammer B, Woodiwiss AJ, Norton GR, Lochner A.** Myocardial susceptibility to ischemic-reperfusion injury in a prediabetic model of dietary-induced obesity. *Am J Physiol Heart Circ Physiol* 294: H2336–H2343, 2008. doi:10.1152/ajpheart.00481.2007.
15. **Edland F, Wergeland A, Kopperud R, Åsrud KS, Hoivik EA, Witsø SL, Æsøy R, Madsen L, Kristiansen K, Bakke M, Døskeland SO, Jonassen AK.** Long-term consumption of an obesogenic high fat diet prior to ischemia-reperfusion mediates cardioprotection via Epac1-dependent signaling. *Nutr Metab (Lond)* 13: 87, 2016. doi:10.1186/s12986-016-0147-1.
16. **Essop MF, Anna Chan WY, Valle A, García-Palmer FJ, Du Toit EF.** Impaired contractile function and mitochondrial respiratory capacity in response to oxygen deprivation in a rat model of pre-diabetes. *Acta Physiol (Oxf)* 197: 289–296, 2009. doi:10.1111/j.1748-1716.2009.02024.x.
17. **Fauconnier J, Andersson DC, Zhang SJ, Lanner JT, Wibom R, Katz A, Bruton JD, Westerblad H.** Effects of palmitate on Ca(2+) handling in adult control and ob/ob cardiomyocytes: impact of mitochondrial reactive oxygen species. *Diabetes* 56: 1136–1142, 2007. doi:10.2337/db06-0739.
18. **Fukushima A, Alrob OA, Zhang L, Wagg CS, Altamimi T, Rawat S, Rebeyka IM, Kantor PF, Lopaschuk GD.** Acetylation and succinylation contribute to maturational alterations in energy metabolism in the newborn heart. *Am J Physiol Heart Circ Physiol* 311: H347–H363, 2016. doi:10.1152/ajpheart.00900.2015.
19. **Gambert S, Vergely C, Filomenko R, Moreau D, Bettaieb A, Opie LH, Rochette L.** Adverse effects of free fatty acid associated with increased oxidative stress in postischemic isolated rat hearts. *Mol Cell Biochem* 283: 147–152, 2006. doi:10.1007/s11010-006-2518-9.
20. **Glatz JF, Luiken JJ, Bonen A.** Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. *Physiol Rev* 90: 367–417, 2010. doi:10.1152/physrev.00003.2009.
21. **Hafstad AD, Boardman N, Aasum E.** How exercise may amend metabolic disturbances in diabetic cardiomyopathy. *Antioxid Redox Signal* 22: 1587–1605, 2015. doi:10.1089/ars.2015.6304.
22. **Hafstad AD, Boardman NT, Lund J, Hagve M, Khalid AM, Wisloff U, Larsen TS, Aasum E.** High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart. *J Appl Physiol (1985)* 111: 1235–1241, 2011. doi:10.1093/cvr/cvp132.
23. **Hafstad AD, Khalid AM, Hagve M, Lund T, Larsen TS, Severson DL, Clarke K, Berge RK, Aasum E.** Cardiac peroxisome proliferator-activated receptor-alpha activation causes increased fatty acid oxidation, reducing efficiency and post-ischaemic functional loss. *Cardiovasc Res* 83: 519–526, 2009. doi:10.1093/cvr/cvp132.
24. **Hafstad AD, Khalid AM, How OJ, Larsen TS, Aasum E.** 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* 292: E1288–E1294, 2007. doi:10.1152/ajpendo.00504.2006.
25. **Hafstad AD, Lund J, Hadler-Olsen E, Höper AC, Larsen TS, Aasum E.** High- and moderate-intensity training normalizes ventricular function and mechanoenergetics in mice with diet-induced obesity. *Diabetes* 62: 2287–2294, 2013. doi:10.2337/db12-1580.
26. **Hjortbak MV, Hjort J, Povlsen JA, Jensen RV, Støttrup NB, Laursen MR, Jespersen NR, Løfgren B, Bøtker HE.** Influence of diabetes mellitus duration on the efficacy of ischemic preconditioning in a Zucker diabetic fatty rat model. *PLoS One* 13: e0192981, 2018. doi:10.1371/journal.pone.0192981.
27. **How OJ, Aasum E, Kunnathu S, Severson DL, Myhre ES, Larsen TS.** Influence of substrate supply on cardiac efficiency, as measured by pressure-volume analysis in ex vivo mouse hearts. *Am J Physiol Heart Circ Physiol* 288: H2979–H2985, 2005. doi:10.1152/ajpheart.00084.2005.
28. **Hütter JF, Piper HM, Spieckerman PG.** Effect of fatty acid oxidation on efficiency of energy production in rat heart. *Am J Physiol* 249: H723–H728, 1985. doi:10.1152/ajpheart.1985.249.4.H723.
29. **Inserte J, Aluja D, Barba I, Ruiz-Meana M, Miró E, Poncelas M, Vilardosa Ú, Castellano J, Garcia-Dorado D.** High-fat diet improves tolerance to myocardial ischemia by delaying normalization of intracellular PH at reperfusion. *J Mol Cell Cardiol* 133: 164–173, 2019. doi:10.1016/j.yjmcc.2019.06.001.
30. **Jansen KM, Moreno S, Garcia-Roves PM, Larsen TS.** Dietary Calanus oil recovers metabolic flexibility and rescues postischemic cardiac function in obese female mice. *Am J Physiol Heart Circ Physiol* 317: H290–H299, 2019. doi:10.1152/ajpheart.00191.2019.
31. **Jaswal JS, Keung W, Wang W, Ussher JR, Lopaschuk GD.** Targeting fatty acid and carbohydrate oxidation–a novel therapeutic intervention in the ischemic and failing heart. *Biochim Biophys Acta* 1813: 1333–1350, 2011. doi:10.1016/j.bbamcr.2011.01.015.
32. **Kuster GM, Lancel S, Zhang J, Communal C, Trucillo MP, Lim CC, Pfister O, Weinberg EO, Cohen RA, Liao R, Siwik DA, Colucci WS.** Redox-mediated reciprocal regulation of SERCA and Na+-Ca2+ exchanger contributes to sarcoplasmic reticulum Ca2+ depletion in cardiac myocytes. *Free Radic Biol Med* 48: 1182–1187, 2010. doi:10.1016/j.freeradbiomed.2010.01.038.
33. **Lacerda L, Opie LH, Lecour S.** Influence of tumour necrosis factor alpha on the outcome of ischaemic postconditioning in the presence of obesity and diabetes. *Exp Diabetes Res* 2012: 502654, 2012. doi:10.1155/2012/502654.
34. **Littlejohns B, Pasdois P, Duggan S, Bond AR, Heesom K, Jackson CL, Angelini GD, Halestrap AP, Suleiman MS.** Hearts from mice fed a non-obesogenic high-fat diet exhibit changes in their oxidative state, calcium and mitochondria in parallel with increased susceptibility to reperfusion injury. *PLoS One* 9: e100579, 2014. doi:10.1371/journal.pone.0100579.
35. **Liu Q, Docherty JC, Rendell JC, Clanachan AS, Lopaschuk GD.** High levels of fatty acids delay the recovery of intracellular pH and cardiac efficiency in post-ischemic hearts by inhibiting glucose oxidation. *J Am Coll Cardiol* 39: 718–725, 2002. doi:10.1016/S0735-1097(01)01803-4.
36. **Lopaschuk GD, Collins-Nakai R, Olley PM, Montague TJ, McNeil G, Gayle M, Penkoske P, Finegan BA.** Plasma fatty acid levels in infants and adults after myocardial ischemia. *Am Heart J* 128: 61–67, 1994. doi:10.1016/0002-8703(94)90010-8.
37. **Lopaschuk GD, Saddik M, Barr R, Huang L, Barker CC, Muzyka RA.** Effects of high levels of fatty acids on functional recovery of ischemic hearts from diabetic rats. *Am J Physiol* 263: E1046–E1053, 1992. doi:10.1152/ajpendo.1992.263.6.E1046.
38. **Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC.** Myocardial fatty acid metabolism in health and disease. *Physiol Rev* 90: 207–258, 2010. doi:10.1152/physrev.00015.2009.
39. **Lund J, Hafstad AD, Boardman NT, Rossvoll L, Rolim NP, Ahmed MS, Florholmen G, Attramadal H, Wisløff U, Larsen TS, Aasum E.** Exercise training promotes cardioprotection through oxygen-sparing action in high fat-fed mice. *Am J Physiol Heart Circ Physiol* 308: H823–H829, 2015. doi:10.1152/ajpheart.00734.2014.
40. **Mjos OD.** Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. *J Clin Invest* 50: 1386–1389, 1971. doi:10.1172/JCI106621.
41. **Nagendran J, Pulinilkunnil T, Kienesberger PC, Sung MM, Fung D, Febbraio M, Dyck JR.** Cardiomyocyte-specific ablation of CD36 improves post-ischemic functional recovery. *J Mol Cell Cardiol* 63: 180–188, 2013. doi:10.1016/j.yjmcc.2013.07.020.
42. **Oliver MF, Kurien VA, Greenwood TW.** Relation between serum-free-fatty acids and arrhythmias and death after acute myocardial infarction. *Lancet* 1: 710–714, 1968. doi:10.1016/S0140-6736(68)92163-6.
43. **Opie LH.** Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction. Relation to myocardial ischemia and infarct size. *Am J Cardiol* 36: 938–953, 1975. doi:10.1016/0002-9149(75)90086-7.
44. **Pælestik KB, Jespersen NR, Jensen RV, Johnsen J, Bøtker HE, Kristiansen SB.** Effects of hypoglycemia on myocardial susceptibility to ischemia-reperfusion injury and preconditioning in hearts from rats with and without type 2 diabetes. *Cardiovasc Diabetol* 16: 148, 2017. doi:10.1186/s12933-017-0628-1.
45. **Pedersen TM, Boardman NT, Hafstad AD, Aasum E.** Isolated perfused working hearts provide valuable additional information during phenotypic assessment of the diabetic mouse heart. *PLoS One* 13: e0204843, 2018. doi:10.1371/journal.pone.0204843.
46. **Povlsen JA, Løfgren B, Dalgas C, Birkler RI, Johannsen M, Støttrup NB, Bøtker HE.** Protection against myocardial ischemia-reperfusion injury at onset of type 2 diabetes in Zucker diabetic fatty rats is associated with altered glucose oxidation. *PLoS One* 8: e64093, 2013. doi:10.1371/journal.pone.0064093.
47. **Smith W, Norton GR, Woodiwiss AJ, Lochner A, du Toit EF.** Dependence of Cardiac Systolic Function on Elevated Fatty Acid Availability in

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Obese, Insulin-Resistant Rats. *J Card Fail* 22: 560–568, 2016. doi:10.1016/j.cardfail.2016.04.012.

48. **Stølen TO, Høydal MA, Kemi OJ, Catalucci D, Ceci M, Aasum E, Larsen T, Rolim N, Condorelli G, Smith GL, Wisløff U.** Interval training normalizes cardiomyocyte function, diastolic Ca2+ control, and SR Ca2+ release synchronicity in a mouse model of diabetic cardiomyopathy. *Circ Res* 105: 527–536, 2009. doi:10.1161/CIRCRESAHA.109.199810.
49. **Stone PH, Muller JE, Hartwell T, York BJ, Rutherford JD, Parker CB, Turi ZG, Strauss HW, Willerson JT, Robertson T, Braunwald E, Jaffe AS; The MILIS Study Group.** The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to the adverse prognosis. *J Am Coll Cardiol* 14: 49–57, 1989. doi:10.1016/0735-1097(89)90053-3.
50. **Tocchetti CG, Caceres V, Stanley BA, Xie C, Shi S, Watson WH, O'Rourke B, Spadari-Bratfisch RC, Cortassa S, Akar FG, Paolocci N, Aon MA.** GSH or palmitate preserves mitochondrial energetic/redox balance, preventing mechanical dysfunction in metabolically challenged myocytes/hearts from type 2 diabetic mice. *Diabetes* 61: 3094–3105, 2012. doi:10.2337/db12-0072.
51. **Webster I, Salie R, Marais E, Fan WJ, Maarman G, Huisamen B, Lochner A.** Myocardial susceptibility to ischaemia/reperfusion in obesity: a re-evaluation of the effects of age. *BMC Physiol* 17: 3, 2017. doi:10.1186/s12899-017-0030-y.
52. **Wensley I, Salaveria K, Bulmer AC, Donner DG, du Toit EF.** Myocardial structure, function and ischaemic tolerance in a rodent model of obesity with insulin resistance. *Exp Physiol* 98: 1552–1564, 2013. doi:10.1113/expphysiol.2013.074948.
53. **Whittington HJ, Harding I, Stephenson CI, Bell R, Hausenloy DJ, Mocanu MM, Yellon DM.** Cardioprotection in the aging, diabetic heart: the loss of protective Akt signalling. *Cardiovasc Res* 99: 694–704, 2013. doi:10.1093/cvr/cvt140.

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