Mechanisms for increased myocardial fatty acid utilization following short-term high-fat feeding

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Aims Diet-induced obesity is associated with increased myocardial fatty acid (FA) utilization, insulin resistance, and cardiac dysfunction. The study was designed to test the hypothesis that impaired glucose utilization accounts for initial changes in FA metabolism.

Methods and results Ten-week-old C57BL6J mice were fed a high-fat diet (HFD, 45% calories from fat) or normal chow (4% calories from fat). Cardiac function and substrate metabolism in isolated working hearts, glucose uptake in isolated cardiomyocytes, mitochondrial function, insulin-stimulated protein kinase B (Akt/PKB) and Akt substrate (AS-160) phosphorylation, glucose transporter 4 (GLUT4) translocation, pyruvate dehydrogenase (PDH) activity, and mRNA levels for metabolic genes were determined after 2 or 5 weeks of HFD. Two weeks of HFD reduced basal rates of glycolysis and glucose oxidation and prevented insulin stimulation of glycolysis in hearts and reduced insulin-stimulated glucose uptake in cardiomyocytes. Insulin-stimulated Akt/PKB and AS-160 phosphorylation were preserved, and PDH activity was unchanged. GLUT4 content was reduced by 55% and GLUT4 translocation was significantly attenuated. HFD increased FA oxidation rates and myocardial oxygen consumption (MVO2), which could not be accounted for by mitochondrial uncoupling or increased expression of peroxisome proliferator-activated receptor-α (PPAR-α) target genes, which increased only after 5 weeks of HFD.

Conclusion Rates of myocardial glucose utilization are altered early in the course of HFD because of reduced GLUT4 content and GLUT4 translocation despite normal insulin signalling to Akt/PKB and AS-160. The reciprocal increase in FA utilization is not due to PPAR-α-mediated signalling or mitochondrial uncoupling. Thus, the initial increase in myocardial FA utilization in response to HFD likely results from impaired glucose transport that precedes impaired insulin signalling.

KEYWORDS
Glucose transport; Glycolysis; Fatty acid metabolism; Insulin resistance; Mitochondria

1. Introduction

Obesity and diabetes are associated with reduced myocardial glucose utilization (transport, glycolysis, and oxidation), and increased myocardial fatty acid (FA) utilization and oxygen consumption (MVO2),1–3 which are believed to contribute to cardiac dysfunction.1,2,4,5 In ob/ob and db/db mouse models of severe obesity and insulin resistance, abnormal cardiac metabolism precedes the onset of hyperglycaemia and impaired cardiac function in vivo.6–9 Similarly, in Zucker fatty rat hearts, glucose utilization is depressed prior to evidence of cardiac dysfunction,10 but at a time when there is left ventricular hypertrophy.11 Proposed mechanisms include reduced expression of glucose transporters, myocardial insulin resistance, and increased sarcolemmal localization of the FA transporter CD36. Activation of peroxisome proliferator-activated receptor-α (PPAR-α) may increase the expression of target genes such as pyruvate dehydrogenase kinase (PDH-K), which will decrease flux through pyruvate dehydrogenase (PDH), while increasing the expression of genes such as acyl-CoA dehydrogenases, mitochondrial and cytosolic thioesterases and uncoupling proteins that will promote FA oxidation.1,2,4,12–15 In addition, recent studies in ob/ob and db/db mice have suggested that FA and ROS-mediated mitochondrial uncoupling could contribute to the observed increase in MVO2.2,16–18

The sequence by which these pathophysiological mechanisms develop and their inter-relationships early in the course...
of obesity-related cardiac dysfunction are not well understood, because most studies have been performed after obesity is established or after prolonged periods of high-fat feeding. Few studies have attempted to elucidate early events. At 4 weeks of age, leptin-deficient or -resistant models such as ob/ob and db/db mice exhibited decreased rates of glucose utilization, increased rates of myocardial FA utilization and increased MVO2 in the absence of increased expression of PPAR-α target genes. Eight weeks of high-fat feeding in rats precipitated cardiac dysfunction, which was associated with impaired insulin signal transduction and increased myocardial FA uptake and triglyceride (TG) esterification, which was attributed to increased sarcosomal translocation of CD36. Although these animals were not obese, they manifested hepatic steatosis, skeletal muscle TG accumulation, and impaired glucose tolerance. Ten days of high-fat feeding in C57BL6 mice reduced insulin-mediated glucose uptake in proportion to reduced glucose transporter 4 (GLUT4), and reduced protein kinase B (Akt/PKB) phosphorylation. In this study, the metabolic fate of FA and glucose in the heart and expression of PPAR-α targets were not determined. Thus it is not known if short-term high-fat feeding alters myocardial FA metabolism or MVO2 and if these changes can be attributed to the activation of PPAR-α target genes. The goal of our study was to determine myocardial substrate metabolism after short-term high-fat feeding in the mouse and the molecular mechanisms responsible for observed changes. Two weeks of high-fat feeding increased myocardial FA utilization and MVO2 and decreased basal and insulin-stimulated rates of glycolysis and glucose oxidation. These metabolic changes were associated with impaired GLUT4 translocation but normal PDH activity. Importantly, there was no increase in PPAR-α target gene expression, no reduction in malonyl CoA concentrations, and no evidence of mitochondrial uncoupling. Thus, impaired glucose utilization might be the initial defect that precipitates altered myocardial substrate utilization following short-term high-fat feeding.

2. Methods

2.1. Animals and diets

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Institutional Animal Care and Use Committee of the University of Utah. Male C57BL/6J mice were fed ad libitum either a standard chow diet which provided 24.5% calories from protein, 4.4% calories from fat, and 54.5% calories from carbohydrate (8656, Harlan Teklad, Madison, WI, USA) or a high-fat, high-sucrose diet (D12451, Research Diets, New Brunswick, NJ, USA), which provided 20% calories from protein (200 gm% casein), 173 gm% sucrose, and 45% calories from fat (178 gm% lard, 25 gm% soybean oil), and 35% of calories from carbohydrates (50% sucrose) (73 gm% corn starch, 100 gm% maltodextrin, and 173 gm% sucrose) for 2 or 5 weeks, starting at ten weeks of age. Serum levels of glucose, insulin, TGs, and free fatty acids (FFA), glucose tolerance tests, and tissue TG levels were measured after a 6 h fast as previously described. The area under the curve is calculated by deriving the sums of the area of a trapezoid for each adjacent time point and glucose measurement, using Microsoft excel. Hearts were excised following anaesthesia with chloral hydrate, and then injected with either saline or 3 U of human regular insulin (Novolin R; Novo Nordisk, Bagsvaerd, Denmark) via the inferior vena cava. Hearts were harvested 5 min after saline or insulin injection and immediately frozen in liquid nitrogen.

2.2. Substrate metabolism, MVO2, and cardiac function in isolated working hearts

Rates of glucose oxidation, glycolysis, palmitate oxidation, and myocardial oxygen consumption (MVO2) were measured in isolated working hearts obtained from random-fed high-fat fed (HFD) and normal chow (NC) mice, perfused with Krebs Henseleit buffer supplemented with 5 mM glucose and 0.4 mM palmitate bound to 3% BSA as previously described.

2.3 Measurement of malonyl CoA concentrations and pyruvate dehydrogenase activity

Malonyl CoA concentrations were measured by HPLC and PDH activity determined using a radioactive enzymatic method in hearts that were isolated after a 6 h fast.

2.4. Respiration and ATP production in saponin-permeabilized fibres

Mitochondrial respiratory parameters were studied in saponin-permeabilized fibres using four independent substrates (in mM): (i) glutamate 5 and malate 2, (ii) pyruvate 10 and malate 5, (iii) palmitoyl-carnitine 0.02 and malate 2, or (iv) succinate 5 and rotenone 10 μM. The measured mitochondrial respiratory parameters were: basal respiration before the addition of ADP (State 2 or VO2b), maximally ADP-stimulated (1 mM) respiration (State 3 or V0Dp), and respiration in the presence of oligomycin (1 μg/mL), which inhibits ATP synthase activity (State 4 or V0a). ATP concentration was determined by a bioluminescence assay using the Enliten Luciferase/Luciferin Reagent (Promega, Madison, WI, USA).

2.5. Isolation of cardiac myocytes and determination of glucose uptake

Insulin-stimulated 2-deoxyglucose uptake was measured in collagenase dissociated mouse cardiomyocytes as previously described.

2.6. Western blot analysis

Total protein lysates were extracted from frozen hearts for immunoblot analysis as described; see Supplementary material online for antibodies.

2.7. Analysis of glucose transporter 4 translocation by sucrose gradient centrifugation

GLUT4 translocation was determined by examining insulin-mediated redistribution of GLUT4 vesicles from intracellular compartments to the plasma membrane using sucrose gradients; see Supplementary material online for details.

2.8. Glucose transporter 4 immunofluorescence

Hearts were rapidly removed and fixed in 4% paraformaldehyde, protected in a sequential series of 10, 20, and 30% sucrose/PBS solutions, oriented in OCT-filled molds (Tissue-Tek, Hatfield, PA, USA), rapidly frozen, and then sectioned at 6 μm on a Cryostat microtome (Leica Instruments, Bannockburn, IL, USA) at −20°C. Sections were washed with PBS, blocked in 1% bovine serum albumin without permeabilization, to analyze the fluorescence associated with the cell surface, and then incubated with a polyclonal rabbit GLUT4 antibody (Millipore) at 1:400 dilution for 2 h at room temperature. Slides were washed with PBS and then incubated...
High-fat feeding and cardiac metabolism

3. Results

3.1. Metabolic characterization and cardiac function

Body weights were increased by 8.0 and 9.4%, respectively, in HFD animals relative to NC mice at 2 and 5 weeks, respectively, and cardiac hypertrophy did not develop in HFD mice (see Supplementary material online, Table S2). After 2 weeks of HFD, fasting serum glucose, TG and FFA concentrations were not different in NC and HFD mice, but insulin concentrations were modestly increased (see Supplementary material online, Table S2). Intraperitoneal glucose tolerance was mildly impaired, as evidenced by a 25% increase in the area under the GTT curve in HFD animals relative to NC mice at 2 and 5 weeks, which is in agreement with previous studies.18,24 Samples from six hearts per condition were analyzed in triplicate. Data were normalized by expressing them relative to 18S rRNA expression and are reported as fold change relative to age-matched NC-fed mice.


data table

Table 1. Basal and insulin-stimulated cardiac performance in isolated working hearts after 2 and 5 weeks of high-fat diet

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2.9. Quantitative RT–PCR

Total RNA was extracted from hearts and reverse transcribed cDNA was analyzed by quantitative real-time PCR as previously described.18,24 Samples from six hearts per condition were analyzed in triplicate. Data were normalized by expressing them relative to β-actin expression and are reported as fold change relative to age-matched NC-fed mice.

2.10. Statistical analysis

Data expressed as mean ± SEM, were analyzed by ANOVA, and significant differences assessed by Fisher’s protected least significant difference or the unpaired t-test, with P < 0.05 accepted as significant (Statview 5.0.1 software package, SAS Institute Inc., Cary, NC, USA).

3.2. Glucose utilization in isolated working hearts

Cardiac substrate metabolism was determined in the presence and absence of 1 nM insulin. After 2 weeks of HFD, basal rates of glycolysis and glucose oxidation were reduced by 25–30% and declined further (by 30–40%, respectively, vs. normal diets) after 5 weeks of HFD (Figure 1). In control hearts, insulin increased glycolysis by 46%, but this response was completely abolished after 2 and 5 weeks of HFD. In control hearts, insulin increased glucose oxidation by 43%. Although basal rates of glucose oxidation were reduced at 2 weeks, the ability of insulin to increase glucose oxidation was still preserved. However, by 5 weeks, the ability of insulin to stimulate glucose oxidation was also abolished.

with Alexa Fluor 488-labelled goat anti–rabbit IgG (Invitrogen) at 1:500 dilution in PBS with 0.1% bovine serum albumin for 1 h and were examined using an Olympus IX71 inverted microscope (Olympus, Center Valley, PA, USA) equipped with a fluorescence filter at 40× magnification.
3.3. Insulin-stimulated glucose uptake in isolated cardiomyocytes

We hypothesized that impaired cellular uptake of glucose accounted for the absence of insulin-stimulated glycolysis. Indeed, insulin-stimulated 2-deoxyglucose uptake in isolated cardiomyocytes was significantly reduced after 2 and 5 weeks of HFD, respectively (Figure 2). We then determined if this could be accounted for by impaired insulin signalling to Akt/PKB. Following in vivo administration of insulin, we observed no defects in phosphorylation of Akt/PKB or its down-stream target AS-160 after 2 or 5 weeks of HFD (Figure 3).

3.4. Glucose transporter 4 translocation

Impaired insulin-mediated glucose uptake despite normal signalling to AS-160 led us to hypothesize that GLUT4 content or translocation was impaired following HFD. Total GLUT4 content was reduced by 55% following 2 weeks of HFD (Figure 4A). Using sucrose gradient centrifugation we observed that insulin reduced the content of intracellular GLUT4 vesicles in NC-fed, but not in HFD mice (Figure 4B). In NC-fed animals, there was a 20–30% increase in plasma membrane GLUT4 in insulin-treated mice, which was absent in the HFD animals (Figure 4C). Using GLUT4

Figure 1  Glucose utilization in isolated working hearts. Glucose metabolism in isolated working hearts after 2 and 5 weeks of high-fat feeding. Data (mean ± SEM) were obtained from perfusion of four high-fat (HF) hearts and five to six normal chow (NC) hearts after 2 weeks and three to five high-fat hearts and four to five normal chow hearts after 5 weeks. **P < 0.01 vs. normal chow perfused under the same conditions. †P < 0.05 vs. basal under similar dietary conditions.

Figure 2  Insulin-stimulated glucose uptake in isolated cardiomyocytes. Data (mean ± SEM) were obtained from five high-fat (HF) hearts and five normal chow (NC) hearts after 2 and 5 weeks, respectively. *P < 0.05 and **P < 0.01 vs. normal chow, at the same insulin concentration. †P < 0.05 vs. no insulin under similar dietary conditions.
immunofluorescence, we observed that insulin led to a clearly visible increase in cell surface GLUT4 staining in NC-fed animals (Figure 4D). In contrast, in HFD mice, there was no consistent change in membrane fluorescence above background fluorescence in insulin-treated heart samples isolated from HFD mice. Thus, three independent approaches support the existence of an HFD-induced defect in GLUT4 translocation.

3.5. Pyruvate dehydrogenase activity

Total PDH activity measured after 3 weeks of NC and HFD were $3.65 \pm 0.45$ and $3.75 \pm 0.27$ nmol/min/mg, respectively ($P = 0.85$), and percentage in the active form was $39.6 \pm 5.0$ and $35.8 \pm 4.1\%$, respectively ($P = 0.58$).

3.6. Fatty acid metabolism in isolated working hearts

Two weeks of HFD increased basal rates of palmitate oxidation by 40% and MVO$_2$ by 20%. After 5 weeks, MVO$_2$ increased further and was 40% higher in hearts from HFD mice relative to NC animals and palmitate oxidation rates remained elevated. In contrast to glucose utilization, the ability of insulin to suppress FA oxidation was not altered by HFD, with insulin reducing palmitate oxidation rates and MVO$_2$ in control and HFD hearts by 15 and 10%, respectively (Figure 5). After 5 weeks, tissue TG content in hearts was similar in HFD and NC-fed mice ($7.7 \pm 0.3$ vs. $7.9 \pm 0.4$ nmol/mg wet heart weight, $P > 0.2$, respectively), and there were no differences in malonyl CoA content ($0.702 \pm 0.032$–HFD vs. $0.767 \pm 0.064$ pmol/mg/wet weight—NC, $P > 0.3$).

3.6. Mitochondrial function in saponin-permeabilized fibres

To determine if mitochondrial uncoupling contributed to the increase in MVO$_2$ following HFD, we examined mitochondrial function in saponin-permeabilized cardiac muscle fibres. After 2 and 5 weeks of HFD, no evidence of mitochondrial dysfunction (normal state 3 respirations and ATP production rates) or increased mitochondrial uncoupling (normal state 4 respirations and ATP/O ratios) was observed (Figure 6). Similar results were obtained in permeabilized fibres obtained after perfusing hearts with 1 mM palmitate, which we previously reported unmasked mitochondrial uncoupling in $ob/ob$ and $db/db$ mouse hearts$^{17,18}$ (data not shown).

3.7. Transcriptional adaptations to high-fat feeding

After 2 weeks of HFD, there were no changes in expression levels of the 10 PPAR-$\alpha$ targets examined. There was also no change in mRNA levels of PGC-1$\alpha$ or $\beta$, PPAR-$\alpha$, GLUT1, or GLUT4. After 5 weeks, expression of MCD, MTE1, PDK4,
UCP2, UCP3, and MCAD were increased (Figure 7), suggesting that transcriptional activation of PPAR-α targets occurred after changes in FA metabolism had developed.

4. Discussion

This study demonstrates that hearts rapidly adapt to caloric excess, developing patterns of substrate utilization that mimic changes observed in longstanding obesity or diabetes (type 1 or type 2). They occur in the absence of significant obesity or hyperlipidaemia, but are associated with mildly abnormal glucose tolerance and a two-fold increase in fasting insulin concentrations. As early as 2 weeks of HFD, MVO₂ and FA oxidation are increased, whereas rates of glucose oxidation and glycolysis are reduced. PDH activity is not altered, but GLUT4 protein content is reduced via post-transcriptional mechanisms. GLUT4 translocation is also impaired independently of changes in insulin-mediated activation of the upstream regulators of glucose transport Akt/PKB and AS160. Increased FA utilization occurs prior to the activation of PPAR-α signalling, without any changes in malonyl CoA content and in the absence of mitochondrial uncoupling. Taken together, these data suggest that the initial molecular defect that alters myocardial substrate metabolism early in the course of high-fat feeding is impaired GLUT4-mediated myocardial glucose utilization.

Contraction-mediated GLUT4 translocation in beating hearts might be the major mediator of basal myocardial glucose utilization. Support for this comes from studies in mice with cardiomyocyte deletion of GLUT4 (G4H⁻/⁻). These animals have increased expression levels of GLUT1, yet after an overnight fast, basal rates of glucose uptake in perfused hearts were negligible. Moreover, in mice with cardiomyocyte-restricted KO of insulin receptors (CIRKO), basal rates of glycolysis in perfused hearts were significantly increased, despite a 50% reduction in levels of GLUT1 protein, but a two-fold increase in the GLUT4 protein. In contrast, in isolated cardiomyocytes, GLUT1 is the major contributor to basal glucose uptake. Thus in CIRKO mice, GLUT1 protein content and basal glucose uptake in cardiomyocytes were proportionately reduced, whereas in GLUT4-deficient cardiomyocytes, basal rates of glucose uptake were unchanged. Thus, the normal rate of basal glucose uptake in isolated cardiomyocytes in the present study was not unexpected given that expression levels of GLUT1 were unchanged.

PDH flux is an important regulator of glucose oxidation in the heart. Prior studies in HFD rats demonstrated reduced...
activity of the active fraction of PDH (PDHa) after 28 days but not after 10 days of high-fat feeding. Moreover, PDH kinase activities were increased at 28 days, but not at 10 days.\textsuperscript{33,34} Other studies in skeletal muscle of humans and rodents have also suggested that the decline in PDH activity with high-fat feeding parallels an increase in PDH kinase activity.\textsuperscript{32,35,36} In our study, we observed no change in the expression levels of pyruvate dehydrogenase kinase (PDK4)
vesicles on microtubules by myosin motors. Moreover, budding, actin polymerization, and movement of GLUT4 complex and incompletely understood, but involve vesicle from the intracellular compartment to the sarcolemma are mechanisms that are responsible for the transit of GLUT4

CPT1 glucose transporter isoform 1 or 4. PPAR-α, peroxisome proliferator activated receptor-alpha; HADhK or β-hydroxy acyl CoA dehydrogenase alpha or beta; MTE1, mitochondrial thioesterase-1; CPT1β, carnitine palmitoyl transferase-1 beta; CPT2, carnitine palmitoyl transferase-2; PDK4, pyruvate dehydrogenase kinase-4; UCP2 or 3, uncoupling protein 2 or 3; MCAD/LCAD, medium or long chain acyl CoA dehydrogenase; ACCβ, acetyl CoA carboxylase-beta; MCD, malonyl CoA decarboxylase; GLUT1/4, glucose transporter isoform 1 or 4.

Figure 7 Expression levels of genes that regulate fatty acid and glucose metabolism. Total RNA from six high-fat and six normal chow hearts at 2 and 5 weeks, respectively, was quantified by real-time PCR and normalized to β-actin. Values represent fold change in mRNA relative to normal chow, which was assigned as 1. Data are mean ± SEM.*P < 0.05 and **P < 0.01 vs. normal chow of similar age. Gene names are: PGC1-α or β-PPAR gamma coactivator 1-alpha or beta; PPAR-α, peroxisome proliferator activated receptor-alpha; HADhK or β-hydroxy acyl CoA dehydrogenase alpha or beta; MTE1, mitochondrial thioesterase-1; CPT1β, carnitine palmitoyl transferase-1 beta; CPT2, carnitine palmitoyl transferase-2; PDK4, pyruvate dehydrogenase kinase-4; UCP2 or 3, uncoupling protein 2 or 3; MCAD/LCAD, medium or long chain acyl CoA dehydrogenase; ACCβ, acetyl CoA carboxylase-beta; MCD, malonyl CoA decarboxylase; GLUT1/4, glucose transporter isoform 1 or 4.

after 2 weeks of high-fat feeding, and consistent with this, we observed no differences in the total or active fraction of PDH, measured at this early time point. We did observe a significant increase in PDK4 activity after 5 weeks of high-fat feeding and would expect that PDH_e (if measured) would be reduced after 5 weeks of high-fat feeding. Taken together, these findings are consistent with the conclusion that reduced GLUT4-mediated glucose uptake may represent the critical mechanism for reduced basal rates of glycolysis and glucose oxidation early in the course of high-fat feeding (2 weeks), but as the duration of high-fat feeding becomes more prolonged, reduced PDH flux will likely contribute to the impairment in glucose oxidation.

High-fat feeding attenuated insulin-mediated glucose uptake in isolated cardiomyocytes and prevented insulin-mediated increases in glycolysis and glucose oxidation in isolated working hearts despite normal insulin signalling to AS-160. We believe this reflects a distal defect in GLUT4 translocation. Evidence for this was obtained by analyzing insulin-mediated redistribution of intracellular GLUT4 vesicles to the sarcolemma and qualitatively by GLUT4 immuno-histochemistry. The dissociation of insulin-mediated GLUT4 translocation from Akt/PKB and AS-160 signalling suggests that high-fat feeding initially impairs key steps in the movement of GLUT4 vesicles from their intracellular compartment to the sarcolemma. Studies in palmitate-exposed L6 myotubes and skeletal muscles of HFD mice illustrated that impaired insulin-mediated glucose uptake can occur in the absence of defects in insulin-stimulated phosphorylation of Akt/PKB or AS-160. Moreover, increased sarcosarcomal cholesterol content, reduced phosphatidylinositol-4,5-bisphosphate (phosphatidylinositol-3,4-bisphosphate) content or disruption of cortical F-actin can impair insulin-mediated GLUT4 translocation in skeletal muscle without reducing insulin-mediated phosphorylation of Akt/PKB. The molecular mechanisms that are responsible for the transit of GLUT4 from the intracellular compartment to the sarcolemma are complex and incompletely understood, but involve vesicle budding, actin polymerization, and movement of GLUT4 vesicles on microtubules by myosin motors. Moreover, there are regulated steps involved in GLUT4 vesicle docking and fusion, which could be perturbed by high-fat feeding in the heart. For example, increased expression of the SNARE protein Munc-18, a negative regulator of GLUT4 vesicle docking, was described in skeletal muscle with lipid-induced insulin resistance on the basis of overexpression of lipoprotein lipase. Thus, additional studies will be required to elucidate the mechanisms by which short-term high-fat feeding impairs GLUT4 trafficking in the heart.

An intriguing aspect of this study is the difference in tempo of impaired insulin-stimulated glycolysis relative to the ability of insulin to stimulate glucose oxidation. Insulin-stimulated glycolysis was completely absent after 2 weeks of HFD at a time when the ability of insulin to stimulate glucose oxidation was relatively preserved, whereas the ability of insulin to stimulate glucose oxidation was abrogated after 5 weeks of HFD. Increased glycolysis following insulin stimulation is due in part to increased GLUT4 translocation, which was clearly impaired as early as 2 weeks of HFD. The oxidative metabolism of glucose while partially dependent on glycolytic flux is also regulated by flux through PDH, which is regulated by PDH phosphatases and kinases whose activities are modulated by allosteric interactions with nucleotides, acetyl CoA, NAD(H), and intracellular [Mg_2+] and [Ca^{2+}]. Thus, the regulatory mechanisms for glycolysis might exhibit differential insulin sensitivity relative to mechanisms that regulate glucose oxidation.

Reduced myocardial glucose utilization and increased myocardial FA utilization after 2 weeks of HFD, although reminiscent of changes described in severe diabetes and obesity occurred in the absence of major changes in the serum concentrations of glucose, FFA, or TGs. It is widely believed that an important mediator of altered myocardial substrate metabolism in obesity and diabetes is activation of PPAR-α signalling via increased delivery of FA ligands to the heart. The present study suggests that the activation of PPAR-α signalling does not occur early in the course of high-fat feeding at a time when myocardial FA utilization is increased. We propose that the initial increase in FA utilization likely results from reduced basal rates of myocardial glucose utilization, which is secondary to reduced GLUT4 content and translocation, which according to Randle’s hypothesis would be predicted to increase FA oxidation. CD36 translocation to the sarcolemma has been described
in the hearts of rats after 8 weeks of high-fat feeding. 15 We did not determine sarcolemmal CD36 content in the present study, thus this mechanism cannot be ruled out. Perfusion of hearts with FA alone could also clarify the mechanism. If impaired glucose uptake was the sole basis for initial metabolic defects within 2 weeks of high-fat feeding, then FA utilization rates in the absence of glucose in the perfusate would not be expected to be changed.

Convincing evidence for the increased activation of PPAR-α signalling pathways was evident only after 5 weeks of HFD. Thus it is likely that the activation of the PPAR-α pathway may sustain the increase in myocardial FA utilization only when increased dietary lipid intake persists. These results are similar to those of Buchanan et al., who noted increased myocardial FA utilization and decreased glucose utilization in hearts from obese 4-week-old ob/ob and db/db mice prior to the onset of diabetes, which was not associated with the activation of PPAR-α signalling in young mice. However, PPAR-α signalling increased as animals aged and after hyperglycaemia developed. 6

HFD caused an early increase in MVO₂, which commonly accompanies increased FA metabolism. Previous studies from our laboratory suggested that mitochondrial uncoupling may contribute to increased MVO₂ in severe obesity. 17,18 However, the present study demonstrated that following short-term high-fat feeding, changes in FA oxidation and oxygen consumption occurred in the absence of mitochondrial uncoupling. Thus, increased MVO₂ is likely a consequence of altered substrate metabolism. FA is a less efficient substrate than glucose, producing less ATP per oxygen consumed. Thus an increase in FA utilization in HFD hearts would be expected to increase myocardial oxygen consumption. 16,46 It is likely that as caloric excess becomes more prolonged, mitochondrial uncoupling could occur as could be sustained by the increased expression of uncoupling proteins (UCP2 and UCP3), which was evident after 5 weeks of HFD. In addition, increased expression of mitochondrial thioesterases at 5 weeks would further reduce myocardial energetic efficiency by promoting futile ATP-wasting FA cycling between the mitochondria and cytosol. 47,48

In conclusion, high-fat feeding causes an early reduction in glucose utilization on the basis of reduced GLUT4 content and GLUT4 translocation, which is independent of coordinate reductions in PDH activity or in insulin-mediated Akt/ PKB and AS-160 phosphorylation. The reciprocal increase in cardiac FA oxidation (Randle effect) is initially independent of PPAR-α activation, and the increase in MVO₂ is not attributable to mitochondrial uncoupling. Thus, cardiac metabolism rapidly adapts to high-fat feeding. These changes precede the development of obesity or diabetes, but recapitulate changes that have classically been associated with longstanding obesity and diabetes.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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