Insulin-Stimulated Cardiac Glucose Oxidation Is Increased in High-Fat Diet–Induced Obese Mice Lacking Malonyl CoA Decarboxylase

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OBJECTIVE—Whereas an impaired ability to oxidize fatty acids is thought to contribute to intracellular lipid accumulation, insulin resistance, and cardiac dysfunction, high rates of fatty acid oxidation could also impair glucose metabolism and function. We therefore determined the effects of diet-induced obesity (DIO) in wild-type (WT) mice and mice deficient for malonyl CoA decarboxylase ($MCD^{-/-}$; an enzyme promoting mitochondrial fatty acid oxidation) on insulin-sensitive cardiac glucose oxidation.

RESEARCH DESIGN AND METHODS—WT and $MCD^{-/-}$ mice were fed a low- or high-fat diet for 12 weeks, and intramyocardial lipid metabolite accumulation was assessed. A parallel feeding study was performed to assess myocardial function and energy metabolism (nanomoles per gram of dry weight per minute) in isolated working hearts (+/– insulin).

RESULTS—DIO markedly reduced insulin-stimulated glucose oxidation compared with low fat–fed WT mice (167 ± 31 vs. 734 ± 125; $P < 0.05$). $MCD^{-/-}$ mice subjected to DIO displayed a more robust insulin-stimulated glucose oxidation (554 ± 82 vs. 167 ± 31; $P < 0.05$) and less incomplete fatty acid oxidation, evidenced by a decrease in long-chain acylcarnitines compared with WT counterparts. $MCD^{-/-}$ mice had long-chain acyl CoAs similar to those of WT mice subjected to DIO but had increased triacylglycerol levels (10.92 ± 3.72 vs. 3.29 ± 0.62 μmol/g wet wt; $P < 0.05$).

CONCLUSIONS—DIO does not impair cardiac fatty acid oxidation or function, and there exists disassociation between myocardial lipid accumulation and insulin sensitivity. Our results suggest that MCD deficiency is not detrimental to the heart in obesity. ***Diabetes*** **58:1766–1775, 2009**

The incidence of obesity, insulin resistance, and diabetes is rapidly increasing (1–4), and by 2025 it is estimated that worldwide the incidence of diabetes will affect nearly 333 million individuals between 20 and 79 years of age (4). Obesity, insulin resistance, and diabetes are characterized, at least in part, by an elevation of circulating plasma fatty acid concentrations. Whereas there is a general consensus that increased circulating fatty acids lead to increased rates of fatty acid uptake in muscle, the contribution of impaired mitochondrial fatty acid oxidation to the accumulation of cytosolic intramuscular fatty acid metabolites implicated in the pathogenesis of insulin resistance (such as long-chain acyl CoAs, triacylglycerols, ceramides, and diacylglycerols) remains controversial (5–8). It has been proposed that an acceleration of mitochondrial fatty acid oxidation may alleviate or attenuate skeletal muscle insulin resistance by preventing the cytosolic accumulation of these fatty acid metabolites (9,10).

We have recently demonstrated that insulin resistance in response to high-fat feeding (diet-induced obesity [DIO]) in skeletal muscle does not arise from an impairment of mitochondrial fatty acid oxidation per se but rather is associated with excessive rates of incomplete fatty acid oxidation (11,12). DIO leads to an accumulation of intramuscular long-chain acyl CoAs, the vast majority of which are localized and oxidized in the mitochondria (13). However, in the absence of high energy demand, often observed in obese sedentary individuals, flux through the tricarboxylic acid (TCA) cycle is unable to increase to such a rate to accommodate the large increase in acyl CoAs oxidized. Interestingly, we also demonstrated in mice that preventing the mitochondrial uptake of fatty acids attenuates the development of fatty acid–induced whole-body insulin resistance (11). This was achieved by genetic deletion of malonyl CoA decarboxylase ($MCD^{-/-}$), which degrades malonyl CoA, a potent endogenous inhibitor of the rate-limiting enzyme in mitochondrial fatty acid uptake (carnitine palmitoyl transferase-1). A similar beneficial effect of MCD inhibition on insulin-stimulated glucose metabolism in skeletal muscle cells was also demonstrated recently (14). In our studies, MCD inhibition was associated with a reduction in the accumulation of long-chain acylcarnitines and a prevention in the downregulation of TCA cycle intermediates observed during DIO (11). It can be argued that such a strategy would increase the intramuscular accumulation of the aforementioned fatty acid metabolites (15). However, there was not any increase in long-chain acyl CoAs and triacylglycerols beyond that induced by high-fat feeding itself.

The accumulation of intramyocardial fatty acid metabolites is also implicated in mediating myocardial dysfunction (16,17). It is not clear what effect modifying fatty acid oxidation has on insulin sensitivity in cardiac muscle. Previous findings in the obese Zucker rat demonstrate an impairment in fasting myocardial fatty acid oxidation compared with lean controls, an effect accompanied by elevated levels of intramyocardial lipid and an inability to increase the expression of peroxisome proliferator–activated receptor (PPAR)-α target genes (17). Conversely, we

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TABLE 1
MCD deficiency does not result in ex vivo cardiac dysfunction following DIO

	WT (LF)	MCD$^{-/-}$ (LF)	WT (DIO)	MCD$^{-/-}$ (DIO)
Cardiac output (ml/min)	8.3 ± 1.0	11.3 ± 1.1	11.2 ± 0.9	10.8 ± 0.3
Cardiac work (ml · mmHg^{-1} · min^{-1})	5.3 ± 0.8	7.4 ± 0.9	7.0 ± 0.5	6.9 ± 0.5

Data are means ± SE. Parameters of cardiac function (cardiac output and cardiac work) were assessed in isolated working hearts obtained from WT and MCD$^{-/-}$ mice subjected to either a low-fat diet (LF) or DIO (n = 5–7).

showed previously no difference in the rates of myocardial fatty acid oxidation in either the fed or fasted states between insulin-resistant JCR:LA-cp rats and lean controls (18). Furthermore, in insulin-resistant JCR:LA-cp rats there was nearly a doubling in myocardial triacylglycerol stores, supporting the observation that the accumulation of intramyocardial fatty acid metabolites is the result of excessive fatty acid supply, opposed to impaired fatty acid oxidation. Also, we have demonstrated that myocardial fatty acid oxidation rates are elevated in transgenic PPAR-α–overexpressing mice, a phenotype resembling that of type 2 diabetes (19). Therefore, debate still exists with regards to the accumulation of fatty acid metabolites, fatty acid oxidation rates, and myocardial insulin resistance.

In this study, we investigated the relationship between myocardial fatty acid metabolite accumulation, fatty acid oxidation, and insulin-stimulated glucose oxidation in wild-type (WT) and MCD$^{-/-}$ mice fed either low- or high-fat diet. We hypothesized that inhibition of mitochondrial fatty acid uptake via ablation of MCD preserves insulin-sensitive myocardial glucose oxidation, despite elevated levels of intramyocardial long-chain acyl CoAs and triacylglycerols.

RESEARCH DESIGN AND METHODS

An expanded RESEARCH DESIGN AND METHODS section is available in the online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0011/DC1. Details on isolated working heart perfusions, ultrasound echocardiography, metabolic profiling, high-energy phosphate assessment, pyruvate dehydrogenase (PDH) activity, and immunoblot analysis is also provided in the online appendix.

All animals received care according to the Canadian Council on Animal Care and the University of Alberta Health Sciences Animal Welfare Committee. Twelve-week-old WT or MCD$^{-/-}$ mice were placed on a standard chow/low-fat diet (4% kcal from lard) or high-fat diet (60% kcal from lard; Research Diets, D12492) for a 12-week period. At the end of week 12, animals were killed via an intraperitoneal injection of sodium pentobarbital (12 mg) either in the fed state in the middle of the dark cycle or after a 6-h fast. Hearts were excised and immediately frozen in liquid N_2 for biochemical analyses or perfused in the isolated working mode (see online appendix).

RESULTS

Effects of DIO on cardiac fatty acid and glucose oxidation in isolated working mouse hearts. Subjecting mice to a 12-week period of DIO did not result in any alteration in isolated working heart function compared with low fat–fed mice (Table 1). Of interest is that cardiac function was also normal in MCD$^{-/-}$ mice regardless of whether they were obtained from the low fat–fed or DIO group. This demonstrates that MCD deficiency does not contribute to cardiac dysfunction in DIO mice. Reinforcing our ex vivo perfusion data, we also showed via ultrasound echocardiography that in vivo cardiac function was unaltered by DIO in WT or MCD$^{-/-}$ mice (Table 2). Despite the lack of functional changes, dramatic differences in cardiac energy metabolism were observed between the experimental groups. Hearts from WT mice subjected to DIO had a significant reduction in glucose oxidation rates compared with low fat–fed mice, with no change in oxidation of exogenously supplied [9,10-^{3}H]palmitate (Fig. 1*B*). Insulin (100 μU/ml) robustly stimulated myocardial glucose oxidation in low fat–fed WT mice (Fig. 1*A*) and decreased fatty acid oxidation (Fig. 1*B*). In contrast, insulin had only a small effect on glucose oxidation in WT DIO mice (Table 3), demonstrating an impaired insulin-stimulated glucose metabolism in these DIO hearts.

Insulin stimulation of glucose oxidation was significantly increased in MCD$^{-/-}$ mice compared with WT mice in both the low fat–fed and DIO groups (Fig. 1*A*). This was accompanied by a significant decrease in fatty acid oxidation in low fat–fed MCD$^{-/-}$ mice and a small nonsignificant decrease in fatty acid oxidation in MCD$^{-/-}$ DIO mice, which may be attributed to a trend toward lower fatty acid oxidation rates in the absence of insulin (Fig. 1*B*). As such, the contribution of glucose oxidation for acetyl CoA production was significantly increased whereas the contribution of fatty acid oxidation for acetyl CoA production was significantly decreased in MCD$^{-/-}$ DIO mice, illustrat-

TABLE 2
MCD deficiency does not result in in vivo cardiac dysfunction following DIO

	WT (LF)	MCD$^{-/-}$ (LF)	WT (DIO)	MCD$^{-/-}$ (DIO)
Tei index	0.42 ± 0.02	0.44 ± 0.02	0.45 ± 0.08	0.46 ± 0.04
Cardiac output (ml/min)	23.89 ± 3.16	24.60 ± 2.12	26.19 ± 2.10	23.07 ± 0.71
LVPW:d (mm)	0.74 ± 0.02	0.83 ± 0.06	0.87 ± 0.02*	0.81 ± 0.01
LVID:d (mm)	4.10 ± 0.10	4.27 ± 0.08	4.32 ± 0.04	4.21 ± 0.03
LVPW:s (mm)	1.04 ± 0.03	1.06 ± 0.07	1.23 ± 0.03*	1.07 ± 0.02†
LVID:s (mm)	2.78 ± 0.14	3.06 ± 0.15	2.72 ± 0.07	2.96 ± 0.05
Body weight (g)	26.33 ± 0.99	28.44 ± 1.78	41.33 ± 0.54	39.68 ± 1.86
LV mass (g)	0.16 ± 0.01	0.17 ± 0.01	0.22 ± 0.02	0.22 ± 0.01
LV mass/body weight	0.60 ± 0.01	0.59 ± 0.01	0.54 ± 0.07	0.57 ± 0.04

Data are means ± SE. In vivo cardiac function and ventricular wall measurements were assessed via ultrasound echocardiography (VisualSonics; Vevo 770) in isoflurane-anesthetized WT and MCD low-fat diet (LF) and DIO mice (n = 5–12). *$P < 0.05$ indicates a significant difference from low-fat diet counterpart. †$P < 0.05$ indicates a significant difference from WT DIO mice. d, diastole; s, systole; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall.

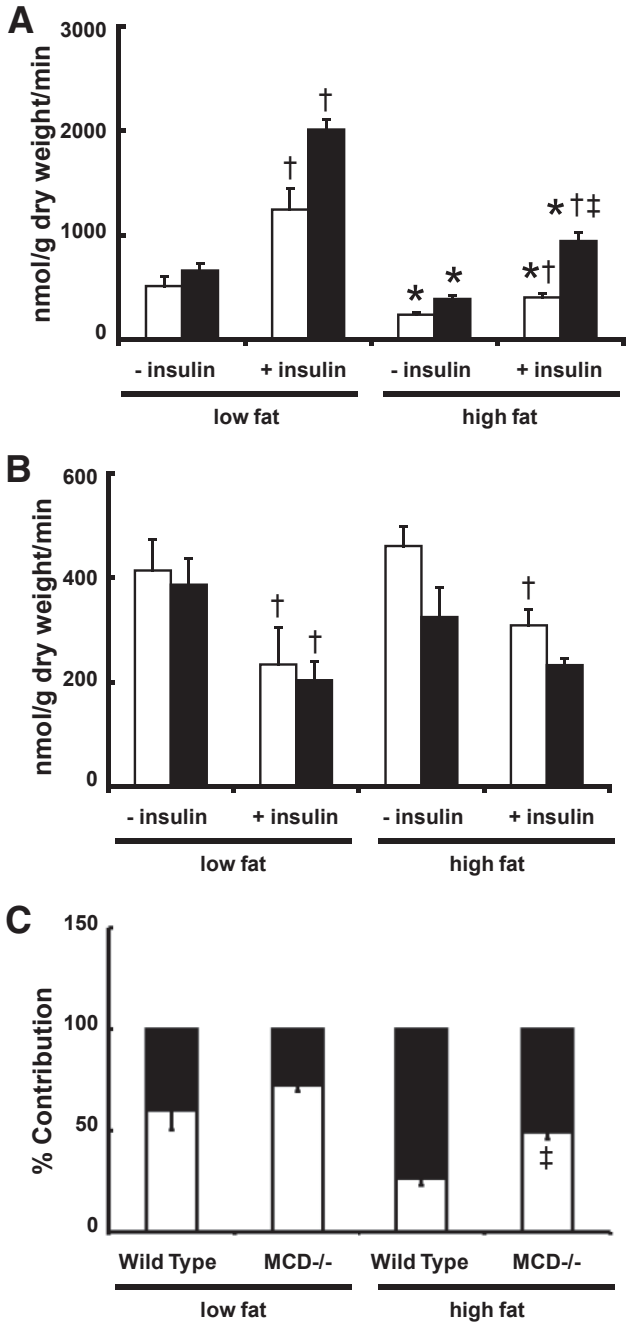

FIG. 1. MCD deficiency improves insulin-stimulated glucose oxidation in DIO mice. *A*: DIO lead to an impairment in insulin-stimulated glucose oxidation in hearts from WT mice (□) that was prevented in hearts from MCD$^{-/-}$ mice (■). *B*: Rates of fatty acid oxidation did not differ in hearts from WT (□) and MCD$^{-/-}$ (■) mice. *C*: However, the contribution of myocardial fatty acid oxidation rates to acetyl CoA production was decreased in MCD$^{-/-}$ DIO versus WT DIO mice. □, Glucose oxidation; ■, fatty acid oxidation. Values represent means ± SE (n = 5–7). Differences were determined using a repeated-measures ANOVA. *P < 0.05, significantly different from low-fat counterpart. †P < 0.05, significantly different from insulin-negative counterpart. ‡P < 0.05, significantly different from insulin-positive high-fat WT.

ing that these mice rely less on fatty acids for energy metabolism versus their WT counterparts (Fig. 1*C*). Paralleling our earlier work in MCD$^{-/-}$ mice whereby compensatory increases in PPAR-α target gene mRNA expression could explain the lack of change in myocardial fatty acid oxidation rates in MCD$^{-/-}$ mice, we show that MCD$^{-/-}$ DIO hearts had higher PPAR-α target gene pyruvate dehydrogenase kinase (PDK)-4 protein expression (Fig. 2*A*). Despite this elevation in PDK4 expression, total PDH activity and the active state of PDH were significantly higher in hearts from MCD$^{-/-}$ DIO mice, but the ratio of active to total PDH activity remained the same in both groups (Fig. 2*B* and *D*). Nonetheless, these findings demonstrate that decreasing MCD can partially overcome the dramatic impairment in insulin-stimulated glucose metabolism seen with DIO, which may be due to a preservation of PDH activity.

TABLE 3
MCD deficiency results in an improved myocardial insulin sensitivity index following DIO

	WT (LF)	MCD$^{-/-}$ (LF)	WT (DIO)	MCD$^{-/-}$ (DIO)
Insulin sensitivity	734 ± 125	1,351 ± 23	167 ± 31	554 ± 82*

Data are means ± SE. Insulin sensitivity index was calculated as the absolute increase (Δ [with insulin − without insulin]) in glucose oxidation (nanomoles per gram of dry weight per minute) in response to insulin (100 μU/ml) treatment in hearts obtained from WT and MCD$^{-/-}$ mice (n = 5–7). *P < 0.05 indicates a significant difference from WT DIO mice. LF, low-fat diet.

Effects of DIO and fasting on cardiac malonyl CoA levels. Because malonyl CoA is a major regulator of cardiac fatty acid oxidation, we examined what effect DIO had on cardiac malonyl CoA levels. Shown in Fig. 3, DIO

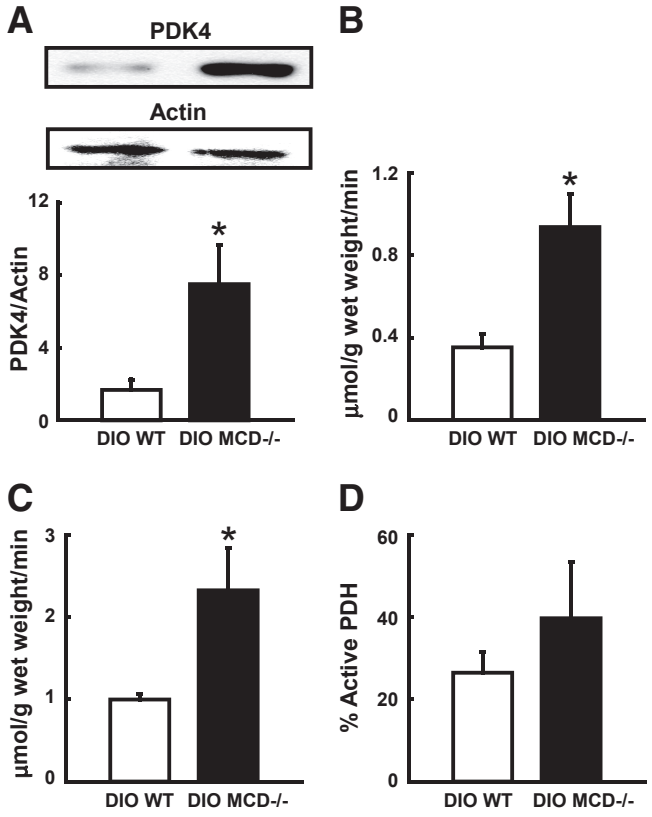

FIG. 2. MCD deficiency improves PDH activity in DIO mice despite an increased expression of PDK4. *A*: MCD$^{-/-}$ DIO mice had an increased expression of the PPAR target gene PDK4. *B*: The active portion of the PDH complex was higher in MCD$^{-/-}$ DIO mice. *C*: Total PDH activity was also higher in MCD$^{-/-}$ DIO mice. *D*: However, the percentage of active PDH did not differ between MCD$^{-/-}$ DIO and WT DIO mice. Values represent means ± SE (n = 6). Differences were determined using a Student's two-tailed t test. *P < 0.05, significantly different from WT DIO mice.

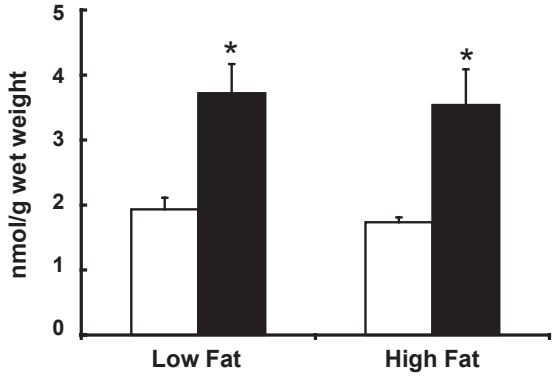

FIG. 3. MCD deficiency increases myocardial malonyl CoA levels in DIO mice. Malonyl CoA levels were higher in MCD$^{-/-}$ (■) than WT (□) mice following low-fat diet or DIO. Values represent means ± SE (n = 4–5). Differences were determined using a two-way ANOVA followed by Bonferroni post hoc analysis. *P < 0.05, significantly different from diet-matched WT mice.

had no effect on malonyl CoA levels in WT mice compared with low fat–fed WT mice. As expected, MCD$^{-/-}$ mice subjected to DIO showed a significant increase in malonyl CoA levels compared with WT DIO mice (Fig. 3). In addition, malonyl CoA levels from fasted WT mice did not differ from their fed counterparts but were significantly lower than those in fasted MCD$^{-/-}$ mice (supplemental Fig. 1*A*).

Effects of DIO and fasting on myocardial long-chain acylcarnitine levels. Following 12 weeks of low-fat feeding or DIO, there was a significant reduction in the accumulation of a number of long-chain acylcarnitine species in hearts from MCD$^{-/-}$ mice compared with hearts from WT littermates (Fig. 4*A*–*D*). In addition, medium-chain fatty acids do not require carnitine palmitoyl transferase (CPT)-1 for entry into the mitochondria, and as such no changes in any medium-chain acylcarnitine species were observed in hearts of MCD$^{-/-}$ DIO mice versus WT DIO mice, except the C10:3 species (4.4 ± 1.3 vs. 15.8 ± 4.1 pmol C10:3 acylcarnitine/mg protein, $P < 0.05$; data not shown). These findings are consistent with an inhibition of CPT1 and a lowering of intramitochondrial fatty acid oxidation intermediates.

In another group, following 12 weeks of low-fat feeding, mice were fasted for 6 h prior to the analysis of myocardial long-chain acylcarnitines, and similar to our DIO findings, we report that long-chain acylcarnitines do not accumulate to the same extent in hearts from fasted MCD$^{-/-}$ mice versus their WT counterparts (supplemental Fig. 1*B*). The medium-chain acylcarnitine profile from fasted mice also paralleled our DIO findings in which no accumulation was observed except for the C10:3 species (4.8 ± 2.2 vs. 17.9 ± 2.0 pmol C10:3 acylcarnitine/mg protein, $P < 0.05$; data not shown), consistent with no CPT-1 requirement for mitochondrial oxidation of medium-chain fatty acids. Interestingly, the fasting-induced increase in β-hydroxybutyrl (C4-OH) carnitine was prevented in the MCD$^{-/-}$ group (223.9 ± 20.8 vs. 104.2 ± 12.7 pmol β-hydroxybutyrl carnitine/mg protein, $P < 0.05$; data not shown), paralleling what we have previously reported in skeletal muscle (11).

Myocardial TCA cycle intermediates are not depleted by DIO or fasting. Previous work in skeletal muscle has suggested that fatty acid overload in response to fasting or DIO results in incomplete fatty acid oxidation by causing a disconnect between fatty acid oxidation and the TCA cycle, an effect prevented by the genetic deletion of MCD (11). Interestingly, myocardial short-chain CoAs and TCA cycle intermediates were not depleted by either fasting (data not shown) or DIO (Fig. 4*E* and *F*) in WT or MCD$^{-/-}$ groups, contrasting what we have reported previously in skeletal muscle. This likely reflects the higher acetyl CoA production from glucose oxidation in the MCD$^{-/-}$ mice; however, total acetyl CoA production from both glucose and palmitate was significantly depressed in WT DIO mice, indicative of mitochondrial impairment (Table 4). Moreover, deficiency of MCD, although associated with a reduced contribution of fatty acids for acetyl CoA production, did not impair mitochondrial function in lean mice and did not exacerbate the impairment observed in DIO WT mice. AMP (0.37 ± 0.08 vs. 0.41 ± 0.06 μmol/g dry wt in WT and MCD$^{-/-}$ low fat–fed mice and 0.31 ± 0.05 vs. 0.33 ± 0.03 μmol/g dry wt in WT and MCD$^{-/-}$ DIO mice) and ATP (1.89 ± 0.15 vs. 1.81 ± 0.35 μmol/g dry wt in WT and MCD$^{-/-}$ low fat–fed mice and 1.63 ± 0.11 vs. 1.77 ± 0.27 μmol/g dry wt in WT and MCD$^{-/-}$ DIO mice) levels were also not altered between any of the experimental groups, consistent with normal TCA cycle activity in these hearts. Myocardial lactate and pyruvate levels were also measured and showed no changes, regardless of diet, between WT and MCD$^{-/-}$ mice (Table 5).

Intramyocardial long-chain acyl CoA and triacylglycerol content following DIO or fasting. It has been suggested that an impairment in the mitochondrial uptake and subsequent oxidation of fatty acids contribute to the accumulation of intramuscular acyl CoAs and the development of insulin resistance (6,8,15) and myocardial dysfunction (16,17,20). Therefore, we investigated the effects of DIO on the myocardial accumulation of acyl CoAs. In low fat–fed mice, MCD deficiency did not alter the levels of the major long-chain acyl CoAs or total long-chain acyl CoAs compared with those in WT mice (Fig. 5*A*). DIO resulted in a marked increase in the major cardiac long-chain acyl CoAs and total long-chain acyl CoAs in WT mice compared with low fat–fed mice (Fig. 4*A*). DIO significantly increased intramyocardial long-chain acyl CoA levels to similar extents in MCD$^{-/-}$ mice compared with WT mice (Fig. 5*A*). This demonstrates dissociation between long-chain acyl CoA accumulation and insulin sensitivity in DIO mice.

MCD deficiency did not alter myocardial triacylglycerol levels in low fat–fed mice (Fig. 5*B*). However, a significant increase in triacylglycerol levels was seen in the DIO MCD$^{-/-}$ mice compared with WT DIO mice. This again demonstrates a disassociation between accumulation of triacylglycerol and insulin resistance in DIO hearts.

Because accumulation of ceramide has also been implicated in cardiac lipotoxicity, we measured ceramide levels in WT and MCD$^{-/-}$ mice subjected to DIO (Fig. 5*C*). No difference was observed in ceramide levels between DIO and low fat–fed mice in either the WT or MCD$^{-/-}$ groups.

Low fat–fasted WT and MCD$^{-/-}$ mice showed results similar to those of DIO WT and MCD$^{-/-}$ mice in that a similar accumulation in long-chain acyl CoA and no accumulation in ceramide were observed. Furthermore, myocardial triacylglycerol levels once again did not accumulate in fasted WT mice but did accumulate in fasted MCD$^{-/-}$ mice (8.42 ± 1.28 vs. 3.72 ± 0.68 μmol/g wet wt, $P < 0.05$; data not shown).

Effects of DIO on expression and phosphorylation of proteins involved in regulating myocardial energy metabolism and insulin signaling. Several proteins including PPAR-γ coactivator 1α (PGC1-α) and uncoupling protein 2 (UCP2) have been proposed to be altered by DIO in both skeletal and cardiac muscle (12,21–24). Neither the

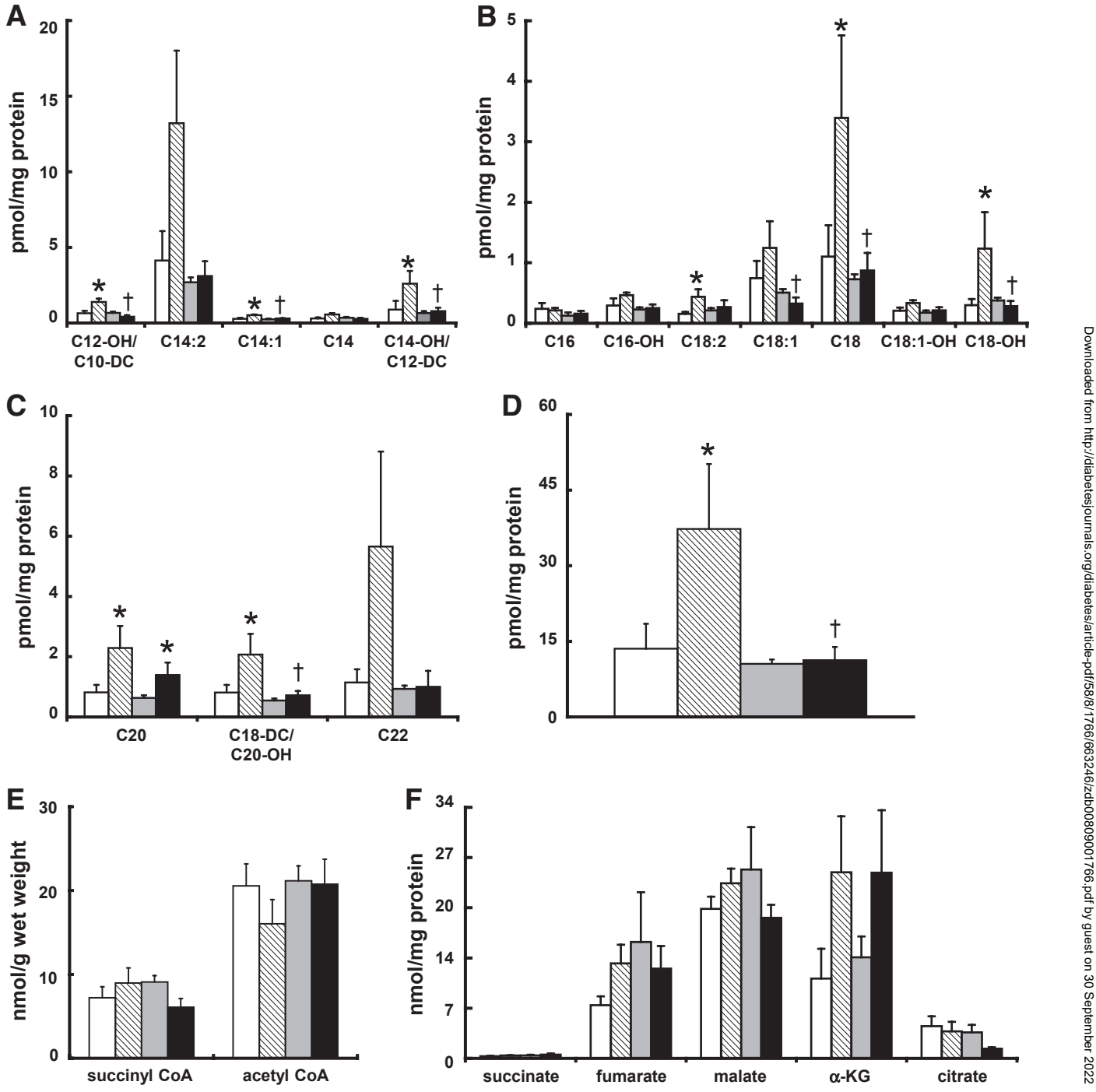

FIG. 4. MCD deficiency decreases the accumulation of fatty acid oxidative intermediates in DIO mice without decreasing TCA cycle intermediates. MCD$^{-/-}$ mice subjected to DIO had less accumulation of myocardial C12–C14 acylcarnitines (*A*), C16–C18 acylcarnitines (*B*), and C20–C22 acylcarnitines (*C*). *D*: Total long-chain acylcarnitines compared with WT DIO mice. *E*: MCD$^{-/-}$ mice subjected to DIO did not alter myocardial short-chain CoA compared with WT DIO mice. *F*: MCD$^{-/-}$ mice subjected to DIO did not have alterations in myocardial TCA cycle intermediates compared with WT DIO mice. Values represent means ± SE (n = 5–11). Differences were determined using a two-way ANOVA followed by Bonferroni post hoc analysis. *P < 0.05, significantly different from WT low fat–fed mice. †P < 0.05, significantly different from WT DIO. □, Wild-type low fat; ▨, wild-type high fat; ▩, MCD$^{-/-}$ low fat; ■, MCD$^{-/-}$ high fat.

expression of PGC1-α (Fig. 6*A*) or UCP2 (Fig. 6*B*) was altered by DIO in either the WT or MCD$^{-/-}$ groups. Supporting improved cardiac insulin sensitivity in MCD$^{-/-}$ DIO mice, we demonstrate an increase in Akt serine 473 phosphorylation and a trend to an increased glycogen synthase kinase 3β serine 9 phosphorylation in MCD$^{-/-}$ DIO hearts (P = 0.09) perfused aerobically with insulin (Fig. 7*A* and *B*). Insulin had no effect on 5′AMP-activated protein kinase (AMPK) phosphorylation at threonine 172 (Fig. 7*C*), suggesting that AMPK likely does not play a role in the improved insulin sensitivity observed in MCD$^{-/-}$ DIO hearts.

DISCUSSION

This study provides a number of important novel findings regarding the effects of DIO on cardiac insulin sensitivity.

TABLE 4
Insulin-stimulated cardiac acetyl CoA production in WT and MCD$^{-/-}$ mice following DIO

	WT (LF)	MCD$^{-/-}$ (LF)	WT (DIO)	MCD$^{-/-}$ (DIO)
Acetyl CoA production from glucose oxidation (nmol · g dry wt^{-1} · min^{-1})	2,491 ± 397	4,023 ± 181	811 ± 71*	1,888 ± 159*†
Acetyl CoA production from palmitate oxidation (nmol · g dry wt^{-1} · min^{-1})	1,868 ± 567	1,629 ± 284	2,471 ± 252	1,866 ± 104
Total acetyl CoA production (glucose + palmitate) (nmol · g dry wt^{-1} · min^{-1})	4,522 ± 464	5,652 ± 416	3,282 ± 216*	3,702 ± 192*

Data are means ± SE. Insulin-stimulated cardiac acetyl CoA production was determined by multiplying the glucose oxidation rate by 2 and the palmitate oxidation rate by 8. These values were used to determine the overall percent contribution to TCA cycle acetyl CoA production in hearts obtained from WT and MCD$^{-/-}$ mice (n = 5–7). *$P < 0.05$ indicates a significant difference from low-fat diet (LF) counterpart. †$P < 0.05$ indicates a significant difference from WT DIO mice.

We demonstrate that *1*) DIO results in a profound impairment in insulin-stimulated glucose metabolism in mouse hearts; *2*) increasing malonyl CoA levels in the heart secondary to deletion of MCD can prevent this impairment; *3*) the DIO-induced impairment in cardiac insulin-stimulated glucose metabolism can be dissociated from the accumulation of myocardial lipid intermediates, contradicting what has been previously proposed in skeletal muscle; *4*) similar to recent evidence in skeletal muscle (11), this impairment in insulin-stimulated glucose metabolism correlates with the accumulation of intermediates of incomplete fatty acid oxidation; and *5*) the insulin-sensitizing effects of MCD deletion are accompanied by a decrease in the accumulation of fatty acid intermediates of incomplete fatty acid oxidation. These data provide important insights into the pathophysiology of cardiac insulin resistance and suggest that inhibition of fatty acid oxidation (as opposed to stimulation of fatty acid oxidation) may have therapeutic potential in preventing obesity-induced impairments on cardiac insulin-stimulated glucose metabolism.

In skeletal muscle, two opposing views of how high fat–induced insulin resistance occurs have been proposed. A widely cited hypothesis is that the accumulation of cytoplasmic fatty acid intermediates impairs insulin signaling (6,8,15). Based on this hypothesis, it has been proposed that stimulation of fatty acid oxidation can lower these intermediates and improve insulin sensitivity. However, recent studies by us (11) and others (14) have challenged this concept. Recent studies have suggested that accumulation of intermediates of incomplete fatty acid oxidation correlate with skeletal muscle insulin resistance (11,25,26). Of importance is that inhibition of fatty acid oxidation secondary to MCD deletion resulted in an

TABLE 5
Myocardial lactate and pyruvate content in WT and MCD low-fat diet and DIO mice

	Lactate	Pyruvate	Lactate + pyruvate	Lactate/ pyruvate
LF				
WT	223.1 ± 27.6	8.0 ± 3.3	231.2 ± 29.5	43.9 ± 12.1
MCD$^{-/-}$	255.9 ± 46.6	4.6 ± 0.9	260.5 ± 46.6	71.8 ± 18.3
DIO				
WT	215.5 ± 18.5	6.6 ± 1.0	221.1 ± 19.1	35.8 ± 6.4
MCD$^{-/-}$	172.4 ± 23.4	25.4 ± 11.7	197.8 ± 22.8	17.7 ± 10.0

Data are means ± SE. Lactate and pyruvate content (nanomoles per milligram of protein) were assessed during the dark cycle in the fed state in hearts obtained from WT or MCD$^{-/-}$ mice on either a low-fat diet (LF) or subjected to DIO (n = 5–11).

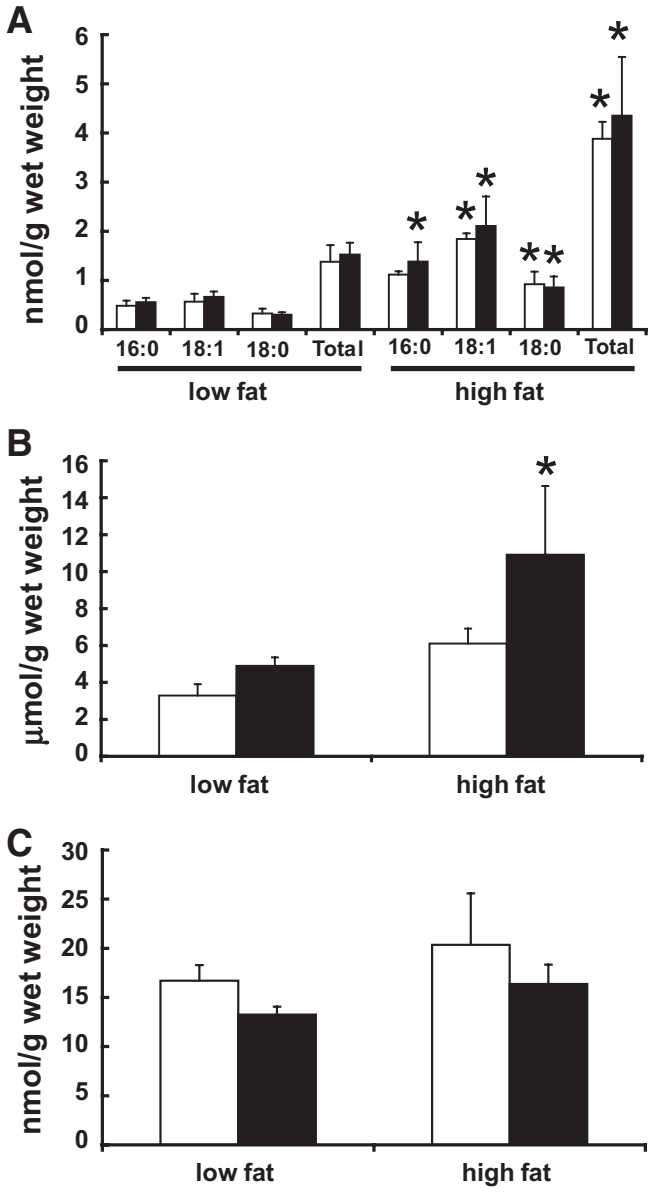

FIG. 5. Improvement of insulin senstivity in MCD$^{-/-}$ mice subjected to DIO does not correlate with the accumulation of myocardial lipid intermediates. *A*: DIO increased long-chain acyl CoAs to similar extents in both WT and MCD$^{-/-}$ mice. *B*: Triacylglycerols only accumulated in MCD$^{-/-}$ mice following DIO and did not accumulate in WT mice. *C*: Ceramides do not accumulate following DIO in hearts from WT and MCD$^{-/-}$ mice. Values represent means ± SE (n = 4–11). Differences were determined using a two-way ANOVA followed by Bonferroni post hoc analysis. *$P < 0.05$, significantly different from low fat–fed counterpart. ■, MCD$^{-/-}$; □, WT.

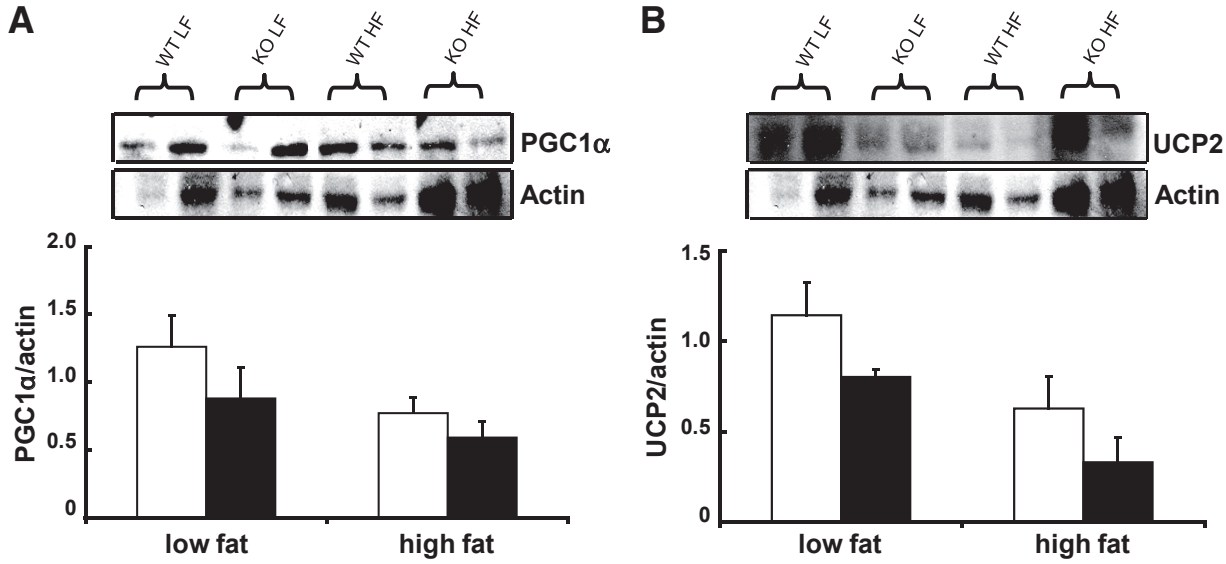

FIG. 6. Neither DIO nor MCD deficiency alters the expression of proteins involved in energy metabolism and mitochondrial function. *A*: PGC1-α expression is not altered by DIO or MCD deficiency. *B*: UCP2 expression is not altered by DIO or MCD deficiency. Values represent means ± SE (n = 4 per group). ■, MCD$^{-/-}$; □, WT. HF, high-fat diet; KO, knockout; LF, low-fat diet.

improvement of whole-body insulin sensitivity determined via glucose/insulin tolerance testing, fasting plasma glucose levels, and indirect calorimetry (11). Using siRNA to knockdown MCD in skeletal muscle cells also results in an increase in insulin-stimulated glucose metabolism (14). In this study we show a similar beneficial effect of MCD deletion in cardiac muscle. MCD$^{-/-}$ mice subjected to DIO showed a significant improvement in insulin-stimulated cardiac glucose oxidation and insulin signaling at the level of Akt phosphorylation. This was associated with a decreased accumulation of a number of long-chain acyl carnitine species in response to DIO. These data clearly suggest that stimulation of cardiac fatty acid oxidation would not be desirable in preventing the impairment in insulin-stimulated glucose metabolism seen with DIO.

The preservation of insulin-stimulated glucose oxidation in hearts from MCD$^{-/-}$ mice may be due to increases in both active and total PDH activity versus that observed in the WT littermate controls. Interestingly, the percentage of active PDH versus total PDH activity remained the same in both MCD$^{-/-}$ and WT DIO hearts. However, the fact that both the active portion and total activity of PDH are higher in the MCD$^{-/-}$ DIO hearts likely explains the preservation of insulin-stimulated glucose oxidation rates in these mice.

Surprisingly, we only observed a trend to a reduction in fatty acid oxidation rates in hearts from MCD$^{-/-}$ mice,

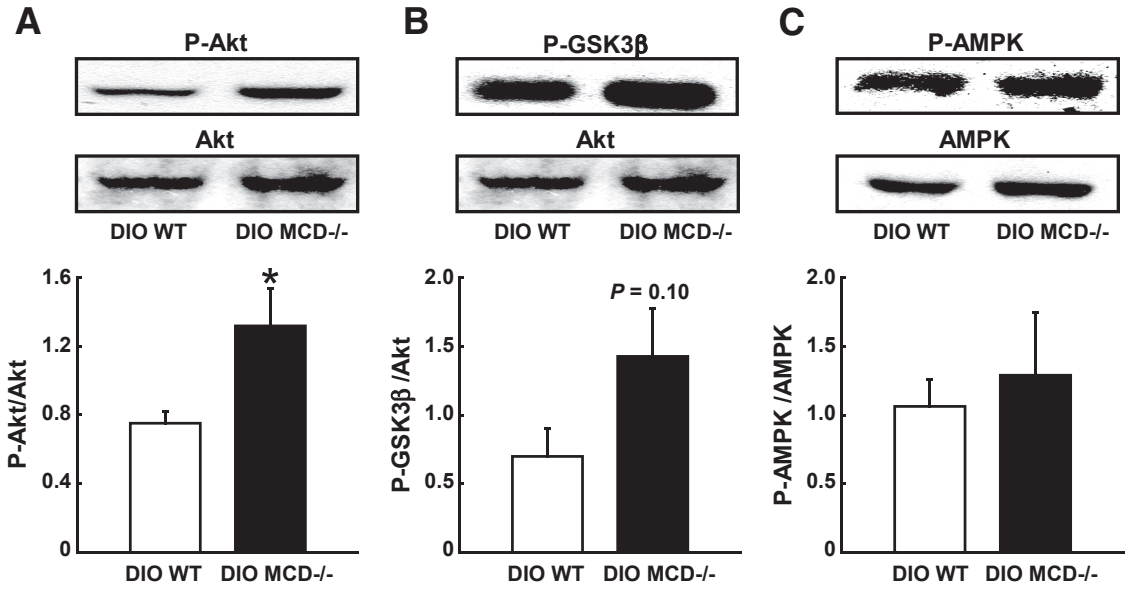

FIG. 7. Insulin-stimulated Akt phosphorylation is increased in hearts from MCD$^{-/-}$ mice following DIO. *A*: Insulin-stimulated Akt phosphorylation at serine 473 is increased in hearts of MCD$^{-/-}$ mice subjected to DIO. *B*: Insulin-stimulated glycogen synthase kinase 3β (P-GSK3β) phosphorylation at serine 9 in WT and MCD$^{-/-}$ mice subjected to DIO. *C*: AMPK phosphorylation is not altered in MCD$^{-/-}$ mice following DIO. Values represent means ± SE (n = 6 per group). Differences were determined using a Student's two-tailed t test. *P < 0.05, significantly different from WT DIO mice.

regardless of diet. Nevertheless, it is important to emphasize that the fatty acid oxidation assay utilized in this study only measures oxidation of a single exogenously supplied fatty acid ([9,10-^{3}H]palmitate). Unfortunately, the assay does not account for incomplete fat oxidation, oxidation of endogenously supplied fatty acids, and oxidation of other fatty acid species (oleate, stearate, etc.). By comparison, the intramyocardial acylcarnitine measurements provide more comprehensive information. The acylcarinitine profile revealed that MCD deficiency had a greater impact on the accumulation of oleyl-carnitine (C18:1), stearoyl-carnitine (C18:0), linoleyl-carnitine (C18:2), and tetradecadienoyl-carnitine (C14:2) as compared with palmitoyl-carnitine (C16:0). Coupled together with the observation that ATP and acetyl CoA levels were similar between nonperfused WT and MCD$^{-/-}$ DIO hearts, despite greater glucose oxidation rates in MCD$^{-/-}$ DIO hearts, it is highly suggestive that fatty acid oxidation is greater in the hearts of WT mice.

In this study DIO increased long-chain acyl CoA levels without affecting triacylglycerol or ceramide levels in hearts from WT mice. MCD deletion did not exacerbate the increase in long-chain acyl CoA levels in DIO mice but did result in an increase in triacylglycerol levels compared with WT DIO mice. DIO increased triacylglycerols in hearts from MCD$^{-/-}$ mice. Whereas DIO severely attenuated insulin-stimulated glucose oxidation in hearts from WT mice, hearts from MCD$^{-/-}$ mice were protected from this decrement. These data demonstrate a clear dissociation between myocardial lipid accumulation and insulin resistance in DIO. The DIO-induced alterations in myocardial fatty acid metabolite accumulation and insulin-stimulated glucose oxidation also occurred independently of changes in the expression of PGC1-α or UCP2 or the expression and phosphorylation of AMPK. These data indicate that inhibition of the mitochondrial uptake of fatty acids via ablation of MCD does not exacerbate the accumulation of intramyocardial fatty acyl carnitines or fatty acyl CoAs and does not induce insulin resistance or myocardial dysfunction. Rather, ablation of MCD prevents the accumulation of intramyocardial acylcarnitines likely by increasing the esterification of available intracellular fatty acids into triacylglycerols, thereby alleviating the detrimental effects of DIO on cardiac insulin-stimulated glucose metabolism.

Supporting our findings, studies in mice with a cardiac-specific overexpression of PPAR-α demonstrate an elevation in cardiac fatty acid oxidation rates and possess a phenotype mimicking that seen in type 2 diabetes (19). These animals have a dramatic impairment in insulin-stimulated glucose oxidation and show a reduction in ^{18}F-fluorodeoxyglucose uptake via positron emission tomography imaging. Furthermore, genetic ablation of the fatty acid transporter CD36 in these animals restored the impairment in glucose oxidation rates, with a strong trend to a reduction in oxidation of exogenously supplied palmitate (27). Elegant studies by Aasum et al. (28) also parallel our findings, where DIO mice and leptin receptor–deficient (*db/db*) diabetic mice have a dramatic impairment in insulin-stimulated glucose oxidation rates (29). Peripheral activation of PPAR-α in DIO mice with fenofibrate improved whole-body glucose tolerance and myocardial recovery from ischemia/reperfusion injury while significantly increasing hepatic fatty acid oxidation rates (28). However, myocardial fatty acid oxidation rates were significantly reduced, and this was associated with a complete restoration of glucose oxidation rates. Treatment of *db/db* mice with the PPAR-γ agonist rosiglitazone produced a similar effect, reducing myocardial fatty acid oxidation rates and restoring glucose oxidation rates to those seen in *db/+* lean nondiabetic control mice, which was also associated with protection against myocardial ischemia/reperfusion injury (29).

Interestingly, in contrast to skeletal muscle (11), there was not an accompanying depletion of TCA cycle intermediates in hearts from WT mice subjected to DIO compared with low fat–fed WT mice. We also did not observe any differences in total high-energy phosphates between groups. This discrepancy may be related to the marked increase in acetyl CoA derived from glucose oxidation in the MCD$^{-/-}$ mice hearts, which may exceed that seen in skeletal muscle (Table 3). Furthermore, potential differences in the anaplerotic capacity of cardiac and skeletal muscle may also partially account for this difference. Pyruvate carboxylation is an important anaplerotic reaction in cardiac muscle (30), and the relative abundance of pyruvate carboxylase is greater in cardiac versus skeletal muscle (31). Taken together these differences may, by mass action, replenish the TCA cycle at the level of malate and oxaloacetate (32–34) and thus account for the lack of TCA cycle intermediate depletion in response to fasting or DIO in hearts from both WT and MCD$^{-/-}$ mice.

As discussed, it has been hypothesized that an acceleration of skeletal muscle fatty acid β-oxidation has the potential to ameliorate insulin resistance by preventing the cytosolic accumulation of fatty acid metabolites (9,10). These findings are of particular importance because this hypothesis has been extrapolated to cardiac muscle, where it is proposed to attenuate myocardial dysfunction in the failing heart (16,17,20). Thus, it was initially anticipated that MCD$^{-/-}$ mice would accumulate intramuscular lipid and become insulin resistant and that this would be exacerbated by DIO. However, this was clearly not the case because MCD$^{-/-}$ mouse hearts were highly insulin sensitive and protected against insulin resistance in response to DIO. We also show that although MCD$^{-/-}$ mice accumulate intramyocardial long-chain acyl CoAs in response to DIO, this accumulation is not exacerbated compared with that seen in WT mice. The intramyocardial content of ceramides was not affected by either fasting or DIO in hearts obtained from WT or MCD$^{-/-}$ mice. The lack of alteration in myocardial ceramide content may be related to the dynamic nature of this sphingolipid pool, the size of which is determined by its rates of both synthesis and degradation (35). The aforementioned effects are likely attributable to the partitioning of intracellular fatty acid intermediates into the triacylglycerol pool in hearts from MCD$^{-/-}$ mice following both fasting and DIO.

Despite the increase in myocardial triacylglycerol in hearts from MCD$^{-/-}$ mice following DIO, myocardial insulin sensitivity was preserved, evidenced from the insulin-stimulated increase in myocardial glucose oxidation compared with hearts from WT mice following DIO. These findings support recent work in skeletal muscle suggesting that triacylglycerol accumulation may not be a mediator of insulin resistance, but may actually serve as a buffer against other potential lipid mediators of insulin resistance, such as ceramide and diacylglycerol (36,37). Further support for this concept lies in the athletes' paradox, in which endurance-trained individuals have greater muscle insulin sensitivity despite greater levels of intramuscular triacylglycerols (38,39). Although no

changes in ceramide were observed in hearts from MCD$^{-/-}$ mice following DIO, it may be possible that the increase in myocardial triacylglycerol buffered against the build up of diacylglycerol in MCD$^{-/-}$ mice. A recent study also demonstrates that acute inhibition of MCD in human skeletal muscle cells via an siRNA approach decreases fatty acid uptake and oxidation, effects that are accompanied by a concomitant increase in glucose uptake and oxidation (14). Previous work from our laboratory demonstrates that the acute inhibition of MCD with novel MCD inhibitors and the genetic deletion of MCD also causes a metabolic shift in myocardial substrate preference toward increased glucose oxidation and improves the recovery of myocardial function following ischemia reperfusion (40,41).

The results of this study are in direct contrast to a recent study published by Essop et al. (42) showing that insulin sensitivity was higher in mice deficient for acetyl CoA carboxylase 2 (ACC2$^{-/-}$). ACC2 produces malonyl CoA from acetyl CoA, hence ACC2$^{-/-}$ mice have lower malonyl CoA levels, and based on the Randle cycle should have decreased glucose oxidation rates at the expense of elevated fatty acid oxidation rates. Interestingly, ACC2$^{-/-}$ mice have elevated glucose and fatty acid oxidation rates and an increased insulin-stimulated 2-deoxyglucose uptake. However, it should be noted in this study that the reported glucose oxidation rates are 10- to 20-fold lower than what is normally reported in the literature and the change in fatty acid oxidation rates is very minimal, showing a smaller difference versus the fatty acid oxidation rates between WT and MCD$^{-/-}$ mice reported in our study.

In regards to AMPK, it has been shown to undergo alterations in both the muscle and hypothalamus following DIO and may account for some of the metabolic changes that take place in response to DIO caused by an impaired response to leptin (43). Despite these findings, our results show no difference between AMPK phosphorylation in WT and MCD$^{-/-}$ DIO hearts; we have also shown previously that leptin does not activate AMPK in isolated perfused working rat hearts (44), suggesting that leptin is likely not mediating an effect on AMPK to alter glucose metabolism in this study.

In conclusion, these findings demonstrate that DIO results in an impaired cardiac insulin sensitivity, which is not correlated with the accumulation of cytoplasmic lipid intermediates. MCD deficiency prevents this impairment independent of lipid accumulation. The beneficial effects of MCD deficiency on improving insulin sensitivity correlate with a decrease in incomplete fatty acid oxidation. Thus, inhibition of mitochondrial fatty acid uptake via MCD inhibition represents a novel mechanism for treating many of the detrimental conditions associated with DIO.

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