

Metformin counters the insulin-induced suppression of fatty acid oxidation and stimulation of triacylglycerol storage in rodent skeletal muscle

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Collier, Cheryl A., Clinton R. Bruce, Angela C. Smith, Gary Lopaschuk, and David J. Dyck. Metformin counters the insulin-induced suppression of fatty acid oxidation and stimulation of triacylglycerol storage in rodent skeletal muscle. *Am J Physiol Endocrinol Metab* 291: E182–E189, 2006. First published February 14, 2006; doi:10.1152/ajpendo.00272.2005.—The present study examined the acute effects of metformin on fatty acid (FA) metabolism in oxidative soleus (SOL) and glycolytic epitrochlearis (EPT) rodent muscle. SOL and EPT were incubated for either 30 or 180 min in the absence or presence of 2 mM metformin and with or without insulin (10 mU/ml). Metformin did not alter basal FA metabolism but countered the effects of insulin on FA oxidation and incorporation into triacylglycerol (TAG). Specifically, metformin prevented the insulin-induced suppression of FA oxidation in SOL but did not alter FA incorporation into lipid pools. In contrast, in EPT metformin blunted the incorporation of FA into TAG when insulin was present but did not alter FA oxidation. In SOL, metformin resulted in a 50% increase in AMP-activated protein kinase $\alpha 2$ activity and prevented the insulin-induced increase in malonyl-CoA content. In both fiber types, basal and insulin-stimulated glucose oxidation were not significantly altered by metformin. All effects were similar regardless of whether they were measured after 30 or 180 min. Because increased muscle lipid storage and impaired FA oxidation have been associated with insulin resistance in this tissue, the ability of metformin to reverse these abnormalities in muscle FA metabolism may be a part of the mechanism by which metformin improves glucose clearance and insulin sensitivity. The present data also suggest that increased glucose clearance is not due to its enhanced subsequent oxidation. Additional studies are warranted to determine whether chronic metformin treatment has similar effects on muscle FA metabolism.

obesity; diabetes; lipid; adenosine 5'-monophosphate-activated protein kinase

PHYSICAL INACTIVITY AND OBESITY are major risk factors for the development of type 2 diabetes. An appropriate lifestyle change, including dietary modifications and increased physical activity, is recommended for both the prevention and treatment of diabetes. In addition, pharmacological intervention, either alone or in combination with lifestyle changes, is an important means of treatment. There are various pharmacological agents available for the treatment of diabetes including sulfonylureas, thiazolidinediones, and biguanides. A commonly prescribed biguanide is metformin, also more commonly known as Glucophage. It is recognized that one of the primary mechanisms by which metformin improves the management of blood glucose in type 2 diabetics is to reduce hepatic glucose production (17, 31). Additionally, metformin increases peripheral glucose clearance (7, 19, 21), which also contributes to the control of

blood glucose. This is mediated through a metformin-induced increase in the activity of AMP-activated protein kinase (AMPK), as recently demonstrated in diabetic human skeletal muscle (26). It is well established that the activation of AMPK results in the translocation of GLUT4 to the plasma membrane and the subsequent increase in glucose uptake (20).

There is also evidence that chronic metformin treatment results in the lowering of lipids in human skeletal muscle (9, 21) as well as in other human cell lines (34). However, there is little information regarding the acute effect of metformin on FA oxidation and deposition into various lipid pools in muscle. Interestingly, there is some prior evidence that metformin may actually decrease FA oxidation in muscle (8). This seems contradictory to metformin's action as an antidiabetic agent and the fact that improved insulin sensitivity in muscle is associated with increases in FA oxidation (5, 13). Furthermore, activation of AMPK results in an increase in FA oxidation mediated by a decrease in malonyl-CoA content and relief of carnitine palmitoyl transferase I (CPT I) inhibition. Indeed, this effect has been demonstrated with pharmacological agents known to activate AMPK, such as 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) (2, 22), as well as insulin-sensitizing adipokines such as leptin (23) and adiponectin (33). It would be expected, therefore, that metformin would increase FA oxidation and reduce lipid storage. Accordingly, the primary objective of this study was to examine the acute effect of metformin on muscle fatty acid (FA) metabolism. Although it is clearly important to ultimately establish the chronic effects of metformin on muscle lipid metabolism, it is logical to first determine the acute effects before initiating longer studies.

Acutely, metformin has been shown to increase AMPK activity in muscle cells (10) and in isolated glycolytic epitrochlearis (EPT) rodent muscle following 3 h of incubation (35), resulting in an increase in glucose uptake (35). However, whether metformin also acutely increases the subsequent oxidation of glucose has not been examined. A recent study from our laboratory (29) demonstrated that AICAR increases glucose oxidation in rat soleus (SOL) muscle. Therefore, we hypothesized that metformin, through the activation of AMPK, would acutely increase glucose oxidation in this muscle. We examined the effects of metformin on SOL metabolism at both an early (initial 30 min) and late (final 30 min) stage of a 3-h incubation to elucidate any time-course effects. Finally, we also examined the effects of metformin on glucose oxidation in the glycolytic epitrochlearis (EPT) muscle following a 30-min

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incubation to extend the only previously published results where this muscle was used (35).

METHODS

Animals

Female Sprague-Dawley rats (180–220 g) were used for all experiments (Charles River, Saint Constant, QC, Canada). Water and standard Purina rat chow were provided ad libitum. Animals were housed in a controlled environment and kept on a reverse 12:12-h light-dark cycle. Ethical consent for all procedures used was obtained from the Animal Care Committee at the University of Guelph. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body mass) prior to all procedures. Upon completion of the muscle dissection, rats were humanely euthanized with an intracardiac injection of pentobarbital sodium.

Incubations

Procurement of muscles. EPT muscles were removed intact at each tendon (~40–45 mg). SOL muscles were stripped into two thin longitudinal pieces of ~25–30 mg. Upon their excision, muscle samples were immediately placed in 2.5 ml of pregassed (95% O₂-5% CO₂) modified Krebs-Heinseleit buffer containing 4% FA-free BSA, 10 mM glucose, and 1 mM palmitate. Muscles were equilibrated for 30 min at 30°C. No radiolabeled substrates were present in the buffer during the equilibration period.

Experimental design. The acute effects of metformin in SOL muscle on glucose oxidation and FA metabolism, as well as AMPK activation and malonyl-CoA content, were measured during a 30-min incubation window, either from 0–30 min or 150–180 min. Four viable SOL strips (2 per leg) can be procured from each rat; however, because the EPT muscles are not stripped, only two samples per animal are obtained. Therefore, as a secondary objective, we also chose to examine the effects of metformin on glucose oxidation and FA metabolism in the glycolytic EPT muscle but did not examine the AMPK-malonyl-CoA axis. Following 30 min of equilibration in buffer at 30°C, muscles were transferred to buffer containing 0 or 2 mM MET (Sigma Chemicals, St. Louis, MO) as well as 0 or 10 mU/ml human insulin (Eli Lilly, Toronto, ON, Canada). Pairs of muscles obtained from each animal were randomly placed in the control (CON) and metformin (MET) conditions, or insulin (INS) and insulin plus metformin (INS + MET) conditions, allowing for the direct determination of metformin's effects in the absence and presence of insulin. In one set of experiments the buffer contained 2 μ Ci of [U-¹⁴C]glucose (Amersham Biosciences, Baie d'Urfe, QC, Canada) to monitor glucose oxidation, and in the other set of experiments it contained 0.5 μ Ci of [1-¹⁴C]palmitate (Amersham Biosciences) to monitor FA oxidation as well as incorporation into the diacyl- (DAG) and triacylglycerol (TAG) pools. Because only two EPT muscles could be sampled per rat, it was necessary to use one rat for the CON vs. MET conditions and another for the INS vs. INS + MET conditions. Therefore, it was not the intention of this design to provide a direct comparison of the effects of insulin vs. control. Rather, the design was intended to provide a paired comparison of metformin vs. control and the additive effects of insulin and metformin vs. insulin alone. This protocol was also used for the SOL strips. Muscle viability was assessed in a separate series of experiments. ATP and phosphocreatine contents remained essentially unchanged from the end of the equilibration period to the end of 3 h of incubation (data not shown), as we have previously reported with this preparation.

Palmitate metabolism and glucose oxidation. Gaseous ¹⁴CO₂ produced from exogenous glucose oxidation during the incubation was captured in a microcentrifuge tube containing 0.4 ml of benzethonium hydroxide that was placed in the sealed vial. In addition, ¹⁴CO₂ remaining in the buffer following the incubation was released by transferring the 2.5 ml of buffer into a sealed flask and acidifying with

2.5 ml of 1 M sulfuric acid and captured in benzethonium hydroxide. The tubes containing benzethonium hydroxide and trapped ¹⁴CO₂ were then counted using standard liquid scintillation counting techniques.

¹⁴CO₂ produced from [1-¹⁴C]palmitate oxidation was captured as described above for glucose oxidation. In addition, to account for label fixation, a portion of the acid-soluble phase obtained during the muscle lipid extraction was measured for ¹⁴C content (see below). Muscle samples were placed in 5 ml of ice-cold chloroform-methanol (2:1) and homogenized. Following centrifugation at 2,000 g (4°C) for 10 min, the supernatant was collected and mixed with 2 ml of distilled water, shaken for 10 min, and centrifuged to separate the aqueous and lipophilic phases. One milliliter of the aqueous phase was quantified by liquid scintillation counting to determine the amount of ¹⁴C-labeled oxidative intermediates resulting from isotopic exchange. The lipid containing chloroform phase was gently evaporated under a stream of N₂ and redissolved in 100 μ l of 2:1 chloroform-methanol containing ~5 mg of lipids (di- and tripalmitin, Sigma) to identify the lipid bands on the silica gel plates. Fifty microliters of each sample were spotted on an oven-dried silica gel plate (Fisher Scientific Canada, Mississauga, ON, Canada) and placed in a sealed tank containing 60:40:3 heptane-isopropyl ether-acetic acid for 50–60 min. Plates were then dried, sprayed with dichlorofluorescein dye (0.02% wt/vol in ethanol), and visualized under long-wave ultraviolet light. The individual lipid bands were marked on the plate with a scalpel and scraped into vials for liquid scintillation counting.

AMPK activity and acetyl-CoA and malonyl-CoA contents. AMPK activity and malonyl-CoA content were determined in SOL muscle in each of the CON, MET, INS, and INS + MET conditions. Because only one leg per rat (2 SOL strips) was used for the determination of either glucose or FA metabolism, the second leg provided two additional SOL samples for measuring AMPK activity and malonyl-CoA content in either the CON and MET or INS and INS + MET conditions. These determinations were not made in the EPT due to the lack of tissue to perform these measurements.

To determine AMPK activity, muscle strips (~25 mg) were homogenized in buffer [50 mM Tris·HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol (DTT), 50 mM NaF, 5 mM Na pyrophosphate, 10% glycerol, 1% Triton X-100, 10 μ g/ml trypsin inhibitor, 2 μ g/ml aprotinin, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride]. The homogenates were incubated with AMPK α 1 and - α 2 (Upstate Scientific, Charlottesville, VA) antibody-bound protein A beads (Sigma) each for 2 h at 4°C. Immunocomplexes were washed with PBS and suspended in 60 μ l of dilution buffer [50 mM Tris (pH 7.5), 1 mM DTT, 10% glycerol, 0.1% Triton-X] for AMPK activity assay (6). Briefly, 20 μ l of sample were combined with 20 μ l of reagent mixture [5 mM HEPES, pH 7.5, 1 mM MgCl₂, 0.5% glycerol, 1 mM DTT, 100 μ M SAMS peptide (Upstate Scientific), 250 μ M ATP with [γ -³²P]ATP (Amersham Biosciences) and 100 μ M AMP]. The reaction proceeded for 15 min, after which 23 μ l of reaction mixture were spotted onto p81 filter paper (Upstate Scientific) and washed three times in 1% phosphoric acid. Filter papers were dried and placed in organic scintillant for counting.

Acetyl-CoA and malonyl-CoA contents were measured in freeze-dried muscle samples by high-performance liquid chromatography, as described elsewhere (27).

Calculations and Statistics

Results are expressed as means \pm SE. Differences between CON and MET pairs and INS and INS + MET pairs were assessed by a two-tailed paired *t*-test. To compare the metabolic effects of insulin treatment to untreated control strips, a one-tailed *t*-test with Welch's correction for unequal variances was implemented. Significance was accepted at *P* < 0.05. The amount of palmitate oxidized or incorporated into lipid pools was calculated from the specific activity of palmitate in the incubation buffer (dpm radiolabeled palmitate/nmol

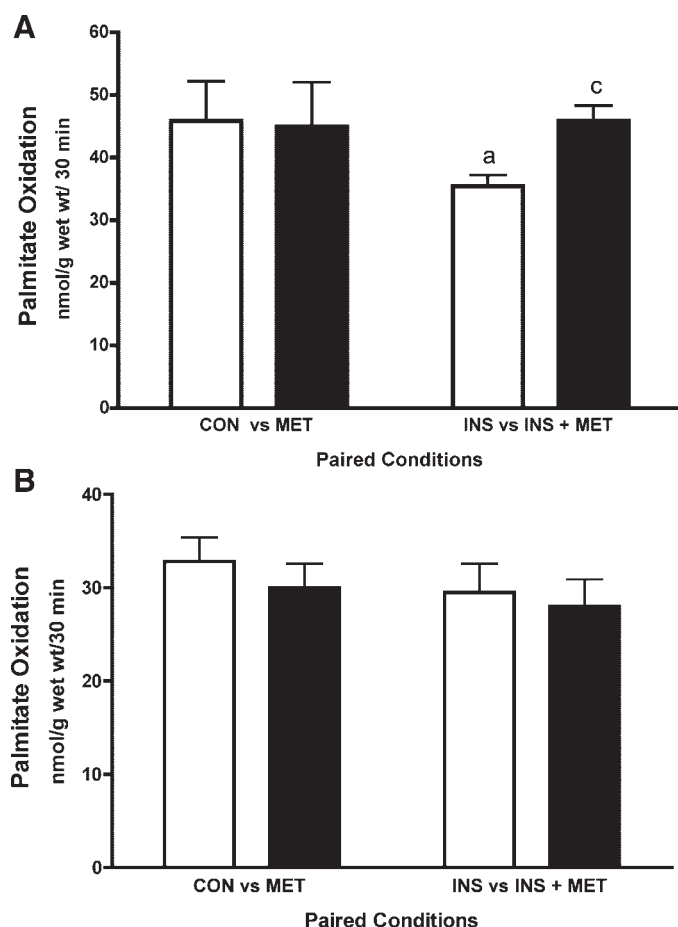


Fig. 1. Palmitate oxidation in soleus and epitrochlearis muscle during 0–30 min of incubation in control (CON) vs. metformin (MET) and insulin (INS) vs. INS + MET conditions. ^aSignificantly different from CON condition; ^csignificantly different from INS condition. $P < 0.05$; $n = 8$ –12 in each group.

total palmitate). Glucose oxidation was calculated in similar fashion using the specific activity of labeled glucose in the buffer. AMPK activity is expressed as picomoles ³²P transferred to SAMS peptide per minute per milligram of protein.

RESULTS

Palmitate Metabolism

Insulin suppressed palmitate oxidation in SOL following both the early and late incubation times, but only after 180 min in EPT (Fig. 1 and Tables 1 and 2). Metformin had no isolated effect on FA oxidation (i.e., in the absence of insulin) but prevented the insulin-induced suppression of FA oxidation in SOL. In EPT, incorporation of FA into TAG

was not significantly elevated by insulin at either time point compared with the CON condition. However, when metformin and insulin were both present, FA incorporation into TAG was blunted by a small but significant amount (–10 to 12%, $P < 0.05$) at both time points compared with insulin alone, indicating that metformin counters insulin's prostorage effect on lipid deposition (Fig. 2 and Table 2). In SOL, insulin increased FA incorporation into TAG (Fig. 2 and Table 1) but was unaffected by metformin. Thus, in SOL, the ratio of palmitate oxidized relative to that incorporated into TAG (i.e., partitioning ratio) was significantly decreased by insulin, but not in EPT (Fig. 3). Furthermore, the presence of metformin prevented the insulin-induced decrease in the partitioning ratio in SOL.

In addition, insulin stimulated the incorporation of FA into the DAG pool in both SOL and EPT muscles (Table 3). The presence of metformin had no influence on DAG formation either in the basal or insulin-stimulated conditions.

AMPK activity and malonyl-CoA content in SOL

AMPK α 1 activity in SOL was unaffected by either insulin, metformin, or their combination during the 0- to 30-min (Fig. 4) or 150- to 180-min periods (data not shown). However, the activity of AMPK α 2 was increased by ~50% in the presence of metformin ($P < 0.05$). Metformin also increased AMPK α 2 activity in the presence of insulin ($P < 0.05$), although not to so great an extent. These effects were similar at both the early (Fig. 4) and late (Table 2) time points.

As expected, insulin resulted in a significant elevation of malonyl-CoA content at both time points (Fig. 5 and Table 4). The insulin-induced increase in malonyl-CoA was completely prevented by metformin. Taking both time points into account, metformin alone had no significant effect on malonyl-CoA content.

Glucose Oxidation

Glucose oxidation was significantly increased in the presence of insulin compared with the CON condition in both SOL and EPT muscles (Fig. 6 and Tables 1 and 2). Metformin did not alter glucose oxidation relative to either the CON or INS conditions.

DISCUSSION

The direct effects of the antidiabetic agent metformin on skeletal muscle substrate metabolism have not been extensively examined. A recent study by Zhou et al. (35) demonstrated that 3 h exposure to metformin in glycolytic (EPT) rat muscle resulted in the activation of AMPK α 1 and - α 2 isoforms and, consequently, glucose uptake. However, there have been

Table 1. Glucose oxidation, palmitate oxidation, and storage in SOL muscle following 180 min of incubation in CON vs. MET and INS vs. INS + MET conditions

Parameter	CON	MET	INS	INS + MET
Glucose oxidation	75.7 \pm 4.1	77.7 \pm 6.0	92.0 \pm 7.2*	81.2 \pm 4.1
Palmitate oxidation	29.9 \pm 3.4	28.4 \pm 4.4	21.0 \pm 0.5*	30.9 \pm 4.0†
Palmitate incorporation	87.2 \pm 10.4	80.6 \pm 10.6	112.1 \pm 11.3*	101.6 \pm 8.3

Values expressed as means \pm SE in nmol \cdot g⁻¹ \cdot 30 min⁻¹. SOL, soleus; CON, control; MET, metformin; INS, insulin; TAG, triacylglycerol. *Significantly different from CON condition; †significantly different from INS condition. $P < 0.05$; $n = 8$ –12 in each group.

Table 2. Glucose oxidation, palmitate oxidation, and TAG storage in EPT muscle following 180 min of incubation in CON vs. MET and INS vs. INS + MET conditions

Parameter	CON	MET	INS	INS + MET
Glucose oxidation	60.6±3.9	56.6±6.0	77.5±4.1*	82.2±8.8
Palmitate oxidation	21.6±2.1	18.8±1.6	16.2±1.2*	16.0±2.0
Palmitate incorporation into TAG	45.2±4.5	40.2±2.8	53.5±6.2*	46.0±5.2*

Values expressed as means ± SE in nmol·g⁻¹·30 min⁻¹. EPT, epitrochlearis. *Significantly different from CON condition; †significantly different from INS condition. *P* < 0.05; *n* = 8–12 in each group.

no determinations of the acute effect of metformin on FA metabolism or glucose oxidation in skeletal muscle. Therefore, a primary purpose of this study was to examine the acute effects of metformin on FA metabolism in both oxidative and glycolytic skeletal muscle under basal and insulin-stimulated conditions and whether this could be explained by changes in malonyl-CoA content (as determined in SOL). We also chose to examine whether metformin acutely altered glucose oxidation in either fiber type. Finally, we expanded upon previous findings by examining the acute effects of metformin at both an early and late period during a 3-h incubation. The novel findings of this study are that 1) FA metabolism, in the absence of insulin, is unaffected by metformin; 2) in oxidative SOL

muscle the suppression of FA oxidation by insulin is prevented with metformin, which is accompanied by a reduction in malonyl-CoA content; 3) AMPKα2, but not -α1, is activated by metformin in SOL; 4) there is a small, but significant, reduction in TAG formation by insulin in EPT when metformin is present; 5) glucose oxidation under basal and insulin-stimulated conditions is not acutely affected by metformin; and finally, 6) these effects were consistent at both the early (0–30 min) and late (150–180 min) time points.

FA Metabolism

A major finding of this study was that metformin restored the insulin-induced suppression of FA oxidation in SOL mus-

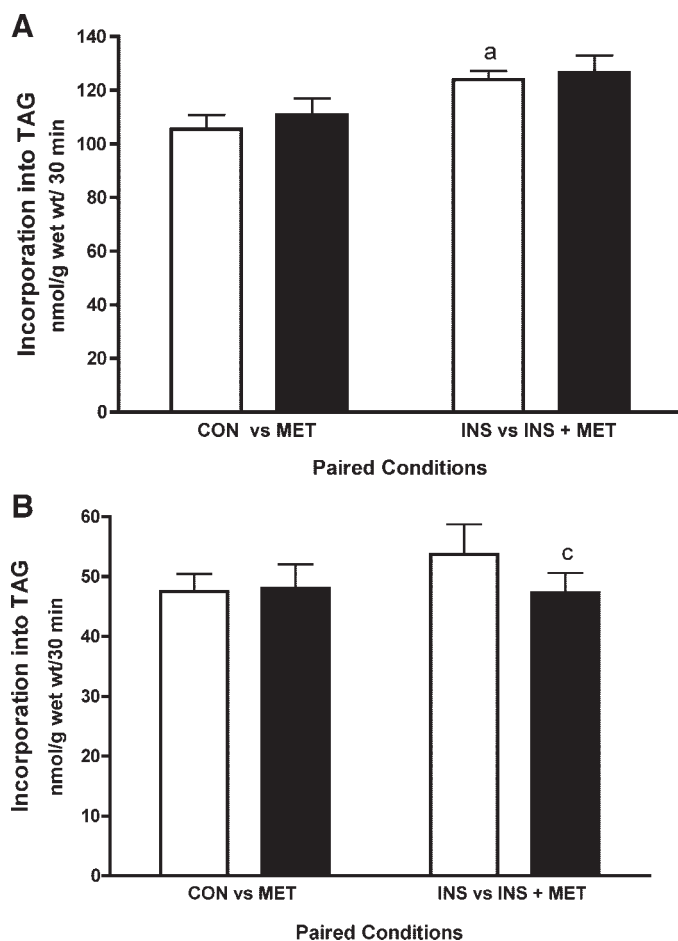


Fig. 2. Palmitate incorporation into triacylglycerol (TAG) in soleus and epitrochlearis muscle during 0–30 min of incubation in CON vs. MET, and INS vs. INS + MET conditions. *Significantly different from CON condition; †significantly different from INS condition. *P* < 0.05; *n* = 8–12 in each group.

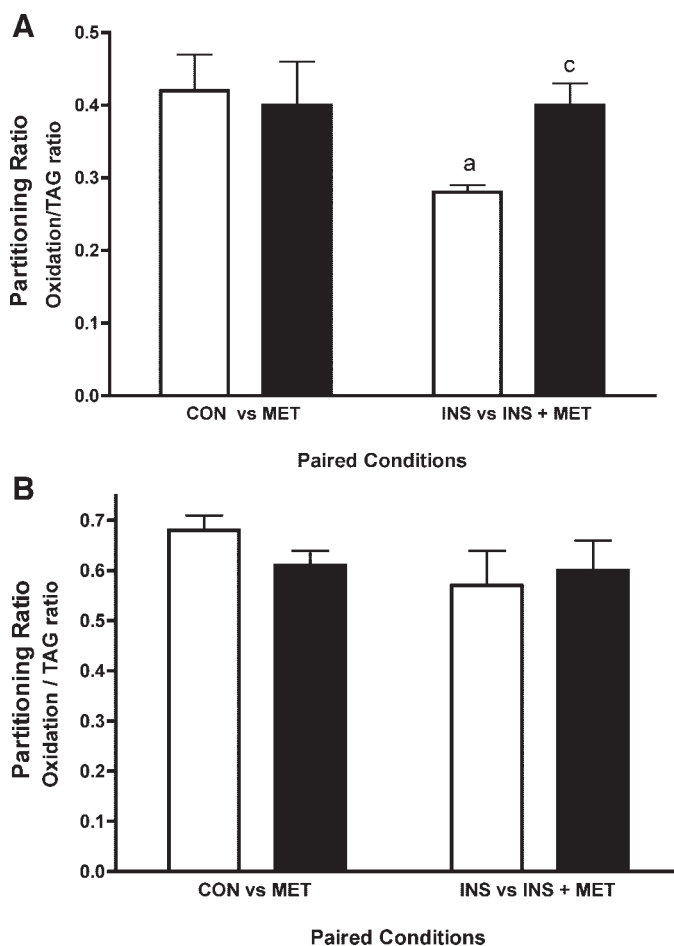


Fig. 3. Partitioning of incorporated palmitate (oxidation relative to TAG storage) in soleus and epitrochlearis muscle during 0–30 min of incubation in CON vs. MET and INS vs. INS + MET conditions. *Significantly different from CON condition; †significantly different from INS condition. *P* < 0.05; *n* = 8–12 in each group.

Table 3. Incorporation of [14 C]palmitate into diacylglycerol in EPT and SOL following incubation in CON, MET, INS, and INS + MET conditions

Time, min	Muscle	CON	MET	INS	INS + MET
0–30	EPT	21.8 ± 1.2	21.1 ± 0.8	26.3 ± 1.3*	24.7 ± 0.8
	SOL	14.5 ± 0.7	14.7 ± 0.7	20.6 ± 1.3*	22.1 ± 1.3
150–180	EPT	14.5 ± 0.7	14.7 ± 0.7	20.6 ± 1.3*	22.1 ± 1.3
	SOL	12.4 ± 1.2	11.6 ± 1.3	16.7 ± 1.6*	13.6 ± 0.6

Values expressed as means ± SE in nmol·g⁻¹·30 min⁻¹ wet wt. *Significantly different from CON condition; *n* = 8–12 in each group.

cle. There has been virtually no contemporary research on the acute effects of metformin on FA metabolism in skeletal muscle. Early research indicated that biguanides, such as metformin, might improve glucose utilization by decreasing FA oxidation (29). This is in keeping with the proposed Randle effect, or glucose-FA cycle, that elevated rates of FA oxidation in various disease states, such as obesity and diabetes, lead to suppressed glucose utilization (11, 12). However, more recent data indicates the converse; that is, rates of FA utilization/oxidation are either similar or impaired in obesity/diabetes (15, 18). Thus increased rates of FA transport/uptake coupled with similar or impaired rates of oxidation lead to lipid deposition in muscle (4). Increased intramuscular lipid accumulation is strongly associated with insulin resistance (28), although the

exact lipid species (TAG, DAG, ceramide, fatty acyl-CoA, etc.) responsible for this is a matter of debate. Improvement in FA oxidation, such as through aerobic training, is also a strong independent predictor of improved insulin sensitivity (5, 14). Thus the ability of metformin to reduce lipid storage and restore FA oxidation in muscle in the presence of insulin may be an important mechanism by which metformin improves insulin sensitivity. Clearly, it is important to establish that these effects persist on a chronic basis. However, the findings from the present study are supported by several reports of decreased muscle lipid content following chronic metformin treatment (9, 21, 34). Furthermore, it remains to be established why metformin appears to exhibit different effects in oxidative (restored FA oxidation) and glycolytic (reduced TAG formation) fiber types.

In the present study, we assessed the ability of metformin to acutely stimulate AMPK and reduce malonyl-CoA content in SOL muscle. A reduction in malonyl-CoA would be expected to relieve CPT I inhibition and increase FA oxidation. In accordance with the lack of effect of metformin on SOL FA oxidation in the basal condition, there was no significant decrease in malonyl-CoA content. However, the lack of change in malonyl-CoA and FA oxidation occurred despite an increase in AMPK activity. There are previous reports (32) of a lag between the activation of AMPK and the subsequent phosphorylation/deactivation of acetyl-CoA carboxylase and the resultant decrease in malonyl-CoA. This may explain our inability to observe an increase in FA oxidation during the initial 30-min period of incubation. However, we also failed to observe an increase in FA oxidation in SOL after 3 h of exposure to

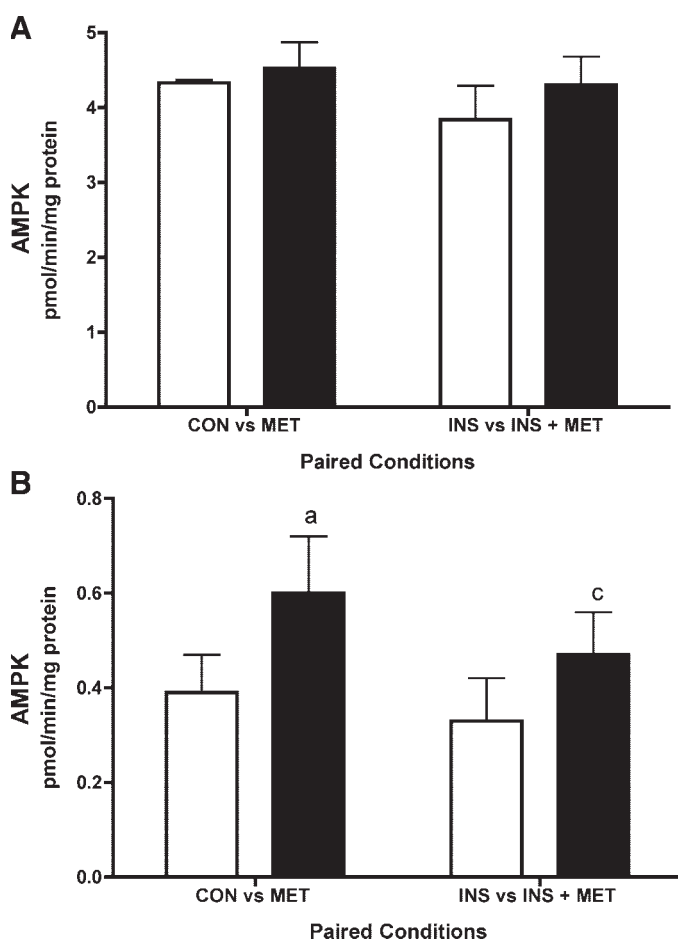


Fig. 4. AMP-activated protein kinase (AMPK) α 1 and α 2 activity in soleus muscle following 0–30 min of incubation in CON vs. MET and INS vs. INS + MET conditions. *Significantly different from CON condition; ^asignificantly different from INS condition. *P* < 0.05; *n* = 7–9 in each group.

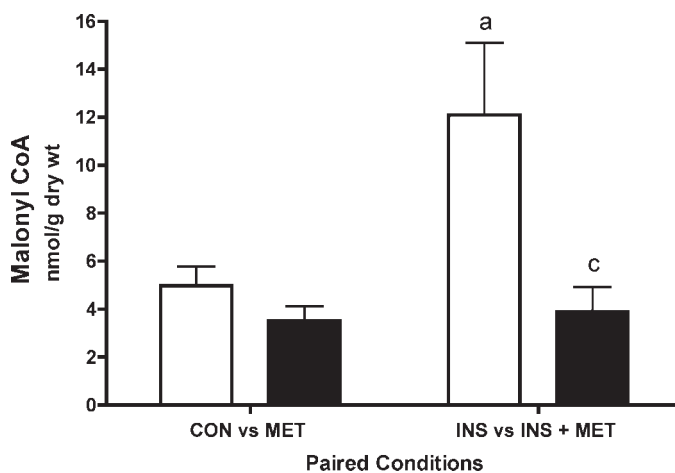


Fig. 5. Malonyl-CoA content in soleus muscle following 0–30 min of incubation in CON vs. MET and INS vs. INS + MET conditions. *Significantly different from CON condition; ^asignificantly different from INS condition. *P* < 0.05; *n* = 7–9 in each group.

Table 4. AMPK α 2 activity and malonyl-CoA content in SOL muscle following 180 min of incubation in CON vs. MET and INS vs. INS + MET conditions

Parameter	CON	MET	INS	INS + MET
AMPK α 2, pmol·min ⁻¹ ·mg protein ⁻¹	0.4±0.2	0.7±0.2*	0.2±0.1	0.3±0.1†
Malonyl-CoA, nmol/g dry wt	7.6±1.1	7.9±1.7	11.5±1.2*	6.7±1.1†

Values expressed as means ± SE. AMPK α 2, AMP-activated protein kinase- α 2. *Significantly different from CON condition; †significantly different from INS condition. $P < 0.05$; $n = 7-9$ in each group.

metformin similar to the time course utilized by Zhou et al. (35). It is, of course, possible that a transient metformin-induced increase in FA oxidation occurred between these time points; this possibility could be addressed in a future study. Our finding that metformin prevented the insulin-induced increase in malonyl-CoA is in agreement with the observed restoration of FA oxidation.

Chronic treatment with metformin in humans decreases muscle lipid content (9, 21). However, to our knowledge, this is the first study to examine the acute effects of metformin on the incorporation of FA into various intramuscular lipids. It has previously been demonstrated that various activators of AMPK, including AICAR (25) and leptin (24, 30), inhibit the incorporation of FA into TAG in muscle. However, this was not consistently observed in the present study with metformin.

Unexpectedly, metformin decreased the incorporation of FA into TAG in the presence of insulin in the glycolytic EPT, but not oxidative SOL muscle. Regardless, the combined data imply a potential lipid-lowering effect of metformin, whether due to increased oxidation or decreased deposition. It is presently a matter of debate which lipid species might be responsible for the impaired insulin signaling observed in obesity/diabetes. Elevated TAG stores may only be a marker of dysfunctional muscle FA metabolism, and accumulations of more reactive lipid species such as long-chain fatty acyl-CoA, DAG, or ceramides may be responsible for the impaired insulin signaling (16). Toward this end, we also monitored the deposition of FA into the DAG pool in the presence of metformin. As expected, insulin increased the deposition of FA into DAG in both fiber types; however, this was not blunted in the presence of metformin. It is possible that a much longer time frame is required to see any effect of metformin. Furthermore, we did not assess the breakdown of DAG in the present study, which might have been affected. Alternatively, metformin may have effects on the formation of other lipid species, such as ceramides, that are implicated in the impairment of insulin signaling. This could be addressed in future studies.

Glucose Metabolism

It is well established that metformin treatment can improve glucose tolerance in diabetic individuals. In addition to its ability to reduce hepatic glucose output (17, 31), metformin also improves peripheral glucose clearance in humans, as demonstrated using the euglycemic hyperinsulinemic clamp (7, 19, 21). Because >80% of insulin-stimulated glucose uptake is mediated by skeletal muscle, it follows that glucose uptake is improved by metformin in this tissue. This has recently been confirmed in isolated glycolytic rodent muscle, in which 3 h of exposure to metformin resulted in an activation of both catalytic units of AMPK (α 1 and α 2) and, subsequently, increased glucose uptake (35). However, it was not determined whether this increase in glucose uptake was associated with increased glycogen formation (i.e., nonoxidative) or glucose oxidation. Studies examining the fate of metformin-induced glucose disposal are inconclusive. There is evidence that chronic metformin treatment in humans results in improved nonoxidative (26), but not oxidative, disposal. A recent study (1) also demonstrated that 8 days of metformin treatment resulted in increased glycogen synthesis in cultured primary human skeletal myotubes from healthy subjects. In mice, chronic metformin treatment increases maximal insulin-stimulated, but not basal glucose, oxidation in SOL from diabetic, but not nondiabetic, mice (3). Thus, although metformin appears to improve nonoxidative glucose disposal, its effect on glucose oxidation is inconclusive. To our knowledge, the acute

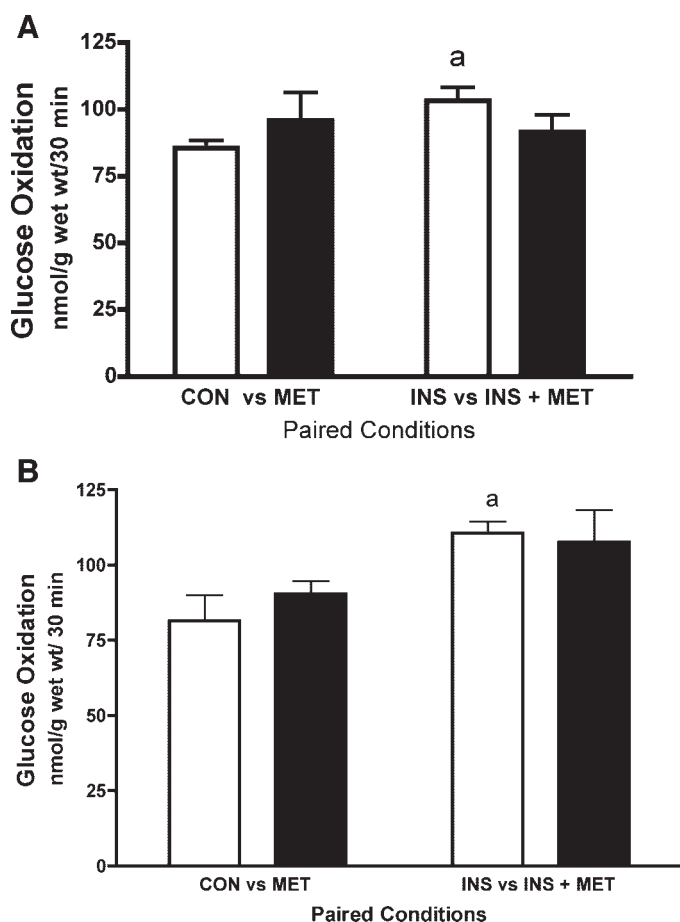


Fig. 6. Glucose oxidation in soleus and epitrochlearis muscle during 0–30 min of incubation in CON vs. MET and INS vs. INS + MET conditions. *Significantly different from CON condition. $P < 0.05$; $n = 9-12$ in each group.

effect of metformin on muscle glucose oxidation has not been investigated.

In the present study, we determined the acute effect of metformin on glucose oxidation in oxidative SOL and glycolytic EPT muscles. Our results, in both muscle types, failed to demonstrate an increase in glucose oxidation with metformin in either the presence or absence of insulin. Thus any increase in glucose uptake due to metformin, as demonstrated by others (7, 9, 21), presumably results in increased incorporation into glycogen (i.e., nonoxidative disposal), although this was not determined in the present study. The reason as to why there is an absence of effect of metformin on glucose oxidation is difficult to interpret. Our finding that metformin stimulates muscle AMPK activity is in agreement with previous studies (10, 35). Specifically, we demonstrated an increase of $\alpha 2$ activity in the SOL. Interestingly, we did not observe an increase in AMPK $\alpha 1$ activity, as Zhou et al. (35) did in the glycolytic EPT, suggesting fiber type differences in the ability of metformin to activate the catalytic subunits of AMPK. Although the stimulatory effect of AMPK on glucose uptake is well documented (20, 22), the effect of AMPK activation on glucose oxidation in skeletal muscle is limited and equivocal. Hulver et al. (15) reported a decrease in glucose oxidation in isolated rat SOL muscle in response to acute treatment with AICAR. However, AMPK activity was not increased in SOL in this study, making it difficult to comment on the direct effect of AMPK activation on glucose oxidation. In contrast, a recent study from our laboratory reported an increase in AMPK $\alpha 2$ activity and glucose oxidation in SOL as a result of acute AICAR treatment. Thus it was our hypothesis that metformin, through the activation of AMPK, would result in an increase in glucose oxidation. However, we have previously demonstrated that 40 to 60 min duration is required for AICAR to significantly increase the activity of pyruvate dehydrogenase (PDH) activity (29), a rate-limiting enzyme of glucose oxidation. Although this might explain the absence of effect on glucose oxidation during the initial 30 min of incubation, again, it does not explain the absence of effect at the latter time point unless the activation of PDH is also transient. The time course used in the present study does not allow us make any conclusions regarding this speculation. Interestingly, our data actually suggest that there may have been a small reciprocal decrease in glucose oxidation when FA oxidation was elevated. Indeed, in both muscle types, the highest rates of glucose oxidation occurred when FA oxidation rates were at or near their lowest.

In summary, the findings of the present study demonstrate that, in both oxidative and glycolytic muscle, metformin counters some of the effects of insulin on FA metabolism. In SOL, metformin prevents the insulin-induced suppression of FA oxidation, an effect that appears to be attributable to a stimulation of AMPK and the reduction of malonyl-CoA content. In the glycolytic EPT muscle, metformin blunts TAG storage in the presence of insulin. Because reductions in FA oxidation and accumulation of intramuscular lipids are associated with the development of insulin resistance, either of these mechanisms might contribute, at least in part, to the antidiabetic properties of metformin. On the basis of these acute findings, studies examining the chronic effects of metformin on muscle lipid metabolism in greater detail appear warranted.

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GRANTS

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