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Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals

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Sharoff CG, Hagobian TA, Malin SK, Chipkin SR, Yu H, Hirshman MF, Goodyear LJ, Braun B. Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals. *Am J Physiol Endocrinol Metab* 298: E815–E823, 2010. First published January 13, 2010; doi:10.1152/ajpendo.00517.2009.—Results from the Diabetes Prevention Program highlight the effectiveness of metformin or regular physical activity in the prevention of type 2 diabetes. Independently, metformin and exercise increase insulin sensitivity, but they have not been studied in combination. To assess the combined effects, insulin-resistant subjects ($n = 9$) matched for weight, body fat, and aerobic fitness were studied before any treatment (B), after 2–3 wk of 2,000 mg/day metformin (MET), and after metformin plus 40 min of exercise at 65% $\dot{V}O_{2peak}$ (MET + Ex). A second group ($n = 7$) was studied at baseline and after an identical bout of exercise with no metformin (Ex). Biopsies of the vastus lateralis were taken at B, after MET, immediately after MET + Ex (*group 1*), or immediately after Ex (*group 2*). Insulin sensitivity was assessed 4 h postexercise with a euglycemic hyperinsulinemic (40 $mU \cdot m^2 \cdot min^{-1}$) clamp enriched with [6,6-^{2}H]glucose. Insulin sensitivity was 54% higher after Ex ($P < 0.01$), but there was no change with Met + Ex. Skeletal muscle AMPKα2 activity was elevated threefold ($P < 0.01$) after Ex, but there was no increase with MET + Ex. These findings suggest that the combination of short-term metformin treatment and an acute bout of exercise does not enhance insulin sensitivity, and the addition of metformin may attenuate the well-documented effects of exercise alone.

TYPE 2 DIABETES IS A GROWING HEALTH EPIDEMIC that is caused by decreased insulin action in skeletal muscle, adipose tissue, and liver. A single bout of exercise dramatically enhances insulin-stimulated glucose uptake in individuals who are insulin resistant (4, 11), an effect that lasts for 3–72 h postexercise (27). The mechanisms by which exercise enhances whole body insulin sensitivity are multifactorial and likely involve alterations in enzymes regulating nonoxidative glucose disposal (2), decreased muscle glycogen (6), and increased skeletal muscle blood flow (9). In addition to the aforementioned factors, the molecular energy sensor AMP-activated protein kinase (AMPK) has been implicated as an important mediator of postexercise insulin sensitivity. Moderate-intensity exercise increases phosphorylation of AMPKα2 in skeletal muscle, and prior activation of AMPK by contraction was associated with increased insulin-stimulated glucose disposal (15). Upregulation of AMPK activity directly stimulates glucose uptake and is associated with elevated fatty acid oxidation in skeletal muscle. These alterations have the potential to increase oxidative and nonoxidative glucose disposal and diminish potential deleterious fatty acid metabolites, such as diacylglycerides, that have been shown to inhibit insulin signaling (21).

The diabetes drug metformin increases whole body insulin sensitivity and delays the onset of type 2 diabetes, effects that mimic the well-known actions of regular exercise. Metformin increases whole body insulin sensitivity by 10–30% in individuals with and without type 2 diabetes in some (13, 32, 39) but not all (8) studies. Like exercise, metformin activates AMPKα2 in skeletal muscle and may, at least partially, explain the increase in whole body insulin sensitivity and nonoxidative glucose disposal found after 10 wk of metformin treatment in individuals with type 2 diabetes (32). Potentially adding to its overall glucose-lowering effects, metformin reduces hepatic glucose production by activating AMPKα2 activity in the liver (43). The effectiveness of metformin to lower blood glucose and decrease hemoglobin A_{1c}, as well as its inexpensive cost, has made metformin the most prescribed drug for individuals with type 2 diabetes.

The Diabetes Prevention Program highlighted the independent success of lifestyle modification (which included regular exercise) and metformin treatment for the prevention of type 2 diabetes (28). To date, there are few data describing how exercise and metformin interact when combined. Our laboratory has reported that, in healthy individuals, short-term metformin treatment slightly lowers peak oxygen consumption ($\dot{V}O_{2peak}$) and raises fat oxidation during submaximal exercise (Ref. 3 and Malin SK, Stephens BR, Hagobian TA, Sharoff CG, Chipkin SR, and Braun B, unpublished observations). Others have reported that metformin and exercise increased skeletal muscle blood flow but failed to increase muscle glucose uptake or insulin signaling (17, 24). In high-fat-fed Zucker rats, exercise training plus metformin treatment had no greater effect than training alone on the progression to hyperglycemia and skeletal muscle lipid content (37). Collectively, these data suggest that the effects of metformin and exercise are not complimentary with respect to glucose metabolism but may have additive effects on blood flow and substrate metabolism, both of which are linked to improvements in whole body insulin action (19, 20). Therefore, the main purpose of this study was to assess the impact of combining a single bout of exercise and metformin treatment on whole body insulin sensitivity, AMPK activity, and substrate metabolism in insulin-resistant individuals.

METHODS

Overview. To compare the impact of combining a single bout of exercise with metformin treatment, we studied two groups of subjects

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that were randomized to either a metformin or placebo group and matched on age, weight, BMI, percent body fat, fasting glucose, fasting insulin, $\dot{V}O_{2peak}$, and whole body insulin sensitivity index score (Table 1). The metformin group was studied before treatment (baseline), after short-term metformin treatment (metformin), and after short-term metformin treatment plus a single bout of exercise (metformin + exercise). The placebo group was studied at baseline and after a single bout of exercise (exercise).

Preliminary testing. Because the effects of exercise and metformin are key independent variables in this study, only sedentary individuals (<60 min of moderate- or high-intensity activity/wk as measured by questionnaire) who were not currently using metformin were enrolled. Other exclusion criteria included weight loss or weight gain >5% of body weight within the past 6 mo, use of pharmaceutical agents or supplements known or suspected to alter glucose metabolism, use of tobacco products, and the presence of other disease states (e.g., exercise-induced asthma, hypo- or hyperthyroidism) that impact energy metabolism and/or exercise capacity. After study procedures were explained verbally, subjects signed a written informed consent document. Our study was approved by the Institutional Review Board at the University of Massachusetts Amherst. Subjects who qualified for the study underwent further screening to assess body composition (Lunar dual-energy X-ray absorptiometry; GE Healthcare) and level of insulin resistance by a 2-h, 75-g oral glucose tolerance test (OGTT). For the OGTT, individuals arrived at the Energy Metabolism Laboratory after a 10- to 12-h overnight fast. Plasma glucose and insulin concentrations were measured before and during 30-min intervals during the OGTT. These values were used to calculate the composite insulin sensitivity index (ISI) (29). Only insulin-resistant subjects (ISI ≤4 on a 12-point scale) were included in the study.

Estimation of $\dot{V}O_{2peak}$. To assess maximal cardiorespiratory fitness ($\dot{V}O_{2peak}$), subjects performed a YMCA submaximal cycle ergometer test. Heart rate was recorded and the workload increased every 3 min by the amount stipulated by the YMCA protocol based on the average heart rate during the last 2 min of each stage. The test continued until heart rate reached 85% heart rate reserve. To determine $\dot{V}O_{2peak}$, heart rate and workload were plotted and a line of best fit was drawn. At the point of intersection between the mean heart rate and estimated maximal heart rate, a perpendicular line was drawn to the *x*-axis to determine (estimated) maximal workload. Estimated maximal workload was then used to calculate predicted $\dot{V}O_{2peak}$ from the American College of Sports Medicine metabolic equation, i.e., $\dot{V}O_{2peak} = 3.5\ ml \cdot min^{-1} \cdot kg^{-1} + 12.24 \cdot (power) \cdot (body\ weight^{-1})$, where power is in watts and body weight is in kilograms.

Metformin and placebo treatment. Metformin treatment began at 500 mg/day and was increased by 500 mg every day to reach 1,000 mg twice/day. Once at the final dosage, all participants continued on that dose for 14 consecutive days. Participants then came in for the metformin-only or metformin-plus-exercise testing session, which was done in balanced order. The testing order was balanced such that in one half of the participants the effect of metformin alone was tested 14 days after metformin treatment began, and in the other half, the effect of metformin plus exercise was tested 14 days after the initiation of treatment. The participants then continued treatment for 7 days and returned for the remaining testing session.

Three participants noted bloating, upset stomach, and/or a metallic taste during the 1st week of treatment. These participants decided to continue with the study and reported that these symptoms were gone after 7 days of treatment.

Placebo treatment matched the metformin group so that participants were taking two pills by *day 4* and continued at this dose for 7–14 days. One participant complained of upset stomach after 3 days of treatment but also opted to continue with the dosing and the study. In this group, the order of the sessions was also balanced. A minimum of 7 days separated the exercise test and the baseline test in the instances where the exercise session was done first. None of the participants took medications on the morning of testing.

Exercise protocol and muscle biopsies. Participants arrived at the laboratory at 8 AM after a 10- to 12-h fast. Participants refrained from any structured exercise, other than walking, for 48 h before the test session and were instructed to eat ≥250 g of carbohydrate for the 3 days prior to testing and counseled about ways to achieve the stipulated dietary requirement.

Before exercise, the vastus lateralis was numbed with lidocaine and a small incision made in preparation for the muscle biopsy. The incision was closed with Steri-strips and bandaged and the participant moved to the cycle ergometer, where they peddled for 2 min with low resistance and then cycled for 30 min at 65% $\dot{V}O_{2peak}$. Oxygen consumption was measured by indirect calorimetry (TrueMax2400 Metabolic Measurement System; Parvomedics, Salt Lake City, UT) during exercise, and the ergometer resistance was adjusted until oxygen consumption was steady around the desired value. After 30 min, exercise was stopped for 6 min while additional lidocaine was applied to the biopsy site. The incision site was rewrapped, and subjects exercised for 10 min more at 85% $\dot{V}O_{2peak}$. Immediately after exercise, the participant was quickly moved to the biopsy table, and the second biopsy was taken within 3 min of the cessation of exercise.

In the no-exercise conditions, the experimental procedures were identical to the exercise condition, but instead of exercise the subjects rested for 30 min before the biopsy.

Euglycemic hyperinsulinemic clamp and stable isotope tracer infusion. Thirty minutes after the muscle biopsy, indwelling catheters were placed in the superficial vein of each forearm for continuous infusion of glucose stable isotope solution ([6,6-^{2}H]glucose) and venous blood sampling. Basal blood samples were collected to determine background levels of isotopic enrichment, glucose, insulin, lactate, and free fatty acids (FFA). A priming bolus of 200 mg [6,6-^{2}H]glucose was given, followed by a 90-min infusion of [6,6-^{2}H]glucose at a rate of 2.5 mg/min delivered by a peristaltic infusion pump (Harvard Apparatus Pump 22; Harvard Apparatus, Holliston, MA). Breath and blood samples were collected at 0, −75, and −90 min to measure basal substrate oxidation. Following the last basal measurement, a primed ($250\ mU \cdot m^{-2} \cdot min^{-1}$) constant infusion ($40\ mU \cdot m^{-2} \cdot min^{-1}$) of insulin diluted in saline containing 3% (vol/vol)

Table 1. *Subject characteristics*

Subject Characteristics	Metformin (n = 9; 6 women, 3 men)	Placebo (n = 7; 5 women, 2 men)	P Value
Age, yr	33 ± 9.5	32 ± 9.9	0.84
Weight, kg	87.1 ± 16.3	90.8 ± 23.1	0.73
BMI, kg/m^2	30.3 ± 4.7	31.0 ± 4.1	0.75
%Body fat	41.3 ± 7.0	36.4 ± 6.1	0.21
Glucose, mmol	5.4 ± 0.8	5.1 ± 0.5	0.65
Insulin, pmol	87.6 ± 40.4	115.8 ± 85.2	0.43
Insulin sensitivity index	2.8 ± 1.0	2.2 ± 1.0	0.26
$\dot{V}O_{2peak}$, $ml \cdot kg^{-1} \cdot min^{-1}$	26.8 ± 2.6	29.2 ± 5.7	0.43

Data are means ± SD for the baseline characteristics of 9 individuals (6 women and 3 men) in the metformin group and 7 individuals (5 women and 2 men) in the placebo group. $\dot{V}O_{2peak}$, peak oxygen consumption.

of the subject's own serum was started. Four minutes later, a variable infusion of 20% glucose plus 2% [6,6-^{2}H]glucose was started to maintain euglycemia at 5 mmol/l for 120 min. Blood samples were collected for glucose analysis every 5 min and for insulin, isotopic enrichment, and FFA at *minutes 15*, *30*, *45*, *60*, *75*, *90*, *105*, *110*, and *120* during the insulin infusion. Breath samples were collected at rest between *minutes 60–70* and *105–120* of the clamp for determination of oxidative and nonoxidative glucose disposal. Values obtained during the last 30 min of the clamp were used for comparison across and between groups.

Blood sample collection and analysis. Blood samples were collected in syringes, transferred to vaccutainers, spun at 3,000 rpm for 15 min, and aliquoted to cryotubes for storage at −80°C. Samples for analysis of isotopic enrichment, glucose, and lactate were transferred to vacutainers containing sodium fluoride. Samples for analysis of insulin and FFA were collected in vacutainers containing EDTA. Plasma glucose and concentrations were determined enzymatically using a glucose/lactate analyzer (GL5 Analyzer; Analox Instruments, Lunenberg, MA). Plasma insulin concentrations were measured by radioimmunoassay (Linco Research, St. Charles, MO).

Glucose isotopic enrichment was measured by high-performance liquid chromatography-mass spectrometry, as described previously (20).

Preparation of skeletal muscle tissue lysates. Muscles were pulverized at the temperature of liquid nitrogen and then homogenized with a Polytron (Brinkmann Instruments) on ice in lysis buffer (20 mM Tris, pH 7.5, 5 mM EDTA, 10 mM Na_3PO_4, 100 mM NaF, 2 mM $NaVO_4$, 1% Nonidet P-40, 10 μM leupeptin, 3 mM benzamidine, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Homogenates were rotated end over end for 1 h at 4°C and centrifuged at 14,000 *g* for 20 min at 4°C. The supernatants were collected and the protein concentrations determined by the Bradford method using a dye reagent from Bio-Rad (Hercules, CA). Muscle lysates were aliquoted, snap-frozen in liquid nitrogen, and stored at −80°C for immunoblots and for the AMPK activity assay.

Immunoblotting. To determine protein concentrations, equal amounts of muscle lysates (20 μg of protein) were resolved by SDS-PAGE (8% polyacrylamide), transferred to nitrocellulose membranes, and blocked for 1 h at room temperature in Tris-buffered saline (10 mM Tris and 150 mM NaCl, pH 7.8) containing 0.05% Tween-20 and either 5% nonfat dry milk or 5% bovine serum albumin. Membranes were then incubated overnight at 4°C in the appropriate primary antibodies against GLUT4, glycogen synthase (GS; Chemicon International), AMPK pan-α (made in the Goodyear Laboratory), and phosphorylated acetyl-CoA carboxylase (phospho-ACC)-Ser^{79} (Upstate). ACC was detected using streptavidin-horseradish peroxidase. Membranes were probed with horseradish peroxidase-conjugated secondary antibody and visualized using an enhanced chemiluminescence system (PerkinElmer, Wellesley, MA). Bands were scanned and quantified by densitometry.

AMPKα2 activity assay. Muscle lysates (200 μg of protein) were immunoprecipitated with isoform-specific antibodies to the α2 catalytic subunits of AMPK. These are antipeptide antibodies made to the amino acid sequence SAAGLHRPRSSVDSS (491–505) of α2. Immnoprecipitates were washed in lysis buffer and wash buffer (240 mM HEPES and 480 mM NaCl). Kinase reactions were performed in 40 mM HEPES (pH 7.0), 0.2 mM SAMS peptide (synthetic substrate for AMPK), 0.2 mM AMP, 80 mM NaCl, 0.8 mM dithiothreiol, 5 mM $MgCl_2$, and 0.2 mM ATP (containing 2 μCi [α-^{32}]ATP) in a final volume of 40 μl for 20 min at 30°C. At the end of the reaction, a 20-μl aliquot was removed and spotted on Whatman P81 paper. The papers were washed six times in 1% phosphoric acid and one time with acetone. Radioactivity was quantified with a scintillation counter. Activity was expressed as picomoles phosphate incorporated per minute per milligram lysate protein immunoprecipitated.

Calculations. Whole body insulin sensitivity is defined as the rate of blood glucose uptake (R_d) per unit plasma insulin concentration. Glucose rates of appearance and disappearance were calculated using the non-steady-state equations derived by Wolfe (42). Carbohydrate and fat oxidation rates were calculated from the oxygen consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) using the formulas of Péronnet and Massicotte (33). Hepatic insulin sensitivity is defined as suppression of basal hepatic glucose production (HGP_{basal}) during the clamp and was calculated as $1 - (HGP_{inf}/HGP_{basal}) \times 100$. HGP_{basal} is the glucose rate of appearance in the basal state. HGP during the infusion (HGP_{inf}) is calculated as (steady-state glucose R_a) − (glucose infusion rate). Percent suppression of FFA during insulin stimulation is defined as suppression of circulating FFA during the last 60 min of the clamp compared with basal values and was calculated as (FFA_{clamp}/FFA_{basal}) × 100.

Statistics. Means and standard deviations are presented for subject (Table 1) and exercise characteristics (Table 2). Data are expressed as means ± SE for all other outcome measures. A repeated-measures model (a.k.a. random effects model) was fitted to each outcome parameter. Differences between parameters were calculated from *Z*-tests, and the representative *P* value is reported. Time and treatment (metformin vs. placebo) were modeled as individual factors. Because we found that baseline whole body insulin sensitivity was quantifiably different, we included baseline insulin sensitivity as a covariate in the model to assess the independent effect of exercise. Tukey's post hoc analysis was used to detect the location of group mean differences when there was a significant interaction or main effect. Appropriate adjustments were made for multiple comparisons. Pearson's product-moment correlation coefficient was used to examine the relationships between FFA, lactate, and basal hepatic glucose production as well as FFA and insulin sensitivity.

RESULTS

Exercise characteristics. $\dot{V}O_2$, heart rate, intensity, and rate of perceived exertion during exercise with metformin and placebo are shown in Table 2. There were no group differences in any of these exercise parameters during *minutes 0–30* (moderate intensity) or the last 10 min (intense) of exercise. Since there were no differences between the metformin and placebo groups in any of the measured exercise characteristics, any differences in the main outcome variables are not likely due to differences in exercise intensity.

Table 2. *Exercise characteristics*

	Metformin	Placebo	*P* Value
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}			
0–30	17.4 ± 3.8	18.9 ± 3.0	0.39
30–40	23.1 ± 4.7	24.0 ± 5.7	0.78
$\dot{V}O_2$, %$\dot{V}O_{2peak}$			
0–30	65.8 ± 3.7	66.1 ± 4.5	0.96
30–40	85.0 ± 9.3	80.2 ± 5.2	0.48
Heart rate, beats/min			
0–30	148.6 ± 13.1	141.0 ± 10.0	0.25
30–40	165.9 ± 15.1	161.3 ± 13.6	0.61
Watts			
0–30	91.6 ± 22.4	99.6 ± 23.0	0.50
30–40	120.4 ± 32.0	121.3 ± 34.2	0.96
RPE			
0–30	14.2 ± 1.4	13.8 ± 2.0	0.71
30–40	16.7 ± 1.6	15.6 ± 2.9	0.49

Data are means ± SD for the exercise responses during steady-state exercise. RPE, rate of perceived exertion. Participants exercised for 30 min at 65% $\dot{V}O_{2peak}$ (0–30) and for 10 min more at 85% $\dot{V}O_{2peak}$ (30–40). Relative $\dot{V}O_2$ was measured during steady-state exercise using indirect calorimetry. Heart rate, watts, and RPE were recorded at the end of each minute during exercise.

Table 3. *Fasting basal insulin, glucose, and lactate*

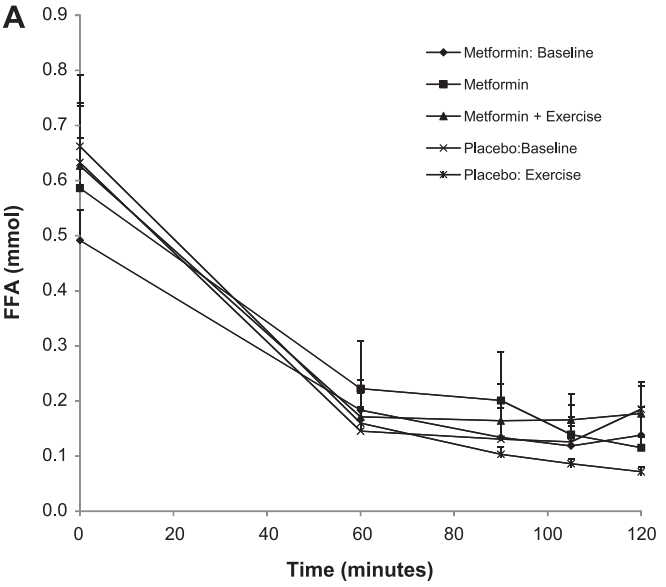
	Metformin			Placebo	
	Baseline	Metformin	Metformin + Exercise	Baseline	Exercise
Glucose, mM	5.4 ± 0.2	5.4 ± 0.2	5.5 ± 0.2	5.3 ± 0.2	4.9 ± 0.2
Insulin, μU/ml	14.6 ± 2.0	13.5 ± 1.6	14.7 ± 2.1	15.4 ± 3.7	14.6 ± 4.6
Lactate, mM	1.0 ± 0.1	1.1 ± 0.1	3.0 ± 0.6*	0.7 ± 0.1	1.2 ± 0.2

Data are means ± SD. Blood samples were measured 30 min after rest (baseline and metformin) or exercise while the participants were still fasting. *Significantly different from baseline, metformin, and exercise ($P < 0.0001$).

Effect of metformin plus exercise on resting and fasting glucose, insulin, and lactate. Resting glucose, insulin, and lactate concentrations were measured 30 min after exercise or at rest in all conditions (Table 3). Plasma glucose and insulin concentrations were not altered by exercise alone, metformin plus exercise, or metformin alone. Exercise alone did not alter plasma lactate in the resting fasted state, but exercise in the metformin-treated group increased lactate concentrations threefold relative to any of the other conditions.

Effect of metformin plus exercise on whole body insulin sensitivity. Unexpectedly, whole body insulin sensitivity at baseline (before treatment) was >50% higher in the metformin group compared with the placebo group (4.8 ± 1.0 vs. 7.5 ± 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$, respectively), although this difference was not statistically significant ($P = 0.65$). Steady-state plasma insulin concentrations were not different between the placebo and metformin groups; therefore, the difference in baseline insulin sensitivity was explained completely by a significantly lower steady-state glucose R_d in the placebo group compared with the metformin groups ($P = 0.001$). Exercise alone increased whole body insulin sensitivity by 52% in the placebo group ($P = 0.01$). However, when exercise was added to the metformin group there was no change in insulin sensitivity (Fig. 1). The same pattern was observed when metabolic clearance rate was calculated by scaling glucose R_d to the prevailing glucose concentration (data not shown).

Effect of metformin plus exercise on suppression of FFA and fat oxidation during the clamp. Resting fasted plasma FFA and steady-state plasma FFA during the clamp were not different between groups or conditions (Fig. 2*A*). Exercise lowered steady-state FFA concentrations during the clamp in the pla-



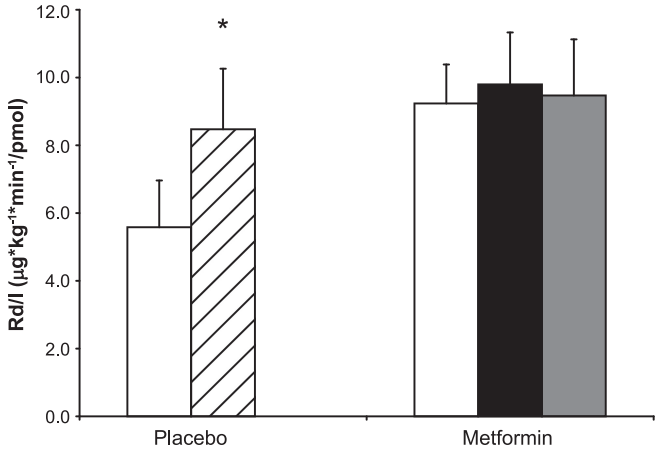


Fig. 1. Effect of metformin + exercise on whole body insulin sensitivity. Insulin sensitivity was measured during the last 30 min of the euglycemic hyperinsulinemic clamp and is defined as the rate of disappearance of glucose per steady-state plasma insulin. Open bars, baseline; hatched bar, exercise only; black bar, metformin; gray bar, metformin + exercise. *Significantly different from placebo baseline ($P < 0.05$). R_d/I, rate of blood glucose uptake per unit plasma insulin concentration.

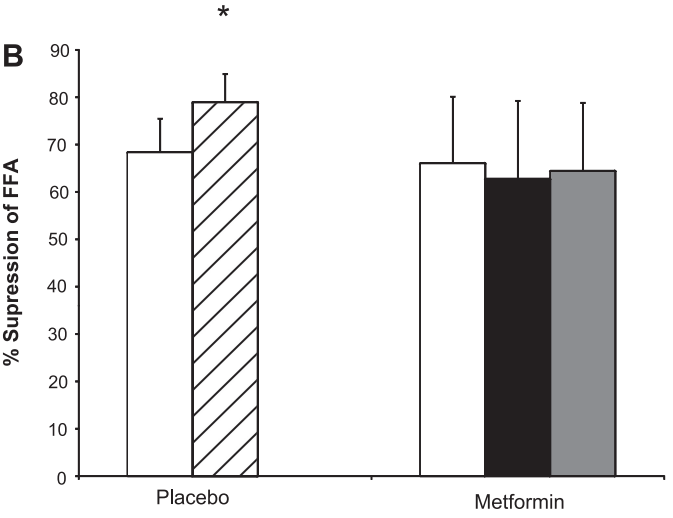


Fig. 2. Effect of metformin + exercise on basal and insulin-stimulated free fatty acid (FFA) concentrations (*A*) and suppression of FFA during the last 30 min of a euglycemic hyperinsulinemic clamp (*B*). Open bars, baseline; hatched bar, exercise only; black bar, metformin; gray bar, metformin + exercise. *Significantly different from placebo baseline ($P < 0.05$).

cebo group but not in the metformin group. Exercise caused a 15% suppression of FFA (an indirect measurement of adipose tissue insulin sensitivity) in the placebo group (Fig. 2*B*). Conversely, metformin plus exercise did not alter the suppression of FFA during insulin stimulation. We measured basal substrate oxidation 30 min after the muscle biopsy and at the end of the 120-min euglycemic hyperinsulinemic clamp. In the placebo group, exercise did not alter basal fat oxidation or carbohydrate oxidation compared with baseline values. However, metformin plus exercise increased basal fat oxidation compared with baseline values ($P = 0.009$; Fig. 3). Fat oxidation at the end of insulin stimulation was not different after exercise alone or metformin plus exercise (data not shown).

Skeletal muscle AMPKα2 activity and glycogen. In the placebo group, exercise alone increased AMPKα2 activity by 300% ($P = 0.0001$; Fig. 4*A*), whereas metformin plus exercise resulted in a smaller (33%) increase in activity. Metformin alone did not alter AMPKα2 activity. The increase in AMPKα2 with exercise was not strongly correlated with changes in whole body insulin sensitivity ($r^2 = 0.22$). Since changes in insulin sensitivity and AMPKα2 activity have been shown to be influenced by muscle glycogen concentrations, we also measured muscle glycogen after metformin treatment and after exercise in both groups. In the placebo group, exercise alone lowered muscle glycogen by 37% (Fig. 4*B*). Similarly, muscle glycogen was reduced by 48% with metformin plus exercise.

Protein content and phosphorylation in muscle. We measured glucose transporter 4 (GLUT4) protein content to determine whether differences in GLUT4 protein content may be responsible for the differences in whole body insulin action. In the metformin group, neither metformin nor metformin plus exercise altered GLUT4 protein content. In the placebo group, there was no effect of exercise on GLUT4 protein content (Table 4). Thus, it does not seem likely that the differences in whole body insulin sensitivity can be explained by differences in GLUT4 protein.

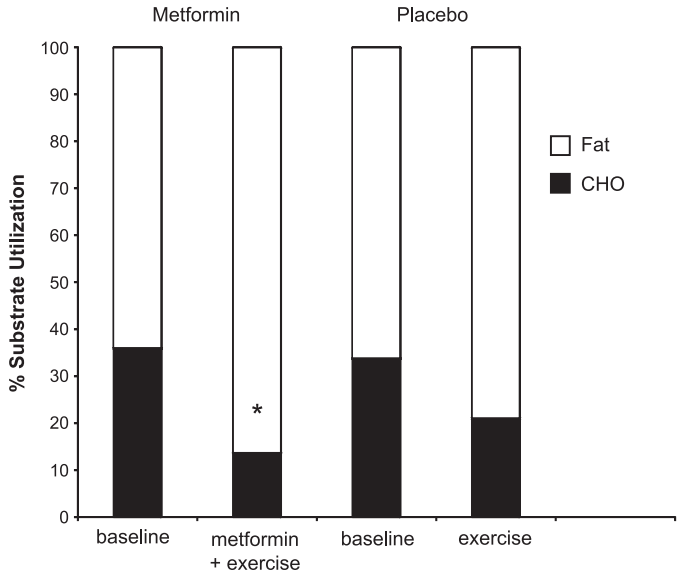


Fig. 3. Basal whole body substrate oxidation after exercise. Substrate oxidation was measured 30 min after exercise using indirect calorimetry. *Significantly different from metformin baseline ($P < 0.05$). CHO, carbohydrate.

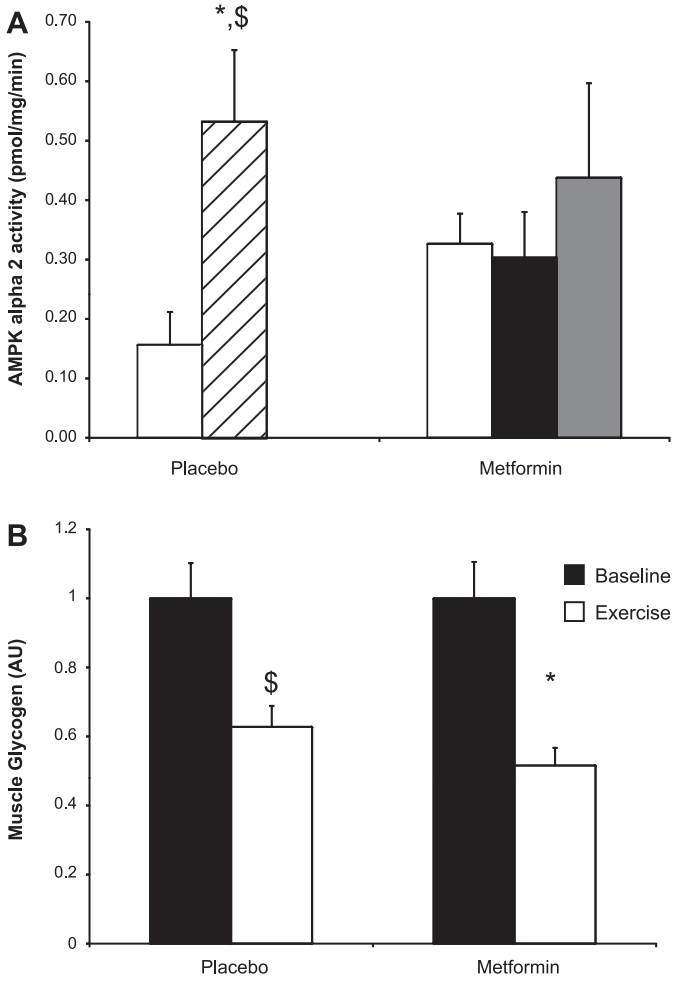


Fig. 4. Skeletal muscle AMP-activated protein kinase (AMPK)α2 activity (*A*) and muscle glycogen concentrations (*B*) measured in skeletal muscle biopsies taken immediately after exercise or rest. Open bars, baseline; hatched bar, exercise only; black bars, metformin; gray bar, metformin + exercise. *Significantly different from placebo baseline ($P < 0.0001$); $significantly different from metformin + exercise ($P < 0.0001$). AU, arbitrary units.

We determined whether differences in basal substrate oxidation could be explained by differences in protein abundance of ACC, phospho-ACC, or GS. There was no effect of metformin or metformin plus exercise on total ACC or GS protein content. Similarly, exercise alone did not alter the protein abundance of total ACC or GS. There was a significant increase in phospho-ACC with exercise compared with baseline values in the placebo group. Again, like AMPKα2 activity, metformin plus exercise attenuated this increase, resulting in only a 50% increase in phospho-ACC compared with baseline and metformin alone. Given that ACC is a downstream substrate of AMPK, it is likely that the group differences in AMPKα2 activity are responsible for the differences in ACC phosphorylation.

Basal hepatic glucose production and hepatic insulin sensitivity. Basal hepatic glucose production was measured 2 h after the muscle biopsy in all conditions. In the metformin group, metformin did not alter basal hepatic glucose production. However, metformin plus exercise increased basal hepatic glucose production by 25% ($P = 0.07$). In the placebo group, exercise decreased basal hepatic glucose production 25% (Fig. 5), an

Table 4. *Muscle protein content and phosphorylation*

	Baseline	Metformin	Metformin + Exercise	Exercise
GLUT4	1.00 ± 0.14	0.89 ± 0.08	0.95 ± 0.06	1.15 ± 0.19
GS	1.00 ± 0.07	1.01 ± 0.09	1.06 ± 0.16	0.94 ± 0.25
ACC	1.00 ± 0.12	1.11 ± 0.23	0.92 ± 0.13	0.93 ± 0.25
ACC-P	1.00 ± 0.13	1.28 ± 0.21	1.56 ± 0.27	2.00 ± 0.35*
AMPK	1.00 ± 0.22	0.96 ± 0.18	0.98 ± 0.11	1.16 ± 0.28

Data are means ± SD and are expressed relative to baseline values for each group. GLUT4, glucose transporter 4; GS, glycogen synthase; ACC, acetyl-CoA carboxylase; ACC-P, phosphorylated ACC; AMPK, AMP-activated protein kinase. Total protein for GLUT4, GS, ACC, AMPK, and ACC-P was measured from skeletal muscle biopsies taken after 30 min of rest or immediately after exercise. *Significantly different from baseline ($P < 0.01$).

effect that was significantly different from and opposite in action to the effect of metformin plus exercise ($P = 0.002$). There was no effect of exercise, metformin, or metformin plus exercise on hepatic insulin sensitivity.

DISCUSSION

The central aim of this study was to assess the combined effect of short-term metformin treatment plus a single bout of exercise on whole body insulin sensitivity in insulin-resistant individuals. We reasoned that, because metformin and exercise target different tissues (i.e., liver vs. skeletal muscle), and because both stimulate AMPKα2, additive effects on insulin action were likely. Instead, we found that the combination of exercise and metformin did not increase whole body insulin sensitivity above baseline values, a finding that is dissimilar from the well-documented effects of exercise alone. There are several potential explanations for this unexpected result. First, the exercise bout may have been inadequate to elicit a change in whole body insulin sensitivity; i.e., the exercise dose may have been too low. This is unlikely since exercise alone increased whole body insulin sensitivity by 54% in the placebo group, mimicking results from other studies where the exercise bout was similar in duration and intensity (4, 9). Our results are most similar to those published by Burstein et al. (4), who reported a 45% increase in insulin action after 1 h of aerobic treadmill walking in obese nondiabetic individuals. Alternatively, the baseline insulin sensitivity in the metformin plus exercise group may have been too high to be accentuated by a single exercise bout. This explanation is also unlikely since a single bout of exercise, similar to that used in our study, consistently improves insulin sensitivity in healthy, lean individuals who are more insulin sensitive at baseline than our metformin plus exercise group (30, 34). Overall, our placebo group data recapitulated the common finding that a single bout of exercise increases whole body insulin sensitivity. The novel result was that this well-known effect disappeared when exercise was performed in conjunction with metformin treatment.

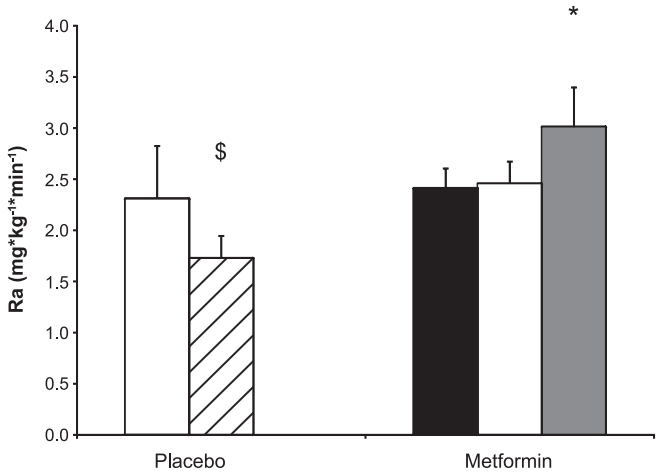


Fig. 5. Basal rate of glucose appearance (R_a). R_a was measured in the 2-h period after exercise. Open bars, baseline; hatched bar, exercise only; black bar, metformin; gray bar, metformin + exercise. $Significantly different from metformin + exercise ($P < 0.005$); *significantly different from metformin baseline and metformin only ($P < 0.05$).

Exercise and metformin independently activate AMPKα2 in skeletal muscle (16, 31). Prior activation of AMPK, either by contraction or stimulation with the chemical 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, increases skeletal muscle insulin sensitivity (15, 23), suggesting that AMPK is a key mediator of postexercise insulin sensitivity (31). We found significantly less AMPKα2 activation with metformin plus exercise compared with exercise alone. It is possible that the absence of improvement in whole body insulin sensitivity was due to lack of AMPK activation. This explanation implies that something about the exercise bout was different when it was performed against a background of metformin compared with placebo. AMPK activation and postexercise insulin sensitivity are both, at least partially, regulated by a change in muscle glycogen concentration (6, 41). However, muscle glycogen concentrations were similarly decreased in the placebo and metformin groups, suggesting a comparable stimulus to AMPK. Other variables such as exercise intensity, body composition, and sex are known to impact AMPK activity during exercise (36, 38), but these are unlikely to be confounding variables because we ensured that they were similar across groups. AMPK activation is dependent on the AMP concentrations in the skeletal muscle and is altered by high glucose and free fatty acid availability (1, 40). It is possible that the combination of metformin and exercise reduced intracellular AMP and/or raised substrate availability during exercise. Unfortunately, we do not have measurements of these variables, so this potential explanation cannot be confirmed or denied.

Metformin treatment alone did not increase whole body insulin sensitivity or AMPK activity in the current study, so it was reasonable to expect little or no additivity when metformin and exercise were combined. In previous studies, metformin alone has had inconsistent effects on insulin sensitivity with increases from 0 (8) to as much as 30% (13, 32, 39). Long-term metformin treatment can induce subtle but potentially relevant weight loss (~5–7 kg) that can contribute to enhanced whole body insulin sensitivity (10). Metformin treatment did not cause weight loss in the current study, which may be a reason why there was no independent effect of metformin on insulin

action. Additionally, the metformin treatment lasted 3 wk, which was shorter than in the few prior studies (32). However, 3 wk of metformin alone was sufficient time to observe measurable increases in nonoxidative glucose disposal and interactions with exercise on AMPKα2 activity, basal hepatic glucose production, and lactate concentrations. These effects suggest that 3 wk of metformin treatment has a measureable impact on metabolic pathways related to insulin sensitivity, and therefore, it is unlikely that the lack of additivity was ascribable to insufficient duration of treatment.

Finding no further increase in insulin sensitivity when metformin is added to a single bout of exercise is not unique to our study. Hallsten et al. (17) reported that metformin treatment combined with isometric exercise did not increase muscle glucose uptake during an insulin infusion compared with exercise and insulin alone in individuals with type 2 diabetes. However, unlike our study, the exercise session in these previous studies had no effect on enhancing glucose disposal over insulin infusion alone, so a direct comparison to our study is impossible. In high-fat-fed Zucker rats, chronic metformin treatment combined with exercise training did not enhance insulin-stimulated glucose uptake more than exercise training alone (37).

Our study shows that not only is there no additivity with exercise, but the characteristic effect of an exercise bout to improve insulin action may be considerably attenuated (or even abolished) in insulin-resistant humans taking metformin. This unexpected result may be related to unpredictable effects of combining medications (e.g., metformin and exercise). In the fields of pharmacology and toxicology, it is common for a drug or intervention to have stimulatory effects at low doses and inhibitory effects at high doses (known as hormesis), resulting in decidedly nonlinear dose-response curves (5). In our case, it is possible that the combined dose of metformin plus exercise was sufficiently high to inhibit the typical exercise effect by desensitizing the pathways where metformin and exercise overlap. We prescribed 1,000 mg twice/day because this dose was shown to activate AMPK in human skeletal muscle. This dose is on the high end of the common clinical range (1,500–2,000 mg/day). Given that both metformin and exercise activate AMPK, the combination may have paradoxically downregulated a pathway that is stimulated with just a single intervention. The combination of metformin and exercise could have inhibited a signaling molecule(s) required for improved insulin sensitivity. A notable example of this hormetic effect was recently reported in a study showing that improvements in whole body insulin sensitivity were completely prevented when exercise training was combined with antioxidant supplementation in individuals with type 2 diabetes (35). Although we are not able to define the mechanism responsible in our study, it is clear that the drug-exercise interactions are poorly understood, complex, and worthy of more systematic study.

Skeletal muscle can readily switch between fatty acids and glucose for oxidation, depending on substrate availability. High concentrations of circulating free fatty acids, while predominantly being shunted toward oxidation, also decrease insulin signaling by downregulating key enzymes responsible for glucose uptake/storage (e.g., pyruvate dehydrogenase and phosphofructokinase) (14, 26). In the present study, circulating concentrations of free fatty acids during the glucose clamp were higher when exercise was combined with metformin compared with exercise alone. We found a significant negative relationship between plasma free fatty acid concentrations and insulin sensitivity, suggesting that elevated circulating free fatty acids could partially explain the decrease in insulin sensitivity with metformin and exercise (data not shown). In one study, metformin decreased the antilipolytic effects of insulin and prevented the decline in fat oxidation typically observed during insulin stimulation (7). These effects may be accentuated after exercise when high circulating catecholamine concentrations cause mobilization of stored lipid and a sharp rise in fat oxidation (18). Elevating circulating free fatty acids decreases insulin action and reduces insulin signaling (25). It is quite possible that combining metformin and exercise increases fatty acid availability after exercise, competitively inhibiting uptake of glucose and blunting whole body insulin sensitivity.

The combination of metformin and exercise significantly elevated circulating plasma lactate concentrations 30 min after exercise. Typically, circulating lactate concentrations would be at or near baseline values by 30 min postexercise. Metformin has been shown to increase lactate concentrations in the fasted state and during a euglycemic hyperinsulinemic clamp (12). In our study, metformin did not increase plasma lactate concentrations in the fasted state, but exercise above 65% $\dot{V}O_{2max}$ increases lactate production and clearance. Thus, the combination of metformin and exercise could have altered muscle lactate production, hepatic clearance, or both. Greater lactate availability provides more gluconeogenic precursors and could raise hepatic glucose production (22). Consistent with this scenario, exercise alone reduced basal hepatic glucose production, but the addition of metformin caused the opposite response. Increased lactate availability may have stimulated gluconeogenesis and driven greater hepatic glucose output. Alternatively, the increase in basal hepatic glucose production with metformin and exercise could be related to the higher circulating free fatty acid concentrations. Elevated free fatty acids, which we observed in the exercise plus metformin condition, also increase hepatic gluconeogenesis. Therefore, it is possible that both higher circulating free fatty acids and elevated lactate concentrations contributed to increased hepatic glucose production.

In summary, we found that when a single bout of exercise was performed by insulin-resistant humans pretreated with metformin, the expected enhancement of insulin sensitivity was attenuated considerably. This result may be explained by parallel attenuation of AMPKα2 activation when exercise was combined with metformin and/or by the increase in postexercise fat oxidation with metformin and exercise. These results suggest that metformin treatment opposed the effects of exercise alone. However, it is important to note that these results are specific to the conditions used in this study, i.e., short-term metformin treatment and single bout of exercise in men and women who are insulin resistant but not hyperglycemic. The results could be different if the metformin and exercise interventions are extended (i.e., long-term drug therapy combined with exercise training) or done in individuals who have diagnosed type 2 diabetes. In addition, we found that metformin and exercise caused a significant elevation in circulating plasma lactate concentrations after exercise, a side effect that may be undesired in individuals with type 2 diabetes. Because

current clinical recommendations focus on increased physical activity and metformin as two cornerstone therapies for prediabetes and type 2 diabetes, results from the current study have direct clinical relevance. Although it would be very premature to adopt the message that metformin treatment renders exercise less effective, our findings strongly imply that the clinical impacts of combining exercise and metformin treatment are likely to be complex. These results reinforce the idea that drug-exercise interactions are not predictable from their individual effects and should be studied systematically to provide important public health information.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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