



The Effect of Metformin on Glucose Homeostasis During Moderate Exercise

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Diabetes Care 2015;38:293–301 | DOI: 10.2337/dc14-1480

OBJECTIVE

We investigated the role of metformin on glucose kinetics during moderate exercise.

RESEARCH DESIGN AND METHODS

Before, during, and after a 45-min bout of exercise at 60% VO_{2max} , glucose kinetics were determined by isotope tracer technique in patients with type 2 diabetes mellitus with metformin treatment (DM2+Met) or without metformin treatment (DM2) and in healthy control subjects (CON) matched for BMI and age. Glucoregulatory hormones and metabolites were measured throughout the study.

RESULTS

Plasma glucose concentration was unchanged during exercise in CON but decreased in DM2. No significant change was found in DM2+Met. Hormones and metabolites showed no differences among the groups except for elevated exercise-induced concentrations of lactate in DM2 (area under the curve [AUC] $31 \pm 1\%$ vs. CON) and glucagon in DM2 (AUC $5 \pm 1\%$ vs. DM2+Met). Free fatty acid levels were lower in DM2+Met than in DM2 (AUC $-14 \pm 1\%$). Absolute values of the baseline glucose rate of appearance (Ra) were elevated in DM2 and DM2+Met, but the increase in glucose Ra relative to baseline was blunted in DM2 ($19 \pm 1\%$) and DM2+Met ($18 \pm 4\%$) compared with CON ($46 \pm 4\%$). Glucose rate of disappearance relative to baseline increased more in CON ($31 \pm 3\%$) than in DM2 ($6 \pm 1\%$) and DM2+Met ($21 \pm 2\%$), showing a small increase caused by metformin. Glucose metabolic clearance rate relative to baseline was similar during exercise in DM2 ($33 \pm 1\%$) and CON ($35 \pm 3\%$) but was improved in DM2+Met ($37 \pm 3\%$) compared with DM2.

CONCLUSIONS

Metformin has a positive effect on glucose homeostasis during exercise.

Exercise and weight loss are the cornerstones of prevention and treatment of type 2 diabetes (1–3). Apart from supporting a reduction in body weight, regularly performed exercise training markedly increases skeletal muscle insulin-mediated glucose uptake (4–7). Consequently, the knowledge of glucose homeostasis in type 2 diabetic patients during exercise is highly relevant when defining treatment guidelines.

In type 2 diabetes, the regulation of hepatic glucose production (HGP) is altered. At rest in the fasting state, the fraction of gluconeogenesis is higher (65–70% of HGP). Glucose tracer studies have reported inconsistent data on the effect of

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Received 18 June 2014 and accepted 27 October 2014.

Clinical trial reg. no. NCT01765894, clinicaltrials.gov.

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-1480/-/DC1>.

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moderate exercise on HGP in patients with type 2 diabetes. In the 1980s, two studies showed that during moderate-intensity exercise, plasma insulin did not decrease; HGP failed to increase; and thus, plasma glucose concentrations declined (8,9). Similar observations were made by Hübinger et al. (10) but without estimation of HGP. In the postprandial state, exercise has been demonstrated not to increase HGP in hyperinsulinemic patients with type 2 diabetes (11). In contrast, other studies found similar increases in HGP in patients with type 2 diabetes and in healthy control subjects during moderate-intensity exercise (12,13).

Metformin is a biguanide and the most prescribed oral antidiabetic drug in the treatment of type 2 diabetes. It has a documented effect on glucose homeostasis by reducing plasma glucose and on HbA_{1c} by reducing gluconeogenesis (14–18). The mechanisms of action are not fully understood, but a primary one is the reduction of a gluconeogenic precursor through diminished lactate uptake by the liver. Another possible action is metformin increasing peripheral insulin sensitivity through AMPK activation in skeletal muscle (19), although many studies failed to observe this (14,20–22). A recent study has documented that metformin inhibits gluca-gon signaling (23).

HGP is decreased by 12–26% in the postabsorptive state of metformin 1 g two times/day (14,15,22). A study of patients with type 2 diabetes already treated with sulfonylureas also showed a decrease in HGP of 64% after adding metformin 500 mg two times/day (19). However, others (24) have found that metformin does not alter basal HGP compared with placebo (after treatment with metformin 1 g two times/day for 3 weeks), and another study (21) found no difference in basal HGP in prediabetic subjects after treatment with metformin 1 g two times/day for 12 weeks.

The influence of metformin on HGP and glucose homeostasis during exercise in patients with type 2 diabetes has only been studied sparsely. A similar HGP during exercise (25) and an increase in HGP after moderate exercise (24) (65% VO_{2max}, 45 min) was observed in patients treated with metformin 1 g two times/day versus placebo/no treatment, respectively. Adding metformin

to exercise is reported to have no additional effect on insulin sensitivity in patients with impaired glucose tolerance (21). Consequently, how the combination of metformin and exercise exerts an effect on glucose homeostasis is not clear, and to our knowledge, no studies have applied isotopic measurement of HGP during a single bout of moderate-intensity exercise in patients with type 2 diabetes treated with and without metformin. Because both treatment modalities are recommended and used extensively, we aimed to investigate whether one treatment negates or amplifies the other, ultimately to avoid the recommendation of combined treatment modalities, which could induce hypoglycemia or worsen glycemic control. We hypothesized that glucose homeostasis during exercise would be perturbed by a diminished increase in HGP in patients with type 2 diabetes compared with healthy control subjects and that HGP would be decreased in patients treated with metformin compared with an exercise condition without concomitant metformin treatment.

RESEARCH DESIGN AND METHODS

Subjects

We recruited 11 male patients with type 2 diabetes treated with metformin twice daily since diagnosis (3.3 ± 1 years). They were studied with metformin treatment (DM2+Met) and without metformin treatment (DM2) were compared with a healthy male control subjects (CON) ($n = 10$).

None of the subjects had any clinical or biochemical evidence of hepatic, renal, or cardiovascular disease and no diabetes-related complications. The order of experiments with and without metformin was random in a unblinded crossover fashion, and there was at least 1 week between the two. Medication in the groups was prescribed by the patients' general practitioner; this study prescribed no new medication. Among the patients who participated in both DM2 and DM2+Met, five received statins, and three received other antidiabetic agents (two liraglutide and one vildagliptin). Two CON and six DM2/DM2+Met received antihypertensive medication. All subjects had stable weight at least 2 months before the study. The study was carried out in accordance with the Declaration of Helsinki, and the protocol was

approved by the local Ethics Committee of Copenhagen (Protocol: H-1-2012-074).

Protocol

All subjects gave written informed consent and participated in 2 test days separated by 1 week. On test day A, a DEXA scan (Lunar iDXA Series; GE Medical Systems, Madison, WI) was performed to measure body composition, and International Physical Activity Questionnaire (IPAQ) scoring was obtained to categorize individual activity level. A clinical examination was performed, including electrocardiogram. Steady-state oxygen consumption, heart rate, and respiratory exchange ratio (RER) were measured during exercise (at 80 and 130 W) on an ergometer stationary bike (Monark 839E; Monark Exercise AB, Vansbro, Sweden) using an oxycon system (Jaeger Oxycon Pro; VIASYS Healthcare, Hoechberg, Germany). This was followed by measurement of VO_{2max} (10-min warm-up period at 75 W + 25 W/min until exhaustion). Criteria for achieving VO_{2max} were plateau in oxygen consumption despite increasing workloads and/or an RER >1.05.

Three days before test day B, all subjects consumed at least 250 g carbohydrates per day and abstained from alcohol and vigorous exercise. All subjects presented in the morning after an 11-h overnight fast. DM2+Met subjects paused all other medications besides metformin for 3 days up to test day B (last metformin dose taken in the morning of the test day [eight subjects 1 g and three subjects 0.5 g]) (Table 1). CON and DM2 were asked to pause all medications 3 days before test day B.

The patients were weighed and positioned sitting in a bed. An antecubital intravenous cannula was placed for infusing 6,6D₂ glucose isotopes (Cambridge Isotope Laboratories by Regional Pharmacy of Copenhagen, Denmark), starting at $t = -150$. Before the isotope infusion, background samples for isotopes were drawn. Subsequently, a bolus of 23 μmol 6,6D₂ glucose/kg fat free mass (FFM) was given followed by a continuous infusion of 0.825 μmol /FFM/min throughout the rest of the study. If the fasting blood glucose concentration was >5 mmol/L, the prime bolus was increased with a factor [blood glucose (mmol/L) $\cdot 5^{-1}$]. An arterial catheter was inserted in the radial or brachial artery for continuous blood pressure monitoring and blood sampling. Baseline

Table 1—Baseline characteristics and measures during exercise ensuring the same work intensity

	Baseline			Exercise (steady state)		
	CON (n = 10)	DM2 (n = 11)	DM2+Met (n = 11)	CON	DM2	DM2+Met
Age (years)	51 ± 1	53 ± 1	53 ± 1	—	—	—
Fasting glucose (mmol/L)	5.9 ± 0.1	9.6 ± 0.8*	8.9 ± 0.7*	—	—	—
HbA _{1c} (%)	5.5 ± 0.1	7.1 ± 0.3*	6.9 ± 0.3*	—	—	—
HbA _{1c} (mmol/mol)	37 ± 1	53 ± 3*	52 ± 3*	—	—	—
HOMA-IR†	1.1 ± 0.1	2.1 ± 0.2*	1.6 ± 0.2*§	—	—	—
BMI (kg/m ²)	30.7 ± 0.8	30.9 ± 0.8	30.9 ± 0.7	—	—	—
Lean body mass (kg)	64.1 ± 2.2	67.5 ± 1.7	67.5 ± 1.7	—	—	—
Visceral fat (kg)	2.1 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	—	—	—
IPAQ (MET-min/week)	2,071 ± 511	2,688 ± 713	2,688 ± 713	—	—	—
VO _{2max} (mL/min)	3,483 ± 194	3,234 ± 136	3,234 ± 136	—	—	—
VO _{2max} (mL/min/kg)	36 ± 1	32 ± 1	32 ± 1	—	—	—
% of VO _{2max}	—	—	—	60 ± 1	59 ± 1	59 ± 1
Workload (W)	—	—	—	112 ± 5	102 ± 4	101 ± 5
VO ₂ (mL/min)	222 ± 7	237 ± 11	248 ± 13	2,071 ± 116	1,915 ± 75	1,912 ± 80
RER (VCO ₂ /VO ₂)	0.84 ± 0.02	0.83 ± 0.02	0.82 ± 0.02	0.90 ± 0.01	0.93 ± 0.02	0.91 ± 0.01
Heart rate (/min)	55 ± 1	60 ± 2	61 ± 3	128 ± 3	131 ± 3	132 ± 3
HRR (%)	—	—	—	77 ± 2	79 ± 2	80 ± 1
MAP (mmHg)	103 ± 3	106 ± 2	107 ± 2	90 ± 2	99 ± 3	94 ± 3
Medication (n)‡						
Metformin 1 g three times/day	—	1	1			
Metformin 1 g two times/day	—	7	7			
Metformin 500 mg two times/day	—	3	3			

Data are mean ± SE or n. Basal RER was measured by canopy-ventilated hood technique, and exercise RER was measured by an oxycon system connected to a nose-and-mouth-covering mask. HOMA-IR, HOMA insulin resistance; HRR, heart rate reserve; IPAQ, International Physical Activity Questionnaire, short version; MAP, mean arterial pressure. *Significant difference from CON ($P < 0.05$). †HOMA2 calculator used with fasting glucose and C-peptide data. §Significant difference between DM2 and DM2+Met ($P < 0.05$). ‡All groups paused all medication 3 days before the test day. DM2+Met only continued with usual metformin medication. All 11 DM2+Met subjects also participated in DM2.

samples were drawn at $t = -120$ and $t = -100$. Resting RER, heart rate, and oxygen consumption was measured using a ventilated hood system (Jaeger Oxycon Pro; In-tramedic, Hoechberg, Germany) while the subjects were in a supine, relaxed, and awake position. At $t = -20$, -10 , and 0 , blood samples were drawn for later calculation of baseline glucose rate of appearance (Ra) and rate of disappearance (Rd).

Each subject next performed 45 min of exercise at 60% VO_{2max} on a bicycle ergometer, beginning with a warm-up period of 5 min at 75 W. During the exercise, arterial blood was drawn frequently for measurements of concentrations of glucose, isotope tracer/tracee ratio, metabolites, and hormones. Plasma glucose concentration, oxygen saturation, and hematocrit were measured every 5–10 min (ABL800 FLEX blood gas analyzer; Radiometer, Copenhagen, Denmark). The workload was controlled by frequent measurements of oxygen uptake. To avoid dehydration, isotonic saline 0.5 L was infused slowly, and the subjects had free access to water. Heart rate and blood pressure were monitored continuously

(LabChart; ADInstruments, Oxford, U.K.). Continuous measurement of gaseous exchange rates was carried out from $t = 30$ –40 min of the 45-min exercise bout (Table 1). After termination of exercise, the subjects were placed in a bed to relax for the next 60 min while isotope infusion and blood sampling continued.

Calculations and Statistics

All data are presented as mean ± SEM. Statistical significance was set at $P < 0.05$. During and after exercise, glucose Ra and Rd were calculated by Steele's non-steady-state equation as previously described (26). Volume of distribution was set to 70 mL/kg coherent to rapid changes in glucose concentrations and, hence, a small pool fraction. Isotope data were smoothed with sliding-fit curves (27). Baseline Ra and Rd were calculated using Steele's steady-state equation (28).

Area under the curve (AUC) and incremental AUC (iAUC) were calculated by the trapezoidal method. Statistical comparisons for baseline data, AUC, and iAUC were made using an unpaired t

test, and paired t test was used for comparison between DM2 and DM2+Met (same subjects). For other variables, comparisons between groups used two-way ANOVA with repeated measures followed by Student-Newman-Keuls post hoc test when a significant interaction was detected. When a normality test or an equal variance test failed, logarithmic transformation of data was applied. Analyses were performed with SigmaPlot 12.3 software (Systat Software Inc., San Jose, CA).

Analytical Methods

All blood samples were drawn from arterial blood and immediately centrifuged for 10 min (4°C, 2,000g). Plasma was frozen at -80°C until assay. The 6,6D₂ glucose isotope tracer/tracee ratio was determined by mass spectrometry (Sciex API 3000; Applied Biosystems, Foster City, CA). Concentrations of hormones in plasma were analyzed by ELISA technique as follows: adrenocorticotrophic hormone (ACTH) (IBL, Hamburg, Germany), cortisol (Demeditec, Kiel-Wellsee, Germany), C-peptide (ALPCO, Salem, NH),

growth hormone (GH) (IBL), glucagon (ALPCO), and insulin (Dako, Glostrup, Denmark). Substrates and metabolites in plasma were measured by spectrophotometry (Cobas 6000 c501; Roche, Glostrup, Denmark) as follows: free fatty acids (FFAs) (Wako, Neuss, Germany); glucose (Roche, Hvidovre, Denmark); glycerol (Randox, Crumlin, U.K.); β -hydroxybutyrate (Randox); and lactate, cholesterol, triglycerides, HDL, and LDL (all from Roche). HbA_{1c} was analyzed on a DCA Vantage Analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Body composition was determined by DEXA scanning (enCORE version 14.10.022 software; GE Medical Systems). Calculation of visceral adipose tissue was done automatically by DEXA (Corescan).

RESULTS

The groups were well matched according to age, BMI, VO_{2max} , percent body fat, lean body mass, visceral fat content, and daily activity level (Table 1). LDL and cholesterol levels were lower in type 2 diabetes patients (LDL: CON 3.7 ± 0.2 , DM2 2.6 ± 0.2 , DM2+Met 2.5 ± 0.2 ; cholesterol: CON 5.0 ± 0.2 , DM2 4.0 ± 0.2 , DM2+Met 4.0 ± 0.3), probably as a result of usual statin treatment. During exercise, all subjects performed the same relative work: 60% VO_{2max} corresponding to $\sim 80\%$ of heart rate reserve. Other parameters of physical stress during exercise were also the same in the three groups (mean arterial pressure, absolute workload, and RER) (Table 1).

Glucose Kinetics

Baseline plasma glucose concentrations were higher in the patients with type 2 diabetes (DM2 9.6 ± 0.8 , DM2+Met 8.9 ± 0.7 mmol/L) compared with CON (5.9 ± 0.1 mmol/L) and remained higher during exercise (Fig. 1A). Absolute plasma glucose concentrations were never different between DM2 and DM2+Met ($P > 0.05$). During exercise, glucose concentrations did not change in CON, whereas a decrease (compared with baseline) was seen with exercise and postexercise in the patients with type 2 diabetes (Fig. 1B). However, this decrease tended to be less pronounced during exercise in DM2+Met than in DM2, showing a small glucose-stabilizing effect of metformin (glucose iAUC DM2 vs. DM2+Met $P = 0.16$) (Fig. 1B). The

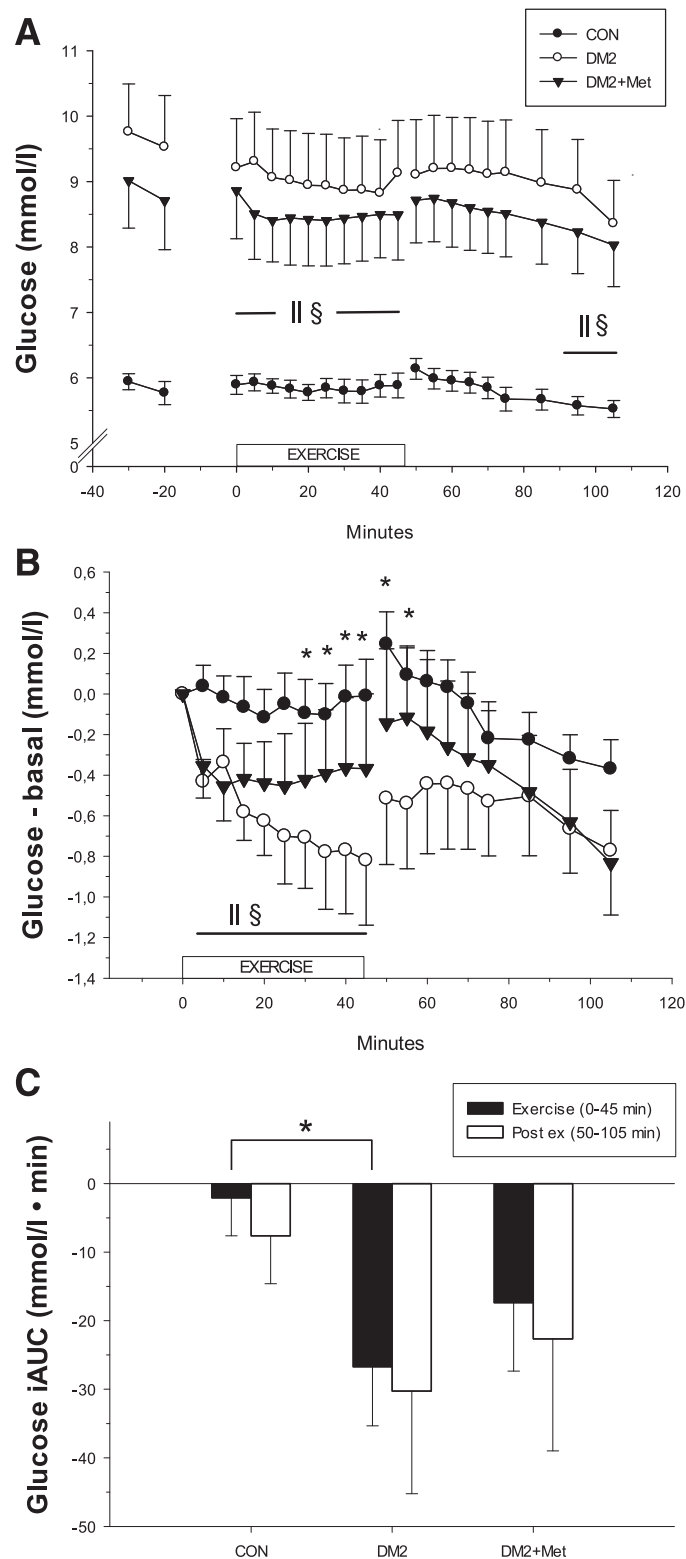


Figure 1—Absolute (A) and relative to baseline (B) plasma glucose concentrations during and after 45 min of moderate exercise in CON ($n = 10$), DM2+Met ($n = 11$), and DM2 ($n = 11$). C: The iAUCs during and after exercise are shown. Data are mean \pm SE. All values in A for CON differed from DM2 and DM2+Met. In A and B, ||DM2 was significantly lower than own baseline/initial value ($P < 0.05$), §DM2+Met was lower than own baseline/initial value ($P < 0.05$), and *CON was different from DM2 ($P < 0.05$). In C, *glucose iAUC differed during exercise between CON and DM2 ($P = 0.03$).

exercise-induced change in glucose (from baseline to steady-state exercise) showed a significant difference among all groups ($P < 0.01$), again with a diminished decrease in glucose in DM2+Met compared with DM2. Glucose Ra was higher in DM2 and DM2+Met than in CON at baseline (20.7 ± 1.2 , 21.1 ± 1.3 , and $15.8 \pm 0.5 \mu\text{mol/FFM/min}$, respectively) (Fig. 2A). With exercise, glucose Ra always increased and then decreased postexercise (Fig. 2A and C), with no significant effect of metformin in the patients with type 2 diabetes ($P > 0.05$).

In response to exercise, Ra increased significantly more in CON than in DM2 and DM2+Met ($P = 0.002$). Postexercise only, DM2+Met had a lower Ra than seen at baseline (Fig. 2A and C).

Glucose Rd increased in response to exercise (Fig. 2B and D), with higher Rd in DM2 and DM2+Met and no effect of metformin treatment in absolute values. However, in response to exercise, baseline Rd increased significantly more in CON than in DM2 and DM2+Met ($P < 0.05$) (Fig. 2D). DM2+Met showed a tendency toward a higher response than

DM2 ($P = 0.07$). With hyperglycemia per se, as seen in the patients with type 2 diabetes (Fig. 1A), some additional effect on peripheral glucose uptake rates (glucose-mediated glucose uptake by mass action) is expected. A gross correction for this can be made by calculating the metabolic clearance rate (MCR) of glucose as $Rd/[glucose]$. MCR was significantly lower in DM2 than in CON, and MCR was improved during exercise in all three groups (Fig. 3). Furthermore, the exercise-induced increase in MCR was the same in CON

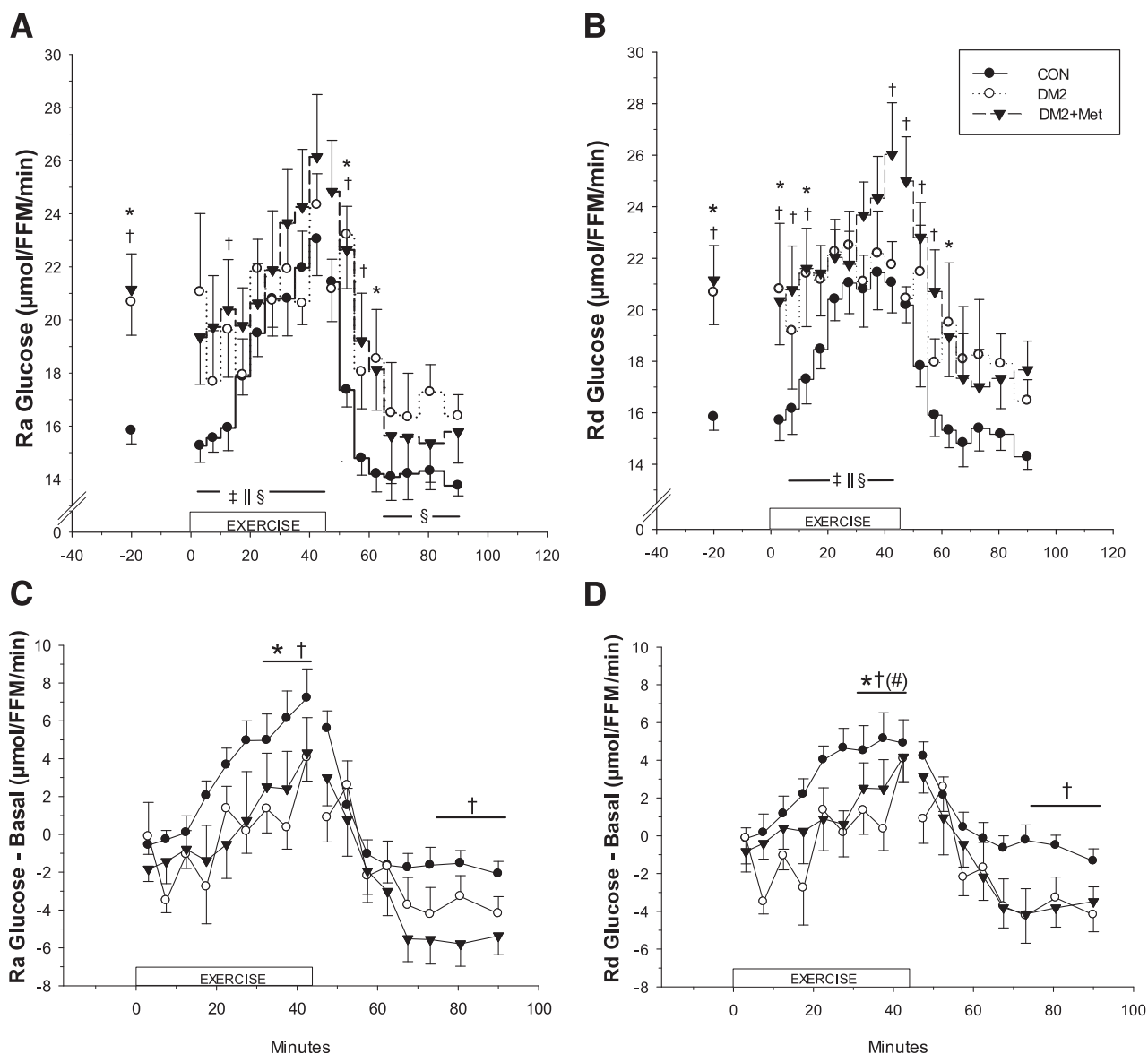


Figure 2—Glucose Ra (A) and glucose Rd (B) before, during, and after 45 min of moderate exercise in CON ($n = 10$), DM2+Met ($n = 11$), and DM2 ($n = 11$). C and D: Changes in Ra and Rd, respectively, relative to baseline values. Data are mean \pm SE. In A and B, *CON different from DM2 ($P < 0.05$) and †CON different from DM2+Met ($P < 0.05$). During exercise, there was a main effect of time on Ra and Rd within †DM2, ‡CON, and §DM2+Met ($P < 0.001$), and postexercise, §DM2+Met had lower levels of Ra compared with baseline. In C and D, *CON had a higher exercise-induced response in Ra – baseline ($P = 0.0002$) and Rd – baseline ($P = 0.003$) than DM2 (calculated as the mean of the three steady-state measurements during exercise) and †DM2+Met ($P = 0.007$ and 0.02 , respectively). (#)DM2 had a tendency to a smaller response in Rd – baseline than DM2+Met ($P = 0.07$).

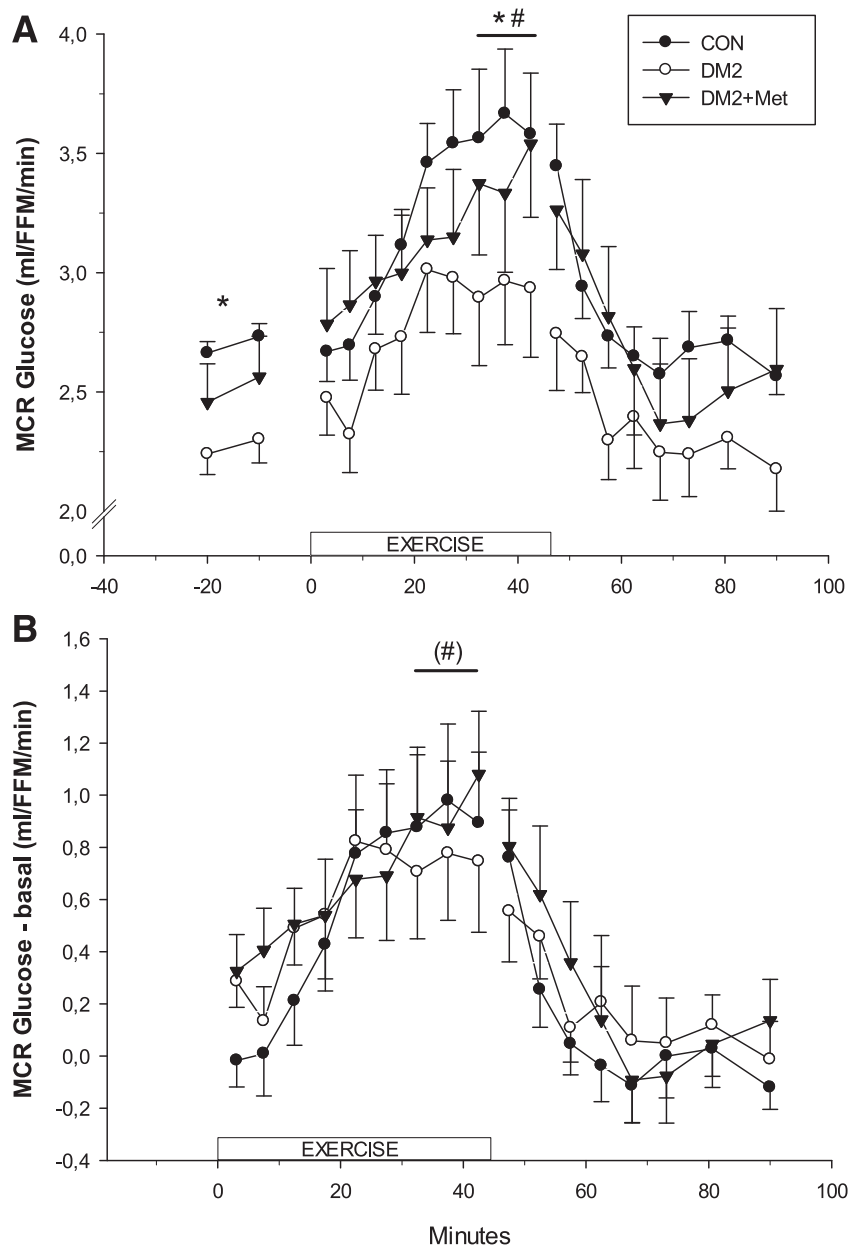


Figure 3—A: MCRs during and after 45 min of moderate exercise in CON ($n = 10$), DM2+Met ($n = 11$), and DM2 ($n = 11$). B: MCR – baseline. Data are mean \pm SE. Exercise-induced response in MCR and MCR – baseline is calculated as the mean of the three steady-state measurements during exercise. In A, *CON > DM2 ($P = 0.002$) and #DM2+Met > DM2 ($P = 0.001$). In B, (#) a tendency to a higher MCR – basal in DM2+Met compared with DM2 ($P = 0.07$).

and DM2+Met ($P = 0.21$) and was higher in DM2+Met than in DM2 ($P = 0.001$), showing an improving effect of metformin. However, when subtracting baseline values, there was only a tendency toward an improvement of MCR due to metformin treatment ($P = 0.07$) (Fig. 3B).

Hormones

Glucoregulatory hormones increased with exercise, as expected, and decreased immediately postexercise (Supplementary Fig. 1). Plasma concentrations of

insulin and C-peptide were higher in the patients with diabetes throughout the experiment (Table 2 and Supplementary Fig. 1A and B). A decrease was seen during exercise (most pronounced for insulin), and for both insulin and C-peptide, a rebound increase was seen immediately following the exercise period (Supplementary Fig. 1A and B).

Other glucoregulatory hormones (glucagon, cortisol, epinephrine, norepinephrine, ACTH, and GH) were generally similar in their response to exercise,

except for glucagon AUC, which was lower in DM2+Met than in DM2 both during and after exercise (Table 2). Norepinephrine showed a blunted response to exercise in DM2 and DM2+Met compared with CON (Supplementary Fig. 1F).

Substrates and Metabolites

Plasma concentrations of FFA were similar in the three experimental groups at baseline and decreased initially with exercise but showed a marked rebound increase postexercise. During exercise, DM2 had a larger AUC than DM2+Met (Table 2 and Supplementary Fig. 2A). The changes in β -hydroxybutyrate (ketone) concentrations reflected the changes in FFA concentrations (Supplementary Fig. 2C). Plasma lactate concentration and AUC were significantly higher during and after exercise in DM2 than in CON, and during recovery, DM2+Met also had a larger AUC than CON (Table 2 and Supplementary Fig. 2D).

CONCLUSIONS

As previously demonstrated (8,12), we observed a slight, but significant exercise-induced decrease in the plasma glucose concentrations in patients with type 2 diabetes. A main finding in the current study was that the combined effect of metformin and exercise improved MCR (Fig. 3A) with no risk of hypoglycemia. We also observed impaired muscle contraction-mediated glucose uptake in type 2 diabetic patients.

The antidiabetic effect of metformin is partly an inhibitory effect on HGP (14,15,17) and partly a direct effect on skeletal muscle (19,29), which enhances the MCR of glucose in the blood. This was seen at baseline in the current study, where MCR in DM2+Met was not significantly different from CON, whereas MCR in DM2 was significantly lower than in CON (Fig. 3A). Potentially, if metformin sustained an inhibitory effect on glucose Ra during exercise and had a peripheral effect in skeletal muscle-enhancing glucose uptake rates, exercise-induced hypoglycemia could be a problem. However, neither mechanism seemed to operate with any significance during moderate-intensity exercise; thus, there is no reason that patients with type 2 diabetes treated with metformin should avoid taking up exercise. The decrease in plasma glucose during moderate-intensity exercise is modest, not influenced by ongoing

metformin treatment, and far from hypoglycemic concentrations. The response of glucose Ra at the onset of exercise was different in control subjects compared with patients with type 2 diabetes. In DM2+Met and DM2, the exercise-induced increase in glucose Ra (Fig. 2C) was delayed, and only after ~20 min of exercise could an increase be seen. The delay may be that the prevailing hyperglycemia in the patients exerted a negative feedback on HGP. This mechanism was proposed in a study showing that ingested carbohydrate almost completely suppressed HGP during exercise (30), albeit in healthy subjects without type 2 diabetes. After exercise, glucose Ra decreased more (relative to baseline values) in DM2+Met than in CON (Fig. 2C), but in absolute terms, glucose Ra was still elevated, and plasma glucose concentrations remained hyperglycemic.

In accordance with other studies, another main conclusion in the current study is that the increase in glucose uptake due to muscle contractions is impaired in type 2 diabetes. In all three groups, the exercise intensity was similar (Table 1), yet the increase in glucose Rd was significantly lower in the patients with type 2 diabetes than in the control subjects, and, more importantly, the glucose MCR was higher in the control subjects than in the patients with type 2 diabetes, with a small improvement in MCR when treated with metformin (Fig. 3A and B). This occurred in the face of significantly higher plasma insulin concentrations during exercise in the patients with type 2 diabetes. Thus, it can be concluded that during moderate-intensity exercise, the contraction-induced glucose uptake rate is impaired in patients with type 2 diabetes, but this impairment is, to some extent, alleviated by concomitant metformin treatment (Fig. 3). However, metformin showed no significant interaction with exercise in a study of the long-term effect of exercise in type 2 diabetes wherein no additional improvement in HbA_{1c} was seen in the metformin-treated group compared with the group not treated with metformin (31).

Baseline and exercise concentrations of glucagon, cortisol, epinephrine, and norepinephrine were similar among the groups (Table 2 and Supplementary Fig. 1), demonstrating that these gluco-regulatory hormones cannot explain the

Table 2—Hormones and metabolites at baseline, during exercise, and during recovery

	Baseline			Exercise (AUC)			Recovery (AUC)		
	DM2	DM2+Met	CON	DM2	DM2+Met	CON	DM2	DM2+Met	CON
Insulin (pmol/L)	72 ± 11	64 ± 10	44 ± 3**	2,565 ± 286	2,608 ± 458	1,566* ± 129	4,963 ± 942	5,239 ± 928	3,735 ± 441
C-peptide (pmol/L)	742 ± 87	621 ± 72	492 ± 43*	30,686 ± 3,544	28,109 ± 3,508	19,461* ± 1,318	42,333 ± 5,405	40,942 ± 5,433	30,364* ± 2,479
Glucagon (pg/mL)	266 ± 97	256 ± 106	297 ± 86	14,362 ± 5,024	13,633# ± 4,936	15,654 ± 4,380	16,411 ± 5,859	14,312# ± 5,505	18,161 ± 5,161
Cortisol (ng/mL)	91 ± 11	78 ± 7	87 ± 14	4,932 ± 424	4,610 ± 450	4,290 ± 481	8,379 ± 811	7,609 ± 973	7,022 ± 730
Epinephrine (pg/mL)	35 ± 5	34 ± 8	43 ± 7	4,221 ± 496	4,513 ± 770	5,451 ± 824	2,542 ± 325	2,440 ± 403	2,621 ± 313
Norepinephrine (pg/mL)	251 ± 21	276 ± 29	264 ± 37	42,220 ± 3,388	46,145 ± 3,210	55,183 ± 6,864	25,778 ± 2,431	25,581 ± 1,858	24,797 ± 2,514
ACTH (pg/mL)	20 ± 4	18 ± 5	22 ± 7	1,952 ± 256	1,825 ± 383	1,536 ± 260	2,773 ± 643	2,446 ± 707	1,613 ± 339
GH (ng/mL)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	81 ± 27	55 ± 14	105 ± 30	72 ± 19	77 ± 19	98 ± 27
FFA (μmol/L)	510 ± 64	459 ± 41	430 ± 41	18,667 ± 3,073	15,978# ± 2,474	15,977 ± 1,577	44,345 ± 6,769	40,103 ± 5,691	40,778 ± 3,681
Glycerol (μmol/L)	41 ± 5	42 ± 3	40 ± 3	4,723 ± 779	4,281 ± 693	5,196 ± 691	3,927 ± 577	3,596 ± 444	4,400 ± 672
β-Hydroxybutyrate (mmol/L)	0 ± 0	0 ± 0	0 ± 0	2 ± 0	2 ± 0	2 ± 0	11 ± 3	9 ± 3	12 ± 3
Lactate (mmol/L)	1 ± 0	2 ± 0#	1 ± 0**	154 ± 19	149 ± 20	106* ± 15	104 ± 11	119 ± 16	70* ± 8

Data are mean ± SE. Supplementary Figs. 1 and 2 show graphs with absolute values. *CON vs. DM2 ($P < 0.05$). †CON vs. DM2+Met ($P < 0.05$). #DM2 vs. DM2+Met ($P < 0.05$).

differences in glucose Ra at rest or during exercise and proving that metformin has no effect on these regulatory hormones. Only C-peptide concentrations changed minimally, but because of a considerably lower MCR than insulin, peripheral C-peptide concentrations may not accurately reflect insulin secretion rates when these change rapidly (32). Concentrations of FFA, glycerol, and ketone bodies (measured by β -hydroxybutyrate) in plasma at rest and during exercise were similar in all groups. The elevated plasma lactate concentrations in the patients with type 2 diabetes is not an unexpected finding in hyperglycemic patients because of an enhanced glycolytic rate in skeletal muscle (Supplementary Fig. 2D).

The strengths of this study are the well-matched groups and the accurate measurements of VO_{2max} and, hence, the tight control of the individual workloads. Furthermore, we measured a large array of hormones, substrates, and metabolites, and thus, excluded differences in concentrations of glucoregulatory hormones can explain the findings.

A limitation of the study is that metformin was withdrawn for only 3 days before the experiment. A residual effect of the last dose of metformin cannot be excluded, even though the half-life is only ~ 5 h (33). However, a prolonged withdrawal of metformin would have resulted in severely dysregulated patients with type 2 diabetes.

In summary, we conclude that metformin and exercise can be taken in combination in patients with type 2 diabetes. HGP is not changed during exercise in patients currently treated with metformin, even though the drug is known to inhibit HGP at rest. However, we found a slight improvement in MCR during exercise in patients treated with metformin, indicating that metformin improves peripheral glucose uptake. The baseline improvement of HOMA insulin resistance indicated that metformin induced an increase in hepatic insulin sensitivity. Furthermore, we demonstrate that muscle contraction-mediated glucose clearance is impaired in patients with type 2 diabetes.

Acknowledgments. The authors thank Stinna Skaaby (Xlab) for helping with recruitment and test days; Jeppe Bach, Thomas Bech, Katrine Qvist, and Regitze Kraunsøe (Xlab) for technical assistance; and Gerrit van Hall (Clinical Metabolism,

Department of Biomedical Sciences, University of Copenhagen) for isotope analysis. The authors also thank the subjects for their cooperation.

Funding. This work was supported by the Nordea Foundation, Aase and Ejnar Danielsens Foundation, Simon Fougnier Hartmann Family Foundation, Torben and Alice Fridomts Foundation, A.P. Møller Foundation, and the Oda and Hans Svenningsens Foundation.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.H. contributed to the study design, clinical test days, data research, and writing of the manuscript. M.K.P. contributed to the clinical test days, data research, and review of the manuscript. J.W.H. contributed to the discussion and review and editing of the manuscript. F.D. contributed to the study design, discussion, and review and editing of the manuscript. M.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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