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ARTICLE

The interaction between metformin and physical activity on postprandial glucose and glucose kinetics: a randomised, clinical trial

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Abstract

Aims/hypothesis The aim of this parallel-group, double-blinded (study personnel and participants), randomised clinical trial was to assess the interaction between metformin and exercise training on postprandial glucose in glucose-intolerant individuals.

Methods Glucose-intolerant (2 h OGTT glucose of 7.8–11.0 mmol/l and/or HbA_{1c} of 39–47 mmol/mol [5.7–6.5%] or glucose-lowering-medication naive type 2 diabetes), overweight/obese (BMI 25–42 kg/m^2) individuals were randomly allocated to a placebo study group (PLA, $n = 15$) or a metformin study group (MET, $n = 14$), and underwent 3 experimental days: BASELINE (before randomisation), MEDICATION (after 3 weeks of metformin [2 g/day] or placebo treatment) and TRAINING (after 12 weeks of exercise training in combination with metformin/placebo treatment). Training consisted of supervised bicycle interval sessions with a mean intensity of 64% of $Watt_{max}$ for 45 min, 4 times/week. The primary outcome was postprandial glucose (mean glucose concentration) during a mixed meal tolerance test (MMTT), which was assessed on each experimental day. For within-group differences, a group × time interaction was assessed using two-way repeated measures ANOVA. Between-group changes of the outcomes at different timepoints were compared using unpaired two-tailed Student's *t* tests.

Results Postprandial glucose improved from BASELINE to TRAINING in both the PLA group and the MET group (ΔPLA: −0.7 [95% CI −1.4, 0.0] mmol/l, $p = 0.05$ and ΔMET: −0.7 [−1.5, −0.0] mmol/l, $p = 0.03$), with no between-group difference ($p = 0.92$). In PLA, the entire reduction was seen from MEDICATION to TRAINING (−0.8 [−1.3, −0.1] mmol/l, $p = 0.01$). Conversely, in MET, the entire reduction was observed from BASELINE to MEDICATION (−0.9 [−1.6, −0.2] mmol/l, $p = 0.01$). The reductions in mean glucose concentration during the MMTT from BASELINE to TRAINING were dependent on differential time effects: in the PLA group, a decrease was observed at timepoint (t) = 120 min ($p = 0.009$), whereas in the MET group, a reduction occurred at t = 30 min ($p < 0.001$). $\dot{V}O_{2peak}$ increased 15% (4.6 [3.3, 5.9] ml kg^{-1} min^{-1}, $p < 0.0001$) from MEDICATION to TRAINING and body weight decreased (−4.0 [−5.2, −2.7] kg, $p < 0.0001$) from BASELINE to TRAINING, with no between-group differences ($p = 0.7$ and $p = 0.5$, respectively).

Conclusions/interpretation Metformin plus exercise training was not superior to exercise training alone in improving postprandial glucose. The differential time effects during the MMTT suggest an interaction between the two modalities.

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Trial registration ClinicalTrials.gov (NCT03316690).

Keywords Exercise · Impaired glucose tolerance · Interaction · Metformin · Mixed meal tolerance test · Postprandial glucose · Prediabetes · Stable isotope glucose tracers · Training

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Abbreviations

AMPK	AMP-activated protein kinase
DXA	Dual x-ray absorptiometry
EGP	Endogenous glucose production
HR	Heart rate
MET	Metformin + exercise training (group)

Research in context

What is already known about this subject?

- Exercise improves glycaemic control in glucose-intolerant individuals
- Metformin improves glycaemic control in glucose-intolerant individuals
- An interaction between metformin and physical activity on peripheral insulin sensitivity has been suggested

What is the key question?

- Is the combination of metformin treatment and exercise training superior to exercise training alone for improving postprandial glucose?

What are the new findings?

- Metformin treatment plus exercise training is not superior to exercise training alone for improving postprandial glucose in glucose-intolerant individuals
- The lack of superiority of metformin treatment plus exercise training vs exercise training alone in improving postprandial glucose may be due to a physiological interaction between metformin and exercise training

How might this impact on clinical practice in the foreseeable future?

- These findings may challenge the provision of metformin treatment and exercise training in combination for glucose-intolerant individuals

MMTT	Mixed meal tolerance test
PLA	Placebo + exercise training (group)
PP	Per protocol
R_{aMMTT}	Rate of glucose appearance from the MMTT
R_{aTOTAL}	Rate of total glucose appearance
R_d	Rate of glucose disappearance
RPE	Rate of perceived exertion

Introduction

The prevalence of prediabetes (in this paper defined as HbA_{1c} of 39–47 mmol/mol [5.7–6.5%] and/or 2 h OGTT glucose concentration of 7.8–11.0 mmol/l (140–199 mg/dl) is rapidly increasing and approximately 25% of individuals with prediabetes will develop type 2 diabetes within 3–5 years [1]. Several studies have however shown that the progression of prediabetes to type 2 diabetes is preventable with lifestyle interventions [2–4]. In the Diabetes Prevention Program trial [2], an intensive lifestyle intervention reduced the incidence of type 2 diabetes by 58% over 3 years compared with the control group whereas metformin treatment resulted in a 31% reduction [2]. Based on these findings, physical activity is broadly recommended for diabetes prevention [5, 6], and for some individuals with prediabetes, the ADA is recommending both metformin treatment and physical activity [5]; a recommendation which also forms the first line treatment for patients with type 2 diabetes [7, 8].

Both metformin and physical activity are effective for improving glycaemic control and other cardiovascular risk factors when evaluated in isolation [9–11], but the combined effect is not well described.

One mechanism by which exercise exerts its acute effects on glucose metabolism is through activation of AMP-activated protein kinase (AMPK) [12]. Moreover, cell and animal studies suggest that AMPK activation also plays an important role in the effect of metformin on glucose metabolism [13]. Despite recent findings that have challenged whether activation of AMPK in human skeletal muscle is seen with metformin treatment [14], interest has gathered around the potential interaction between metformin and physical activity [15]. Evidence is, however, conflicting. Some studies have shown that metformin mitigates the improvement of physical activity on glycaemic control [16–18], some that metformin amplifies the improvement of physical activity [19–21], and other studies have shown that the combination of metformin and physical activity does not impact exercise-induced improvements in glycaemic control [22]. All these studies were either observational [17, 18, 22] or assessed single exercise bouts [19–21]. Therefore, a randomised trial assessing this potential interaction between metformin and an exercise intervention is warranted.

To our knowledge, only one RCT assessing the interaction between metformin and exercise training in this context has been performed [16], in which it was reported that adding metformin to exercise training did not accentuate improvements in insulin sensitivity, and potentially even blunted the training-induced improvements on this variable [16]. The effects on postprandial glucose levels and fluxes during a physiological meal test have not been assessed but are of high clinical relevance.

As such, the aim of the current study was to assess interactions between metformin and exercise training on postprandial glucose and glucose kinetics. We hypothesised that metformin treatment plus exercise training would not lead to greater improvements in postprandial glucose levels, compared with exercise training alone.

Methods

Design

This was a parallel-group, randomised clinical trial. Participants were randomly allocated using a computer-based algorithm (randomizer.org) to one of two arms (placebo + exercise training [PLA] or metformin + exercise training [MET]), with an allocation ratio 1:1 in a block size of six. Randomisation was performed by an individual who did not participate in the experimental work, and this individual also kept the allocation sequence. Both investigators and participants were blinded to the treatment.

All data were collected at Trygfondens Centre for Physical Activity Research at Rigshospitalet, Copenhagen (detailed information about sample size calculation, randomisation, allocation and blinding can be found in electronic supplementary material [ESM] Methods).

The primary outcome was changes in postprandial glucose, measured by mean glucose concentration during a 4 h mixed meal tolerance test (MMTT). For further details, see ClinicalTrials.gov (NCT03316690).

Participants

Participants (recruited by advertisements in newspapers and social media) underwent medical screening. Glycaemic control was measured by HbA_{1c} and 2 h OGTT where participants consumed 75 g of glucose dissolved in 300 ml of water. Inclusion criteria were: impaired glucose tolerance (2 h OGTT glucose concentration of 7.8–11.0 mmol/l and/or HbA_{1c} of 39–47 mmol/mol [5.7–6.5%]) or glucose-lowering-medication naive type 2 diabetes; white; age 18–70 years; BMI 25–42 kg/m^2; and habitual ≤90 min of structured physical activity/week. Exclusion criteria were: pregnancy; smoking; prior and current glucose-lowering treatment; treatment with steroids and/or other immunomodulating drugs; contraindication to increased levels of physical activity [23]; liver disease (alanine aminotransferase elevated more than three times above upper normal limit, or reduced levels of plasma albumin [<35 g/l] and coagulation factors II + VII + X [<0.6 U/l]); and self-reported prior history of lactic acidosis.

Written informed consent was obtained from all participants. The trial was approved by the ethical committee of the Capital Region of Denmark (H-17012307).

Experimental days

Participants reported to the laboratory for 3 experimental days: 'BASELINE' where randomisation to metformin/placebo treatment was performed; 'MEDICATION' after 3 weeks of metformin/placebo treatment without training; and 'TRAINING' after 12 weeks of training +15 weeks of metformin/placebo treatment (ESM Fig.1).

The experimental days consisted of MMTT, a dual x-ray absorptiometry (DXA) scan and a $\dot{V}O_{2peak}$ exercise test.

To ensure standardisation, participants were instructed to keep diet records 2 days prior to the BASELINE experimental day. Afterwards a copy of each individual record was handed out, and participants were instructed to mirror this diet prior to experimental days on MEDICATION and TRAINING. Participants were instructed not to perform vigorous physical activity within 48 h prior to each experimental day. The last training session was scheduled to be completed 48–72 h prior to the TRAINING experimental day.

MMTT with stable isotope glucose tracers

Following overnight fasting (≥8.5 h) and 2 h prior to arriving in the laboratory, participants were instructed to ingest a standardised breakfast (60 g bun with 20 g cheese [220 kcal: fat 8.8 g, carbohydrates 24.7 g, protein 9.9 g]) along with the metformin/placebo treatment, in order to minimise potential side effects to metformin treatment. After arriving in the laboratory, body weight was measured by standard procedures, and bilateral venous lines for tracer infusion and blood sampling were inserted. A baseline tracer blood sample was drawn and a primed (20 μmol/kg body weight multiplied by fasting glucose divided by 5 mmol/l), continuous (0.3 μmol [kg body weight]$^{-1}$ min^{-1}) infusion of [6,6-$^2H_2$]glucose tracer was initiated. Two hours after initiation of the tracer infusion, a 4 h liquid MMTT (400 ml Nestle Resource, 3.138 kJ, [macronutrient composition: 64% energy from carbohydrate, 24% energy from fat, 12% energy from protein] spiked with 2 g of [U-$^{13}C_6$]glucose) was initiated.

One hour prior to the MMTT (i.e. 3.5 h after completion of the breakfast), blood samples were drawn for measurement of HbA_{1c}, insulin and lipids (lithium heparin tubes), glucose and lactate (heparinised syringes) and tracers (NaF tubes). After this, blood samples for glucose, lactate and tracers were repeatedly drawn every 15th min for 4 h (glucose and lactate for 5 h, every 30th min in the last hour). Samples for measurement of insulin were taken every 30th min from 1 h prior to the MMTT until termination of the test. Glucose and lactate were analysed immediately (ABL 7 series, Radiometer, Denmark), whereas all other blood samples were placed on ice and subsequently centrifuged at 2000 *g* for 10 min at 4°C. Samples were stored at −80°C until analysis. Cholesterol and triacylglycerol levels were determined using an enzymatic colorimetric assay (P-

Modular; Roche, Stockholm, Switzerland), HbA_{1c} by HPLC (Tosch G7 Analyzer, San Francisco, CA, USA), and insulin by electrochemiluminescence immunoassay (E-Modular; Roche). Tracers were quantified using LC-MS/MS (Thermo Vantage LC/MS/MS System, Thermo Scientific, USA) performed on a hexobenzoyl derivative of plasma glucose [24]. Rate of total glucose appearance (R_{aTOTAL}), endogenous glucose production (EGP), exogenous rate of glucose appearance from the MMTT (R_{aMMTT}), and rate of glucose disappearance (R_d) were calculated using non-steady-state assumptions [25].

Body composition

After completion of the MMTT, a DXA scan (Lunar Prodigy Advance; GE Healthcare, Madison, WI, USA) was performed to obtain information about body composition, including lean body mass and fat mass.

$\dot{V}O_{2peak}$ test and $Watt_{max}$

After the DXA scan, an incremental exercise test was performed on a cycle ergometer (Monark 739E, Varberg, Sweden) using indirect calorimetry (Cosmed Quark, Rome, Italy) to assess $\dot{V}O_{2peak}$. After 12 min of warm-up below 100 W, the load was increased by 25 W every minute until at least one of the following criteria were met: plateauing of heart rate (HR) and $\dot{V}O_2$ with incremental workloads, respiratory exchange ratio >1.1, or volitional exhaustion. Breath-by-breath values of oxygen uptake were recorded, averaged over 30 s, and $\dot{V}O_{2peak}$ was defined as the highest value. The $Watt_{max}$ was calculated from the incremental test and was the basis for the initial intensity in the training intervention.

MRI scan

MRI scan (3 Tesla, Siemens Magnetom Prisma, Erlangen, Germany) was performed before (between BASELINE and MEDICATION) and after (minimum 24 h and maximum 4 days after the last training session) the training intervention, using a phased array body coil and high-performance gradients. A single axial image at vertebra L3 was used to estimate the amount of visceral fat in the abdomen (by manual delineation of visceral fat tissue) [26] using UTHSCA-RII Mango 3.5 software package (Research Imagine Institute, Texas, USA).

Interventions

Medication Pills were distributed in prepacked pill boxes. To reduce the commonly seen gastrointestinal discomfort with metformin treatment, participants initiated treatment at 500 mg × 2/day and gradually increased the dose during the next 9 days to 1000 mg × 2/day. This dose was maintained throughout the study. Participants were instructed to report any pills missed and were asked about compliance during training sessions. Compliance was calculated by the number of times the participants self-reported a missing dose.

Exercise training The exercise training intervention consisted of supervised bouts of interval-type exercise (ergometer cycling) for 45 min, 4 times/week for 12 weeks. There were four different exercise protocols (each protocol completed every week, ESM Fig. 2), which all had the same mean workload. The relative intensity was increased successively during the intervention (ESM Table 1). A $Watt_{max}$ test (described above) was performed at the beginning of the training intervention and thereafter every 4th week, and absolute exercise intensity was adjusted accordingly.

The HR was recorded during each training session, and the mean and maximal HR determined. The rate of perceived exertion (RPE) [27] was obtained after each training session.

Statistical methods

Outcomes were analysed according to the per protocol (PP) principle. The PP population was defined as participants completing all visits, compliant with the study placebo or active medication (taking ≥80% of the prescription) and compliant with the exercise intervention (completing ≥75% of the training sessions).

For within-group differences, a group × time interaction was assessed using two-way repeated measures ANOVA with the outcome as dependent variable and group (two levels) and time (three levels) as independent variables (fixed effects) and with the unique patient identifier as random effect. Standard model diagnostics were used to assess the adequacy of the model. The within-group differences are presented as least squares means with Bonferroni-corrected (three comparisons according to the different timepoints) 95% CIs and p values. Between-group changes (post-measure minus pre-measure) of the outcomes at different time points were compared using unpaired two-tailed Student's t tests.

All statistical analyses were performed by Prism version 8 (GraphPad). Statistical significance was accepted with $p < 0.05$ (two-sided).

Results

Participants

A total of 34 participants were included in the trial. Five participants discontinued the trial, resulting in 29 participants who completed the trial (14 in the MET group, 15 in the PLA group) (Fig. 1). All 29 participants fulfilled the PP criteria. No serious adverse events were observed. One participant in the MET group complained of gastrointestinal side effects,

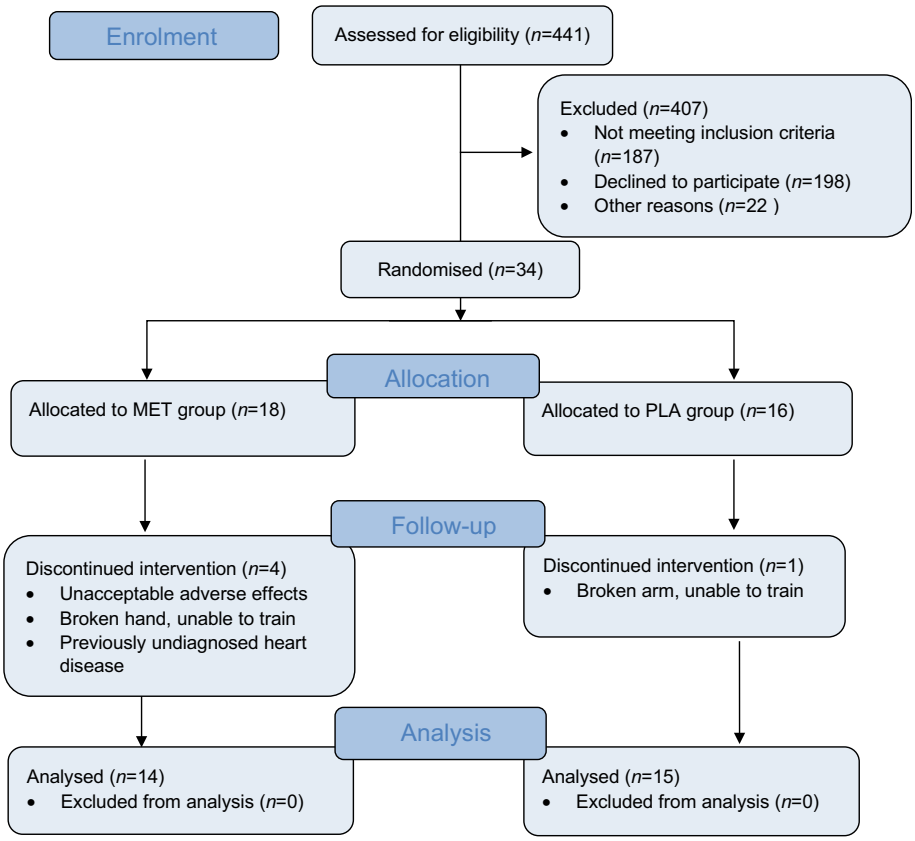

Fig. 1 Flow diagram

therefore the metformin dose for this participant was reduced to 500 mg × 2/day.

No difference in baseline characteristics between groups (Table 1) or in medicine compliance were detected (PLA 99.6 ± 0.6%, MET 99.3 ± 1.1%, $p = 0.4$). No within- or between-group differences in dietary intake prior to each experimental day were seen (data not shown).

Table 1 Baseline characteristics

Variable	PLA $n = 15$	MET $n = 14$
Participants with type 2 diabetes (*n*)	1	1
Female (*n*)	10	5
Age (years)	51 ± 13	48 ± 7
Height (cm)	172 ± 2	177 ± 2
Body weight (kg)	109 ± 15	108 ± 16
BMI (kg/m^2)	37 ± 4	34 ± 5
$\dot{V}O_{2peak}$ absolute (l/min)	2.6 ± 0.5	3.0 ± 0.6
$\dot{V}O_{2peak}$ relative (ml kg^{-1} min^{-1})	24 ± 4	28 ± 6
Fasting glucose (mmol/l)	5.7 ± 0.8	5.9 ± 1.0
2 h OGTT glucose (mmol/l)	9.5 ± 2.3	8.3 ± 3.0
HbA_{1c} (mmol/mol)	40.6 ± 4.4	39.7 ± 5.0
HbA_{1c} (%)	5.9 ± 0.4	5.7 ± 0.5

Baseline characteristics are presented as mean ± SD

Exercise training

The total planned exercise volume was 48 exercise training sessions per participant. The compliance with training did not differ between groups (PLA 98.5 ± 5.4%, MET 97.8 ± 8.1% $P = 0.8$). Mean exercise intensity increased throughout the training intervention, with no differences between groups: week 1–2: PLA 57.7 ± 2.9% $Watt_{max}$, MET 56.6 ± 2.5% $Watt_{max}$, $p = 0.3$; week 3–5: PLA 64.0 ± 2.8% $Watt_{max}$, MET 63.3 ± 3.3% $Watt_{max}$, $p = 0.5$; week 6–12: PLA 67.3 ± 1.8% $Watt_{max}$, MET 66.6 ± 2.7% $Watt_{max}$, $p = 0.4$ (ESM Table 1).

There was no difference in mean HR or RPE either during the entire training intervention, or during the last part (HR week 6–12 [PLA 146.4 ± 13.9 beats per minute, MET 149.8 ± 10.7 beats per minute, $p = 0.5$]; RPE week 6–12 [PLA 15.7 ± 1.8, MET 17.0 ± 0.9, $p = 0.7$]).

Physical fitness

$\dot{V}O_{2peak}$ increased on average by 15% (4.6 [95% CI 3.3, 5.9] ml kg^{-1} min^{-1}, $p < 0.0001$) from MEDICATION to TRAINING with no between-group difference (ΔPLA 4.8 [3.0, 6.6] ml kg^{-1} min^{-1} and ΔMET 4.3 [2.5, 6.2] ml kg^{-1} min^{-1}, $p = 0.7$) (Tables 2 and 3).

Table 2 Absolute values and within-group changes in glycaemic control, insulin, lipids, body composition, physical fitness and lactate

	BASELINE	MEDICATION	TRAINING	ΔTRAINING-BASELINE		ΔMEDICATION-BASELINE		ΔTRAINING-MEDICATION	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value
Physical fitness									
$\dot{V}O_{2peak}$, absolute (l/min)[b]									
PLA	2.6 (0.1)	2.6 (0.2)	3.0 (0.1)	0.3 (0.2, 0.5)	<0.001	−0.0 (−0.2, 0.1)	>0.9	0.4 (0.2, 0.6)	<0.001
MET	3.0 (0.2)	2.9 (0.2)	3.2 (0.2)	0.2 (0.0, 0.5)	0.04	−0.1 (−0.2, 1.6)	0.05	0.4 (0.0, 0.6)	0.009
$\dot{V}O_{2peak}$, relative (ml kg^{-1} min^{-1})[b]									
PLA	24.2 (1.2)	23.5 (1.3)	28.3 (1.2)	4.3 (2.3, 5.9)	<0.001	−0.7 (−2.5, 1.1)	>0.9	4.8 (3.0, 6.6)	<0.001
MET	28.4 (1.8)	27.5 (1.9)	31.9 (2.2)	3.5 (1.6, 5.4)	<0.001	−0.8 (−2.7, 1.1)	0.9	4.3 (2.5, 6.2)	0.001
Body composition									
Body weight (kg)[b]									
PLA	108.6 (15)	108.7 (14)	105.0 (14)	−3.6 (−5.8, −1.3)	0.002	0.1 (−0.7, 1.0)	>0.9	−3.7 (−5.9, −1.5)	0.001
MET	107.7 (16)	106.9 (17)	103.4 (15)	−4.4 (−7.1, −1.7)	0.002	−0.9 (−2.2, 0.3)	0.2	−3.5 (−6.0, −0.9)	0.008
Lean body mass (kg)									
PLA	57.1 (10.3)	57.5 (11.5)	56.8 (11.1)	−0.3 (−1.5, 0.9)	>0.9	0.4 (−1.6, 0.8)	>0.9	−0.7 (−1.9, 0.5)	0.5
MET	61.7 (10.8)	61.5 (10.9)	60.9 (9.5)	−0.8 (−2.0, 0.5)	0.4	−0.2 (−1.4, 1.0)	>0.9	−0.6 (−1.8, 0.6)	0.7
Total fat mass (kg)[b]									
PLA	47 (8.5)	47 (8.9)	44 (8.9)	−3.0 (−4.4, −1.5)	<0.001	0.0 (−1.0, 1.0)	>0.9	−3.0 (−4.2, −1.8)	<0.001
MET	42 (12.5)	41 (13.5)	38 (12.8)	−3.5 (−5.9, −1.3)	0.003	−0.7 (−2.0, 0.7)	0.6	−3.0 (−4.8, −1.0)	0.003
Visceral fat content (cm^2)									
PLA	NA	323 (83.8)	314 (76.5)	NA	NA	NA	NA	−9.0 (−65.9, 47.9)	>0.9
MET	NA	362 (70.8)	347 (81.8)	NA	NA	NA	NA	−14.9 (−71.8, 42.0)	>0.9
Glycaemic control									
Fasting glucose (mmol/l)[a,b]									
PLA	5.7 (0.6)	5.8 (0.5)	5.6 (0.5)	−0.1 (−0.5, 0.2)	>0.9	0.0 (−0.3, 0.4)	>0.9	−0.2 (−0.5, 0.2)	0.8
MET	5.9 (0.7)	5.4 (0.6)	5.4 (0.4)	−0.5 (−1.0, −0.0)	0.03	−0.5 (−0.8, −0.3)	<0.001	0.0 (−0.4, 0.4)	>0.9
HbA_{1c} (mmol/mol)[a,b]									
PLA	40.6 (4.4)	40.3 (4.3)	40.7 (3.2)	0.1 (−1.4, 1.7)	>0.9	−0.3 (−1.1, 0.4)	0.7	0.5 (−1.2, 2.1)	>0.9
MET	39.7 (5.0)	38.5 (4.9)	38.0 (3.8)	−1.7 (−2.9, −0.5)	0.01	−1.2 (−2.5, 0.1)	0.1	−0.5 (−1.8, 0.8)	>0.9
HbA_{1c} (%)[a,b]									
PLA	5.9 (0.4)	5.8 (0.4)	5.9 (0.3)	0.0 (−0.1, 0.2)	>0.9	−0.0 (−0.1, 0.0)	0.7	0.0 (−0.1, 0.2)	>0.9
MET	5.8 (0.5)	5.7 (0.5)	5.6 (0.4)	−0.2 (−0.3, 0.0)	0.01	−0.1 (−0.2, 0.0)	0.07	−0.0 (−0.2, 0.1)	>0.9
Insulin									
Fasting insulin (pmol/l)[b]									
PLA	146 (71)	156 (101)	115 (65)	−31 (−62, 1)	0.06	10 (−22, 41)	>0.9	−40 (−72, −9)	0.008
MET	131 (72)	96 (56)	85 (51)	−51 (−85, −17)	0.002	−36 (−70, −2)	0.03	−15 (−49, 19)	0.9
Mean MMTT insulin (pmol/l)[a,b]									
PLA	1066 (484)	1076 (377)	923 (414)	−143 (−298, 11)	0.07	10 (−185, 206)	>0.9	−154 (−354, 47)	0.2
MET	1011 (710)	613 (423)	585 (336)	−427 (−713, −141)	0.004	−399 (−741, −57)	0.02	−28 (−228, 173)	>0.9
Lipids									
Triacylglycerols (mmol/l)									
PLA	2.1 (0.8)	2.4 (0.9)	2.0 (0.7)	−0.2 (−0.6, 0.3)	>0.9	0.3 (−0.4, 0.9)	>0.9	−0.4 (−1.1, 0.3)	0.4
MET	1.9 (0.7)	2.0 (0.9)	2.1 (0.9)	0.2 (−0.2, 0.7)	0.4	0.1 (−0.3, 0.5)	>0.9	0.1 (−0.3, 0.6)	>0.9
Fasting NEFA (mmol/l)									
PLA	0.51 (0.20)	0.48 (0.17)	0.54 (0.03)	0.03 (−0.05, 0.10)	>0.9	−0.03 (−0.15, 0.10)	>0.9	0.05 (−0.02, 0.13)	0.3
MET	0.45 (0.12)	0.45 (0.13)	0.50 (0.14)	0.10 (−0.03, 0.14)	0.4	0.00 (−0.10, 0.10)	>0.9	0.05 (−0.04, 0.14)	0.5
Mean MMTT NEFA (mmol/l)[a]									
PLA	0.35 (0.12)	0.33 (0.08)	0.31 (0.03)	−0.04 (−0.08, 0.00)	0.08	−0.03 (−0.08, 0.03)	0.6	−0.01 (−0.04, 0.02)	0.8

Table 2 (continued)

	BASELINE	MEDICATION	TRAINING	ΔTRAINING-BASELINE		ΔMEDICATION-BASELINE		ΔTRAINING-MEDICATION	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value
MET	0.28 (0.06)	0.31 (0.09)	0.30 (0.09)	0.02 (−0.03, 0.06)	>0.9	0.03 (−0.02, 0.08)	0.4	−0.01 (−0.08, 0.05)	>0.9
Total cholesterol (mmol/l)[b]									
PLA	5.6 (1.1)	5.8 (1.3)	5.3 (1.1)	−0.3 (−0.7, 0.1)	0.3	0.2 (−0.2, 0.6)	0.4	−0.5 (−1.0, −0.0)	0.1
MET	4.9 (0.7)	4.7 (0.6)	4.6 (0.7)	−0.3 (−0.6, 0.0)	0.1	−0.3 (−0.6, 0.1)	0.2	−0.0 (−0.5, 0.4)	>0.9
HDL-cholesterol (mmol/l)									
PLA	1.3 (0.3)	1.3 (0.3)	1.3 (0.4)	0.0 (−0.1, 0.1)	>0.9	0.0 (−0.1, 0.1)	>0.9	0.0 (−0.1, 0.1)	>0.9
MET	1.1 (0.3)	1.2 (0.4)	1.1 (0.3)	0.0 (−0.0, 0.1)	0.6	0.1 (−0.0, 0.1)	0.2	−0.0 (−0.1, 0.1)	>0.9
LDL-cholesterol (mmol/l)[a,b]									
PLA	3.7 (0.9)	3.8 (1.1)	3.4 (1.0)	−0.3 (−0.6, 0.1)	0.2	0.1 (−0.2, 0.4)	0.8	−0.4 (−0.8, 0.0)	0.08
MET	3.2 (0.5)	2.9 (0.5)	2.9 (0.5)	−0.3 (−0.5, −0.1)	<0.001	−0.2 (−0.4, −0.1)	0.01	−0.1 (−0.3, 0.1)	0.9
Lactate (mmol/l)									
Fasting lactate (mmol/l)[a,b]									
PLA	0.9 (0.3)	0.9 (0.2)	0.9 (0.3)	−0.0 (−0.2, 0.2)	>0.9	0.0 (−0.1, 0.2)	>0.9	−0.1 (−0.2, 0.1)	0.8
MET	0.9 (0.4)	1.3 (0.4)	1.3 (0.5)	0.4 (0.1, 0.8)	0.01	0.4 (0.3, 0.6)	<0.001	−0.0 (−0.2, 0.2)	>0.9
Mean MMTT lactate (mmol/l)[a,b]									
PLA	1.3 (0.3)	1.3 (0.2)	1.2(0.2)	−0.0 (−0.2, 0.1)	>0.9	−0.0 (−0.1, 0.1)	>0.9	−0.0 (−0.1, 0.1)	>0.9
MET	1.2 (0.3)	1.7 (0.5)	1.6 (0.5)	0.4 (0.1, 0.7)	0.004	0.5 (0.2, 0.8)	0.009	−0.1 (−0.3, 0.1)	0.8

Within-group differences between timepoints adjusted for multiple comparison by Bonferroni (ΔTRAINING-BASELINE, ΔMEDICATION-BASELINE and ΔTRAINING-MEDICATION)

[a] $p < 0.05$ (group × time interaction)

[b] $p < 0.05$ (main effect of time)

NA, not applicable

Body composition

Body weight decreased on average by 4.0 [95% CI −5.2, −2.7] kg ($p < 0.0001$) from BASELINE to TRAINING with no between-group difference (ΔPLA −3.6 [−5.8, −1.3] kg and ΔMET −4.4 [−7.1, −1.7] kg, $p = 0.5$, Table 3). Total fat mass decreased in both groups from BASELINE to TRAINING (ΔPLA −3.0 [−4.2, −1.8] kg, $p < 0.001$, and ΔMET −3.0 [−4.8, −1.0] kg, $p = 0.003$), with no between-group difference ($p = 0.5$, Table 3). Neither lean body mass nor visceral fat content changed over time in either group (Table 2).

Glycaemic control

MMTT glucose concentration at BASELINE, MEDICATION and TRAINING, respectively, are shown in Fig. 2.

Mean glucose concentration during the MMTT decreased from BASELINE to TRAINING in both groups (ΔPLA −0.7 [95% CI −1.4, 0.0] mmol/l, $p = 0.05$ and ΔMET −0.7 [−1.5, −0.0] mmol/l, $p = 0.03$), with no between-group difference (−0.0 [−0.9, 0.8] mmol/l, $p = 0.92$, Table 3). Adjustment for BASELINE glucose values did not result in between-group differences from BASELINE to TRAINING.

The comparable reductions in mean MMTT glucose concentration between BASELINE and TRAINING for the MET and PLA study groups were seen at different timepoints (Fig. 2c, d): in the PLA group, no reduction was observed from BASELINE to MEDICATION (0.1 [95% CI −0.6, 0.8] mmol/l, $p > 0.9$), whereas the entire reduction occurred from MEDICATION to TRAINING (−0.8 [−1.3, −0.1] mmol/l, $p = 0.01$). Conversely, in the MET group, the entire reduction was observed from BASELINE to MEDICATION (−0.9 [−1.6, −0.2] mmol/l, $p = 0.01$), with no reduction from MEDICATION to TRAINING (0.1 [−0.6, 0.8] mmol/l, $p > 0.9$). Moreover, significant between-group differences were observed from BASELINE to MEDICATION (−1.0 [−1.9, −0.2] mmol/l, $p = 0.02$) and MEDICATION to TRAINING (1.0 [0.2, 1.7] mmol/l, $p = 0.01$, Table 3). If adjusted for values at MEDICATION, no between-group difference from MEDICATION to TRAINING was seen (−0.5 [−1.2, 0.3] mmol/l, $p = 0.2$).

The intervention-induced reductions in mean glucose concentration during the MMTT were dependent on differential time effects (Fig. 2a, b). In the PLA group, the reductions were mainly dependent on reductions in the last part of the MMTT, whereas in the MET group, the reduction was mainly dependent on reductions in the first part of the MMTT.

Table 3 Between-group difference in change (MET vs PLA)

	ΔTRAINING-BASELINE		ΔMEDICATION-BASELINE		ΔTRAINING-MEDICATION	
	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value	Mean (95% CI)	*p* value
Physical fitness						
$\dot{V}O_{2peak}$, absolute (l/min)	−0.1 (−0.3, 0.1)	0.3	−0.0 (−0.2, 0.1)	0.5	−0.0 (−0.3, 0.2)	0.7
$\dot{V}O_{2peak}$, relative (ml kg^{-1} min^{-1})	−0.6 (−2.8, 1.6)	0.6	−0.1 (−1.6, 1.4)	0.9	−0.5 (−3.1, 2.1)	0.7
Body composition						
Body weight (kg)	−0.7 (−3.2, 1.8)	0.5	−1.0 (−2.1, 0.2)	0.09	0.2 (−2.3, 2.7)	0.8
Lean body mass (kg)	−0.5 (−2.0, 1.0)	0.5	−0.6 (−1.8, 0.6)	0.3	0.1 (−1.5, 1.7)	0.9
Total fat mass (kg)	−0.6 (−2.6, 1.4)	0.5	−0.7 (−1.9, 0.5)	0.3	0.1 (−1.6, 1.7)	0.9
Visceral fat content (cm^2)	NA	NA	NA	NA	5.9 (−63.3, 75.2)	0.9
Glycaemic control						
Fasting glucose (mmol/l)	−0.4 (−0.8, 0.1)	0.1	−0.6 (−0.9, −0.2)	0.002	0.2 (−0.2, 0.6)	0.4
Mean MMTT glucose (mmol/l)	−0.0 (−0.9, 0.8)	0.92	−1.0 (−1.9, −0.2)	0.02	1.0 (0.2, 1.7)	0.01
MMTT glucose (mmol/l), t = 30	−0.9 (−1.8, −0.0)	0.05	−1.8 (−3.0, −0.7)	0.003	0.9 (−0.2, 2.1)	0.1
MMTT glucose (mmol/l), t = 120	0.2 (−1.0, 1.4)	0.7	−1.2 (−2.9, 0.5)	0.2	1.4 (0.1, 2.8)	0.04
HbA_{1c} (mmol/mol)	−1.8 (−3.3, −0.4)	0.02	−0.9 (−2.0, 0.2)	0.1	−1.0 (−2.6, 0.6)	0.2
HbA_{1c} (%)	−0.2 (−0.3, 0.0)	0.02	−0.1 (−0.2, 0.0)	0.1	−0.1 (−0.2, 0.1)	0.2
Glucose kinetics						
Mean R_{aTOTAL}—MMTT (mg kg^{-1} min^{-1})	−0.2 (−0.7, 0.3)	0.5	−0.6 (−1.1, −0.2)	0.01	0.5 (−0.1, 1.0)	0.07
Mean EGP—fasting (mg kg^{-1} min^{-1})	0.4 (0.1, 0.7)	0.007	0.4 (0.2, 0.6)	0.002	0.0 (−0.2, 0.2)	0.9
Mean EGP—MMTT (mg kg^{-1} min^{-1})	0.1 (−0.2, 0.3)	0.5	−0.1 (−0.3, 0.2)	0.6	0.2 (0.0, 0.3)	0.04
Mean R_{aMMTT}—MMTT (mg kg^{-1} min^{-1})	−0.3 (−0.6, 0.1)	0.2	−0.6 (−1.0, −0.1)	0.01	0.3 (−0.2, 0.8)	0.2
Mean R_d—MMTT (mg kg^{-1} min^{-1})	−0.3 (−0.9, 0.3)	0.4	−0.8 (−1.3, −0.2)	0.01	0.5 (−0.0, 1.0)	0.06
Insulin						
Fasting insulin (pmol/l)	−20 (−51, 10)	0.2	−45 (−86, −3)	0.004	26 (−15, 66)	0.2
Mean MMTT insulin (pmol/l)	−284 (−523, −45)	0.02	−409 (−699, −119)	0.01	126 (−88, 339)	0.2
Lipids						
Triacylglycerols (mmol/l)	0.4 (−0.1, 0.9)	0.1	−0.2 (−0.8, 0.4)	0.6	0.6 (−0.1, 1.2)	0.09
Fasting NEFA (mmol/l)	0.03 (−0.06, 0.11)	0.6	0.03 (−0.08, 0.15)	0.6	−0.00 (−0.09, 0.08)	0.9
Mean MMTT NEFA (mmol/l)	0.05 (0.01, 0.10)	0.03	0.05 (0.00, 0.11)	0.05	−0.00 (−0.05, 0.05)	>0.9
Total cholesterol (mmol/l)	−0.0 (−0.4, 0.4)	0.9	−0.5 (−0.8, −0.1)	0.02	0.4 (−0.1, 1.0)	0.1
HDL-cholesterol (mmol/l)	0.0 (−0.1, 0.1)	0.7	0.1 (−0.0, 0.1)	0.3	−0.0 (−0.1, 0.1)	0.5
LDL-cholesterol (mmol/l)	−0.1 (−0.4, 0.2)	0.6	−0.4 (−0.7, −0.1)	0.01	0.3 (−0.1, 0.7)	0.09
Lactate						
Fasting lactate (mmol/l)	0.5 (0.2, 0.7)	0.002	0.4 (0.3, 0.6)	<0.0001	0.0 (−0.2, 0.2)	0.8
Mean MMTT lactate (mmol/l)	0.4 (0.2, 0.7)	0.001	0.5 (0.3, 0.7)	<0.0001	−0.1 (−0.2, 0.1)	0.4

t, timepoint (minutes) during MMTT

Supporting this, in the PLA group, no difference in mean glucose from BASELINE to TRAINING was observed at t = 30 min, whereas a decrease in mean glucose at t = 120 min ($p = 0.009$) was seen. In contrast, in the MET group, a decrease in mean glucose at t = 30 min occurred from BASELINE to TRAINING ($p < 0.001$), whereas no difference was seen at t = 120 min.

No changes in HbA_{1c} occurred in the PLA group, whereas HbA_{1c} decreased from BASELINE to TRAINING in the MET group ($p = 0.01$, Table 2).

No changes in fasting glucose occurred in the PLA group, whereas fasting glucose significantly decreased in the MET group ($p = 0.03$) from BASELINE to TRAINING, and from BASELINE to MEDICATION ($p < 0.0001$), the latter with a significant between-group difference ($p = 0.002$, Tables 2 and 3).

Glucose kinetics

Glucose kinetics during the MMTT are illustrated in Fig. 3. In the PLA group, no differences in R_{aTOTAL} between any

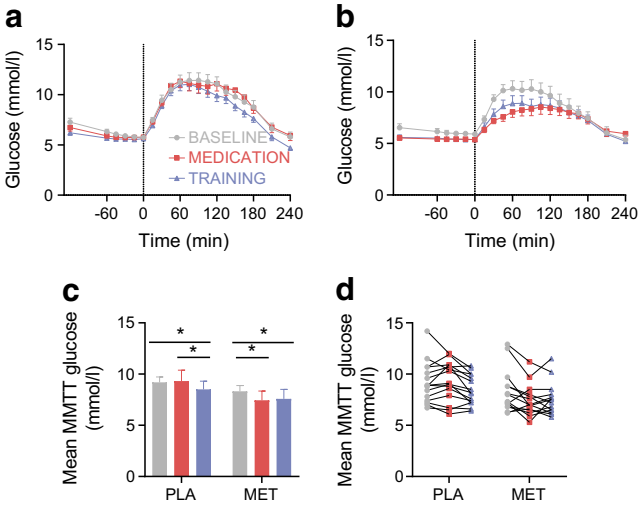

Fig. 2 Glucose concentration during the MMTT. (**a**, **b**) Glucose concentration curves ± SEM during the MMTT in the PLA (**a**) and MET (**b**) group at BASELINE, MEDICATION and TRAINING. MMTT ingested at timepoint = 0 (marked as a dotted line). (**c**) Mean glucose concentration ± SEM during the MMTT at BASELINE, MEDICATION and TRAINING. In the two-way repeated measures ANOVA, a main effect of time and a group × time interaction was seen ($p < 0.05$ for both). *$p < 0.05$ for within-group difference between timepoints. (**d**) Individual glucose concentration during the MMTT at BASELINE, MEDICATION and TRAINING. For detailed statistical analysis, see text and Table 3

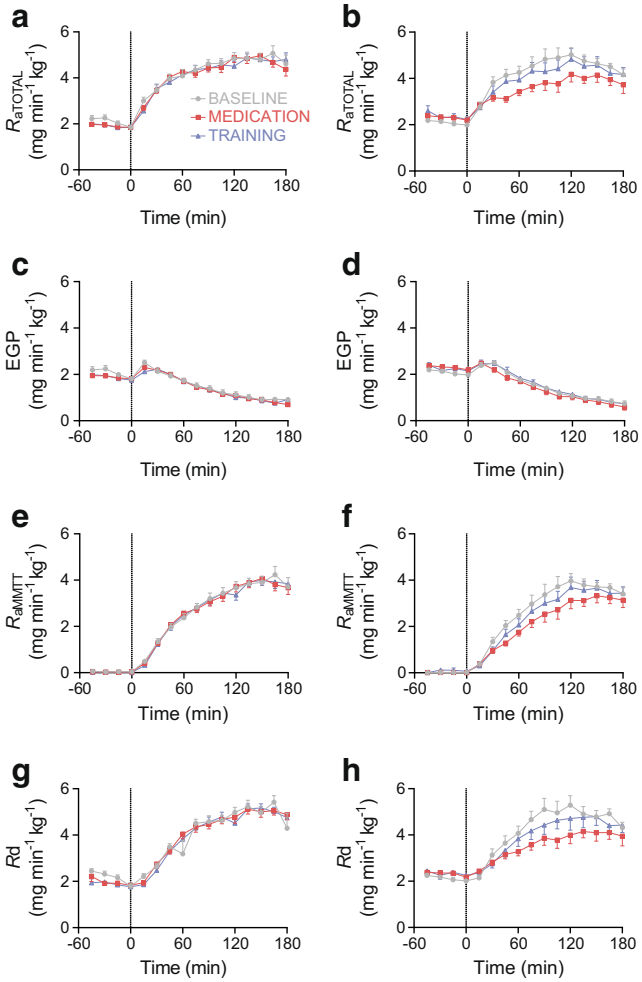

Fig. 3 Glucose kinetics during the MMTT. (**a**, **b**) R_{aTOTAL} in the PLA (**a**) and MET (**b**) group. (**c**, **d**) EGP in the PLA (**c**) and MET (**d**) group. (**e**, **f**) R_{aMMTT} in the PLA (**e**) and MET (**f**) group. (**g**, **h**) R_d in the PLA (**g**) and MET (**h**) group. In the two-way repeated measures ANOVA, a main effect of time and a group × time interaction was seen in R_{aTOTAL} and R_{aMMTT} ($p < 0.05$ for both), and a main effect of time was seen in EGP (fasting state) and R_d ($p < 0.05$ for both). Data are shown as mean ± SEM. MMTT consumed at timepoint = 0 (marked as a dotted line) in all figure parts. For statistical analysis, see text and Table 3

experimental days were seen. In the MET group, R_{aTOTAL} decreased from BASELINE to MEDICATION ($p = 0.003$) with a significant between-group difference ($p = 0.01$), whereas no changes occurred from BASELINE to TRAINING or from MEDICATION to TRAINING (Fig. 3a, b and Table 3).

Regarding fasting EGP in the PLA group, this was unchanged throughout the intervention. In the MET group, fasting EGP numerically increased from BASELINE to TRAINING ($p = 0.06$) and increased from BASELINE to MEDICATION ($p = 0.04$). A significant between-group difference was observed from BASELINE to TRAINING ($p = 0.007$) and from BASELINE to MEDICATION ($p = 0.002$, Table 3). When adjusting fasting EGP for insulin concentration (by multiplication), fasting EGP numerically decreased from BASELINE to TRAINING in the PLA group ($p = 0.06$), whereas a significant ($p = 0.02$) decrease was seen in the MET group during the same period.

During the MMTT, EGP increased from MEDICATION to TRAINING in the MET group only ($p = 0.04$), with a significant between-group difference ($p = 0.04$) (Fig. 3c, d and Table 3). When adjusting EGP for insulin concentration (by multiplying EGP with insulin levels), a significant decrease from BASELINE to MEDICATION ($p = 0.007$) and from BASELINE to TRAINING ($p = 0.008$) occurred in the MET group only.

Regarding R_{aMMTT}, no differences between any experimental days were seen in the PLA group. In the MET group, R_{aMMTT} decreased from BASELINE to MEDICATION ($p = 0.03$), whereas no changes occurred from BASELINE to TRAINING or from MEDICATION to TRAINING. A significant between-group difference was observed from BASELINE to MEDICATION ($p = 0.01$) (Fig. 3e, f and Table 3).

Regarding R_d, no differences between any experimental days were seen in the PLA group. In the MET group, R_d decreased from BASELINE to MEDICATION ($p = 0.002$) with a significant between-group difference ($p = 0.01$), whereas no changes occurred from BASELINE to TRAINING or from MEDICATION to TRAINING (Fig. 3g, h and Table 3). When adjusting R_d for insulin concentration (by dividing R_d with insulin levels), a numerical increase from MEDICATION to TRAINING was seen in the PLA group ($p = 0.06$), whereas a significant increase was seen from

BASELINE to MEDICATION ($p = 0.03$) and from BASELINE to TRAINING ($p = 0.0002$) in the MET group.

Insulin

In the PLA group, fasting insulin numerically decreased from BASELINE to TRAINING ($p = 0.06$), and decreased from MEDICATION to TRAINING ($p = 0.008$). In the MET group, fasting insulin concentration decreased from BASELINE to TRAINING ($p = 0.003$) and MEDICATION ($p = 0.02$).

Mean insulin concentration during the MMTT numerically decreased in the PLA group ($p = 0.07$), and decreased from BASELINE to TRAINING in the MET group ($p = 0.004$). No changes from BASELINE to MEDICATION was seen in the PLA group, but in the MET group a decrease was observed in this period ($p = 0.02$) (Tables 2 and 3, ESM Fig. 3a, b).

Lipids

Triacylglycerols, total cholesterol and HDL-cholesterol did not change over time in either group. NEFA numerically decreased from BASELINE to TRAINING in the PLA group ($p = 0.08$). LDL-cholesterol numerically decreased from MEDICATION to TRAINING in the PLA group ($p = 0.08$) and decreased from BASELINE to TRAINING ($p < 0.001$) and from BASELINE to MEDICATION ($p = 0.01$) in the MET group.

Discussion

The main finding of this study is that metformin plus exercise training is not superior to exercise training alone for improving postprandial glucose in glucose-intolerant individuals. The successful training intervention with solid and comparable improvements in physical fitness and body composition in the MET and PLA study groups, in addition to the high medicine compliance, indicates a blunting effect of metformin on training-induced improvements in postprandial glucose.

In the MET group, we found a decrease in mean glucose concentration during the MMTT following 3 weeks of metformin treatment but no further improvements with 12 weeks of exercise training. Conversely, in the PLA group, 12 weeks of training resulted in a robust decrease in mean glucose concentration, supporting the argument that the training intervention itself was effective. Hence, the lack of improvement in postprandial glucose with exercise training in the MET group warrants further discussion. Specifically, it must be considered whether the lack of training-induced improvements is dependent on a flooring effect or due to a 'true' (pharmacological/physiological) interaction between metformin and physical activity.

The fact that participants had only moderately impaired postprandial glucose at baseline supports a flooring effect. After initiation of metformin treatment, postprandial glucose was further improved, leaving little room for additional improvements with the training intervention. Also indicative of a flooring effect is that no difference in changes in postprandial glucose between the groups were seen from MEDICATION to TRAINING, when analyses were adjusted for glucose values at MEDICATION.

Supporting a true interaction between metformin and physical activity on postprandial glucose is the fact that mean glucose levels achieved after 3 weeks of metformin treatment (7.4 ± 0.4 mmol/l) did not fully reach normoglycaemic levels, indicating that there was room for a further decrease. This is supported by a meta-analysis by Hrubeniuk et al. [28], where the ability of exercise to improve glycaemic control in individuals with prediabetes was assessed. In the meta-analysis, 2 h plasma glucose values of 6.9 and 7.2 mmol/l after an OGTT were reported after a 12 week training intervention. In comparison, the 2 h MMTT plasma glucose levels after the training intervention in the present study were considerably higher (PLA 9.9 ± 0.6 mmol/l, MET 8.7 ± 0.7 mmol/l), thus further improvements should be possible. Moreover, the fact that the reductions in mean glucose concentration during the MMTT were dependent on differential time effects supports a true interaction between metformin and physical activity. Finally, the apparently differential effects between groups on glucose kinetics (see discussion below) supports a physiological interaction. To further investigate this issue, studies with a higher number of dysglycaemic participants are warranted.

Even though our primary endpoint (postprandial glucose) indicated an interaction between metformin and exercise training, other markers of glycaemic control (HbA_{1c} and fasting glucose) did not. As such, it may be argued that the overall effect of metformin treatment plus exercise training on glycaemic control was superior to the effect of exercise training alone. A potential explanation for the lack of improvement in HbA_{1c} with the training intervention in the PLA group is that the 12 week training period was too short [9]. Regarding the lack of improvements of fasting glucose with training, this is consistent with previous studies [16, 29], and suggests little or no improvement in hepatic insulin resistance with training. This is consistent with the lack of decrease in EGP seen in the present study.

Glucose kinetics

We observed a decreased R_{aMMTT} with metformin treatment, and this effect was partially abolished with exercise training. Explanations for this metformin-induced reduction in R_{aMMTT} could be either higher glucose uptake by the intestinal cells, slower gastric emptying rate or higher glucose uptake by the liver. It has previously been reported that the gut might serve

as an important site of action for metformin pharmacodynamics [30]. As such, an increased glucose uptake by enterocytes and subsequently increased lactate concentration within the enterocytes as a result of increased glycolysis was reported. This led to decreased glucose entering the circulation, and thereby improved postprandial glucose. In continuation of this, a study by Buse et al. indicated that the effect of metformin on glucose metabolism should at least partially be found in the enterocytes [31]. Our data, despite not powered or designed for such analyses, might suggest that the effect of metformin on glucose metabolism in the gut is influenced by physical activity, but further studies are needed in this field.

In the MET group, we saw an increase in fasting EGP with metformin treatment. This finding challenges the existing paradigm that metformin primarily acts in the liver by inhibiting EGP [32, 33]. However, the increase in EGP with metformin treatment is supported in a trial by Gormsen et al. [34]. Similarities between the present study and the Gormsen trial is that participants had better glycaemic control than those in previous studies [35], which may potentially explain this finding. Moreover, it should be noted that, if adjusting for insulin concentration, EGP was robustly decreased, indicating that metformin improved central insulin sensitivity.

Exercise training is believed to increase R_d and peripheral insulin sensitivity [36]. Surprisingly, only when adjusted for insulin concentration, R_d numerically improved with exercise training in the PLA group, despite the solid training-induced improvements in postprandial glucose. The reason may be imprecision and/or inaccuracy in the measurement of R_d in a dynamic test such as an MMTT [37]. In this context, the application of a dual-tracer approach instead of a triple-tracer approach may have played a role [38].

Strengths and limitations

We consider the randomised design and the strict standardisation prior to and during every experimental day a strength. In addition, the training intervention was efficient with solid and comparable improvements in physical fitness and body composition. Hence, the comparisons within and between groups are sound and fair.

A limitation of the present study is the small number of participants, which may lead to both type 1 and type 2 statistical errors.

It must be noted that this study is an efficacy study with high volume of intensive, supervised training, and it is unlikely that such a training intervention can be undertaken and maintained by the majority of individuals with dysglycaemia in the 'real world'. Hence, it is unknown if effectiveness studies would result in the same potential interaction, but this should be investigated.

In summary, this study suggests that metformin plus exercise training was not superior to exercise training alone in improving postprandial glucose in glucose-intolerant individuals. Whether this is dependent on a true interaction between the two modalities or a flooring effect on postprandial glucose remains unclear. Given that current diabetes guidelines [5, 6] recommend both metformin and exercise training as first line treatment for patients with type 2 diabetes, further studies in this population are needed to elucidate the potential interaction between metformin and exercise training.

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Data availability Data from the study are available from the corresponding author on reasonable request, if this does not interfere with the regulations from the Danish Data Protection Agency.

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Contribution statement NSP wrote the manuscript. NSP, KK and MRL performed the statistical analyses. KK conceptualised and designed the analysis with contributions from NSP, KBH and BKP. NSP and KK obtained the funding. NSP, ML, LO, IE, CPB, KaKo, CS, HE and KaKe contributed to data collection, data analysis/processing and/or data quality control procedures. GvH contributed to data analysis/processing and/or data quality control procedures of glucose kinetics and JA and CL to analysis of MRI scans. All authors contributed to drafting the article and/or revising it critically for important intellectual content. All authors approved the final version of the manuscript. All authors accept responsibility for all aspects of the work insofar as ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. KK is responsible for the integrity of the work as a whole.

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