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ARTICLE

Glucose effectiveness, but not insulin sensitivity, is improved after short-term interval training in individuals with type 2 diabetes mellitus: a controlled, randomised, crossover trial

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Abstract

Aims/hypothesis The role of glucose effectiveness (S_G) in training-induced improvements in glucose metabolism in individuals with type 2 diabetes is unknown. The objectives and primary outcomes of this study were: (1) to assess the efficacy of interval walking training (IWT) and continuous walking training (CWT) on S_G and insulin sensitivity (S_I) in individuals with type 2 diabetes; and (2) to assess the association of changes in S_G and S_I with changes in glycaemic control.

Methods Fourteen participants with type 2 diabetes underwent three trials (IWT, CWT and no training) in a crossover study. Exclusion criteria were exogenous insulin treatment, smoking, pregnancy, contraindications to structured physical activity and participation in recurrent training (>90 min/week). The trials were performed in a randomised order (computerised-generated randomisation). IWT and CWT consisted of ten supervised treadmill walking sessions, each lasting 60 min, over 2 weeks. IWT was performed as repeated cycles of 3 min slow walking and 3 min fast walking (aiming for 54% and 89% of $\dot{V}O_{2peak}$, respectively, which was measured during the last minute of each interval), and CWT was performed aiming for a moderate walking speed (73% of $\dot{V}O_{2peak}$). A two-step (pancreatic and hyperinsulinaemic) hyperglycaemic clamp was implemented before and after each trial. All data were collected in a hospitalised setting. Neither participants nor assessors were blinded to the trial interventions.

Results Thirteen individuals completed all procedures and were included in the analyses. IWT improved S_G (mean ± SEM: 0.6 ± 0.1 mg kg^{-1} min^{-1}, $p < 0.05$) but not S_I ($p > 0.05$), whereas CWT matched for energy expenditure and time duration improved neither S_G nor S_I (both $p > 0.05$). Changes in S_G, but not in S_I, were associated with changes in mean ($\beta = -0.62 \pm 0.23$, $r^2 = 0.17$, $p < 0.01$) and maximum ($\beta = -1.18 \pm 0.52$, $r^2 = 0.12$, $p < 0.05$) glucose levels during 24 h continuous glucose monitoring.

Conclusions/interpretation Two weeks of IWT, but not CWT, improves S_G but not S_I in individuals with type 2 diabetes. Moreover, changes in S_G are associated with changes in glycaemic control. Therefore, increased S_G is likely an important mechanism by which training improves glycaemic control in individuals with type 2 diabetes.

Trial registration: ClinicalTrials.gov NCT02320526

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Keywords Continuous glucose monitoring · Exercise interventions · Glucose effectiveness · Hyperglycaemic clamp · Hyperinsulinaemic clamp · Insulin sensitivity · Lifestyle intervention(s) · Mass action of glucose · Pancreatic clamp · Training

Abbreviations

CGM	Continuous glucose monitoring
CWT	Continuous walking training
GIR	Glucose infusion rate
IWT	Interval walking training
R_a	Rate of glucose appearance
R_{aENDO}	Endogenous rate of glucose appearance
R_{aTOTAL}	Total rate of glucose appearance
R_d	Rate of glucose disappearance
RER	Respiratory exchange ratio
S_G	Glucose effectiveness
S_I	Insulin sensitivity

Introduction

Exercise training improves glycaemic control in individuals with type 2 diabetes [1], with reductions in postprandial glucose levels being the most pronounced [2, 3]. These reductions are most often ascribed to increased peripheral tissue glucose disposal [4, 5], and may be dependent on increased peripheral insulin sensitivity (S_I) and/or increased glucose effectiveness [6] (S_G; defined as the ability of glucose per se to stimulate its own uptake and to suppress its own production [7]). However, the relative contribution of these mechanisms towards improved glycaemic control is unknown.

It has been known for many years that acute exercise improves S_I [8] and that training improves S_I in individuals with type 2 diabetes [9]. However, since S_I increases with weight loss, some of the training-induced increases in S_I may be ascribed to training-induced changes in body composition and not to training per se. Supporting this, studies that have compared training with and without weight loss have found much greater improvements in S_I when weight loss is seen [10, 11]. Moreover, training may fail to improve S_I in individuals with type 2 diabetes who have severe insulin resistance [12].

Like S_I, S_G typically deteriorates with the development of glucose intolerance and type 2 diabetes [13, 14]. However, studies have shown that improvements in S_G over time occur alongside improved glucose tolerance [13], allowing S_G to compensate for insulin resistance and thereby maintaining good glycaemic control [15]. The effect of training on S_G has only been examined in a few studies, which have indicated that training can increase S_G in both healthy [16, 17] and obese [18, 19] individuals. To our knowledge, the effects of training on S_G have not previously been assessed in individuals with diabetes. Furthermore, all studies that have evaluated training-induced improvements in S_G have used frequently-sampled IVGTT. This method estimates S_I and S_G by modelling assumptions. While the frequently-sampled IVGTT approach is typically considered a useful tool in healthy, insulin-sensitive individuals, it may be less ideal in insulin-resistant individuals owing to the dynamic nature of the test with transient very high glucose concentrations, which can lead to nonsensical negative values for S_I and concomitant overestimation of S_G [20].

We have previously found that aerobic interval walking training (IWT) is more effective in improving glycaemic control in individuals with type 2 diabetes than energy-expenditure-matched continuous walking training (CWT) [2, 21]. Moreover, we found that IWT-induced improvements in glycaemic control were dependent on improved peripheral tissue glucose disposal [5]. However, since we used hyperglycaemic clamp methodology, it was not possible to determine whether the IWT-induced increments in glucose disposal were dependent on improvements in S_I or S_G, or both. As such, the aim of this controlled, crossover study was to assess the efficacy of IWT and CWT in improving S_I and S_G in individuals with type 2 diabetes using direct measurements derived from a two-step (pancreatic and hyperinsulinaemic) hyperglycaemic clamp (Fig. 1). We hypothesised that, in opposition to CWT, IWT would improve the combination of S_I and S_G.

Methods

Fourteen individuals with type 2 diabetes [22] were recruited to the study. Exclusion criteria were exogenous insulin treatment, smoking, pregnancy, contraindications to structured physical activity [23] and participation in recurrent training (>90 min/week). All participants underwent a screening visit including a $\dot{V}O_{2peak}$ test with portable indirect calorimetry equipment (Cosmed K4B2, Cosmed, Rome, Italy). Details of the experimental design have been published elsewhere [21, 24]. All participants gave informed consent. The study was approved by the regional ethical committee (H-6-2014-043) and registered at ClinicalTrials.gov (registration no. NCT02320526).

Study design Participants underwent an experimental day before and after three trials, with each trial including a 2-week intervention. The trials were performed in a randomised order, with computer-generated randomisation carried out using www.randomization.com, accessed 6 November 2014 (for reproduction, use seed no. 18512). Wash-out periods (8 weeks after the training interventions and 4 weeks after the non-exercise control intervention) were included to ensure that any intervention-induced effects on glucose metabolism had disappeared before the initiation of the next intervention.

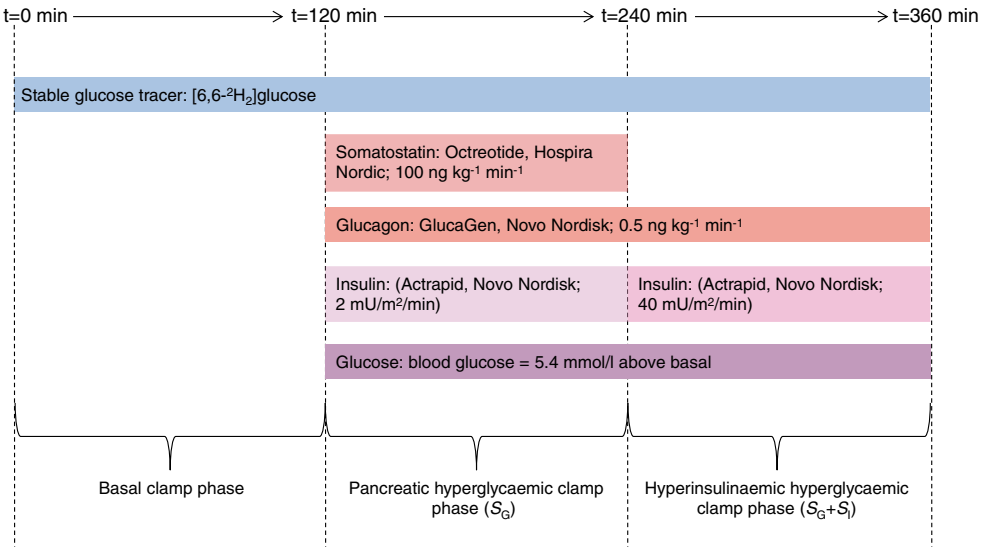

Fig. 1 The two-step (pancreatic and hyperinsulinaemic) hyperglycaemic clamp procedure used in the study. The pancreatic hyperglycaemic clamp phase is considered to represent S_G, and the hyperinsulinaemic hyperglycaemic clamp phase is considered to represent $S_G + S_I$. As such, S_I is derived by subtracting the results of the pancreatic hyperglycaemic clamp phase from the results of the hyperinsulinaemic hyperglycaemic clamp phase

Interventions The CWT and IWT interventions consisted of ten supervised treadmill (Katana Sport, Lode, Groningen, the Netherlands) walking sessions. CWT consisted of continuous walking, aiming for 73% of the $\dot{V}O_{2peak}$, whereas IWT consisted of repeated cycles of 3 min slow walking and 3 min fast walking (aiming for 54% and 89% of $\dot{V}O_{2peak}$ measured during the last minute of each interval, respectively), as previously described [21]. To find the correct walking speeds, oxygen uptake was measured continuously during the first and sixth session of each training intervention using stationary indirect calorimetry equipment (Quark, Cosmed). The walking speeds determined in these sessions were repeated for the following four sessions. Training was performed with a heart-rate monitor (Polar RC3, Polar, Kempele, Finland). During the control intervention, participants were instructed to continue with their life unaltered.

Experimental day For 2 days prior to each experimental day, participants were instructed to avoid strenuous physical activity (except the prescribed CWT or IWT) and alcohol. On the day prior to the experimental day, a standardised diet with individually calculated energy content (based on the Mifflin–St Jeor equation [25] multiplied by a physical-activity level of 1.4 metabolic equivalents) was provided. The diet consisted of nutritional drinks (Resource Komplett 1.5, Nestlé, Vevey, Switzerland) divided into three equal-sized portions, with a mixed macronutrient composition providing 15% of energy from protein, 55% from carbohydrate and 30% from fat. Glycaemic control was assessed using continuous glucose monitoring (CGM; Enlite sensor and iPro2 monitor, Medtronic, Fridley, MN, USA) during the standardised dietary intake in the 24 h preceding each experimental day. Experimental days after the intervention periods were planned so that the last exercise bout was performed 39–43 h before initiation of the clamp procedure.

On the experimental days, participants arrived at the laboratory at 07:00 after approximately 12 h of fasting (water intake was allowed). After voiding, bilateral antecubital vein catheters were inserted and participants were placed in a bed in a temperature-controlled (20°C) and quiet room, where they stayed for the 360 min clamp procedure (Fig. 1). A primed (20 μmol/kg multiplied by fasting glucose, divided by 5 mmol), continuous (0.3 μmol kg^{-1} min^{-1}) infusion of stable [6,6-$^2H_2$]glucose tracer was initiated at 0 min (t = 0). At 120 min, a basal blood sample was obtained and the pancreatic hyperglycaemic clamp phase began. Infusion of a drug cocktail consisting of somatostatin (100 ng kg^{-1} min^{-1}; Octreotide, Hospira Nordic, Stockholm, Sweden), insulin (2 mU/m^2/min; Actrapid, Novo Nordisk, Bagsværd, Denmark) and glucagon (0.5 ng kg^{-1} min^{-1}; GlucaGen, Novo Nordisk) commenced and blood glucose levels were increased to 5.4 mmol/l above the individual's pre-intervention basal blood glucose level [26] by a square-wave glucose infusion (100 mg/ml Glucos Fresenius Kabi, Fresenius Kabi, Uppsala, Sweden) for a 15 min duration. Blood glucose was kept at this hyperglycaemic level for the remainder of the clamp procedure, with glucose infusion rate (GIR) adjustments performed according to a computerised algorithm derived from DeFronzo et al [27] and based on blood glucose measurements obtained every 5 min (ABL 7 series, Radiometer, Herlev, Denmark). At 240 min, the hyperinsulinaemic hyperglycaemic clamp phase commenced. Somatostatin infusion was terminated, glucagon infusion continued unchanged and insulin infusion was initially increased (120 mU/m^2/min) and then exponentially decreased (every 1 min) over 10 min to 40 mU/m^2/min where it remained for the rest of the clamp. At 360 min, the clamp was terminated. Urine from the entire clamp was collected.

Respiratory exchange ratios (RERs) were obtained using indirect calorimetry (Quark, Cosmed) with a ventilated hood.

Measurements were performed between the 60th and 90th min in each of the three clamp phases (basal; pancreatic hyperglycaemic; hyperinsulinaemic hyperglycaemic), with participants placed in a supine position.

Blood samples for tracer analyses (NaF tubes) were obtained during fasting (at 0 min/t = 0) and every 10 min during the last 30 min of each clamp phase. Blood samples for analyses of insulin and C-peptide (lithium-heparin tubes), glucagon (EDTA tubes coated with aprotinin [50 kIU/ml]) and NEFA (EDTA tubes) were obtained at the end of each clamp phase. Blood samples were centrifuged (2000 *g*, 15 min, 4°C) and plasma was kept at −80°C until analyses.

After termination of the clamp, participants underwent a dual-energy x-ray absorptiometry scan (Lunar Prodigy Advance, GE 253 Healthcare, Madison, WI, USA).

Analyses Plasma sample tracer enrichment was performed and analysed as previously described [28]. Analyses were performed using an electrochemiluminescence immunoassay (Cobas 8000, Roche Diagnostics, IN, USA) for insulin and C-peptide, an RIA (GL-32K, Merck Millipore, Darmstadt, Germany) for glucagon and a commercial kit (HR series NEFA-HR(2), Wako Diagnostics, Richmond VA, USA) for NEFA. Urine volume, glucose concentration (ABL 7 series, Radiometer) and urea were measured using absorption photometry (Cobas 8000, Module c702, Roche Diagnostics).

Calculations Mean overall oxygen consumption rates and training-associated RER were calculated, in addition to the mean of the last minute of every fast/slow IWT interval during the first and sixth CWT/IWT sessions for the same variables. Mean heart rates and the mean of the last minute of every fast/slow IWT interval were calculated for all interventions.

Mean GIR was calculated during the last 30 min of the pancreatic hyperglycaemic and hyperinsulinaemic hyperglycaemic clamp phases. GIR measured during the pancreatic hyperglycaemic clamp phase represents S_G [15], whereas GIR measured during the hyperinsulinaemic hyperglycaemic clamp phase represents a combination of S_G and S_I [26, 29]. As such, S_I was calculated by subtracting GIR measured during the pancreatic hyperglycaemic clamp phase from GIR measured during the hyperinsulinaemic hyperglycaemic clamp phase.

Total rates of glucose appearance (R_{aTOTAL}) and disappearance (R_d) were assessed using non-steady-state calculations as previously described [30]. Endogenous glucose production (R_{aENDO}) was considered equal to R_a during the basal clamp phase, and was calculated as R_{aTOTAL} − GIR during the pancreatic and hyperinsulinaemic hyperglycaemic clamp phases. If this resulted in negative values, R_{aENDO} was set to zero [31].

RER levels obtained during the clamp were used to calculate carbohydrate and lipid oxidation rates, according to the equations by Frayn [32]. Protein oxidation rates were considered stable throughout the clamp procedure and were estimated from the urinary nitrogen excretion derived from urea excretion.

Statistics To assess potential baseline differences, pre-intervention variables were compared using one-way repeated-measures ANOVA. Variables obtained during the CWT and IWT interventions were compared using Student's paired t test. Variables obtained pre- and postintervention were analysed by two-way repeated-measures ANOVA (intervention × time) and by one-way repeated-measures ANOVA of the delta (post – pre intervention) values. Comparison of variables between clamp stages was performed using one-way repeated-measures ANOVA. In all ANOVAs, Bonferroni-corrected post hoc tests were applied to access specific differences. Regression analyses were used to determine associations between intervention-induced changes in either S_G or S_I and intervention-induced changes in glycaemic control. In the regression analyses including all interventions (where each participant contributed three measurements), adjustments for repeated measures were performed using random-intercept models including participants as random effects. Normality was confirmed using the Shapiro–Wilk test on residuals. Data are reported as means ± SEM. Statistical tests were performed using Prism v6.03 (GraphPad Software, La Jolla, CA, USA) or Stata v13.1 (StataCorp, College Station, TX, USA), with statistical significance accepted when $p < 0.05$.

Results

All 14 participants (11 men, three women; age 65.3 ± 1.7 years; time since diabetes diagnosis 8.6 ± 1.3 years; BMI 31.6 ± 1.1 kg/m^2; HbA_{1c} 6.5 ± 0.2% [48 ± 2 mmol/mol]) continued their usual glucose-lowering treatment (metformin [n = 14], sulfonylureas [n = 3], glucagon-like peptide 1 analogues [n = 3]) throughout the study period. The standardised diets were consumed as prescribed prior to each experimental day, resulting in a mean energy intake of 10,117 ± 418 kJ with no differences within or between interventions (data not shown). All participants completed all interventions and experimental days. One participant, however, developed fever during the post-IWT experimental day (ear temperature 37.2°C before initiation of the clamp and 38.6°C at the end of the pancreatic hyperglycaemic clamp phase), and so the clamp was stopped prematurely. As such, this participant was excluded from the analyses and the results include only 13 participants.

Training Adherence to training was high (99% sessions completed in both the CWT and IWT interventions) and training sessions were, in all cases, completed at the intended

walking speeds. The CWT and IWT interventions were well matched regarding mean oxygen consumption and heart rates (Table 1), as previously reported [21]. Conversely, mean walking speed was higher and RER was lower in the CWT compared with the IWT intervention.

Body composition and glycaemic control Pre-intervention levels of body composition variables (body mass, fat mass and lean body mass) did not differ between trials. No significant changes within or differences between trials were seen for any body composition variable.

Pre-intervention glycaemic control variables (mean, minimum and maximum CGM glucose levels measured over 24 h) did not differ between trials and remained unchanged following the control intervention and CWT. While IWT did not affect minimum 24 h CGM glucose levels, it resulted in a non-significant decrease in mean 24 h CGM glucose levels ($\Delta = -0.7 \pm 0.2$ mmol/l, $p = 0.07$) and significantly decreased maximum 24 h CGM glucose levels ($\Delta = -1.8 \pm 0.5$ mmol/l, $p < 0.05$).

Body composition and CGM-derived glycaemic control data have been published previously [21].

Table 1 Training variables

Variable	CWT	IWT
Oxygen uptake (ml/min)		
Mean	1524 ± 78	1513 ± 78
Slow-walking intervals		1190 ± 62†
Fast-walking intervals		1814 ± 99†
RER (fraction)		
Mean	0.83 ± 0.01	0.90 ± 0.01*
Slow-walking intervals		0.91 ± 0.01†
Fast-walking intervals		0.90 ± 0.01†
Heart rate (beats per min)		
Mean	108 ± 3	109 ± 3
Slow-walking intervals		100 ± 2†
Fast-walking intervals		119 ± 3†
Walking speed (km/h)		
Mean	5.0 ± 0.1	4.7 ± 0.1*
Slow-walking intervals		3.4 ± 0.1†
Fast-walking intervals		6.0 ± 0.1†

Data are means ± SEM

Slow-/fast-walking intervals include data from the last minute of each interval

Oxygen uptake and RER data are means of the first and sixth training sessions; all other data are means of all training sessions

Variables were compared using Student's paired *t* tests

*$p < 0.05$ CWT vs IWT; †$p < 0.05$ IWT slow/fast intervals vs CWT

Clamp details including hormones and metabolites Pre-intervention levels of blood glucose, insulin, C-peptide, glucagon and NEFA did not differ between trials (Table 2).

Neither basal nor pancreatic/hyperinsulinaemic hyperglycaemic clamp blood glucose concentrations changed with any of the trials, nor were there any intervention-induced differences between trials. Blood glucose concentrations during the clamp were generally stable (CV < 5%) with less than 5% error from the target clamp concentration (Table 2; Fig. 2a).

Insulin concentrations were maintained at basal levels during the pancreatic hyperglycaemic clamp phase with no changes within, nor differences between, trials. Conversely, insulin concentrations were on average approximately sevenfold higher in the hyperinsulinaemic hyperglycaemic clamp phase compared with basal and pancreatic hyperglycaemic clamp phases (both $p < 0.001$), with no changes within or differences between trials (Fig. 2b).

C-peptide concentrations were reduced in the pancreatic and hyperinsulinaemic hyperglycaemic clamp phases compared with basal (both $p < 0.001$), with no differences within or between trials and with no differences between the pancreatic and hyperinsulinaemic hyperglycaemic clamp phases (Fig. 2c).

Glucagon concentrations were reduced sequentially from basal to the pancreatic and hyperinsulinaemic hyperglycaemic clamp phases ($p < 0.001$ for all comparisons) with no differences within or between trials (Fig. 2d).

NEFA concentrations did not differ within or between trials during any clamp phase. Moreover, NEFA concentrations were not different between baseline and the pancreatic hyperglycaemic clamp phase, but declined from the pancreatic hyperglycaemic clamp phase to the hyperinsulinaemic hyperglycaemic clamp phase ($p < 0.001$).

GIRs and kinetics Pre-intervention GIR, R_{aENDO} and R_d did not differ between trials in any clamp phase (Table 2).

GIR (Fig. 3a) in the pancreatic hyperglycaemic clamp phase (S_G) was increased after compared with before IWT ($p < 0.05$), with no intervention-induced effects of the control intervention or CWT and no intervention-induced differences between trials. In the hyperinsulinaemic hyperglycaemic clamp phase ($S_G + S_I$), GIR was increased after compared with before IWT ($p < 0.01$), with no intervention-induced effects of the control intervention or CWT and no intervention-induced differences between trials. When subtracting GIR in the pancreatic hyperglycaemic clamp phase from GIR in the hyperinsulinaemic hyperglycaemic clamp phase (S_I), the significant increase after IWT disappeared, while a non-significant increase in GIR after the control intervention was seen ($p = 0.10$); there were still no intervention-induced differences between trials.

In the pancreatic hyperglycaemic clamp phase, no changes within or differences between trials were seen in R_d (Fig. 3b). Conversely, R_{aENDO} was reduced with CWT ($p < 0.05$) and

Table 2 Clamp variables

Variable	Control[a]		CWT		IWT	
	Pre	Post	Pre	Post	Pre	Post
Basal						
Glucose (mmol/l)	6.9 ± 0.3	7.3 ± 0.6	7.2 ± 0.4	6.9 ± 0.4	7.4 ± 0.6	7.0 ± 0.4
Insulin (pmol/l)	106 ± 12	105 ± 12	96 ± 10	94 ± 15	108 ± 15	98 ± 13
C-peptide (pmol/l)	1126 ± 104	1177 ± 111	1133 ± 78	1132 ± 89	1160 ± 120	1187 ± 114
Glucagon (ng/l)‡	94 ± 10	88 ± 10	92 ± 8	85 ± 10	93 ± 10	86 ± 8
R_{aENDO} (mg kg^{-1} min^{-1})	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
NEFA (mmol/l)	0.62 ± 0.03	0.62 ± 0.05	0.67 ± 0.06	0.63 ± 0.04	0.60 ± 0.05	0.57 ± 0.05
RER	0.82 ± 0.01	0.82 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.82 ± 0.01	0.81 ± 0.01
Pancreatic hyperglycaemic phase						
Glucose (mmol/l)	12.4 ± 0.3	12.4 ± 0.3	12.8 ± 0.4	12.6 ± 0.4	12.6 ± 0.3	12.4 ± 0.3
CV (%)	1.4 ± 0.2	1.1 ± 0.2	1.4 ± 0.3	1.4 ± 0.2	1.2 ± 0.2	1.0 ± 0.1
Deviation from clamp goal (%)	1.4 ± 0.4	1.9 ± 0.5	3.6 ± 1.2	1.3 ± 0.6	2.5 ± 0.6	1.0 ± 0.5
Insulin (pmol/l)	95 ± 18	87 ± 13	92 ± 14	84 ± 13	93 ± 14	90 ± 13
C-peptide (pmol/l)	707 ± 99	711 ± 89	741 ± 96	690 ± 76	744 ± 88	759 ± 99
Glucagon (ng/l)‡	82 ± 7	72 ± 7*	85 ± 8	76 ± 8	85 ± 6	81 ± 6
GIR (mg kg^{-1} min^{-1})†	1.2 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.1	1.0 ± 0.2	1.5 ± 0.2*
R_{aENDO} (mg kg^{-1} min^{-1})‡	1.7 ± 0.2	1.6 ± 0.2	1.8 ± 0.2	1.4 ± 0.2*	1.7 ± 0.2	1.4 ± 0.2§
R_d (mg kg^{-1} min^{-1})	2.9 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.9 ± 0.1
NEFA (mmol/l)	0.65 ± 0.06	0.57 ± 0.04	0.60 ± 0.05	0.58 ± 0.04	0.60 ± 0.05	0.56 ± 0.07
RER	0.78 ± 0.01	0.78 ± 0.01	0.79 ± 0.01	0.77 ± 0.01	0.78 ± 0.01	0.78 ± 0.01
Hyperinsulinaemic hyperglycaemic phase						
Glucose (mmol/l)	12.3 ± 0.4	12.2 ± 0.4	12.4 ± 0.5	12.5 ± 0.4	12.3 ± 0.4	12.2 ± 0.3
CV (%)	2.1 ± 0.4	2.2 ± 0.4	2.0 ± 0.3	2.1 ± 0.5	2.3 ± 0.4	2.4 ± 0.4
Deviation from clamp goal (%)	−0.2 ± 0.5	−1.1 ± 0.6	−1.4 ± 0.8	−1.1 ± 0.4	−0.3 ± 0.6	−0.9 ± 0.5
Insulin (pmol/l)	729 ± 38	733 ± 33	734 ± 30	713 ± 33	728 ± 33	739 ± 35
C-peptide (pmol/l)	698 ± 101	706 ± 88	669 ± 98	712 ± 92	720 ± 99	745 ± 104
Glucagon (ng/l)	68 ± 6	68 ± 5	75 ± 7	67 ± 7	71 ± 6	68 ± 6
GIR (mg kg^{-1} min^{-1})†‡	4.5 ± 0.4	5.0 ± 0.7	4.8 ± 0.5	5.1 ± 0.6	5.1 ± 0.5	6.2 ± 0.7**
R_{aENDO} (mg kg^{-1} min^{-1})	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
R_d (mg kg^{-1} min^{-1})	3.7 ± 0.2	3.6 ± 0.3	3.6 ± 0.2	3.6 ± 0.3	3.9 ± 0.3	4.3 ± 0.3*
NEFA (mmol/l)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
RER	0.85 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	0.84 ± 0.02	0.84 ± 0.01	0.86 ± 0.01

Data are means ± SEM during the second-to-last (RER) and last 30 min of the clamp stages (glucose, CV, error from clamp goal, GIR, R_a and R_d) or at the end of the clamp stage (insulin, C-peptide, glucagon and NEFA)

[a] Non-exercising control intervention

*$p < 0.05$, **$p < 0.01$ (within group, pre vs post); † $p < 0.05$ (time × group interaction); ‡ $p < 0.05$ (main effect of time); § $p = 0.07$ (within group, pre vs post)

was non-significantly decreased with IWT ($p = 0.07$). No changes were seen within the control intervention or between trials (Fig. 3c).

In the hyperinsulinaemic hyperglycaemic clamp phase, R_d increased following IWT ($p < 0.05$), whereas no changes within CWT or the control intervention and no differences between trials were seen (Fig. 3b). R_{aENDO} was maximally suppressed in almost all participants in the hyperinsulinaemic hyperglycaemic clamp phase, and no differences within or between trials were seen (Fig. 3c).

When subtracting R_d in the pancreatic hyperglycaemic clamp phase from R_d in the hyperinsulinaemic hyperglycaemic clamp phase, the significant improvement as a result of IWT disappeared, and no differences within or between trials were seen (Fig. 3b). Since the calculated R_{aENDO} was negative in several hyperinsulinaemic hyperglycaemic clamp phases and therefore

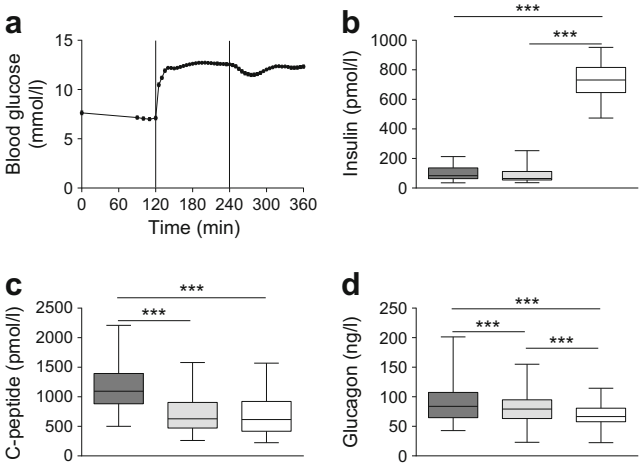

Fig. 2 Blood glucose (**a**), insulin (**b**), C-peptide (**c**) and glucagon (**d**) concentrations during the hyperglycaemic clamp. Dark grey boxes, basal (0–120 min); light grey boxes, pancreatic hyperglycaemic clamp phase (120–240 min); white boxes, hyperinsulinaemic hyperglycaemic clamp phase (240–360 min). Since no differences were seen within or between trials, data are presented as means ± SEM (**a**) and box and whisker plots (**b**–**d**) of all clamps. Statistical differences were analysed using one-way repeated-measures ANOVA between clamp stages. ***$p < 0.001$

set to zero, the subtraction of R_{aENDO} during the pancreatic hyperglycaemic clamp phase from the hyperinsulinaemic hyperglycaemic phase was not performed (Fig. 3c). Since S_G, as described above, is defined as the ability of glucose per se to stimulate its own uptake and suppress its own production [7], we subtracted R_{aENDO} from R_d in the pancreatic hyperglycaemic clamp phase. This resulted in an increase after IWT and an intervention-induced difference between the control intervention and IWT ($p < 0.05$ for both).

Clamp RER and substrate oxidation rates Pre-intervention levels of RER and substrate oxidation rates did not differ in any clamp phase between trials, and no changes within or differences between trials were seen in basal levels or during the pancreatic hyperglycaemic clamp phase (Table 2, Fig. 3d, e). In the hyperinsulinaemic hyperglycaemic clamp phase, carbohydrate oxidation rates non-significantly increased with IWT ($p = 0.10$), whereas fat oxidation rates decreased ($p < 0.05$). No IWT-induced changes were seen in protein oxidation rates, and no intervention-induced changes in RER or any substrate oxidation rates were seen in the control intervention or CWT. When subtracting the pancreatic clamp phase from the hyperinsulinaemic clamp phase, no changes within or differences between trials were seen for RER or substrate oxidation rates.

Associations between intervention-induced changes in glycaemic control and S_G or S_I When evaluating all trials collectively, significant associations between changes in GIR in the pancreatic hyperglycaemic clamp phase (S_G) and changes in mean ($\beta = -0.62 \pm 0.23$, $r^2 = 0.17$, $p < 0.01$, Fig. 4a) and maximum ($\beta = -1.18 \pm 0.52$, $r^2 = 0.12$, $p < 0.05$, Fig. 4b) 24 h CGM glucose were seen, whereas no association was found for minimum

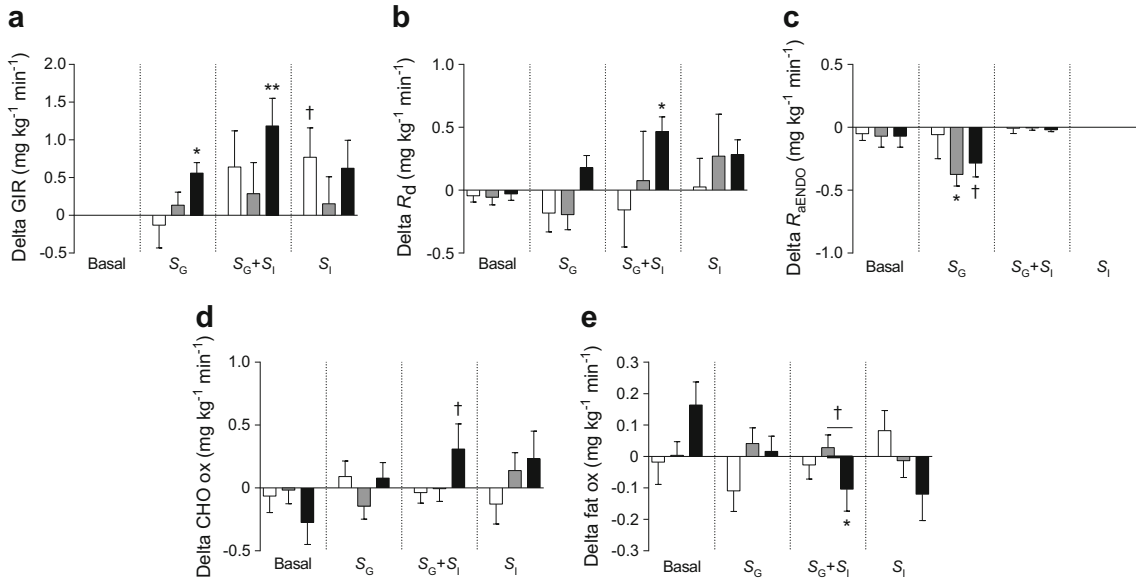

Fig. 3 Delta (post – pre intervention) values for GIR (**a**), R_d (**b**), R_{aENDO} (**c**), and carbohydrate oxidation (CHO ox; **d**) and fat oxidation (fat ox; **e**) rates. Each graph is subdivided into the basal clamp phase, the pancreatic hyperglycaemic (S_G) clamp phase, the hyperinsulinaemic hyperglycaemic ($S_G + S_I$) clamp phase and the hyperinsulinaemic minus pancreatic (S_I) clamp phase. Since the calculated R_{aENDO} was negative in several hyperinsulinaemic hyperglycaemic clamp phases and, therefore, set to zero, subtraction of R_{aENDO} during the pancreatic hyperglycaemic clamp phase from the hyperinsulinaemic hyperglycaemic phase was not performed. White bars, control (no exercise); grey bars, CWT; black bars, IWT. Data are presented as mean delta values ± SEM. Statistical differences were analysed using two-way (intervention × time) repeated-measures ANOVA for within-intervention, within-clamp-phase comparisons and by one-way repeated-measures ANOVA for between-intervention, within-clamp-phase comparisons (indicated by a connecting line between bars). *$p < 0.05$, **$p < 0.01$, †$p \leq 0.10$

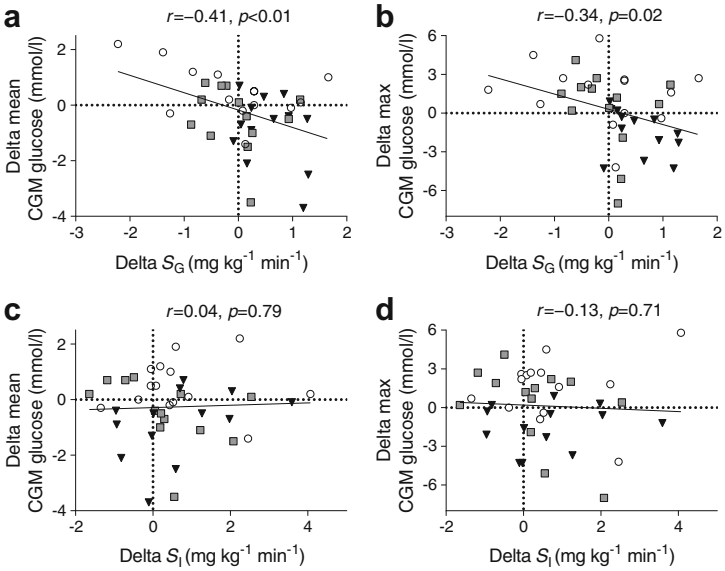

Fig. 4 Associations between delta (post – pre intervention) values in S_G and mean and maximum 24 h CGM glucose levels (**a**, **b**) and delta values in S_I and mean and maximum 24 h CGM glucose levels (**c**, **d**). Circles, control intervention; squares, CWT; triangles, IWT. Associations were examined using linear regression analyses, with r and p values given in the figure

24 h CGM glucose ($p > 0.05$). Conversely, no associations were seen between changes in GIR in the hyperinsulinaemic hyperglycaemic clamp phase minus the pancreatic hyperglycaemic clamp phase (S_I) and any glycaemic control variable (Fig. 4c, d).

When evaluating the IWT trial alone, the associations between changes in S_G and changes in mean ($\beta = -0.89 \pm 0.71$, $r^2 = 0.13$, $p > 0.05$) and maximum ($\beta = -1.40 \pm 0.96$, $r^2 = 0.16$, $p > 0.05$) 24 h CGM glucose retained β-coefficients and r^2 values comparable with those of the analyses including all trials, but were no longer significant.

Discussion

This is the first study to assess training-induced changes in both S_G and S_I in individuals with type 2 diabetes. Several findings are to be highlighted. First, S_G increased significantly following 2 weeks of IWT in individuals with type 2 diabetes, whereas S_I did not. Second, intervention-induced changes in S_G but not S_I were associated with changes in CGM-derived glycaemic control. Third, the IWT-induced increase in S_G was dependent on the combination of increased glucose disposal and decreased endogenous glucose production. Fourth, whereas 2 weeks of CWT reduced endogenous glucose production in response to hyperglycaemia with basal levels of insulin, no overall CWT-induced changes in S_G or S_I were seen.

The associations seen between intervention-induced changes in S_G and the mean and maximum 24 h CGM glucose levels indicate that S_G is an important determinant of glycaemic control in individuals with type 2 diabetes. Since no associations were observed between changes in S_I and either the mean or the maximum 24 h CGM glucose level, increases in S_G may be the most important determinant of short-term training-induced improvements in glucose tolerance in individuals with type 2 diabetes. Interestingly, S_G only increased with IWT but not with CWT, highlighting that peak exercise intensity is important for improvements in S_G, which is in accord with previous findings [33]. Whereas the associations between changes in S_G and CGM glucose levels were not significant when evaluating only the IWT trial, the associations retained comparable β-coefficients and r^2 values as when all trials were included in the regression analyses. As such, at least parts of the IWT-induced improvements in glycaemic control were probably mediated via S_G.

The IWT-induced improvements in S_G were reflected neither by significantly reduced endogenous glucose production nor by increased glucose disposal, but rather by the combined effects of the two. Whereas this may be related to low power, we speculate that individual differences may be responsible, with some individuals responding to hyperglycaemia with reduced endogenous glucose production and other individuals with increased glucose disposal. Type 2 diabetes is a highly heterogeneous disease [34], and responses to exercise are therefore expected to be heterogeneous, as previously demonstrated [16, 35, 36].

The mechanisms underlying the IWT-induced improvements in S_G cannot readily be determined from this study. It has been proposed that impaired S_G in individuals with type 2 diabetes is dependent on increased systemic concentrations of NEFA [37], and that normalisation of NEFA concentrations near-normalises S_G [14, 38]. However, we did not find any effect of the training interventions on NEFA concentrations, suggesting that NEFA is not important for the IWT-improved S_G. Other mechanistic suggestions of importance for S_G include the mass action effect of glucose, glucose-induced stimulation of enzymatic activity in skeletal muscle and liver

and a direct effect of glucose in promoting GLUT4 translocation to the cell membrane in skeletal muscle [7, 15]. Since liver enzyme content and activity can hardly be assessed in human in vivo studies, future studies evaluating training-induced effects on S_G should consider using animal models.

It is generally accepted that physical activity can acutely increase peripheral tissue glucose disposal for up to 48 h, and that this may be mediated both via insulin-dependent and insulin-independent mechanisms [39, 40]. Several insulin-independent mechanisms responsible for acute exercise-induced peripheral tissue disposal have been identified, with AMP-activated protein kinase (AMPK) [41] and Ras-related C3 botulinum toxin substrate 1 [42] being key players. Since our clamp procedure was initiated 39–43 h after the last CWT/IWT exercise bout, we cannot rule out that acute exercise effects might have played a role in the IWT-induced improvements seen in S_G, since these mechanisms are known to be dependent on exercise intensity [43].

Another factor that might have played a role in the difference in S_G after CWT vs IWT is the difference in RER during the training sessions. The increased RER during IWT indicates that a larger fraction of the substrates used during IWT was derived from carbohydrates. Therefore, glycogen depletion in skeletal muscle was probably higher following IWT than CWT. Since skeletal muscle glycogen depletion provides a strong stimulus for increased peripheral tissue glucose disposal [44], this may have influenced the differences found. In addition, since carbohydrate oxidation rates during the pancreatic hyperglycaemic clamp phase were not increased by IWT (Fig. 3b), non-oxidative glucose disposal (glycogen synthesis and storage) might have been increased. However, the approximately 40 h time window between the last exercise bout and the clamp procedure, during which participants consumed a mixed diet, should have been sufficient to replenish skeletal muscle glycogen levels. This is, however, speculation and should be confirmed prospectively.

The lack of significant improvements in S_I with the training interventions must be noted. Since both CWT and IWT numerically increased both S_I and the associated glucose disposal, the lack of significance may potentially be ascribed to low power and the unexpected (and unexplainable) borderline significant increase in S_I after the control intervention. Nonetheless, the increases in S_I following our training interventions were small, particularly when compared with other studies of short-term training interventions [45, 46]. Since training interventions without changes in body composition improve S_I less than training interventions with improved body composition [10], and since training-induced improvements in S_I are typically undetectable 48 h after the last exercise bout when no changes in body composition are seen [47, 48], our study design likely explains the lack of improvement in S_I. Of interest, previous findings have indicated that, whereas training-induced improvements in S_I are short-lived, training-induced improvements in S_G are maintained for at least 1 week after training cessation [17].

It must be noted that the one-way repeated-measures ANOVA analyses performed on the delta values did not result in any between-trial differences in S_G or S_I. Given the observed within-trial differences in the two-way repeated-measures ANOVA analyses, we hypothesise that the small sample size may explain the lack of between-trial differences. As such, future studies must use larger sample sizes in order to assess potential differences between interventions. Moreover, it must be noted that the non-simultaneous measurement of S_G and $S_G + S_I$ may potentially be a problem when S_I is derived. This may be explained by the fact that S_I (assessed by GIR in a euglycaemic–hyperinsulinaemic clamp) typically increases with increasing clamp duration [49]. Although we are not aware of studies evaluating the effect of increased time duration on S_G, it may be questioned whether S_G in the hyperinsulinaemic hyperglycaemic clamp phase was the same as during the pancreatic hyperglycaemic clamp phase. That said, by using state-of-the-art and high-quality glucose clamp methodology, we believe that our study advances the field with novel observations regarding glycaemic adaptations following exercise in diabetes.

In conclusion, this study has shown that short-term IWT improves S_G but not S_I in individuals with type 2 diabetes. Moreover, changes in S_G but not S_I are associated with changes in glycaemic control. As such, S_G is an important yet underappreciated mechanism by which training may influence glucose metabolism, and future studies should evaluate the importance and underlying mechanisms of training-induced improvements in S_G.

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Data availability The data that support the findings of this study are available on request from the corresponding author (KK), given that this does not violate the laws of the Danish Data Protection Agency.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement KK designed the study and obtained the funding. TPJS and BKP contributed to the study design. KK, MAC, IJ and SHK acquired the data. KK, MAC, GvH and TPJS analysed and interpreted the data. KK wrote the manuscript. All authors reviewed and revised the manuscript and approved the final version. KK is the guarantor for the work as a whole.

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