High-intensity interval training combining rowing and cycling improves but does not restore beta-cell function in type 2 diabetes

Houborg Petersen, Maria; Stidsen, Jacob Volmer; Eisemann de Almeida, Martin; Kleis Wentorf, Emil; Jensen, Kurt; Ørtenblad, Niels; Højlund, Kurt

Published in:
Endocrine Connections

DOI:
10.1530/EC-23-0558

Publication date:
2024

Document version:
Final published version

Society for Endocrinology
European Society of Endocrinology

Endocrine Connections (2024) **13** e230558
https://doi.org/10.1530/EC-23-0558

Received 3 January 2024
Accepted 21 March 2024
Available online 21 March 2024
Version of Record published 12 April 2024

RESEARCH

High-intensity interval training combining rowing and cycling improves but does not restore beta-cell function in type 2 diabetes

Maria Houborg Petersen[1,2], Jacob Volmer Stidsen[1], Martin Eisemann de Almeida[1,3], Emil Kleis Wentorf[3], Kurt Jensen[3], Niels Ørtenblad[3] and Kurt Højlund[1,2]

[1]Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark
[2]Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[3]Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark

Correspondence should be addressed to K Højlund: kurt.hoejlund@rsyd.dk

Abstract

Aim: We investigated whether a high-intensity interval training (HIIT) protocol could restore beta-cell function in type 2 diabetes compared with sedentary obese and lean individuals.

Materials and methods: In patients with type 2 diabetes, and age-matched, glucose-tolerant obese and lean controls, we examined the effect of 8 weeks of supervised HIIT combining rowing and cycling on the acute (first-phase) and second-phase insulin responses, beta-cell function adjusted for insulin sensitivity (disposition index), and serum free fatty acid (FFA) levels using the Botnia clamp (1-h IVGTT followed by 3-h hyperinsulinemic–euglycemic clamp).

Results: At baseline, patients with type 2 diabetes had reduced insulin sensitivity (~40%), acute insulin secretion (~13-fold), and disposition index (>35-fold), whereas insulin-suppressed serum FFA was higher (~2.5-fold) compared with controls (all $P < 0.05$). The HIIT protocol increased insulin sensitivity in all groups (all $P < 0.01$). In patients with type 2 diabetes, this was accompanied by a large (>200%) but variable improvement in the disposition index ($P < 0.05$). Whereas insulin sensitivity improved to the degree seen in controls at baseline, the disposition index remained markedly lower in patients with type 2 diabetes after HIIT (all $P < 0.001$). In controls, HIIT increased the disposition index by ~20–30% (all $P < 0.05$). In all groups, the second-phase insulin responses and insulin-suppressed FFA levels were reduced in response to HIIT (all $P < 0.05$). No group differences were seen in these HIIT-induced responses.

Conclusion: HIIT combining rowing and cycling induced a large but variable increase in beta-cell function adjusted for insulin sensitivity in type 2 diabetes, but the disposition index remained severely impaired compared to controls, suggesting that this defect is less reversible in response to exercise training than insulin resistance.

Trial registration: ClinicalTrials.gov (NCT03500016).

Keywords: beta-cell function; botnia clamp (intravenous glucose tolerance test (IVGTT) and hyperinsulinemic–euglycemic clamp); high-intensity interval training (HIIT); obesity; type 2 diabetes

Published by Bioscientifica Ltd.
https://ec.bioscientifica.com

Endocrine Connections (2024) **13** e230558
https://doi.org/10.1530/EC-23-0558

Introduction

Type 2 diabetes is typically characterized by insulin resistance and failure of the pancreatic beta cells to compensate for this abnormality (1). Exercise training is essential in diabetes management (2), and the beneficial effects of regular exercise training on insulin sensitivity, cardiorespiratory fitness, body composition, glycemic control, and lipid profile in patients with type 2 diabetes are well documented (3, 4). Furthermore, there is evidence supporting a beneficial effect of exercise training on beta-cell function in patients with type 2 diabetes (5, 6, 7, 8, 9). Most studies investigating beta-cell function have evaluated the effect of endurance training at moderate intensity involving mainly lower body muscle groups by either using cycle ergometers or treadmills (5, 7, 10, 11, 12, 13, 14). Thus, little is known about effects of high-intensity interval training (HIIT) recruiting upper and lower body muscle groups on beta-cell function in patients with type 2 diabetes.

When evaluating the effect of exercise training on beta-cell function, it is important to adjust for changes in insulin sensitivity (15, 16, 17). Thus, there is general acceptance that the relationship between insulin secretion and insulin sensitivity is hyperbolic (17, 18), and, therefore, when insulin sensitivity is improved in response to exercise training, less insulin secretion is needed. It is, therefore, preferable that insulin sensitivity and insulin secretion are evaluated on the same day after the last bout of exercise has subsided (19, 20). This is often done using oral glucose tolerance test (OGTT)-derived surrogate markers of insulin secretion and insulin sensitivity (5, 12, 13, 14), which is, however, associated with variability due to differences in the rate of gastric emptying and glucose absorption (21, 22, 23). A better alternative to the OGTT could be the Botnia clamp, which consists of an intravenous glucose tolerance test (IVGTT) followed by a hyperinsulinemic–euglycemic clamp, the gold standard for evaluating insulin sensitivity (24, 25, 26, 27, 28). The Botnia clamp has been validated for same-day independent assessment of insulin secretion and insulin sensitivity in patients with type 2 diabetes (24, 29). However, to our knowledge, the Botnia clamp has not previously been used to evaluate the effect of exercise training on beta-cell function adjusted for insulin sensitivity in patients with type 2 diabetes. Furthermore, it remains to be established to what extent exercise training can restore beta-cell function in patients with type 2 diabetes compared to sedentary non-diabetic individuals.

Recent studies provide evidence that HIIT induces similar or even larger metabolic responses compared to training at moderate intensity (30, 31, 32, 33). Correspondingly, HIIT for 6–8 weeks has been reported to increase beta-cell function adjusted for insulin sensitivity as evaluated by OGTT-derived indices in patients with type 2 diabetes (8, 9). Interestingly, a recent acute exercise study, using the one-leg technique, demonstrated that while insulin sensitivity increased in the exercised muscles, it actually decreased at the whole-body level (34). This finding suggests that the recruitment of more muscle groups during exercise training could enhance the effect on beta-cell function adjusted for insulin sensitivity. Taken together, these data suggest that HIIT combining upper and lower body muscle groups may enhance the beneficial effect of aerobic training on beta-cell function adjusted for insulin sensitivity.

Increased plasma FFA often accompanies the development of obesity and type 2 diabetes and may cause lipotoxicity in pancreatic beta-cells, resulting in reduced beta-cell function (35). Recently, endurance exercise training in healthy males was shown to improve adipose tissue insulin sensitivity measured as the product of fasting insulin and FFA levels (36), also known as the adipose tissue insulin resistance index (Adipo-IR). This suggests that exercise training may improve beta-cell function by reducing lipotoxicity. However, it is unknown if HIIT improves both Adipo-IR and insulin-suppressed FFA and whether such changes correlate with changes in beta-cell function.

We have recently shown that an 8-week HIIT protocol combining cycling and rowing markedly improved insulin sensitivity, body composition, and VO_2max in men with type 2 diabetes and glucose-tolerant obese and lean men (37). In this secondary analysis, we hypothesized that this HIIT protocol would markedly improve beta-cell function adjusted for insulin sensitivity (disposition index) as well as insulin-suppressed FFA levels in patients with type 2 diabetes evaluated by the Botnia clamp and that this would restore beta-cell function in patients with type 2 diabetes compared to sedentary obese and lean individuals.

Materials and methods

Study cohort

Fifteen obese (BMI 27–36) sedentary middle-aged (40–65 years) men with type 2 diabetes, 15 age- and BMI-matched sedentary obese (BMI 27–36) glucose-tolerant men, and 18 age-matched sedentary lean (BMI 20–25) glucose-tolerant men were included in this prespecified secondary analysis. See Supplementary Table 1 (see section on supplementary materials given at the end of this article) for clinical and metabolic characteristics, pre- and post-training. Further details about the participants, including medication and eligibility criteria, are given in Supplementary Materials and methods. This study is part of a larger controlled trial from which other results have been published recently (37, 38, 39). In this study, we report the prespecified second outcome beta-cell function adjusted for insulin sensitivity. At inclusion, oral and written informed consent was obtained from the participants, and the study was approved by the Regional Scientific Ethical Committees for Southern Denmark (project ID: S-20170142) and performed in accordance with the Helsinki Declaration.

Study design

Before and after 8 weeks of supervised HIIT combining rowing and cycling, participants underwent examinations on two separate experimental days: Experimental days 1 and 2 were scheduled before the HIIT protocol approximately 1 week apart, while experimental day 3 was scheduled approximately 60 h after the final HIIT session and experimental day 4 was scheduled 48 h after experimental day 3. Experimental days 1 and 3 were identical and consisted of a DXA scan and a VO_2max test, while experimental days 2 and 4 were identical and consisted of a Botnia clamp (see below) and measures of plasma glucose, Hb1Ac, lipids, serum insulin, and FFA (Supplementary Materials and methods). It was assumed that the final VO_2max test maintained the long-lasting effects of the HIIT protocol, whereas the acute effects of the VO_2max test subsided before the final clamp. Participants attended after overnight fasting on all examination days and were instructed to refrain from physically demanding activities 48 h prior to examinations, as well as alcohol and caffeine 24 h prior to examinations. Furthermore, participants were informed to continue their habitual diet during the study period. The VO_2max test and DXA scan are described in the Supplementary Materials and methods, whereas the Botnia clamp is described below. Participants with type 2 diabetes were requested to withdraw all medication 1 week before clamp studies on experimental days 2 and 4 but otherwise to continue their medication during the study period.

As reported (37), the adherence to the training sessions was high with an attendance rate of >95% in all groups, and the average maximum heart rate (HR_{max}) was above 85% during the training intervals in all groups. No participants sustained injuries, and only four participants dropped out during the project. One lean man did not start the training period due to a new knee injury, and one lean man and two men with type 2 diabetes dropped out during the training period due to lack of time.

Botnia clamp

The Botnia clamp consists of an IVGTT and a hyperinsulinemic–euglycemic clamp (24, 29). [3-3H]-glucose tracer was used throughout the Botnia clamp to assess whole-body glucose disposal rates (GDR) and hepatic glucose production (HGP) at the basal and insulin-stimulated steady-state periods (Supplementary Materials and methods) (40). After a basal 2-h tracer equilibration period, a 60-min IVGTT was performed using a bolus of 20% glucose solution (0.3 g/kg body weight, maximum 25 g glucose). The first-phase insulin response (FPIR) was determined as the incremental and total insulin secretion during the first 10 min, and the second-phase insulin response (SPIR) as the incremental and total insulin secretion during the following 10–60 min (24, 29). The acute insulin response to glucose (AIRg) was calculated as the mean increase in serum insulin above baseline insulin in the first 10 min (41). Insulin-stimulated glucose infusion rates (GIR) were determined as the average amount of glucose (mg/min/m^2) needed to maintain euglycemia (5.0–5.5 mmol/L) during the last 40 min of the clamp. GDR and HGP were calculated using Steele's non-steady-state equations during the final 40 min of the basal and insulin-stimulated steady-state periods (40). Further details are given in the Supplementary Materials and methods.

To calculate the beta-cell function adjusted for insulin sensitivity, also termed the disposition index (DI), we multiplied the acute insulin secretion (assessed as the AIRg) by insulin sensitivity measured as insulin-stimulated GDR adjusted for the insulin levels observed at the end of the clamp (GDR/I) (18, 41). Serum FFA levels were measured at the end of the basal and insulin-stimulated steady-state periods.

HIIT protocol

The HIIT protocol consisted of 3 weekly supervised training sessions (Monday, Wednesday, and Friday in the afternoon) performed at high intensity (≥85% of HR_{max}) for 8 weeks on rowing and cycling ergometers with the number of training blocks increasing from two to five per session as described in detail previously (37). After a 10 min warm-up period, each training session included training blocks of five times 1 min training at high intensity (≥85% of HR_{max}) interspersed with a 1 min period of active or passive recovery. In weeks 1–2, the training sessions consisted of two training blocks, and an extra training block was added after every second week completed, ending at five training blocks in weeks 7–8. Training blocks were performed alternately on rowing (Concept2 Model E, Morrisville, Vermont, USA) and cycle ergometer (Wattbike Pro/Trainer, Nottingham, UK), and were separated by a 4 min break. Heart rate was monitored during all training sessions (Polar H7, Polar team, Kempele, Finland) to ensure training at the targeted intensity. One training session was replaced by a midway test of VO_2max to adjust workload.

Statistical analysis

Statistical analysis was performed by STATA/IC 17.0 (StataCorp LLC, TX, USA), while visual presentations were applied by the GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). The sample size was estimated to detect lower insulin-stimulated GDR in men with type 2 diabetes and an increase in insulin-stimulated GDR in response to HIIT, providing a power of >80% when including 13 individuals in each group (37). Mixed model linear regression was used to compare pre- and post-training data within and between groups. The regression model was modified to adjust for different variabilities in the outcome measurements between the three groups. Correlation analyses were performed by the Pearson's correlation coefficient. All data were tested for normality. Baseline characteristics of the four dropouts

were included in the analyses, but the regression model did not include these data in the analyses of the HIIT-induced effects. Data are presented as means ± S.E.M. for each group. A two-sided *P*-value below 0.05 was defined as statistically significant.

Results

Baseline characteristics

The clinical, biochemical, and clamp metabolic characteristics of the study cohort are presented in Supplementary Table 1 and have been reported in detail previously (37). In line with the reported data on total fat mass (FM) and lean body mass (LBM) (37), the regional levels of FM (android, gynoid, trunk, arms, and legs) were higher in obese men with and without type 2 diabetes compared with lean men (all $P < 0.001$), and the regional levels of LBM were higher in obese men with and without type 2 diabetes compared to lean men (all $P < 0.05$) except for a lack of difference in arm LBM between the diabetic and lean groups (Table 1).

At baseline, the acute insulin secretion (AIRg and the incremental FPIR) was markedly lower (~13-fold) in men with type 2 diabetes compared with lean and obese controls (all $P < 0.001$), and the incremental SPIR was two-fold lower in men with type 2 diabetes compared with lean controls ($P < 0.05$) (Table 2). Moreover, the beta-cell function adjusted for insulin sensitivity (estimated as the DI) was >35-fold lower, and the insulin-suppressed serum FFA levels were ~2.5-fold higher in men with type 2 diabetes compared with controls at baseline (all $P < 0.01$) (Table 2). The fasting levels of serum FFA did not differ between groups, but Adipo-IR was ~2.5-fold higher in men with type 2 diabetes compared with lean and obese men at baseline (all $P < 0.05$) (Table 2).

Effects of HIIT on the regional body composition

As reported (37), the HIIT protocol induced a reduction in total FM (1.6–2.3 kg) in all three groups (all $P < 0.001$) (Table 1). All regional levels of FM were reduced in all three groups (all $P < 0.05$), except for arm FM in obese men (Table 1). Interestingly, ~13–16% of the HIIT-induced reduction of total FM was explained by reduced android FM (all $P < 0.05$). Although the total LBM increased (0.6–1.5 kg) in response to the HIIT protocol in all three groups, an increase in truncal LBM was only significant in men with type 2 diabetes ($P < 0.05$) (Table 1). Similarly, the gynoid LBM only increased (200–400 g) in obese men with and without type 2 diabetes (all $P < 0.05$). The HIIT-induced responses on total and regional body composition did, however, not differ significantly between the three groups.

Effects of HIIT on first- and second-phase insulin responses

Plasma glucose and serum insulin levels in response to the IVGTT are presented in Fig. 1. In men with type 2 diabetes, the AIRg and the incremental FPIR were almost numerically doubled after the HIIT protocol, which appeared to be explained by lower levels of insulin prior

Table 1 HIIT-induced changes in regional body composition. Data are presented as mean ± S.E.M.

	Lean		Obese		T2D	
	Pre	Post	Pre	Post	Pre	Post
N	18	16	15	15	15	13
Weight (kg)	78.9 ± 2.0	77.3 ± 2.2[a]	100.0 ± 2.9[f]	98.5 ± 2.6[a,f]	103.1 ± 3.7[f]	102.5 ± 4.1[a,f]
BMI (kg/m^2)	24.0 ± 0.4	23.7 ± 0.4[a]	30.8 ± 0.7[f]	30.3 ± 0.6[a,f]	31.2 ± 0.8[f]	30.8 ± 0.9[a,f]
Fat mass (kg)						
Total	20.1 ± 1.0	18.2 ± 1.2[c]	32.0 ± 1.9[f]	29.7 ± 1.8[c,f]	34.8 ± 2.3[f]	33.0 ± 2.5[c,f]
Legs	5.2 ± 0.2	4.8 ± 0.3[b]	8.5 ± 0.4[f]	8.0 ± 0.4[c,f]	7.8 ± 0.7[e]	7.6 ± 0.8[a,e]
Arms	1.9 ± 0.1	1.8 ± 0.1[b]	2.8 ± 0.2[f]	2.8 ± 0.2[f]	3.1 ± 0.2[f]	2.9 ± 0.2[a,f]
Truncal	12.1 ± 0.7	10.8 ± 0.8[c]	19.7 ± 1.4[f]	18.1 ± 1.3[c,f]	22.8 ± 1.4[f]	21.1 ± 1.6[c,f]
Android	2.1 ± 0.1	1.8 ± 0.2[c]	3.7 ± 0.3[f]	3.3 ± 0.3[c,f]	4.4 ± 0.3[f]	4.1 ± 0.4[b,f,g]
Gynoid	2.9 ± 0.1	2.6 ± 0.2[b]	4.5 ± 0.2[f]	4.2 ± 0.2[c,f]	4.5 ± 0.4[f]	4.3 ± 0.4[b,f]
Lean body mass (kg)						
Total	56.9 ± 1.3	57.1 ± 1.4[a]	65.3 ± 1.3[f]	66.2 ± 1.2[b,f]	64.8 ± 1.7[f]	66.8 ± 2.0[b,f]
Legs	19.1 ± 0.5	19.0 ± 0.5	22.5 ± 0.6[f]	22.9 ± 0.6[d,f]	22.1 ± 0.7[e]	22.6 ± 0.8[f]
Arms	7.1 ± 0.2	7.1 ± 0.2	7.9 ± 0.3[e]	8.1 ± 0.3[e]	7.6 ± 0.4	8.0 ± 0.4[e]
Trunk	27.2 ± 0.6	27.5 ± 0.8	31.2 ± 0.6[f]	31.6 ± 0.7[f]	31.3 ± 0.9[f]	32.4 ± 1.0[a,f]
Android	4.2 ± 0.1	4.1 ± 0.1	4.9 ± 0.1[f]	4.9 ± 0.1[f]	5.0 ± 0.2[f]	5.2 ± 0.2[f]
Gynoid	8.6 ± 0.2	8.6 ± 0.2	10.2 ± 0.2[f]	10.4 ± 0.2[a,f]	9.9 ± 0.3[e]	10.3 ± 0.4[a,f]

[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$; [d]$P < 0.10$ vs pre-training. [e]$P < 0.05$; [f]$P < 0.001$ vs lean. [g]$P < 0.05$.
T2D, type 2 diabetes; Pre, pre-training; Post, post-training.

Endocrine Connections (2024) **13** e230558
https://doi.org/10.1530/EC-23-0558

Table 2 Data from the Botnia clamp pre- and post-training. Data are mean ± S.E.M.

	Lean		Obese		T2D	
Characteristics	**Pre**	**Post**	**Pre**	**Obese**	**Pre**	**Post**
N	18	16	15	15	15	13
Glucose, basal (mmol/L)	5.4 ± 0.1	5.3 ± 0.1	5.5 ± 0.1	5.3 ± 0.1	8.7 ± 0.7[f,i]	8.1 ± 0.7[a,f,i]
Glucose, clamp (mmol/L)	5.4 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.5 ± 0.2	5.4 ± 0.1[k]	5.2 ± 0.1[k]
Insulin, basal (pmol/L)	48 ± 8	43 ± 7	51 ± 7	43 ± 4	106 ± 21[eh]	79 ± 12[d]
Insulin, clamp (pmol/L)	695 ± 40[k]	671 ± 35[k]	660 ± 29[k]	691 ± 34[k]	740 ± 77[k]	707 ± 65[k]
FFA, basal (mmol/L)	0.46 ± 0.02	0.44 ± 0.03	0.49 ± 0.04	0.48 ± 0.04	0.50 ± 0.04	0.50 ± 0.05
FFA, clamp (mmol/L)	0.02 ± 0.01[k]	0.01 ± 0.00[a,k]	0.02 ± 0.00[k]	0.01 ± 0.00[a,k]	0.05 ± 0.01[f,i,,k]	0.03 ± 0.01[a,e,h,k]
Adipo-IR	21 ± 3	18 ± 3	24 ± 4	19 ± 2	55 ± 13[e,h]	39 ± 8[d,g,j]
GDR basal (mg/min/m^2)	75 ± 2	78 ± 2	78 ± 2	81 ± 5	82 ± 2	82 ± 5
GDR clamp (mg/min/m^2)	356 ± 30[k]	463 ± 36[c,k]	351 ± 26[k]	447 ± 29[b,k]	210 ± 24[e,h,k]	317 ± 36[c,e,h,k]
GDR/I, clamp (mg/min/m^2 per pmol/L)	0.56 ± 0.06	0.74 ± 0.08[b]	0.56 ± 0.06	0.66 ± 0.05[a]	0.31 ± 0.04[e,h]	0.49 ± 0.07[c,e,h]
Incremental glucose (0–10 min)	69 ± 3	71 ± 3	68 ± 3	69 ± 3	63 ± 2	65 ± 3
Total glucose (0–10 min)	112 ± 3	114 ± 3	112 ± 3	112 ± 4	133 ± 5[e,h]	130 ± 7[e,h]
AIRg	371 ± 53	354 ± 61	346 ± 60	388 ± 79	26 ± 16[f,i]	47 ± 28[f,i]
Incremental FPIR (pmol/L)	3363 ± 474	3226 ± 538	3193 ± 558	3609 ± 746	220 ± 124[f,i]	413 ± 239[f,i]
Total FPIR (pmol/L)	3839 ± 505	3655 ± 584	3705 ± 573	4141 ± 768	1278 ± 261[f,h]	1130 ± 304[f,i]
Incremental FPIR/glucose	45 ± 7	37 ± 6	41 ± 7	43 ± 8	2.8 ± 1.6[f,i]	5.2 ± 2.7[f,i]
Total FPIR/glucose	30 ± 4	26 ± 4	28 ± 4	30 ± 5	8.4 ± 1.9[f,i]	7.8 ± 2.3[f,i]
Incremental SPIR (pmol/L)	11,257 ± 2986	7875 ± 2311[d]	7551 ± 894	6503 ± 658[a]	5603 ± 1656[g]	5077 ± 1612
Total SPIR (pmol/L)	13,634 ± 3108	10,025 ± 2445[a]	10,111 ± 889	9253 ± 835[d]	11,017 ± 2420	8661 ± 2028[a]
Incremental SPIR/glucose	48 ± 11	34 ± 9[a]	34 ± 4	36 ± 6	22 ± 6[e]	20 ± 6[h]
Total SPIR/glucose	27 ± 6	20 ± 5[a]	20 ± 2	20 ± 2	17 ± 4	14 ± 4
DI (AIRg × GDR, clamp/L)	171 ± 22	218 ± 30[b]	181 ± 25	237 ± 40[a]	4.7 ± 2.5[f,i]	15 ± 6[b,f,i]

[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$; [d]$P < 0.10$ vs pre-training. [e]$P < 0.05$; [f]$P < 0.001$; [g]$P < 0.10$ vs lean. [h]$P < 0.05$; [i]$P < 0.001$; [j]$P < 0.10$ vs obese. [k]$P < 0.01$ clamp vs basal.

Adipo-IR, adipose tissue insulin resistance index; AIRg, acute insulin response to glucose; DI, disposition index; FPIR, first-phase insulin response; GDR, glucose disposal rate; I, serum insulin; Pre, pre-training; Post, post-training; SPIR, second-phase insulin response; T2D, type 2 diabetes.

to the IVGTT post-training (Table 2). However, due to large interindividual variations in the responses, these increases in measures of acute insulin secretion were not significant. In lean and obese men, the AIRg and the incremental FPIR remained unaltered in response to the HIIT protocol (Table 2). Moreover, no HIIT-induced changes in total FPIR were observed in any of the groups. Adjustments for glucose levels (incremental FPIR/glucose and total FPIR/glucose) did not change the results for the acute insulin responses (Table 2). The HIIT protocol induced a reduction in the incremental SPIR (14%) in obese men ($P < 0.05$) and tended to reduce incremental SPIR (32%) in lean men ($P = 0.053$), whereas the incremental SPIR remained unaltered in men with type 2 diabetes (Table 2). Furthermore, the HIIT protocol induced reductions in total SPIR in the lean (27%) and type 2 diabetes (17%) groups (all $P < 0.05$), while a tendency to reduction (8%) was observed in the obese group ($P = 0.05$) (Table 2). However, when the incremental and total SPIR were adjusted for glucose levels, the HIIT-induced reductions only remained significant in lean men (all $P < 0.05$). There were no significant differences in the HIIT-induced effects on the acute insulin responses (AIRg and FPIR) or SPIR between the groups. The post-training levels of AIRg and the incremental FPIR remained markedly lower (13–14-fold) in men with type 2 diabetes compared with controls (all $P < 0.001$), and total FPIR also remained three-fold lower post-training as compared with controls (all $P < 0.05$).

Insulin sensitivity and free fatty acids

As reported (37), insulin-stimulated GDR increased markedly (~27–42%) in all groups after the HIIT protocol (all $P < 0.01$) with no differences in the HIIT-induced responses between the groups (Table 2). In men with type 2 diabetes, insulin-stimulated GDR remained lower (~30%) after the HIIT protocol as compared with both

Endocrine Connections (2024) **13** e230558
https://doi.org/10.1530/EC-23-0558

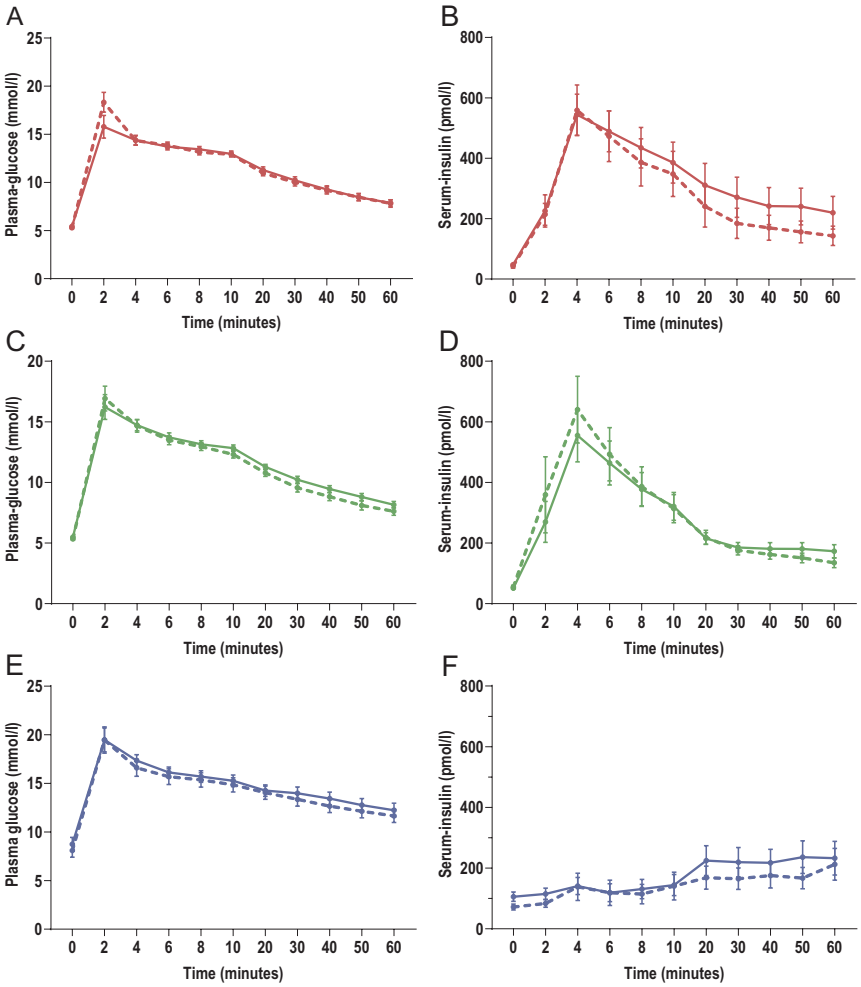

Figure 1

Plasma glucose (A, C, E) and serum insulin levels (B, D, F) during an IVGTT performed before (solid lines) and after (dashed lines) 8 weeks of HIIT in patients with type 2 diabetes (blue lines) and glucose-tolerant, obese (green lines) and lean (red lines) controls. Data are mean ± S.E.M.

lean and obese controls (all $P < 0.01$). However, post-training insulin-stimulated GDR in men with type 2 diabetes was not different from insulin-stimulated GDR in the lean and obese controls at baseline (all $P \geq 0.40$). The fasting (basal) serum FFA levels did not change in response to HIIT in any of the groups, but there was a tendency to a reduction (30%) in the surrogate marker of adipose tissue insulin resistance, Adipo-IR, in men with type 2 diabetes ($P = 0.053$) (Table 2). The HIIT protocol reduced the insulin-suppressed serum FFA levels in all three groups (all $P < 0.05$) with a 29% in men with type 2 diabetes, 35% in obese controls, and 48% in lean controls, respectively (Table 2), and the insulin-suppressed serum FFA levels remained two-fold higher in men with type 2 diabetes compared to controls post-training (all $P < 0.001$). There were no differences in the HIIT-induced responses on FFA between the groups.

Effects of HIIT on beta-cell function adjusted for insulin sensitivity

Figure 2 shows the acute insulin secretion (AIRg) versus insulin sensitivity (GDR/I) in men with type 2 diabetes compared with glucose-tolerant obese and lean men both before and after HIIT (Fig. 2A and B). The HIIT protocol induced beneficial changes in the DI in both men with type 2 diabetes and obese and lean men. These changes were mainly driven by improvements in insulin sensitivity (Fig. 2A and B). Beta-cell function adjusted for insulin sensitivity (assessed as the DI) increased >200% in men with type 2 diabetes ($P < 0.01$), 19% in obese men ($P < 0.05$), and 27% in lean men ($P < 0.01$) in response to the HIIT protocol (Table 2 and Fig. 3A). Although the percentage increase in DI was markedly higher in patients with type 2 diabetes, there were no significant differences in the HIIT-induced changes in DI between the three groups. This was likely explained by the high variability in this response, particularly in patients with type 2 diabetes (Fig. 3B). In contrast to insulin-stimulated GDR, the DI achieved after HIIT in men with type 2 diabetes was still far from the DI observed in the obese and lean men at baseline (Table 2), and DI remained ~15-fold lower in men with type 2 diabetes compared with lean and obese controls post training (all $P < 0.001$).

Figure 2C and D illustrates the relationship between the SPIR and insulin sensitivity in the three groups pre- and post training. The HIIT-induced increase in insulin sensitivity was accompanied by a reduction in the SPIR

Endocrine Connections (2024) **13** e230558
https://doi.org/10.1530/EC-23-0558

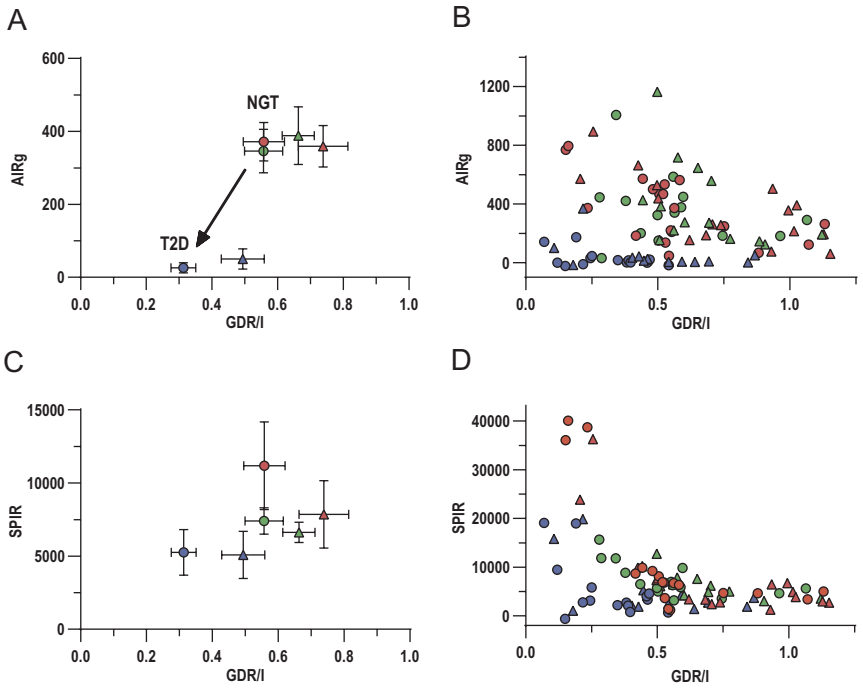

Figure 2

Insulin sensitivity (insulin-stimulated GDR/I) versus (A, B) acute insulin response to glucose (AIRg) and (C, D) second-phase insulin response (SPIR) before (circles) and after (triangles) 8 weeks of HIIT in patients with type 2 diabetes (blue symbols) and glucose-tolerant, obese (green symbols) and lean (red symbols) controls, Data are mean ± S.E.M.

in lean men and to a lesser extent in obese men, while the increase in insulin sensitivity in men with type 2 diabetes is accompanied by an almost unaltered SPIR.

Correlation analyses

To explore the potential role of glucose and FFA levels and FM composition on beta-cell function, we examined the association of fasting (basal) plasma glucose, HbA1c, serum FFA, Adipo-IR, android FM, and gynoid FM with DI at baseline and the changes induced by HIIT. Before HIIT, basal plasma glucose and HbA1c correlated inversely with DI in the pooled cohort (r= −0.45 to −0.60; all $P < 0.05$). The inverse association between basal plasma glucose and DI was present in each group (r= −0.53 to −0.58, all $P < 0.05$), whereas the inverse correlation between HbA1c and DI was only observed in men with type 2 diabetes (r= −0.58, $P < 0.05$). The android FM at baseline also correlated inversely with the DI in the pooled cohort (r= −0.41, $P < 0.01$), whereas no correlation was observed between the gynoid FM and DI. Fasting FFA (baseline) did not correlate with DI, and only a weak inverse correlation was seen between Adipo-IR and DI in the pooled cohort (r= −0.33, $P < 0.05$), whereas the insulin-suppressed serum FFA correlated inversely with the DI in the pooled cohort (r= −0.54, $P < 0.001$). However, the HIIT-induced reductions in the basal plasma glucose levels and HbA1c, which were seen in particular in the diabetic group, did not correlate with the DI in any group. Moreover, the HIIT-induced reductions in android FM and insulin-suppressed serum FFA did not correlate with the increase in DI, and neither did the HIIT-induced changes in Adipo-IR or gynoid FM correlate with the improvement in DI.

Discussion

In this study, we aimed to investigate whether an 8-week HIIT protocol recruiting both upper and lower body muscle groups improves acute and second-phase insulin responses and beta-cell function adjusted for insulin sensitivity (DI) in patients with type 2 diabetes

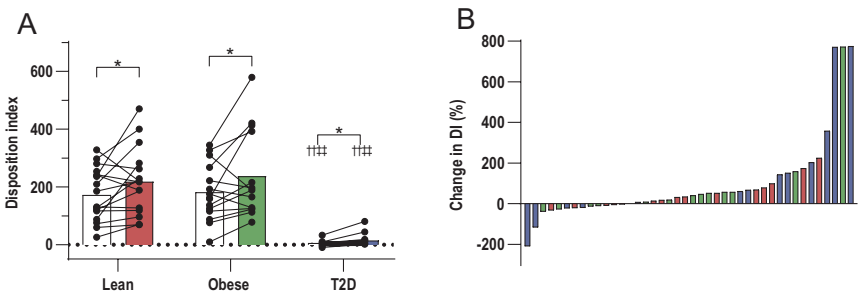

Figure 3

The disposition index (A) before (white bars) and after (colored bars) 8 weeks of HIIT in patients with type 2 diabetes (T2D) and glucose-tolerant, obese, and lean controls, and (B) the interindividual variability in the HIIT-induced changes (%) in DI in patients with T2D (blue bars) and glucose-tolerant, obese (green bars) and lean (red bars) control. Data are mean ± S.E.M.
*$P < 0.05$ pre- vs post-training, ††$P < 0.001$ vs lean, ‡‡$P < 0.001$ vs. obese.

compared with glucose-tolerant, obese, and lean individuals using the Botnia clamp. The main finding is that our HIIT protocol induced an increase in the DI in all three groups with a magnitude that was numerically, but not significantly, larger (>200%) in men with type 2 diabetes than in obese and lean men (~20–30%). This is likely explained by the high variability in the response of the DI to exercise training, particularly in patients with type 2 diabetes. The improved DI was mainly driven by a marked increase in insulin sensitivity rather than an increase in the acute insulin secretion. Importantly, although the HIIT protocol induced a marked increase in the DI in the patients with type 2 diabetes, the beta-cell function even when adjusted for the improved insulin sensitivity, remained severely reduced compared to obese and lean controls post-training, suggesting that this component of type 2 diabetes is less reversible in response to exercise training than insulin resistance.

Other studies, evaluating the effect of more than 6 weeks of moderate or high-intensity aerobic exercise training (cycle ergometer, cross-fit, or treadmill) on same-day indices of beta-cell function and insulin sensitivity from an OGTT or a hyperglycemic clamp in patients with type 2 diabetes, have reported increases in DI in the range of ~35–62% (6, 8, 9). Moreover, a recent study reported increases in the late-phase DI by ~100–140% after 16 weeks of moderate to high-dose exercise training and calorie restriction in patients with type 2 diabetes (42). In that study, late-phase insulin secretion and insulin sensitivity were determined by a hyperglycemic clamp, the gold standard for determining insulin secretion (42). In our study, we found an even higher improvement in the DI of ~200% in response to 8 weeks of HIIT in patients with type 2 diabetes. This suggests that HIIT combining rowing and cycling is more effective in improving beta-cell function. However, the use of different methods to assess the DI in our and previous studies makes comparisons between the effect of exercise training on the DI very difficult (6, 8, 9, 42), and we cannot exclude the possibility that the apparent superior improvement in the DI in patients with type 2 diabetes in our study is explained by the use of the Botnia clamp.

To the best of our knowledge, the Botnia clamp has not previously been used to evaluate the effect of exercise training on beta-cell function adjusted for insulin sensitivity in patients with type 2 diabetes and glucose-tolerant obese and lean controls. Since same-day measurements of insulin secretion and insulin sensitivity cannot be obtained by the gold standard methods of a hyperglycemic clamp and a hyperinsulinemic–euglycemic clamp, respectively, the Botnia clamp is a suggested alternative (24, 29). The IVGTT has shown reproducibility (24, 43), and has been suggested as the most precise method to determine first-phase insulin secretion in response to a carbohydrate challenge (16, 44). However, in contrast to the hyperglycemic clamp, the glucose levels at the initiation of an IVGTT affect the measurement of the acute insulin secretion and thereby the estimates of DI (45). In addition, the IVGTT can be criticized for being non-physiological from several aspects since it bypasses the effects of the incretin hormones, the nutrient stimulus is carbohydrate alone, and the determination of the insulin secretion is based on an immediately markedly supraphysiological glucose stimulus (44).

Consistent with other studies, the improvement in our estimate of beta-cell function seems primarily to be driven by the improvement in insulin sensitivity rather than an increase in insulin secretion *per se* (5, 6, 8, 42). However, when insulin sensitivity increases in response to exercise training, lower insulin levels will be sufficient to maintain glucose homeostasis, and, therefore, it is mandatory to include measurement of insulin sensitivity when assessing beta-cell function (15, 16, 17, 44). In this study, we did see a marked increase in insulin sensitivity in all three groups, and in men with type 2 diabetes, the HIIT protocol increased insulin sensitivity to the same degree as seen in lean and obese glucose-tolerant men at baseline. Although the HIIT protocol, on average, induced a (non-significant) two-fold increase in the estimates of first-phase insulin secretion (AIRg and incremental FPIR) and a very large increase in the DI in men with type 2 diabetes, these measures of acute insulin secretion and DI were still markedly lower compared with both lean and obese controls after the training period. This is in line with other studies (7, 8) indicating that the defects causing beta-cell dysfunction are far less reversible to exercise training than the defects causing insulin resistance in patients with type 2 diabetes. On the other hand, we cannot exclude the possibility that exercise training for a longer duration is necessary to achieve a reversible effect on beta-cell function in patients with type 2 diabetes. This needs to be examined in future studies.

Our study does not explain possible metabolic or molecular mechanisms behind the improvements seen in beta-cell function adjusted for insulin sensitivity. However, the increased insulin sensitivity and improved glycemic control seen in patients with type 2 diabetes in this study could reduce the beta-cell dysfunction proposed to be induced by exposure to high glucose levels (46). Moreover, 10 weeks of endurance exercise training was recently reported to reduce Adipo-IR in young healthy men (36). This was associated with increased protein abundance of the insulin receptor in subcutaneous fat, suggesting a mechanism for improved adipose tissue insulin sensitivity (36). However, while Adipo-IR is a clinically relevant measure of adipose tissue insulin resistance (47), insulin-suppressed plasma FFA correlates much more strongly with the reference method for assessing insulin-mediated suppression of lipolysis (48). Thus, the observed HIIT-induced reduction in insulin-suppressed FFA levels in all three groups in our study extends this beneficial effect of exercise training to middle-aged, sedentary men with and without obesity or type 2 diabetes. This reduction

in insulin-suppressed FFA levels may reflect reduced exposure of the pancreatic beta-cells to lipotoxicity, which is believed to induce beta-cell dysfunction (35). However, we were unable to demonstrate that the HIIT-induced increase in DI correlated with the reduction in insulin-suppressed FFA levels. This suggests that other mechanisms are also involved or that a longer duration of exercise intervention is needed.

The strengths of our study include the use of the Botnia clamp, which has been validated as a method to obtain same-day measurements of acute insulin secretion and insulin sensitivity, and hence DI in type 2 diabetes (24). Moreover, the comparison of the effect of our HIIT protocol on beta-cell function included both patients with type 2 diabetes and obese and lean glucose-tolerant individuals allowing us to distinguish between the effects of type 2 diabetes and obesity *per se*, and to what extent beta-cell function is restored compared to the non-diabetic state. Furthermore, we applied and tested the effects of a novel HIIT protocol involving both upper and lower-body muscle groups to study the effect of exercise training on beta-cell function. This has, to our knowledge, not previously been reported in either patients with type 2 diabetes or glucose-tolerant individuals.

The study also has some limitations. First, the hyperglycemic clamp is considered by many to be the gold standard for the measurement of insulin secretion, and assessment of insulin secretion by this method would have been superior to the determination by an IVGTT. Secondly, the use of the DI as an estimate of beta-cell function adjusted for insulin sensitivity is based on a hyperbolic relationship between the acute insulin secretion and insulin sensitivity and a constant of the product (18, 41). However, in cases where the pathogenesis of type 2 diabetes is mainly an intrinsic beta-cell defect, insulin secretion may be low at any degree of insulin sensitivity (49). Thirdly, although there were no significant correlations between the increase in DI and the reductions in HbA1c, fasting plasma glucose, insulin-suppressed FFA levels, or android FM, we cannot rule out a possible influence of these changes on the HIIT-induced changes in the DI. Finally, we only included men, which precludes the opportunity to conclude whether the same results would have been found in women. However, several studies of the effect of exercise training on beta-cell function in patients with type 2 diabetes have included both men and women and have reported improvements in beta-cell function in pooled data (5, 6, 6, 7, 8, 9).

In summary, this study demonstrates that a novel HIIT protocol combining rowing and cycling markedly improves beta-cell function adjusted for insulin sensitivity in men with type 2 diabetes when evaluated by same-day measurements of insulin secretion and insulin sensitivity using the Botnia clamp. The improved beta-cell function was mainly driven by an increase in insulin sensitivity and not an increase in insulin secretion *per se*. Interestingly, we observed a large interindividual variation in the HIIT-induced effect on beta-cell function, especially in men with type 2 diabetes. However, although our HIIT protocol in obese men with type 2 diabetes improved insulin sensitivity to the same degree seen in obese and lean controls at baseline, beta-cell dysfunction seems to be a much more irreversible defect in response to exercise training in type 2 diabetes.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-23-0558.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

Funding

This study was supported by grants from the Region of Southern Denmark, Odense University Hospital, the Novo Nordisk Foundation, University of Southern Denmark, Christenson-Cesons Family Fund, and from The Sawmill Owner Jeppe Juhl and wife Ovita Juhl Memorial Foundation.

Author contribution statement

MHP, KJ, NØ, and KH contributed to the conception and design of the study. MHP recruited the eligible participants and conducted the metabolic studies. MEA and EKW performed the supervised training, VO_2max tests, and DXA scanning. MHP, JVS, MEA, EKW, NØ, and KH analyzed and interpreted data, and MHP and KH wrote the manuscript. All authors revised the manuscript critically for important intellectual content and gave final approval of the version to be published. KH and NØ are guarantors of this work and as such had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements

We would like to thank L Hansen and C B Olsen at the Steno Diabetes Center Odense, Odense University Hospital for their skilled technical assistance. The Odense Patient data Explorative Network (OPEN) is acknowledged for statistical support and access to data storage in REDCap.

References

1 Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H & Martín C. Pathophysiology of type 2 diabetes mellitus. *International Journal of Molecular Sciences* 2020 **21**. (https://doi.org/10.3390/ijms21176275)

2 Davies MJ, Aroda VR, Collins BS, Gabbay RA, Green J, Maruthur NM, Rosas SE, Del Prato S, Mathieu C, Mingrone G, *et al.* Management of hyperglycaemia in type 2 diabetes, 2022. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 2022 **65** 1925–1966. (https://doi.org/10.1007/s00125-022-05787-2)

3 Vind BF, Pehmoller C, Treebak JT, Birk JB, Hey-Mogensen M, Beck-Nielsen H, Zierath JR, Wojtaszewski JF & Hojlund K. Impaired

insulin-induced site-specific phosphorylation of TBC1 domain family, member 4 (TBC1D4) in skeletal muscle of type 2 diabetes patients is restored by endurance exercise-training. *Diabetologia* 2011 **54** 157–167. (https://doi.org/10.1007/s00125-010-1924-4)

4 O'Hagan C, De Vito G & Boreham CA. Exercise prescription in the treatment of type 2 diabetes mellitus: current practices, existing guidelines and future directions. *Sports Medicine* 2013 **43** 39–49. (https://doi.org/10.1007/s40279-012-0004-y)

5 Johansen MY, Karstoft K, MacDonald CS, Hansen KB, Ellingsgaard H, Hartmann B, Wewer Albrechtsen NJ, Vaag AA, Holst JJ, Pedersen BK, *et al.* Effects of an intensive lifestyle intervention on the underlying mechanisms of improved glycaemic control in individuals with type 2 diabetes: a secondary analysis of a randomised clinical trial. *Diabetologia* 2020 **63** 2410–2422. (https://doi.org/10.1007/s00125-020-05249-7)

6 Karstoft K, Winding K, Knudsen SH, James NG, Scheel MM, Olesen J, Holst JJ, Pedersen BK & Solomon TP. Mechanisms behind the superior effects of interval vs continuous training on glycaemic control in individuals with type 2 diabetes: a randomised controlled trial. *Diabetologia* 2014 **57** 2081–2093. (https://doi.org/10.1007/s00125-014-3334-5)

7 Malin SK & Kirwan JP. Fasting hyperglycaemia blunts the reversal of impaired glucose tolerance after exercise training in obese older adults. *Diabetes, Obesity and Metabolism* 2012 **14** 835–841. (https://doi.org/10.1111/j.1463-1326.2012.01608.x)

8 Madsen SM, Thorup AC, Overgaard K & Jeppesen PB. High intensity interval training improves glycaemic control and pancreatic beta cell function of type 2 diabetes patients. *PLoS One* 2015 **10** e0133286. (https://doi.org/10.1371/journal.pone.0133286)

9 Nieuwoudt S, Fealy CE, Foucher JA, Scelsi AR, Malin SK, Pagadala M, Rocco M, Burguera B & Kirwan JP. Functional high-intensity training improves pancreatic beta-cell function in adults with type 2 diabetes. *American Journal of Physiology Endocrinology and Metabolism* 2017 **313** E314–E320. (https://doi.org/10.1152/ajpendo.00407.2016)

10 Dela F, von Linstow ME, Mikines KJ & Galbo H. Physical training may enhance beta-cell function in type 2 diabetes. *American Journal of Physiology Endocrinology and Metabolism* 2004 **287** E1024–E1031. (https://doi.org/10.1152/ajpendo.00056.2004)

11 Burns N, Finucane FM, Hatunic M, Gilman M, Murphy M, Gasparro D, Mari A, Gastaldelli A & Nolan JJ. Early-onset type 2 diabetes in obese white subjects is characterised by a marked defect in beta cell insulin secretion, severe insulin resistance and a lack of response to aerobic exercise training. *Diabetologia* 2007 **50** 1500–1508. (https://doi.org/10.1007/s00125-007-0655-7)

12 Lee SF, Pei D, Chi MJ & Jeng C. An investigation and comparison of the effectiveness of different exercise programmes in improving glucose metabolism and pancreatic β cell function of type 2 diabetes patients. *International Journal of Clinical Practice* 2015 **69** 1159–1170. (https://doi.org/10.1111/ijcp.12679)

13 Eriksen L, Dahl-Petersen I, Haugaard SB & Dela F. Comparison of the effect of multiple short-duration with single long-duration exercise sessions on glucose homeostasis in type 2 diabetes mellitus. *Diabetologia* 2007 **50** 2245–2253. (https://doi.org/10.1007/s00125-007-0783-0)

14 Michishita R, Shono N, Kasahara T & Tsuruta T. Effects of low intensity exercise therapy on early phase insulin secretion in overweight subjects with impaired glucose tolerance and type 2 diabetes mellitus. *Diabetes Research and Clinical Practice* 2008 **82** 291–297. (https://doi.org/10.1016/j.diabres.2008.08.013)

15 Hannon TS, Kahn SE, Utzschneider KM, Buchanan TA, Nadeau KJ, Zeitler PS, Ehrmann DA, Arslanian SA, Caprio S, Edelstein SL, *et al.* Review of methods for measuring β-cell function: design considerations from the Restoring Insulin Secretion (RISE) Consortium. *Diabetes, Obesity and Metabolism* 2018 **20** 14–24. (https://doi.org/10.1111/dom.13005)

16 Ferrannini E & Mari A. Beta cell function and its relation to insulin action in humans: a critical appraisal. *Diabetologia* 2004 **47** 943–956. (https://doi.org/10.1007/s00125-004-1381-z)

17 Curran M, Drayson MT, Andrews RC, Zoppi C, Barlow JP, Solomon TPJ & Narendran P. The benefits of physical exercise for the health of the pancreatic β-cell: a review of the evidence. *Experimental Physiology* 2020 **105** 579–589. (https://doi.org/10.1113/EP088220)

18 Bergman RN, Ader M, Huecking K & Van Citters G. Accurate assessment of beta-cell function: the hyperbolic correction. *Diabetes* 2002 **51**(Supplement 1) S212–S220. (https://doi.org/10.2337/diabetes.51.2007.s212)

19 Devlin JT, Hirshman M, Horton ED & Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. *Diabetes* 1987 **36** 434–439. (https://doi.org/10.2337/diab.36.4.434)

20 Mikines KJ, Sonne B, Farrell PA, Tronier B & Galbo H. Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *American Journal of Physiology* 1988 **254** E248–E259. (https://doi.org/10.1152/ajpendo.1988.254.3.E248)

21 Libman IM, Barinas-Mitchell E, Bartucci A, Robertson R & Arslanian S. Reproducibility of the oral glucose tolerance test in overweight children. *Journal of Clinical Endocrinology and Metabolism* 2008 **93**. 4231–4237. (https://doi.org/10.1210/jc.2008-0801)

22 Utzschneider KM, Prigeon RL, Tong J, Gerchman F, Carr DB, Zraika S, Udayasankar J, Montgomery B, Mari A & Kahn SE. Within-subject variability of measures of beta cell function derived from a 2 h OGTT: implications for research studies. *Diabetologia* 2007 **50** 2516–2525. (https://doi.org/10.1007/s00125-007-0819-5)

23 Bacha F, Gungor N & Arslanian SA. Measures of beta-cell function during the oral glucose tolerance test, liquid mixed-meal test, and hyperglycemic clamp test. *Journal of Pediatrics* 2008 **152** 618–621. (https://doi.org/10.1016/j.jpeds.2007.11.044)

24 Tripathy D, Wessman Y, Gullström M, Tuomi T & Groop L. Importance of obtaining independent measures of insulin secretion and insulin sensitivity during the same test: results with the Botnia clamp. *Diabetes Care* 2003 **26** 1395–1401. (https://doi.org/10.2337/diacare.26.5.1395)

25 Pacini G & Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best Practice and Research. Clinical Endocrinology and Metabolism* 2003 **17** 305–322. (https://doi.org/10.1016/s1521-690x(03)00042-3)

26 Tura A, Sbrignadello S, Succurro E, Groop L, Sesti G & Pacini G. An empirical index of insulin sensitivity from short IVGTT: validation against the minimal model and glucose clamp indices in patients with different clinical characteristics. *Diabetologia* 2010 **53** 144–152. (https://doi.org/10.1007/s00125-009-1547-9)

27 Bergman RN, Phillips LS & Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *Journal of Clinical Investigation* 1981 **68** 1456–1467. (https://doi.org/10.1172/jci110398)

28 Croymans DM, Paparisto E, Lee MM, Brandt N, Le BK, Lohan D, Lee CC & Roberts CK. Resistance training improves indices of muscle insulin sensitivity and β-cell function in overweight/obese, sedentary young men. *Journal of Applied Physiology* 2013 **115** 1245–1253. (https://doi.org/10.1152/japplphysiol.00485.2013)

29 Lehto M, Tuomi T, Mahtani MM, Widén E, Forsblom C, Sarelin L, Gullström M, Isomaa B, Lehtovirta M, Hyrkkö A, *et al.* Characterization of the MODY3 phenotype. Early-onset diabetes caused by an insulin secretion defect. *Journal of Clinical Investigation* 1997 **99** 582–591. (https://doi.org/10.1172/JCI119199)

30 MacInnis MJ & Gibala MJ. Physiological adaptations to interval training and the role of exercise intensity. *Journal of Physiology* 2017 **595** 2915–2930. (https://doi.org/10.1113/JP273196)

31 De Nardi AT, Tolves T, Lenzi TL, Signori LU & Silva AMVD. High-intensity interval training versus continuous training on physiological and metabolic variables in prediabetes and type 2 diabetes: a meta-analysis. *Diabetes Research and Clinical Practice* 2018 **137** 149–159. (https://doi.org/10.1016/j.diabres.2017.12.017)

32 Lora-Pozo I, Lucena-Anton D, Salazar A, Galán-Mercant A & Moral-Munoz JA. Anthropometric, cardiopulmonary and metabolic benefits of the high-intensity interval training versus moderate, low-intensity or control for type 2 diabetes: systematic review and meta-analysis. *International Journal of Environmental Research and Public Health* 2019 **16**. (https://doi.org/10.3390/ijerph16224524)

33 Jelleyman C, Yates T, O'Donovan G, Gray LJ, King JA, Khunti K & Davies MJ. The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. *Obesity Reviews* 2015 **16** 942–961. (https://doi.org/10.1111/obr.12317)

34 Steenberg DE, Hingst JR, Birk JB, Thorup A, Kristensen JM, Sjøberg KA, Kiens B, Richter EA & Wojtaszewski JFP. A single bout of one-legged exercise to local exhaustion decreases insulin action in nonexercised muscle leading to decreased whole-body insulin action. *Diabetes* 2020 **69** 578–590. (https://doi.org/10.2337/db19-1010)

35 Plötz T & Lenzen S. Mechanisms of lipotoxicity-induced dysfunction and death of human pancreatic beta cells under obesity and type 2 diabetes conditions. *Obesity Reviews* 2024 e13703. (https://doi.org/10.1111/obr.13703)

36 Riis S, Christensen B, Nellemann B, Møller AB, Husted AS, Pedersen SB, Schwartz TW, Jørgensen JOL & Jessen N. Molecular adaptations in human subcutaneous adipose tissue after ten weeks of endurance exercise training in healthy males. *Journal of Applied Physiology* 2019 **126** 569–577. (https://doi.org/10.1152/japplphysiol.00989.2018)

37 Petersen MH, de Almeida ME, Wentorf EK, Jensen K, Ørtenblad N & Højlund K. High-intensity interval training combining rowing and cycling efficiently improves insulin sensitivity, body composition and VO2max in men with obesity and type 2 diabetes. *Frontiers in Endocrinology* 2022 **13** 1032235. (https://doi.org/10.3389/fendo.2022.1032235)

38 de Almeida ME, Ørtenblad N, Petersen MH, Schjerning AN, Wentorf EK, Jensen K, Højlund K & Nielsen J. Acute exercise increases the contact between lipid droplets and mitochondria independently of obesity and type 2 diabetes. *Journal of Physiology* 2023 **601** 1797–1815. (https://doi.org/10.1113/JP284386)

39 de Almeida ME, Nielsen J, Petersen MH, Wentorf EK, Pedersen NB, Jensen K, Højlund K & Ørtenblad N. Altered intramuscular network of lipid droplets and mitochondria in type 2 diabetes. *American Journal of Physiology* 2023 **324** C39–C57. (https://doi.org/10.1152/ajpcell.00470.2022)

40 Hother-Nielsen O, Henriksen JE, Holst JJ & Beck-Nielsen H. Effects of insulin on glucose turnover rates in vivo: isotope dilution versus constant specific activity technique. *Metabolism* 1996 **45** 82–91. (https://doi.org/10.1016/s0026-0495(96)90204-8)

41 Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC & Palmer JP. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes* 1993 **42** 1663–1672. (https://doi.org/10.2337/diab.42.11.1663)

42 Legaard GE, Lyngbæk MPP, Almdal TP, Karstoft K, Bennetsen SL, Feineis CS, Nielsen NS, Durrer CG, Liebetrau B, Nystrup U, *et al.* Effects of different doses of exercise and diet-induced weight loss on beta-cell function in type 2 diabetes (DOSE-EX): a randomized clinical trial. *Nature Metabolism* 2023 **5** 880–895. (https://doi.org/10.1038/s42255-023-00799-7)

43 Ganda OP, Day JL, Soeldner JS, Connon JJ & Gleason RE. Reproducibility and comparative analysis of repeated intravenous and oral glucose tolerance tests. *Diabetes* 1978 **27** 715–725. (https://doi.org/10.2337/diab.27.7.715)

44 Cersosimo E, Solis-Herrera C, Trautmann ME, Malloy J & Triplitt CL. Assessment of pancreatic β-cell function: review of methods and clinical applications. *Current Diabetes Reviews* 2014 **10** 2–42. (https://doi.org/10.2174/1573399810666140214093600)

45 Henquin JC, Dufrane D, Kerr-Conte J & Nenquin M. Dynamics of glucose-induced insulin secretion in normal human islets. *American Journal of Physiology* 2015 **309** E640–E650. (https://doi.org/10.1152/ajpendo.00251.2015)

46 Robertson RP, Harmon J, Tran PO & Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 2004 **53**(Supplement 1) S119–S124. (https://doi.org/10.2337/diabetes.53.2007.s119)

47 Søndergaard E, Espinosa De Ycaza AE, Morgan-Bathke M & Jensen MD. How to measure adipose tissue insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 1193–1199. (https://doi.org/10.1210/jc.2017-00047)

48 Ter Horst KW, van Galen KA, Gilijamse PW, Hartstra AV, de Groot PF, van der Valk FM, Ackermans MT, Nieuwdorp M, Romijn JA & Serlie MJ. Methods for quantifying adipose tissue insulin resistance in overweight/obese humans. *International Journal of Obesity* 2017 **41** 1288–1294. (https://doi.org/10.1038/ijo.2017.110)

49 Ferrannini E & Mari A. β-cell function in type 2 diabetes. *Metabolism* 2014 **63** 1217–1227. (https://doi.org/10.1016/j.metabol.2014.05.012)