Supplementary appendix

Effects of aerobic training and semaglutide treatment on pancreatic β-cell function in patients with type 2 diabetes.

Contents

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Methods

Two-stepped hyperglycemic clamp procedure

We instructed the participants to note all food consumed the day before the experiment to replicate the diet the day before follow-up experiments, and we instructed the participants not to perform rigorous physical activity on these days.

Participants reported to the laboratory at 8.00 in the morning after an overnight fast of approximately 10 hours. Blood pressure and resting heart rate were measured when the participant rested and was lying with the head elevated in a hospital bed. The patients voided before the procedure.

An intravenous catheter (18 G, Vasofix Safety, BRAUN, Kronberg Im Taunus, Germany) was placed in a large antecubital vein for later glucose infusion (Glucose monohydrate, respond. Glucose. 200mg/ml, Fresenius Kabi, Sweden). A second intravenous catheter (18G) was placed in a dorsal hand vein, with the tip of the catheter advanced as close to the metacarpophalangeal joint as possible. The hand was placed inside a heating blanket (60 ° C) to obtain arterialized blood samples. Fasting blood samples were drawn and immediately analyzed for glucose, K^+ Na^+, lactate, and hemoglobin (ABL 800 Radiometer, Brønshøj, Denmark) or stored for later analysis. To avoid hypokalemia, 2x750 mg potassium chloride extended-release tablets (Orifarm, Odense, Denmark) were administered before each clamp step, and potassium levels were monitored throughout the procedure.

A two-stepped sequential hyperglycemic clamp (20 mM and 30 mM, 2 hours at each step) was initiated by the administration of a priming infusion based on the fasting plasma glucose, the participant's size, and target plasma glucose (calculation described in detail by Mikines et al.[1]). We used this algorithm until the target plasma glucose was reached (first step 20 mM, second step 30 mM). Blood samples were drawn and analyzed for glucose concentration every third minute during

the priming infusion (approximately the first 15 minutes) and every fifth minute for the remainder of the clamp. Every 15 minutes, additional samples were collected for later analysis of insulin, C-peptide, and proinsulin. Before initiation of the second step, urine was collected for glucose concentration analysis and urinary glucose excretion calculation. The procedure was repeated for the second hyperglycemic clamp step. If sodium concentration decreased below 128 mM, we reduced the glucose infusion rate and allowed for a slower increase in blood glucose. The latter was an issue only when priming the 30 mM step in patients treated with the combination of semaglutide and training.

Metformin was halted one day before testing; SGLT-inhibitor treatment, three days before investigation. Antihypertensive and lipid-lowering medications were paused only on the day of investigation.

In follow-up experiments, the hyperglycemic clamp procedure was consistently performed 40-48 hours after the last exercise bout. Patients treated with semaglutide were always investigated at day 7 of the last dosage. The following dosage was postponed until the oral glucose tolerance test was performed the following day.

Body composition

Before the hyperglycemic clamp procedure, body fat, fat-free mass, and visceral fat content were determined by dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Scanex).

Oral glucose tolerance test

Participants reported to the laboratory at 8.00 in the morning on the day following the hyperglycemic clamp procedure. An intravenous catheter (18G) was placed in a dorsal hand vein with the tip of the catheter advanced as close to the metacarpophalangeal joint as possible. The hand was placed inside a heating blanket (60 ° C). When basal blood samples had been obtained,

participants ingested 75 g of glucose dissolved in 400 ml of water. Samples were drawn every 15th minute for 180 minutes.

Maximal oxygen uptake

Following the oral glucose tolerance test, the participants underwent a graded exercise test on a cycle ergometer until they experienced exhaustion (Monark 839E, Monark Exercise AB, Vansbro, Sweden). The female participants warmed up for 5 minutes at 60 Watt followed by 20 Watt increments every minute, while male participants warmed up at 75 Watt for 5 minutes, followed by 25 Watt increments every minute. Pulmonary gas exchange was measured breath-by-breath and sampled every 10th seconds, using an automated online system (Jaeger Oxycon Pro, VIASYS healthcare, Hoechberg, Germany). We accepted O_2 uptake values as the $\dot{V}O_2$max when a plateau or an attenuation of increasing values was found despite increasing workloads and when the respiratory exchange ratio was above 1.15. Before each test, the online system was calibrated using a 15 % O_2 and 6 % CO_2 in N_2 gas mixture.

Exercise performance test

During the first and last week of the training intervention, participants underwent a 45 minutes work test, performed at a variable intensity adjusted to correspond to an oxygen consumption corresponding to 75 % of the individual VO_2max, measured by the automated online system throughout the test (Jaeger Oxycon Pro, VIASYS healthcare, Hoechberg, Germany). Before the test and at the last minute, a venous blood sample was obtained to analyse glucoregulatory markers and inflammatory response to the exercise bout. (Figure 4).

Exercise intervention

Participants exercised on a cycle ergometer (Lode Corrival, Lode BV, Netherlands) three times per week and went through three different workouts (Figure S3) designed to yield an average work intensity of 75 % of heart rate reserve (HRR) at every session. The HRR was calculated as the maximum heart rate (obtained from the $\dot{V}O_2$max test) subtracted by the lowest resting heart rates

measured on the experimental days preceding the training intervention. In case a higher maximum heart rate was obtained during a training session, the HHR calculation was adjusted. During the intervention, we did not adjust for changes in resting heart rate. Three participants completed six weeks of the 12-week training intervention at home during the Covid-19 lockdown in the spring of 2020. These sessions were evaluated by online communication (HHR, Garmin vívoactive®) with the investigators. Patients randomized to initial semaglutide treatment were asked to maintain their habitual daily activity level throughout the first 20 weeks of the study period.

Blood and plasma samples

Arterialized blood samples were immediately centrifuged for 10 minutes (4°C, 2000*g,* Centrifuge Hettich Universal 30 RF; Hettich), and plasma was stored at -80°C until assay. Plasma concentrations of insulin, C-peptide, FFA, triglyceride, glycerol, HDL, LDL, total-cholesterol, hs-CRP, ASAT, ALAT, ALP, and bilirubin were analyzed using COBAS 6000 Analyzer 501C; Roche. ELISA technique was used to analyze plasma concentrations of adrenaline (LDN, BA E 5400 R), noradrenaline (LDN BA E 5200R), and intact proinsulin (Biovendor). Plasma concentrations of GIP (ab 867) and total GLP-1 (ab 89390) were measured by RIA[2,3]. Plasma concentrations of interferon-γ, interleukines-2,-6,-10,-12p70 -17A, and TNF-α were analyzed by mesoscale S-plex (MSD). Concentrations of glucose, lactate, sodium, potassium, and hemoglobin were analyzed by ABL 800 (Radiometer, Brønshøj, Denmark). HbA1c was analyzed on a DCA Vantage Analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY).

Study eligibility criteria

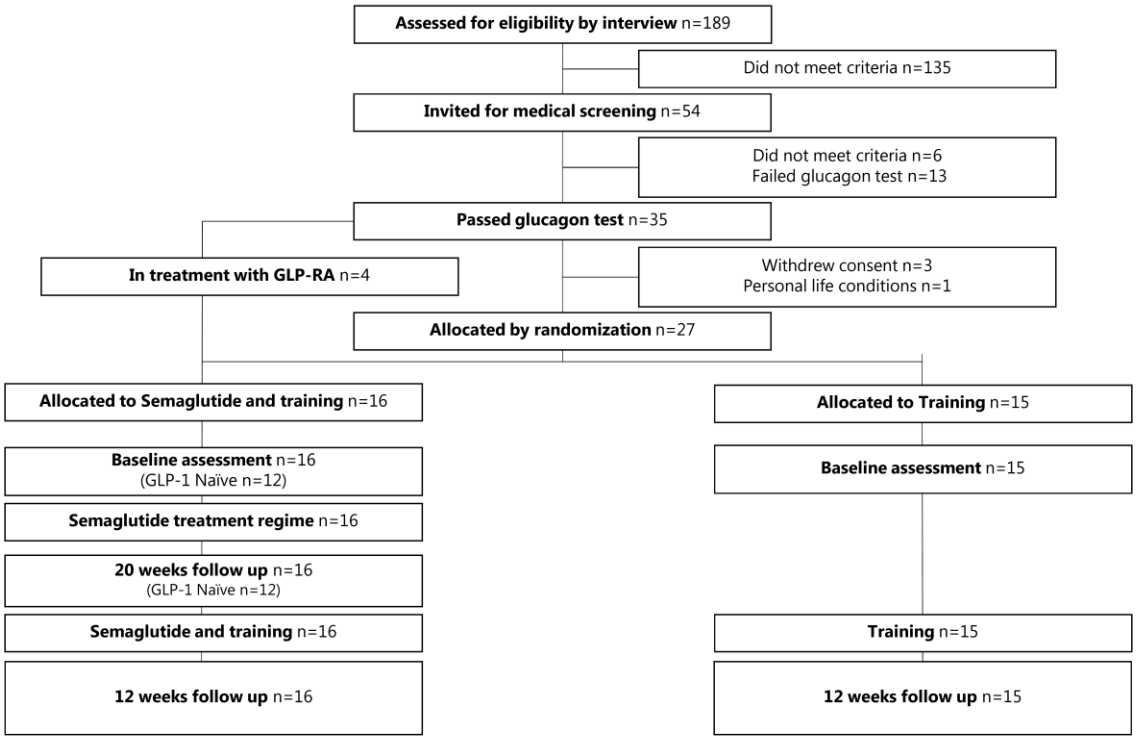
Participants met the following inclusion criteria:

1) Males and females aged between 40 and 70.
2) Diagnosed with type 2 diabetes (HbA1c > 48 mmol/mol, if untreated)
3) Body mass index > 28 kg/m^2
4) Moderately preserved β-cell secretory function, defined by fasting C-peptide concentration equal to or above 1nM along with an increase in C-peptide concentration equal to or above 0.5 nM 6 minutes after intravenous administration of 1 mg glucagon.
5) Less than 2 kg change in body weight six months before inclusion

Exclusion criteria were

1) Insulin treatment
2) Hypertension grade 3
3) Heart disease
4) Medical history of pancreatitis
5) Diagnosed with neuropathy
6) Excessive alcohol consumption
7) Any condition that would interfere with the protocol

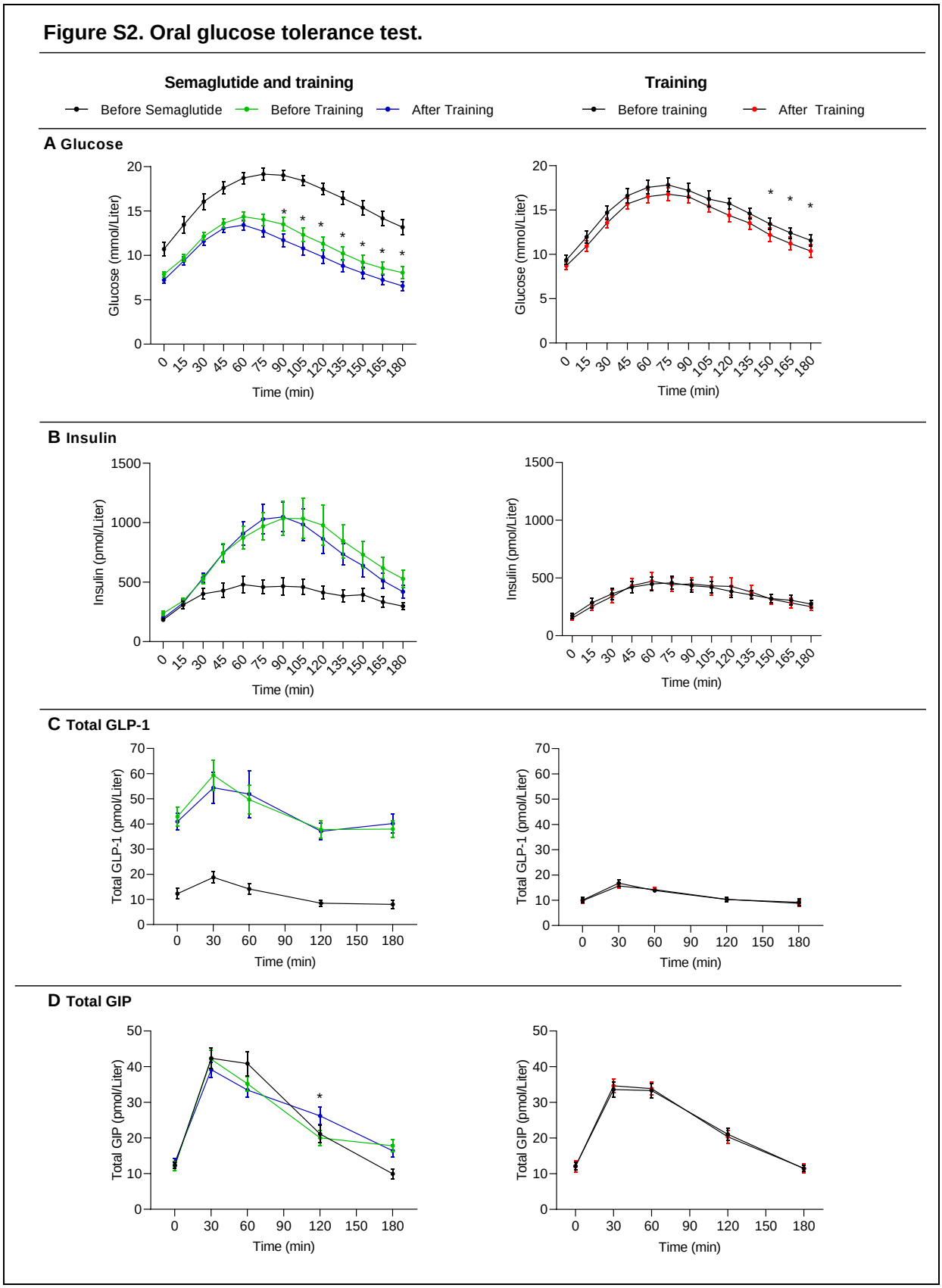
Figure S1. CONSORT Flowchart



Legend Figure S1

Flowchart of the participants.

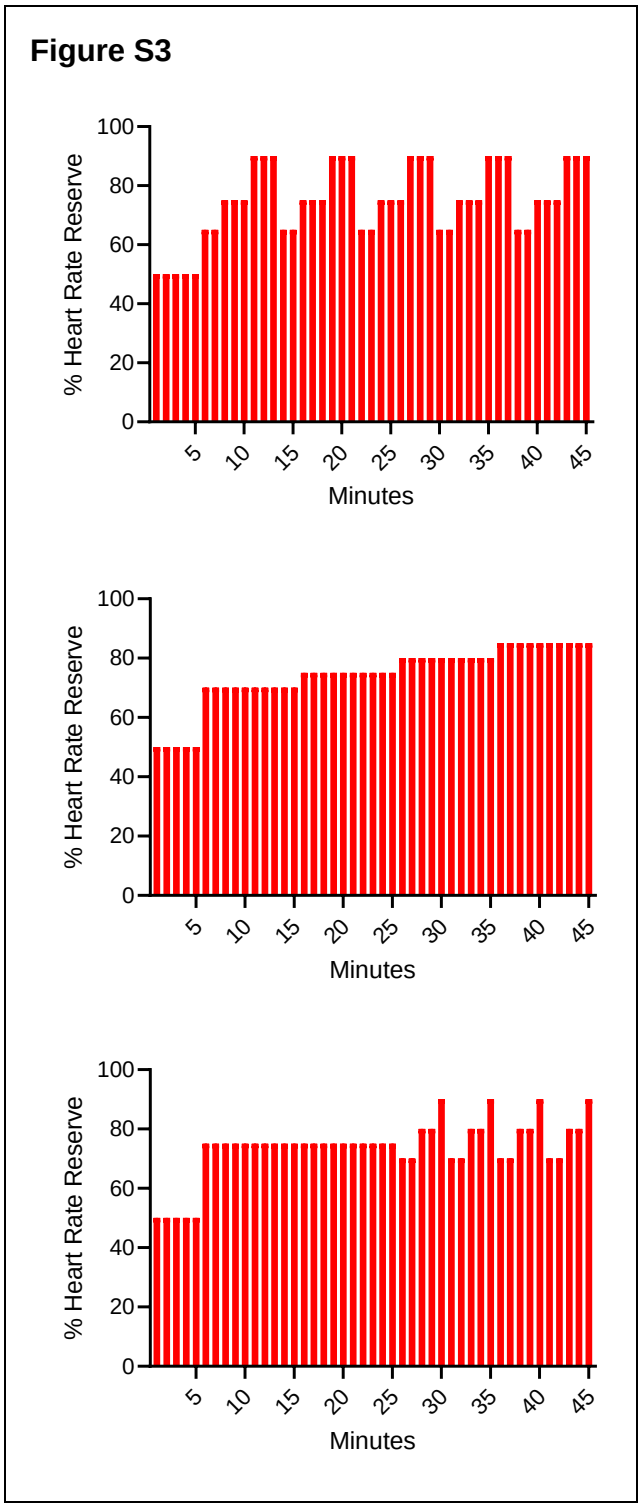
Figure S2. Oral glucose tolerance test



Legend Figure S2:

Results from the oral glucose tolerance test. The left panels show the response to the oral glucose tolerance test before 20 weeks of semaglutide treatment (black line), before 12 weeks of training in combination with semaglutide (green line), and after (blue line). The right panels show the response before 12 weeks of training alone (black lines) and after (red lines). Asterisks indicate differences before and after training ($P<0.05$) in concentrations within the timepoint within the groups, adjusted for multiple comparisons. No statistics are presented for the change with semaglutide alone. All panels present mean values with standard errors (I-bars).

Figure S3. Exercise sessions.



Legend Figure S3

The intensity profile of the three different exercise sessions. The overall aim for each session was to obtain an average intensity of 75 % of heart rate reserve; thus, the workload was adjusted accordingly. All sessions were supervised by study personnel.

Table S1. Inflammatory markers.

Table S1.					
	Group		**Intervention**		
Variable	**Training** (n=15)	**Semaglutide and training** (n=16)	**Semaglutide** (n=12)	**Semaglutide and training** (n=16)	**Training** (n=15)
(fg/mL)	Geometric mean (95 % CI)	Geometric mean (95 % CI)	Change (95 % CI)	Change (95 % CI)	Change (95 % CI)
IL-6	2953 (2121 to 4113)	3547 (2636 to 4772)	162 (-191 to 549)	-752 (-1301 to -67)	-385 (-903 to 26)
IL-2	171(142 to 206)	158 (139 to 178)	-28 (-65 to 21)	3 (-30 to 43)	30 (-12 to 83)
IL-10	896 (705 to 1139)	665 (545 to 811)	-37 (-97 to 29)	78 (-100 to 313)	-56 (-263 to 219)
TNF-α	905 (801 to 1024)	927 (799 to 1077)	-15 (-92 to 70)	-47 (-112 to 23)	-19 (-86 to 54)
INF-γ	804 (529 to 1221)	711 (499 to 1013)	-19 (-231 to 270)	-38 (-114 to 229)	-102 (-249 to 82)
IL-12p70	466 (376 to 578)	375 (309 to 454)	-1 (-29 to 30)	-9 (-69 to 63)	0 (-78 to 95)
IL-17a	558 (426 to 732)	594 (418 to 844)	-13 (-139 to 152)	-42 (-159 to 106)	116 (23 to 378)

0

1

Legend Table S1.

Changes the inflammatory profile with semaglutide, training, and the combination. Baseline data are geometric means and changes are converted ratios of geometric means with 95 % confidence intervals (CI). Changes with semaglutide alone (20 weeks) were assessed in a subgroup (n=12), an evaluated by a two-sided paired students t-test. Within group effects of training with or without semaglutide were evaluated by repeated measures two-way analysis of covariance d the Šidák method was used to adjust for multiple comparisons. No difference between combined semaglutide and training and training alone was detected when tested by unpaired analysis of the delta values from before and after the intervention(Estimated treatment difference, data not shown).

References

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3. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 1994;43(4):535-9. DOI: 10.2337/diab.43.4.535.