Electronic supplementary material

Methods

Exercise test (\(\dot{V}O_2\text{peak}\)) Before and after the 10 week training period, the participants performed a graded maximal cycle test (Model 824E; Monark, Varberg, Sweden) to determine \(\dot{V}O_2\text{peak}\) as previously described [1, 2]. Participants were instructed to avoid exhaustive exercise and to abstain from alcohol 24 h prior to the exercise tests. Before, during and after the test, room temperature was kept at 21°C.

Training programme The exercise-training programme consisted of 10 weeks of cycling on stationary bikes as described previously [1, 2]. In brief, heart-rate monitors and training diaries were used to monitor and ensure training intensity and frequency. Target heart rates were adjusted during week 5, where individuals underwent a midway \(\dot{V}O_2\text{peak}\) test. Participants trained four to five times per week, with two sessions per week under supervision (laboratory training) and two to three sessions performed at home (home-training). Training sessions increased from 20 to 35 min per session during the 10 weeks and were performed at an average exercise intensity of \(\sim 65\%\) of \(\dot{V}O_2\text{peak}\). As described in detail previously [1, 2], laboratory training consisted of interval training and was on average set to elicit \(\sim 60\%\) of \(\dot{V}O_2\text{peak}\) during weeks 1 to 6 and \(\sim 70\%\) \(\dot{V}O_2\text{peak}\) during weeks 7 to 10. Home-training consisted of continuous training (constant exercise intensity) set to elicit \(\sim 60\%\) of \(\dot{V}O_2\text{peak}\) during weeks 1 to 6. During weeks 7 to 10, home-training was changed to interval training and set to elicit \(\sim 70\%\) of \(\dot{V}O_2\text{peak}\). Patients with type 2 diabetes continued their medication throughout the training period, except for one, who discontinued his glucose-lowering treatment after 6 weeks.
Previous studies have indicated that insulin sensitivity is increased for up to 48 h after a single bout of exercise in healthy [3] and insulin-resistant individuals [4]. To estimate the isolated effects of a chronic adaptation to endurance training, rather than an acute effect, our study was carefully designed to ensure that any residual effect of the latest bout of exercise was washed out before the post-training assessments were performed.

**Euglycaemic–hyperinsulinaemic clamp** Within 1 to 2 weeks before commencement of the training programme and again approximately 48 h after the last bout of exercise, participants were admitted to Odense University Hospital at 07:30 hours after an overnight fast. They were instructed to refrain from strenuous activity for the last 48 h before the examination. In diabetic patients, all drugs were withdrawn 1 week prior to the clamp studies and then resumed. After a 30 min rest in the supine position, baseline blood samples were drawn and an euglycaemic–hyperinsulinaemic clamp was performed using a 2 h basal tracer equilibration period, followed by a 3 h insulin infusion period (80 mU min⁻¹ m⁻²) as described before [5]. Using this protocol, glucose levels at 5.0 to 5.5 mmol/l and high physiological hyperinsulinaemia at ~900 pmol/l were obtained in both groups during the insulin-stimulated period. Total $R_d$ and HGP were calculated using Steele’s non-steady-state-equations adapted for labelled glucose infusates as described [6]. Distribution volume of glucose was taken as 200 ml/kg body weight and pool fraction as 0.65. Glycolytic flux was calculated as described [7] from the generation rates of plasma $^3$H₂O from [3-$^3$H]glucose, assuming that all tritium in the C-3 position is lost to water during the glycolytic process [8]. Rates of glucose storage were calculated as the difference between $R_d$ and glycolytic flux. Plasma glucose, plasma lipids, serum insulin, C-peptide and NEFA were measured as described previously [9].

Biopsies were obtained from the vastus lateralis muscle before the 2 h basal equilibration period and after the 3 h insulin infusion period. A modified Bergström needle
was used, with suction under local anaesthesia with 4 to 5 ml lidocaine hydrochloride (10 mg/ml). Muscle samples were immediately blotted free of blood, fat and connective tissue and snap-frozen in liquid nitrogen within 20 to 30 s.

Muscle tissue preparations  Homogenates and lysates were prepared from freeze-dried muscle that had been dissected free of visible fat, blood and connective tissue [10]. Protein concentrations in the lysates were determined using the bicinchoninic acid method (Pierce, Rockford, IL, USA).

Glycogen synthase activity  Muscle glycogen synthase activity in the presence of 8, 0.17 or 0.02 mmol/l glucose 6-phosphate was measured in triplicate using a 96-well plate assay (Unifilter 350 Plates; Whatman, Cambridge, UK) [11]. Glycogen synthase activity in the presence of a saturated concentration of glucose 6-phosphate (8 mmol/l) is termed total glycogen synthase activity. Glycogen synthase activities are reported either as per cent of glucose 6-phosphate-independent glycogen synthase activity (%I form) (100 \times \text{activity in the presence of 0.02 mmol/l glucose 6-phosphate divided by total glycogen synthase activity}) or as %FV (100 \times \text{activity in the presence of 0.17 mmol/l glucose 6-phosphate divided by total glycogen synthase activity}).

SDS-PAGE and western blotting  Western blot analyses were performed as previously described [10]. Membranes used for detection of phosphorylated Akt, TBC1D4 or AMPK were stripped as previously described [12] and re-probed for the corresponding protein using an antibody that recognises the protein independently of phosphorylation.
**Antibodies**  To determine the phosphorylation status of Akt and AMPK, the following phospho-specific antibodies were used: Akt Thr$^{308}$ (Upstate Biotechnologies, Waltham, MA, USA), Akt Ser$^{473}$ (Cell Signaling Technologies, Danvers, MA, USA) and AMPK Thr$^{172}$ (Cell Signaling). Phosphorylation of TBC1D4 was detected using the PAS antibody and phospho-specific antibodies against Ser$^{318}$, Ser$^{341}$, Ser$^{588}$, Thr$^{642}$ and Ser$^{751}$ as previously described [12, 13]. Phosphorylation of glycogen synthase was detected using phospho-specific antibodies against site 2+2a (Ser$^7$+Ser$^{10}$) and 3a (Ser$^{640}$) as previously described [14]. The following antibodies were used to determine total protein expression: Akt1 and Akt2 (Upstate Cell Signaling Solutions, Lake Placid, NY, USA), TBC1D4 (Abcam, Cambridge, UK), GLUT4 (ABR-Affinity Bioreagents, Rockford, IL, USA), hexokinase II (Cell Signaling), $\alpha_2$-AMPK [15] and glycogen synthase (kindly provided by O. Pedersen, Steno Diabetes Center, Gentofte, Denmark). Horseradish peroxidise-conjugated secondary antibodies were purchased from Dako (Glostrup, Denmark).

**References**


glycogen synthase in skeletal muscle of women with polycystic ovary syndrome is reversed by pioglitazone treatment. J Clin Endocrinol Metab 93:3618–3626


