Effect of physical training on insulin secretion and action in skeletal muscle and adipose tissue of first-degree relatives of type 2 diabetic patients

Flemming Dela and Bente Stallknecht
Center for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Submitted 22 December 2009; accepted in final form 17 April 2010

Dela F, Stallknecht B. Effect of physical training on insulin secretion and action in skeletal muscle and adipose tissue of first-degree relatives of type 2 diabetic patients. Am J Physiol Endocrinol Metab 299: E80–E91, 2010. First published April 20, 2010; doi:10.1152/ajpendo.00765.2009.—Physical training affects insulin secretion and action, but there is a paucity of data on the direct effects in skeletal muscle and adipose tissue and on the effect of training in first-degree relatives (FDR) of patients with type 2 diabetes. We studied insulin action at the whole body level and peripherally in skeletal muscle and adipose tissue as well as insulin-secretory capacity in seven FDR and eight control (CON) subjects before and after 12 wk of endurance training. Training improved physical fitness. Insulin-mediated glucose uptake (GU) increased (whole body and leg; \( P < 0.05 \)) after training in CON but not in FDR, whereas glucose-mediated GU increased \(( P < 0.05)\) in both groups. Adipose tissue GU was not affected by training, but it was higher (abdominal, \( P < 0.05 \); femoral, \( P = 0.09 \)) in FDR compared with CON. Training increased skeletal muscle lipolysis \(( P < 0.05)\), and it was markedly higher \(( P < 0.05)\) in subcutaneous abdominal than in femoral adipose tissue and quadriceps muscle with no difference between FDR and CON. Glucose-stimulated insulin secretion was lower in FDR compared with CON, but no effect of training was seen. Glucagon-like peptide-1 stimulated insulin secretion five- to sevenfold. We conclude that insulin-secretory capacity is lower in FDR than in CON and that there is dissociation between training-induced changes in insulin secretion and insulin-mediated GU. Maximal GU rates are similar between groups and increases with physical training.

Glucose- and arginine-stimulated \( \beta \)-cell secretion decreases with a training-induced increase in insulin sensitivity (9, 28, 33, 35) in young, healthy subjects. However, in patients with type 2 diabetes this relationship does not necessarily exist. Patients with a well-preserved insulin-secretory capacity (typically those with the shortest duration of the disease) display an increased rather than a decreased insulin secretion in response to training (11). For this reason and because a potential \( \beta \)-cell adaptation to physical training to our knowledge never has been investigated in FDR, the present study included a test of the \( \beta \)-cell response to stimulation. Glucagon-like peptide-1 (GLP-1) was used as a secretagogue to be superimposed upon hyperglycemia to elicit a maximal insulin response with the purpose of unmasking possible differences between CON and FDR, since GLP-1 is the most potent insulinotropic hormone known that is well tolerated in humans (60).

The inverse relationship between training-induced changes in insulin action and secretion has usually, but not exclusively (33), been demonstrated on the basis of cross-sectional and observational data. In the present study, we sought to elucidate the effect of endurance training on insulin action in both skeletal muscle and adipose tissue, as well as on the whole body level, together with measurements of insulin-secretory capacity, in a comprehensive interventional study in male subjects stratified according to their genetic disposition to type 2 diabetes.

We hypothesized that endurance training in both groups of subjects would increase insulin-mediated glucose uptake rates across the whole body, skeletal muscle, and adipose tissue. Furthermore, we hypothesized that glucose- and GLP-1-stimulated insulin secretion would decrease after training in both groups, i.e., that insulin action and secretion adapt to physical training similarly in FDR and CON.

MATERIALS AND METHODS

Subjects

After ethical approval by the Ethics Committee for Copenhagen and Frederiksberg (Project no. 01-289/00) healthy men with \( n = 7 \) and without \( n = 8 \) a family history of type 2 diabetes, respectively, were recruited via advertisements in newspapers. The study was conducted in accordance with the Declaration of Helsinki, and informed written consent was obtained from each subject. The subjects...
with a family history of type 2 diabetes (FDR) had at least one first-degree relative with confirmed (via hospital or general practitioner records) type 2 diabetes. All subjects had normal glucose tolerance as assessed by a 75-g oral glucose tolerance test. The ages of the subjects were 40 ± 5 and 41 ± 6 yr (means ± SE) in FDR and CON, respectively. Characteristics of the subjects are given in Table 1.

Training Protocol

The training protocol included a home-based bicycle ergometer (Monark 828E or 818E; Monark Exercise, Varberg, Sweden) training for 12 wk, 6 days/wk, 45 min/day at 70% of VO$_{2\text{max}}$. At every training session, the participants filled out a training diary showing date, workload and heart rate (POLAR S610i; Polar Electro Oy, Kempele, Finland), which was mailed to the laboratory one or two times per week. The participants were instructed in adjustments of the workload according to the gradual increase in fitness. According to training diaries, adherence to training sessions was 87 ± 4% in CON and 82 ± 9% in FDR (P > 0.05).

Experimental Protocol

The study consisted of three experimental days and a physical training period followed by a repetition of the three experimental days. In addition, cardiorespiratory fitness [submax ergometer bicycle test with incremental 2-min steps of increasing workloads (50 W-step)] was measured before, during, and at the end of the training period. Pulmonary oxygen consumption (VO$_2$) and carbon dioxide excretion (VCO$_2$) were measured using a Jaeger Champion (Intra-Medic, Lyngby, Denmark). This test was followed by 5-min rest and thereafter measurements of maximal VO$_2$ (VO$_{2\text{max}}$) using the plateau criterion. On separate days, body composition was determined: thigh circumference 20 cm proximal to the patella on both legs; leg volume by water displacement; abdominal skinfold by calipers (model 01127; Lafayette Instruments, IN); and percent body fat by DEXA scanning (LUNAR DPX-IQ; Lunar, Madison, WI). Three days before any experiment, the subjects consumed a diet containing at least 250 g of carbohydrates per day, and one day before, they abstained from alcohol and caffeine intake.

The three experimental days consisted of a frequently sampled intravenous glucose tolerance test (FSIVGTT), a hyperglycemic clamp, and a hyperinsulinemic euglycemic clamp. All experimental days were separated by 2 days. All experiments started at 8:00 in the morning, with the subjects in a 10-h fasted state and in the posttraining experiments 16 h after the last training bout.

FSIVGTT. An arterial catheter was inserted in the radial artery for blood sampling. A venous cubital catheter was used for injection of a bolus (300 mg/kg body wt; max 25 g) of glucose (20%). The bolus was given within 1 min, immediately followed by 50 ml of saline flush of the catheter. Blood was drawn for measurements of plasma glucose and insulin concentration at depicted time points.

Hyperglycemic clamp. Glucose (20%) was infused (using exponentially decreasing infusion rates) into a cubital vein to reach a 20 mM steady-state glucose concentration within 7–12 min, which was maintained via frequent blood sampling (heated hand vein) and glucose analysis and subsequent adjustment of the glucose infusion. At t = 90 min, 2.5 nmol (20 μg) GLP-1 diluted in 50 ml of sterile saline was injected over 1 min in the cubital vein in the cubital vein. Blood samples were drawn for measurements of plasma insulin, C-peptide, and proinsulin.

Hyperinsulinemic euglycemic clamp. Microdialysis catheters were inserted in subcutaneous abdominal and femoral adipose tissue and in the quadriceps muscle (vastus lateralis) as described below. Subsequently, biopsies were obtained from vastus lateralis and the subcutaneous abdominal adipose tissue using a Bergström needle. A venous catheter for infusion of glucose (20%) and insulin was inserted in a cubital vein. A catheter was inserted in the radial or the brachial artery for drawing of blood samples and for monitoring and recording of blood pressure. In a femoral vein a catheter was inserted for later measurements of blood flow (by thermodilution) and drawing of blood samples. A two-step (insulin infusion rates: 28 and 480 mU·min$^{-1}$·m$^{-2}$) sequential clamp was performed, with each step

Table 1. Subject characteristics of FDR and CON subjects before and after 3 mo of ergometer bicycle training

<table>
<thead>
<tr>
<th></th>
<th>FDR (n = 7) Before Training</th>
<th>FDR (n = 7) After Training</th>
<th>CON (n = 8) Before Training</th>
<th>CON (n = 8) After Training</th>
<th>Two-Way RM-ANOVA Group Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg$^a$</td>
<td>93.5 ± 4.3</td>
<td>91.1 ± 4.6</td>
<td>90.8 ± 2.9</td>
<td>89.5 ± 3.8</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>27.4 ± 1.3</td>
<td>26.8 ± 1.5</td>
<td>28.1 ± 0.8</td>
<td>27.7 ± 1.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>24.8 ± 1.7</td>
<td>23.4 ± 2.2</td>
<td>26.8 ± 2.3</td>
<td>26.0 ± 1.7</td>
<td>0.072</td>
</tr>
<tr>
<td>Leg volume, liters$^c$</td>
<td>150 ± 0.5</td>
<td>152.0 ± 0.7</td>
<td>145 ± 0.9</td>
<td>147 ± 0.8</td>
<td>0.399</td>
</tr>
<tr>
<td>Thigh circumference, cm$^d$</td>
<td>58.8 ± 1.6</td>
<td>58.4 ± 1.6</td>
<td>59.9 ± 1.7</td>
<td>59.1 ± 1.9</td>
<td>0.993</td>
</tr>
<tr>
<td>Abdominal skinfold, mm$^e$</td>
<td>27 ± 4</td>
<td>21 ± 3</td>
<td>21 ± 4</td>
<td>18 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Subcutaneous abdominal adipocyte volume, pl</td>
<td>775 ± 144</td>
<td>598 ± 109</td>
<td>886 ± 75</td>
<td>673 ± 35</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Cardiorespiratory fitness

|                     |                           |                           |                             |                             |                                   |
| Work load, max, W   | 314 ± 12                  | 330 ± 14                  | 260 ± 21                   | 293 ± 27                   | 0.012                             | 0.124                             | 0.347                             |
| VO$_{2\text{max}}$, ml·min$^{-1}$·kg$^{-1}$ | 36.4 ± 2.9                | 39.5 ± 3.6                | 32.0 ± 2.0                 | 35.1 ± 2.3                 | 0.002                             | 0.253                             | 0.993                             |
| Heart rate max, f/min | 187 ± 6                   | 183 ± 6                   | 180 ± 7                   | 180 ± 7                   | 0.026                             | 0.658                             | 0.473                             |
| Lactate, max, mM    | 12.6 ± 1.3                | 13.5 ± 1.9                | 11.9 ± 0.7                 | 12.8 ± 0.9                | 0.063                             | 0.706                             | 0.704                             |
| Vt$_{\text{max}}$, l/min | 156 ± 11                  | 176 ± 24                  | 141 ± 9                   | 147 ± 11                  | 0.005                             | 0.134                             | 0.064                             |
| Heart rate, 150 W, l/min | 133 ± 7                   | 131 ± 4                   | 146 ± 3                   | 135 ± 4                   | 0.017                             | 0.174                             | 0.072                             |
| Lactate, 150 W, mM  | 3.0 ± 0.7                 | 2.4 ± 0.8$^*$             | 4.6 ± 0.8                 | 2.8 ± 0.7$^*$             | <0.001                             | 0.301                             | 0.021                             |
| VE, 150 W, l/min    | 55 ± 2                    | 58 ± 8                    | 65 ± 5                    | 50 ± 8                    | 0.190                             | 0.518                             | 0.009                             |

Muscle biopsy analyses

|                     |                           |                           |                             |                             |                                   |
| CS activity, μmol·min$^{-1}$·mg protein$^{-1}$ | 0.26 ± 0.01               | 0.30 ± 0.02               | 0.24 ± 0.02                | 0.29 ± 0.02                | 0.004                             | 0.715                             | 0.466                             |
| β-HAD activity, μmol·min$^{-1}$·mg protein$^{-1}$ | 0.39 ± 0.02               | 0.42 ± 0.03               | 0.36 ± 0.02               | 0.41 ± 0.03               | 0.057                             | 0.671                             | 0.510                             |
| Glycerogen, nmol/mg dry wt | 486 ± 25                  | 590 ± 23                  | 500 ± 23                  | 600 ± 47                  | 0.007                             | 0.794                             | 0.967                             |

Data are means ± SE. FDR, first-degree relatives of type 2 diabetic; CON, control. CS: Citrate synthase; β-HAD: β-hydroxyacyl-CoA-dehydrogenase.

*Measured on 2 different occasions; $^a$average of both legs; $^b$measured 20 cm proximal to the patella; $^c$measured laterally to the umbilicus. Statistical analysis by 2-way ANOVA for repeated measures. $^d$Effect (P < 0.05) of training in subgroup analysis.
last 120 min. Before and at the end of each step, arterial and femoral-venous blood was sampled for determination of substrate and hormone concentrations. Unless otherwise noted, data are given as the mean of the duplicate baseline and the mean of the triplicate end-clamp values. Leg blood flow was measured three to five times immediately after each blood sampling. VO2 and VCO2 were measured by the ventilated hood technique (Jaeger Champion; IntraMedic, Lyngby, Denmark) at baseline and during the final 30 min of each clamp step.

Microdialysis

Microdialysis was performed in principle as described previously (53). At least 60 min before start of the basal period and after local anesthesia, three microdialysis catheters (CMA 60; CMA/Microdialysis, Stockholm, Sweden) were placed in the subcutaneous abdominal and femoral adipose tissue and in the vastus lateralis part of the quadriceps muscle. Catheters were perfused at a rate of 1.0 μl/min. The perfusate consisted of Ringer acetate with 2 mM glucose, 5 kBq/ml [14C]glucose (NEN, Zaventem, Belgium), and 5 kBq/ml [3H]glycerol (NEN).

The in vivo relative recovery (RR) for glucose and glycerol was determined by the internal reference calibration technique (48). This technique assumes that the relative loss of the labeled metabolite from the interstitial fluid equals the RR of the unlabeled metabolite from the interstitial fluid to the microdialysis catheter. The RR of glucose and glycerol was calculated as

\[
\text{RR} = \frac{D_{pmd}}{D_{pmd} - D_{pmd}^*},
\]

where \(D_{pmd}\) is 14Co or 3H disintegrations per minute in 1 of dialysate. Interstitial concentrations of glucose and glycerol were calculated as

\[
C_i = C_p \times \text{RR} + C_p^*,
\]

where \(C_p\) is perfusate concentration.

Subcutaneous Adipose Tissue Blood Flow

Subcutaneous adipose tissue blood flow (ATBF) was measured with the local 133Xe washout technique (30). At least 30 min before the start of the basal period, 0.5–1 MBq gaseous 133Xe (Amersham Health UK) in a volume of 0.05–0.1 ml was injected in the subcutaneous abdominal and femoral adipose tissue. The washout rate of 133Xe was measured continuously with a scintillation counter system (Oakfield Instruments, Eynsham, UK) strapped to the skin surface above the 133Xe depot. ATBF was determined in 30-min periods and calculated as

\[
\text{ATBF} = \frac{-k \cdot \lambda \cdot 100}{(\text{m} \cdot \text{l} \cdot \text{g})^{-1} \cdot \text{min}^{-1}},
\]

where \(k\) is the rate constant of the washout and \(\lambda\) is the tissue-to-blood partition coefficient for 133Xe at equilibrium, which was taken to be 10 ml/g.

Muscle Enzymes And Glycogen

Before biochemical analysis, muscle samples were freeze-dried. With the use of a stereomicroscope, connective tissue, visible fat, and blood were removed from the muscle samples. Muscle glycogen concentration was determined as glucose residues after hydrolysis of the muscle sample in 1 M HCl at 100°C for 2 h. Citrate synthase and β-hydroxyacyl-CoA-dehydrogenase (HAD) activities were measured spectrophotometrically at 37°C. Enzyme activities are expressed as micromoles substrate per minute per gram dry weight muscle tissue.

Adipocyte Size and In Vitro Glucose Incorporation

The adipose tissue was washed with sodium chloride, and adipocytes were isolated by collagenase treatment (0.6 mg/ml, 3.5% albumin, 0.55 mM glucose, pH 7.4, 37°C, 60 min), filtered, and washed three times with glucose-free buffer. Fifty microliters of adipocytes (lipocrit 20%) were mixed with 2 ml of osmium 2% and left for 24–72 h. The radius (r) of each of 200 cells was measured in a stereomicroscope with scaled oculars, and adipocyte volume was calculated as

\[
\frac{4}{3}\pi r^3.
\]

The remaining adipocytes were incubated in buffer (3.5% albumin, pH 7.4, 37°C, lipocrit 1–2%) with trace amounts of 3-[1-14C]glucose (1.5 μmol/l) with or without addition of insulin (1,200 μU/ml). After 60 min, the lipid phase was extracted with heptane, and the glucose incorporation in lipid was determined by scintillation counting.

Sampling and Analyses

Dialysate was collected in 200-μl capped microvials for 30 min during the basal period and for the last 30 min of each of the two clamp steps, immediately frozen, and kept at −20°C until analysis. Dialysate sampling was delayed by 2 min relative to the rest of the experimental protocol to compensate for the transit time in the outlet tubing. Blood was sampled into iced tubes. Blood for determination of insulin, C-peptide, and proinsulin was stabilized with 500 KIE trasytol and 1.5 mg EDTA per milliliter of blood and centrifuged immediately at 4°C. All blood samples were kept at −20°C until analysis. Microdialyse glucose and glycerol as well as plasma glycerol concentrations were determined using a CMA 600 microdialysis analyzer (CMA/Microdialysis, Stockholm, Sweden). Plasma glucose and lactate concentrations were analyzed with an automatic analyzer (ABL 700; Radiometer, Copenhagen, Denmark). Plasma concentrations of insulin, C-peptide, and proinsulin were determined using commercially available ELISA kits (DAKO, Glostrup, Denmark). Plasma free fatty acids (FFA) were measured photometrically (Roche/Hitachi 912 System; Roche, Glostrup, Denmark) using a Wako NEFA-C test kit (Wako Chemical, Neuss, Germany). All analyses were carried out in duplicate.

Calculations

The first-phase insulin response (FPIR) to a glucose bolus in the FSIVGTT experiments was calculated as the mean of the incremental plasma insulin concentrations from 0–10 min following the intravenous glucose bolus.

Glucose infusion rates (GIR) were averaged over 5-min intervals throughout the two sequential 120-min hyperinsulinemic euglycemic clamps. The steady-state GIR minus glucose space correction in the final part (30 min) of each clamp step was taken as the M value. GIR data are presented per fat free mass (FFM).

Insulin action (on glucose uptake rates) and β-cell secretory capacity vary inversely with each other in a hyperbolic relationship. An index of this relationship was calculated as FPIR/M (clamp step 1).

Leg glucose uptake was calculated as the arteriovenous blood glucose concentration difference multiplied by leg blood flow, and data are expressed relative to leg mass, assuming that one liter of leg (water displacement) equals one kilogram of leg.

Estimates of adipose tissue and skeletal muscle venous metabolite concentrations from interstitial metabolite concentrations were based on various assumptions as described previously (53). Metabolite exchange was calculated as arteriovenous metabolite concentration multiplied by blood flow (53).

Statistical Analysis

Results are presented as means ± SE. Data obtained during hyperglycemic and during hyperinsulinemic glucose clamping were analyzed by two-way ANOVA for repeated measurements (i.e., before training, CON vs. FDR; after training, CON vs. FDR; CON, before vs. after training; FDR, before vs. after training). Data on body composition, fitness, and muscle biopsy analyses (i.e., data containing one-factor repetition) were also analyzed by two-way ANOVA for repeated measurements. The Student-Newman-Keuls test was used post hoc to locate differences if statistical interaction was present. Other data were tested by means of nonparametric ranking tests, Mann-Whitney’s for unpaired data and Wilcoxon’s for paired data as appropriate. Correlation between variables was tested by Pearson’s correlation test. P < 0.05 was considered significant in a two-tailed test.
RESULTS

Body Composition and Training Markers

The training program had a significant impact on body composition in both FDR and CON. The training-induced weight loss was primarily due to loss of fat as evidenced by a lower body fat percentage, less abdominal skinfold thickness, and a decrease in subcutaneous abdominal adipocyte size (Table 1). Leg volume and thigh circumference were not changed by training (Table 1), which may have been due to opposite effects on leg skeletal muscle mass and subcutaneous adipose tissue. VO_{2max} increased similarly in FDR (+9 ± 3%) and in CON (+10 ± 3%), and in all measured parameters of cardiorespiratory fitness a main effect of physical training was seen, with some minor differences between the two groups (Table 1). A decreased heart rate and blood lactate concentration during the submaximal, incremental exercise test after training (data not shown), as well as the changes in glycogen content, CS, and HAD activity, also revealed the effects of the training program (Table 1). At maximal exercise, a slight decrease in maximal heart rate and a profound increase in blood lactate (data not shown) indicated increased fitness as a result of the training program.

Whole Body and Leg Insulin Action

During the hyperinsulinemic euglycemic clamp experiments, insulin concentrations were not different before and after training or between the groups (Table 2). Insulin-mediated whole body glucose uptake rates (M value) increased (P > 0.006) in response to training in CON, whereas a significant change was not seen in FDR (Fig. 1). Glucose infusion rates were not different between FDR and CON at clamp step I, but at the maximal insulin concentration (clamp step II) glucose infusion rates were higher in FDR compared with CON (P < 0.05). In the legs, representing mostly skeletal muscle, a similar pattern in glucose uptake rates were seen in CON (increase with training) and in FDR (no effect of training) (Fig. 1). The training-induced increase in glucose uptake rates in CON was primarily due to an effect on leg blood flow, as arteriovenous glucose extraction rates did not change significantly with training (data not shown). Leg glucose uptake rates were not significantly different between CON and FDR, either before or after training.

The effect of training in CON was predominantly due to increases in nonoxidative glucose disposal (in clamp steps I and II, both P < 0.05) and to a minor extent a switch from glucose to lipid oxidation (clamp step II, P < 0.05) (data not shown).

M values correlated positively (P < 0.05) with VO_{2max} in CON (clamp step II: R^2 = 0.57) but not in FDR (clamp step II: R^2 < 0.1) (data from before and after training in one analysis). Likewise, a significant correlation was found between ΔM value and ΔVO_{2max} in CON (clamp step II, R^2 = 0.38) but not in FDR (clamp step II, R^2 < 0.1).

During hyperglycemic clamping, the glucose infusion rates necessary to maintain hyperglycemia at ≈20 mM increased significantly in both groups with training (Fig. 2). This infusion rate is considered a measure of the combined effect of hyperinsulinemia and hyperglycemia on whole body glucose uptake rates, given that plasma glucose concentrations are stable.

Adipose Tissue and Skeletal Muscle Metabolism

In vivo glucose uptake (Fig. 3) was higher (P < 0.05) in subcutaneous abdominal adipose tissue and tended to be higher (P = 0.09) in femoral adipose tissue in FDR compared with CON, but quadriceps muscle glucose uptake did not differ (P > 0.05) between groups. No effect of physical training on in vivo glucose uptake was detected in any of the tissues (all, P < 0.05). In adipose tissue, glucose uptake was increased significantly only at a high insulin concentration (clamp step II), whereas in skeletal muscle glucose uptake was increased (P < 0.05) already at a low insulin concentration (clamp step I). Insulin-stimulated glucose uptake was markedly higher (P < 0.05) in quadriceps muscle than in subcutaneous abdominal and femoral adipose tissue, but insulin-stimulated glucose uptake did not differ (P > 0.05) between the two adipose tissue depots.

Physical training increased (P < 0.05) basal quadriceps muscle in vivo lipolysis (Fig. 4), but physical training did not influence (P > 0.05) adipose tissue or quadriceps muscle lipolysis during insulin infusion. Group (FDR/CON) did not influence (P > 0.05) adipose tissue or quadriceps muscle lipolysis. In subcutaneous abdominal and femoral adipose

Table 2. Plasma insulin and metabolite concentrations at baseline and during 2-step hyperinsulinemic euglycemic clamp in FDR and CON subjects before and after 3 mo of ergometer bicycle training

<table>
<thead>
<tr>
<th></th>
<th>FDR (n = 7)</th>
<th></th>
<th></th>
<th>CON (n = 8)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Clamp Step I</td>
<td>Clamp Step II</td>
<td>Basal</td>
<td>Clamp Step I</td>
<td>Clamp Step II</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>51 ± 6</td>
<td>307 ± 26*</td>
<td>14,130 ± 764*</td>
<td>55 ± 6</td>
<td>333 ± 19*</td>
<td>12,760 ± 914*</td>
</tr>
<tr>
<td>After</td>
<td>45 ± 5</td>
<td>292 ± 18*</td>
<td>13,687 ± 787*</td>
<td>50 ± 6</td>
<td>303 ± 24*</td>
<td>11,751 ± 1,067*</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>5.4 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>After</td>
<td>5.3 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.6 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td>Glycerol, μmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>52 ± 5</td>
<td>19 ± 2*</td>
<td>22 ± 1</td>
<td>51 ± 6</td>
<td>20 ± 4*</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>After</td>
<td>55 ± 7</td>
<td>17 ± 1*</td>
<td>19 ± 1</td>
<td>41 ± 4</td>
<td>21 ± 3*</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>FFA, μmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>481 ± 22</td>
<td>40 ± 10*</td>
<td>14 ± 3*</td>
<td>612 ± 110</td>
<td>57 ± 13*</td>
<td>13 ± 2*</td>
</tr>
<tr>
<td>After</td>
<td>500 ± 53</td>
<td>29 ± 6*</td>
<td>16 ± 4*</td>
<td>466 ± 37</td>
<td>48 ± 12*</td>
<td>11 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Within the clamp steps, insulin concentrations were not different between groups, but basal concentrations decreased with training (main effect, P = 0.048). There was a significant suppression of FFA and glycerol concentrations with increasing insulin concentrations, but this effect was not changed with training. *Significant (P < 0.05) difference from previous value.
tissue, insulin suppressed lipolysis significantly already at a low insulin concentration (step I), but insulin did not \((P < 0.05)\) suppress lipolysis in quadriceps muscle. Lipolysis was markedly higher \((P < 0.05)\) in subcutaneous abdominal adipose tissue than in femoral adipose tissue and quadriceps muscle.

In vitro glucose incorporation into triacylglycerol in adipocytes did not differ \((P > 0.05)\) between FDR and CON subjects in either the basal state [before training: 7.6 ± 2.4 fmol·min⁻¹·μl lipid⁻¹ (FDR) vs. 5.8 ± 0.8 fmol·min⁻¹·μl lipid⁻¹ (CON); after training: 5.3 ± 1.3 (FDR) vs. 4.5 ± 0.6 fmol·min⁻¹·μl lipid⁻¹ (CON)], or during insulin stimulation [before training: 9.1 ± 2.3 (FDR) vs. 7.1 ± 1.0 fmol·min⁻¹·μl lipid⁻¹ (CON); after training: 6.7 ± 1.4 (FDR) vs. 5.3 ± 0.8 fmol·min⁻¹·μl lipid⁻¹ (CON)]. Physical training did not influence \((P > 0.05)\) in vitro adipocyte glucose incorporation into triacylglycerol when expressed per amount of lipid (and hence corrected for the training-induced decrease in adipocyte volume), but physical training decreased \((P < 0.05)\) basal [before training: 4.9 ± 0.6 amol·min⁻¹·adipocyte⁻¹ (FDR) vs. 5.7 ± 0.9 amol·min⁻¹·adipocyte⁻¹ (CON); after training: 3.1 ± 0.4 (FDR) vs. 3.2 ± 0.3 amol·min⁻¹·adipocyte⁻¹ (CON)] and insulin-stimulated [before training: 6.2 ± 1.3 (FDR) vs. 6.7 ± 0.9 amol·min⁻¹·adipocyte⁻¹ (CON); after training: 3.7 ± 0.7 (FDR) vs. 3.8 ± 0.4 amol·min⁻¹·adipocyte⁻¹ (CON)] glucose incorporation when expressed per adipocyte in both FDR and CON. Insulin increased \((P < 0.05)\) in vitro glucose incorporation into triacylglycerol in adipocytes with no difference \((P > 0.05)\) between groups.

**Insulin Secretion**

FPIR \((P < 0.05; \text{Fig. 5})\) and the insulin area under the curve for the entire 40-min FSIVGTT test \((P = 0.051; \text{data not shown})\) was lower in FDR compared with CON, and training did not change these parameters significantly in either group.

During hyperglycemic clamping, plasma glucose concentration was rapidly increased from fasting levels to ~20 mM (Fig. 2), and the coefficient of variation (CV%) of plasma glucose concentrations throughout the clamp was not different between the study groups or before and after 3 mo of training (CV% from \(t = 15\) min and onward, before and after training, respectively: FDR, 5.2 ± 0.8 and 4.8 ± 0.6%; CON, 6.2 ± 0.7 and 3.8 ± 0.5%).
During the first 90 min, when only glucose was infused, plasma insulin and C-peptide concentrations always increased significantly ($P < 0.05$), and no plateau was seen (Fig. 2). At $t = 90$ min, when a GLP-1 bolus was added, a substantial further increase was always seen occurring within 2 min after the bolus was given (Fig. 2). Plasma insulin concentrations peaked at $5.8 \pm 0.7$ and $6.3 \pm 0.8$ min (FDR, before and after training, respectively) and $5.6 \pm 0.8$ and $6.1 \pm 0.7$ min (CON, before and after training, respectively) after the GLP-1 bolus was given, but the time to peak was not different ($P > 0.05$) between groups or with training. The GLP-1-induced increase in insulin concentrations was marked (fold increase: $6.3 \pm 1.1$ and $5.2 \pm 0.9$ in FDR before and after training, respectively; $6.9 \pm 1.1$ and $6.8 \pm 1.2$ in CON before and after training, respectively), but the fold increase was not different ($P > 0.05$) between groups or with training. Proinsulin concentrations mirrored insulin and C-peptide, with no difference between groups at baseline ($1.8 \pm 0.2$ pmol/l), during hyperglycemia ($12.3 \pm 1.2$ pmol/l) and during additional GLP-1 stimulation ($38.4 \pm 4.2$ pmol/l) (pooled mean data from both groups before and after training).

Plasma insulin concentration and secretion (as judged by C-peptide and proinsulin concentrations) did not change significantly with training in FDR or in CON subjects. However, before training, plasma insulin concentrations during hyperglycemia alone tended ($P = 0.066$) to be lower in FDR than in CON, and this difference became significant ($P < 0.05$) after training. In support, after training, plasma C-peptide concentrations during hyperglycemia alone were also lower ($P < 0.05$) in FDR than in CON. During the combined GLP-1 stimulation and hyperglycemia, insulin and C-peptide concentrations tended ($P = 0.08$ and $P = 0.06$, respectively) to be lower in FDR than in CON (Fig. 2).

The integrated index of the $\beta$-cell capacity for insulin production relative to peripheral insulin action on glucose uptake rates, i.e., FPIR/M, did not change from before to after training (FDR, $655 \pm 157$ and $562 \pm 125$; CON, $1,076 \pm 118$ and $909 \pm 146$ pmol·litr$^{-1}$·10 min$^{-1}$)/(mg·min$^{-1}$·kg FF$^{-1}$), respectively), but the index was lower in FDR than in CON before training ($P < 0.05$) and tended to be lower after training ($P = 0.099$).

**Blood Metabolites**

Fasting arterial glycerol and FFA concentrations did not differ between the groups and did not change with training (Table 2). With hyperinsulinemic euglycemic clamping, glycerol and FFA concentrations decreased ($P = 0.05$) in both groups, and the decrease in response to hyperinsulinemia was similar in the two groups and did not change significantly with training (Table 2).

**DISCUSSION**

The main findings of the present study, which is relatively small in number of subjects but experimentally comprehensive, are that healthy men who are first-degree relatives to patients with type 2 diabetes do not improve their insulin-mediated glucose uptake at the whole body level or in skeletal muscle and adipose tissue in response to physical training. However, with combined hyperinsulinemia and hyperglycemia (i.e., hyperglycemic clamping) marked improvements of maximal glucose uptake rates were seen at the whole body level. Neither FDR nor the healthy control subjects changed insulin-secretory capacity in response to physical training. Experiments on metabolite turnover of subcutaneous adipose tissue revealed regional differences and also that in vivo insulin-stimulated glucose uptake was higher in FDR than in CON, while insulin inhibition of lipolysis was similar in both groups. The FDR subjects were metabolically characterized by a decreased insulin-secretory capacity, which was present in parallel with a normal to supranormal peripheral insulin action. In these subjects, a decrease in insulin action would therefore most likely result in the development of overt type 2 diabetes, because there is no reserve capacity in insulin secretion to compensate for a reduced insulin action. This relationship between insulin secretion and action can be expressed as the ratio of FPIR and the M value, which indeed was lower in FDR compared with CON.

**Insulin Action**

Despite similar adherence to the training intervention program and similar increases in cardiorespiratory fitness, FDR subjects did not improve insulin-mediated glucose uptake significantly on the whole body level or specifically in the leg, whereas improvements were seen in the CON subjects. Thus, at clamp steps I and II in CON, training increased whole body glucose uptake rates by $34 \pm 6$ and $12 \pm 5\%$, respectively, and leg glucose uptake rates by $88 \pm 55$ and $35 \pm 13\%$, respectively. The lack of training effect on insulin-mediated glucose uptake rates in FDR subjects are in keeping with a recent bed rest study in which CON subjects displayed significantly larger adaptations to (in)activity compared with FDR subjects (49).

During a hyperglycemic clamp, glucose is infused to maintain hyperglycemia. Thus, the glucose infusion rate during hyperglycemic clamping may also be taken as a measure of whole body glucose uptake (36) mediated partly by the accompanying increase in endogenous insulin and partly by the mass action of glucose per se. In contrast to insulin sensitivity, which is typically evaluated at low to medium insulin concentrations during a hyperinsulinemic euglycemic clamp (26), during the hyperglycemic clamp we found similar training-induced increases in glucose infusion rates between FDR and CON. These rates were achieved in the face of insulin concentrations of “only” 4–5,000 pmol/l (which is high, but still less than half of what was seen during step II of the hyperinsulinemic clamp). Seemingly conflicting results between the hyperinsulinemic and the hyperglycemic clamps, but the extra glucose uptake is explained by the accompanying hyperglycemia, which, by mass-action, increases glucose uptake, i.e., the glucose uptake that is due to the hyperglycemia per se. Our hypothesis is that the training-induced increase in stimulated glucose uptake rates are due to a mixture of insulin- and glucose-mediated glucose uptake in CON, whereas in FDR the training effect is exclusively related to the glucose-mediated glucose uptake. This interpretation is compatible with an augmentation of glucose-mediated glucose uptake in FDR, as shown in some (19, 20) but not all (39) previous studies.

The lack of a training effect on insulin-mediated glucose uptake (whole body and specifically in the leg) in FDR points to the possibility that the exercise-induced changes in proteins and enzymes in the insulin signaling cascade in skeletal muscle is impaired in people with a genetic disposition toward insulin...
resistance. In the literature, most, but not all (32), have reported impaired insulin signaling in skeletal muscle in FDR compared with CON subjects [e.g., insulin receptor kinase activity, Akt activation, PI 3-kinase activity, glycogen synthase activity (18, 27, 37, 54, 56)]. The effect of exercise training on these parameters has not been measured in FDR, but in subjects without familiar disposition to type 2 diabetes as well as in patients with type 2 diabetes, strength (21) and endurance (16)
training increases skeletal muscle protein content of key proteins in insulin signaling. However, the predominant effect is a training-induced enhancement of leg blood flow (16, 21), as also found in CON, but not in FDR, in the present study. The latter observation is in accord with recent findings of diminished insulin-mediated forearm blood flow in FDR (50). In opposition to the present findings, in a recent study in women, training increased an insulin sensitivity index in FDR

Fig. 2. Plasma glucose concentrations, glucose infusion rates, plasma insulin, and C-peptide concentrations during hyperglycemic clamps in 7 FDR and 8 CON subjects. Data are from before (open symbols) and after (filled symbols) 3 mo of endurance training. At basal and during a 2-step hyperinsulinemic euglycemic clamp (step I and step II), glucose uptake was estimated in subcutaneous abdominal and femoral adipose tissue and in quadriceps muscle. Insulin increased glucose uptake in subcutaneous abdominal and femoral adipose tissue and in quadriceps muscle. Insulin increased glucose uptake in subcutaneous abdominal (P < 0.05; post hoc, basal vs. step II) and femoral (P < 0.01; post hoc, basal vs. step II) adipose tissue as well as in quadriceps muscle (P < 0.001, post hoc: basal vs. step II; step I vs. step II). Insulin-stimulated glucose uptake was higher (P < 0.05) in quadriceps muscle than in subcutaneous abdominal and femoral adipose tissue. Glucose uptake was significantly higher in subcutaneous abdominal adipose tissue of FDR than in CON. Values are means ± SE. #P < 0.05 vs. CON (main effect).

Fig. 3. Glucose uptake (by microdialysis technique) in 7 FDR and 8 CON subjects, before (open bars) and after (filled bars) 3 mo of endurance training. At basal and during a 2-step hyperinsulinemic euglycemic clamp (step I and step II), glucose uptake was estimated in subcutaneous abdominal and femoral adipose tissue and in quadriceps muscle. Insulin increased glucose uptake in subcutaneous abdominal and femoral adipose tissue and in quadriceps muscle. Insulin increased glucose uptake in subcutaneous abdominal and femoral adipose tissue and in quadriceps muscle. Insulin increased glucose uptake in subcutaneous abdominal adipose tissue and in quadriceps muscle. Insulin increased glucose uptake in subcutaneous abdominal adipose tissue and in quadriceps muscle. Insulin increased glucose uptake in subcutaneous abdominal adipose tissue and in quadriceps muscle. Values are means ± SE. #P < 0.05 vs. CON (main effect).
but not in CON subjects despite similar increases in $V_O^{2max}$ (3), but similar increases in insulin-mediated glucose uptake in FDR and CON subjects have also been shown (40). In the latter study (40), insulin sensitivity correlated positively with $V_O^{2max}$ in CON but not in FDR, an observation that is confirmed by the present study. The lack of a significant correlation between insulin sensitivity and $V_O^{2max}$ in FDR has been speculated (40) to be due to an impaired oxidative capacity in the mitochondria (i.e., a mitochondrial dysfunction) in FDR. However, skeletal mitochondrial respiration is not decreased in insulin resistance when the content of mitochondria is taken into account (4, 31, 45). Furthermore, mitochondrial biogenesis was stimulated in both groups in the present study, as evidenced by training-induced increases in CS activity (Table 1).

In this study, the FDR and CON groups were carefully matched, their adherence to the training program was not different, and the fitness outcome was identical. The fact that at baseline FDR subjects were not insulin resistant compared with CON cannot explain the lack of improvements in insulin-mediated glucose uptake rates in FDR in response to the training program. A wide heterogeneity in insulin sensitivity in CON and FDR has been shown before (61). We did not measure glucose transporter proteins, insulin signaling molecules, intramyocellular lipids, etc., in muscle biopsies from the present subjects, wherefore it cannot be excluded a priori that differences between groups in these parameters may explain the differences in insulin sensitivity outcomes. For example, accumulation of intramyocellular lipids may impair insulin action, and in FDR such an accumulation has previously been demonstrated (23, 37, 42, 43, 55). Likewise, protein and gene expression of phosphoprotein enriched in astrocytes 15 (PEA15), which interacts with the ERK-PI 3-kinase signaling
found less efficient inhibition of in vivo lipolysis by insulin in subcutaneous abdominal adipose tissue of FDR compared with CON, and it thus seems that subcutaneous abdominal adipose tissue metabolite turnover (glucose, lactate, lipids) in general is increased in FDR. We found no significant differences in in vitro basal and insulin-stimulated glucose incorporation into triacylglycerol in adipocytes between FDR and CON, and a previous in vitro study also did not detect metabolic defects in adipocytes from FDR compared with CON (14). Apparently, factors not present in vitro, e.g., blood flow, are of importance for the differences in adipose tissue metabolism between FDR and CON.

Endurance training increased in vivo skeletal muscle lipolysis, but no other clear effects of training were seen in adipose tissue or skeletal muscle metabolism with the microdialysis technique in either FDR or CON. This indicates that the training program used in the present study was not sufficient to markedly change resting metabolism in skeletal muscle or adipose tissue.

Insulin Secretion

Glucose-stimulated insulin secretion was lower in FDR than in CON. This is in accord with some (12, 13, 15, 25, 38, 41, 58), but not all (2, 24, 44, 59), previous reports. The addition of GLP-1 stimulation to the hyperglycemic stimulus resulted in a marked increase (5- to 7-fold) in insulin concentrations and secretion in both groups; a fold increase that was somewhat higher than previously seen in older healthy subjects and patients with type 2 diabetes also stimulated with 2.5 nmol GLP-1 and 15 mmol/l glucose, but the time to peak was similar (60). Patients with type 2 diabetes have a reduced response to combined hyperglycemia and GLP-1 stimulation (60), and in the present study the response tended (P < 0.1) to be lower in FDR than in CON subjects (Fig. 2). Thus, the β-cell response to glucose is clearly diminished in FDR, and the response to GLP-1 may be diminished. However, an isolated effect of GLP-1 cannot be evaluated with the presently used experimental protocol.

It should be noted that the two independent tests of β-cell secretion (IVGTT and hyperglycemic clamp) showed essentially the same results, i.e., lower insulin secretion in FDR than in CON, and no effect of training. As for CON, the latter point is in contrast to previous studies in young, healthy subjects (9, 28, 33, 35). In light of the observed increase in insulin action on leg and whole body glucose uptake rates, we had expected an adaptation of the glucose-stimulated insulin secretion. Furthermore, we did not find an effect of training on the GLP-1-stimulated insulin response. This is in contrast to the training effect, i.e., lower insulin response, that we previously found with another non-glucose secretagogue, arginine (9). However, a possible effect of training on GLP-1-stimulated insulin secretion may have been masked by the fact that insulin secretion was already markedly stimulated by the hyperglycemia. A likely explanation to the lack of training effect on insulin secretion might be that CON subjects in the present study were on average approximately 20 years older than those in the previous studies (9, 35), and it is known that basal insulin release declines with age (22) and that older individuals do not exhibit the same compensatory increase in β-cell secretion as young individuals after eccentric exercise training (29). In

Adipose Tissue and Skeletal Muscle Metabolism

Adipose tissue and skeletal muscle are important targets for insulin action, and tissue-specific action can be examined in vivo by the microdialysis technique and in vitro in tissue biopsies. We found that increasing doses of insulin stimulate in vivo glucose uptake in skeletal muscle in a stepwise fashion but also that it takes a supraphysiological dose of insulin (clamp step II) to stimulate subcutaneous adipose tissue glucose uptake. The relatively limited ability of insulin to stimulate glucose uptake in human adipose tissue has also been found in previous studies using microdialysis (17) as well as arteriovenous flux (5) techniques in subcutaneous abdominal adipose tissue. In contrast, subcutaneous adipose tissue lipolysis is very sensitive to insulin and is suppressed at a physiological insulin concentration, whereas lipolysis in skeletal muscle is not affected by insulin.

Interestingly, we found the in vivo glucose uptake in subcutaneous abdominal (P < 0.05) and femoral (P < 0.1) adipose tissue to be higher in FDR than in CON. In vivo lactate release from subcutaneous abdominal adipose tissue has previously been found to be higher in FDR than in CON (47), and these findings suggest that an increased uptake and turnover of glucose in adipose tissue is a primary defect in the development of type 2 diabetes. Furthermore, Eriksson et al. (14)
FDR subjects, the β-cell adaptation to a training program is more difficult to predict. In the present study, we found no change in the response (in accord with the finding of no increase in insulin-mediated glucose uptake rates); on the other hand, it has previously been shown that in type 2 diabetic patients with a relatively well-preserved β-cell capacity (i.e., “high-secretors”), the β-cell response to training is, in fact, an increase in the β-cell secretory capacity (11). Thus, the training intervention would tend to pull the β-cell secretory response toward a decrease, whereas the genetic disposition to type 2 diabetes might pull the response toward an increase. Further studies are needed to clarify this issue.

Summary

We found that endurance exercise training for three months in 40-year-old healthy men without a genetic disposition to type 2 diabetes increased insulin-mediated glucose uptake rates in whole body as well as the leg and that this increase was dissociated from a change in the β-cell response to hyperglycemia with or without additional GLP-1 stimulation. In 40-year-old men with a genetic disposition to type 2 diabetes, no effect of training on insulin-mediated glucose uptake rates or on the stimulated β-cell secretion was seen, but glucose-mediated glucose uptake rates were preserved and increased with training. Offspring subjects displayed impaired stimulated β-cell secretion and increased glucose uptake in subcutaneous adipose tissue but not in quadriceps muscle.

ACKNOWLEDGMENTS

We thank Regitze Kraunsøe, Jeppe Bach, and Lisbeth Kall for dedicated technical assistance. We also thank Claus Neumann MD for assistance with the initial experiments.

GRANTS

The financial support from the Novo Nordisk Foundation, the Danish Diabetes Association, the Lundbeck Foundation, the Jacob Madsens and Olga Madsens Foundation, the Foundation of 1870, the Kathrine and Vigo Skovgaard’s Foundation, the Aase and Ejnar Danielsen Foundation, the Simon Fougner Hartmanns Family Foundation, and a European Union grant (6th Framework LSHM-CT-2004-005272, EXGENESIS) gratefully acknowledged.

DISCLOSURES

No conflicts of interest are reported by the authors.

REFERENCES


