0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(12):4903–4910

doi: 10.1210/jc.2006-1142

Exercise-Induced Improvement in Vasodilatory Function Accompanies Increased Insulin Sensitivity in Obesity and Type 2 Diabetes Mellitus

Elena De Filippis, Kenneth Cusi, Gloria Ocampo, Rachele Berria, Susan Buck, Agostino Consoli, and Lawrence J. Mandarino

Center for Metabolic Biology (E.D.F., L.J.M.), Arizona State University, Tempe, Arizona 85287; Department of Medicine (K.C., G.O.), University of Texas Health Science Center, San Antonio, Texas 78229; Department of Obstetrics and Gynecology (R.B.), Case Western Reserve University, Cleveland, Ohio 44106; Texas Diabetes Institute (S.B.), San Antonio Texas 78207; and Department of Medicine and Aging Sciences (A.C.), University of Chieti, 66013 Chieti, Italy

Objective: The present study was undertaken to determine whether improved vasodilatory function accompanies increased insulin sensitivity in overweight, insulin-resistant subjects (OW) and type 2 diabetic patients (T2DM) who participated in an 8-wk exercise training regimen.

Design: Before and after training, subjects had euglycemic clamps to determine insulin sensitivity. Brachial artery catheterization was done on another occasion for measurement of vasodilatory function. A lean, healthy, untrained group was studied as nonexercised controls.

Results: Training increased oxygen consumption (VO_2) peak [OW, 29 ± 1 to 37 ± 4 ml/kg fat-free mass (FFM)·min; T2DM, 33 ± 2 to 43 ± 3 ml/kg FFM·min; $P < 0.05$] and improved insulin-stimulated glucose disposal (OW, 6.5 ± 0.5 to 7.2 ± 0.4 mg/kg FFM·min; T2DM, 3.8 ± 0.3 to 4.2 ± 0.3 mg/kg FFM·min; $P < 0.05$) in insulin resistance. OW and T2DM, before training, had decreased acetylcholine chloride (ACh)- and sodium nitroprusside-mediated vasodilation and decreased reactive hyperemia compared with lean controls. Training increased the vasodilatory response to ACh [OW (30 μg ACh/min), 12.2 ± 3.4 to 19 ± 4.2 ml/100 g·min; T2DM (30 μg ACh/min), 10.1 ± 1.5 to 14.2 ± 2.1 ml/100 g·min; $P < 0.05$] in both groups without affecting nitroprusside response.

Conclusion: Because vasodilatory dysfunction has been postulated to contribute to insulin resistance, the exercise-induced improvement in vasodilatory function may signify changes in the endothelium that could contribute to the improvement in insulin sensitivity observed after aerobic exercise training. **(*J Clin Endocrinol Metab* 91: 4903–4910, 2006)**

AEROBIC EXERCISE IS commonly used as a treatment for insulin resistance. Exercise training increases GLUT-4 protein expression and glycogen synthase activity in skeletal muscle (1–4) and may (5–7) or may not (8) increase AMP kinase expression and activity. Clearly, these events can have a major impact on glucose and fat metabolism. However, it is not clear how these effects lead to enhanced insulin sensitivity, because exercise training has not been found consistently to improve insulin signaling in skeletal muscle from insulin-resistant obese and type 2 diabetic patients (9, 10). Therefore it is likely that there are additional mechanisms by which exercise training influences insulin sensitivity.

Endothelial dysfunction and insulin resistance often coexist (11). Mildly overweight subjects with a strong family history of type 2 diabetes who do not yet exhibit typical clinical abnormalities of the metabolic syndrome such as impaired glucose tolerance, hypertension, or dyslipidemia already have endothelial dysfunction, suggesting that the latter may precede the development of many aspects of the metabolic syndrome and perhaps insulin resistance itself (12). Endothelial dysfunction also characterizes individuals with impaired glucose tolerance (12–14), insulin resistance (15), and type 2 diabetes mellitus (16), suggesting that insulin resistance and endothelial (vasodilatory) dysfunction are intimately linked (12).

Moreover, vasodilation in itself is an insulin-mediated event (17), so it is likely that insulin-stimulated glucose disposal and insulin-mediated vasodilation share at least some common signaling pathways (although these probably are in different tissues). Therefore, a training-induced improvement in insulin action may ultimately lead to improvements in glucose disposal and vasodilatory function.

In addition to its tight relationship with insulin resistance, endothelial dysfunction is considered to be an early event in atherogenesis and precedes the development of detectable cardiovascular disease (18, 19). Investigators have used several interventions designed to arrest or reduce the progression of endothelial dysfunction. One of these, exercise training, has been demonstrated to improve vascular function in some groups of patients (20, 21) and is associated with a reduction in primary (22–24) and secondary (25) cardiovascular events. In particular, aerobic and/or resistance exercise training increase the acetylcholine chloride (ACh)-mediated

First Published Online October 3, 2006

Abbreviations: ACh, Acetylcholine chloride; BMI, body mass index; EGP, endogenous glucose production; FBF, forearm blood flow; HbA_{1c}, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NO, nitric oxide; SNP, sodium nitroprusside; VO_2, oxygen consumption.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

vasodilatory response in patients with type 2 diabetes (26). Conversely, several groups have failed to show that exercise training increases endothelial or vasodilatory function in healthy subjects despite a demonstrably increased capacity for peak blood flow (20). The lack of benefit may have several explanations, one being that exercise training of healthy subjects, who do not have endothelial dysfunction, may not further increase the already normal system, whereas benefit may be possible in those with antecedent endothelial dysfunction (20). It is also not clear whether exercise training improves vasodilatory function in insulin resistance in general, independent of blood glucose (26). Therefore, this study was undertaken to determine whether improved vascular function accompanies an exercise-induced enhancement of insulin sensitivity in profoundly insulin-resistant subjects.

Subjects and Methods

Subjects

Five lean control, nine overweight nondiabetic, and 14 type 2 diabetic subjects, all of Mexican-American ethnicity, participated in the study. The study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and informed written consent was obtained from all subjects. Studies were conducted at the Texas Diabetes Institute Clinical Research Center. A history and physical examination were obtained. No subjects had risk factors (other than obesity or diabetes) for vascular disease. None of the type 2 diabetics had diabetic complications. Subjects were normotensive, did not smoke, and did not participate in regular exercise. As part of the physical examination, subjects had a resting 12-lead electrocardiogram and a complete chemistry panel. A 75-g oral glucose tolerance test was performed using American Diabetes Association criteria to exclude nondiabetic subjects with impaired glucose tolerance. Nondiabetic subjects had no family history of type 2 diabetes. Six of the 14 diabetic subjects were taking glyburide, which was withdrawn 3 d before clamp studies. No subject was taking any other medication known to affect glucose metabolism.

Training program

The training program was undertaken by the obese and diabetic subjects only. Subjects were given a choice of stationary bicycle or treadmill exercise, but all subjects chose the cycle ergometer except one diabetic patient who preferred the treadmill. Because leg exercise decreases forearm blood flow (FBF) (27), while performing leg exercise, subjects squeezed a spring-loaded handgrip 30 times per minute every 5 min (nondominant arm). The training program was based on the guidelines of the American College of Sports Medicine and on the relationship between heart rate and oxygen consumption (VO_2). Subjects started training at 60% of measured VO_2 peak for 20 min three times per week and progressively increased the workload and the frequency of the exercise to 70% of VO_2 for 45 min four times per week; all exercise sessions were supervised. Subjects were instructed to make no attempt to alter their diet during the training period. The total length of the training program was 8 wk. All subjects randomly repeated blood flow studies and glucose clamps within 48 h after the last training session.

Hydrostatic weighing

Body composition was determined using a submerged-weight electronic tank system (Ergo Science, Inc., Charlestown, MA), calculating body density by the method of Brozek and Anderson (28) and residual lung volume using equations provided (Ergo Science).

Peak aerobic capacity (VO_2 peak)

VO_2 peak was determined using an electrically braked cycle ergometer and a Sensormedics model V29 metabolic measurement system (Sensormedics, Savi Park, CA). The test was performed twice, before starting 8 wk of exercise training and at the end of the training. Exercise was started at a workload of 40 W and increased by 10 W per minute until perceived exhaustion or a respiratory quotient of 1.10 was reached. The highest VO_2 observed during a 30-sec period was defined as VO_2 peak. Heart rate and rhythm were monitored using a 12-lead electrocardiogram. Heart rate, work in watts, VO_2, and carbon dioxide production (VCO_2) were continuously recorded throughout the test, and the relationship between heart rate and work was used to design the training program (see below). During the pretraining VO_2 peak test, VO_2 and heart rate achieved at 60 W were recorded as measures of submaximal exercise responses. After training, the VO_2 and work rate at the pretraining 60-W heart rate were measured for comparison.

Euglycemic-hyperinsulinemic clamp

Subjects reported to the clinic for the euglycemic-hyperinsulinemic clamp study after consuming nothing but water after 2200 h the previous night. An antecubital vein was catheterized for infusion of 3-[^{3}H]glucose, insulin, and 20% dextrose. Euglycemic-hyperinsulinemic clamps were performed using an insulin infusion rate of 40 mU/m^2·min, as previously described (29, 30). Glucose-specific activity was determined using barium hydroxide/zinc sulfate deproteinization of plasma samples, and rates of glucose appearance and disappearance were calculated using steady-state or non-steady-state equations, as appropriate (30).

Assessment of vasodilatory function

On another day, the patient was admitted after consuming nothing but water after 2200 h the previous night to assess forearm vasodilatory function. The brachial artery in the nondominant arm was cannulated using a 20-gauge needle connected to an epidural catheter under local anesthesia (1% lidocaine). After 60 min of rest in the supine position, basal FBF and arterial blood pressure were measured using a mercury-filled silastic strain-gauge plethysmograph (EC-4; D. E. Hokanson, Inc., Bellevue WA). After baseline measurements, the effect of the endothelium-dependent vasodilator ACh (7.5, 15, and 30 μg/min) and endothelium-independent vasodilator sodium nitroprusside (SNP) (6 and 10 μg/min) was investigated in the nondominant arm and in the dominant arm, which was identified to be used as control (26). Drugs were continuously infused for 5 min, and data were collected in the last 3 min of infusion. Reactive hyperemia also was assessed as a reflection of endothelium-dependent vasodilation. The response to hyperemia was obtained by inflating a blood pressure cuff placed on the upper arm up to 250 mm Hg for 5 min; after releasing the cuff, FBF measurements were performed for 3 min after release. Drug infusions and reactive hyperemia were started only after allowing the FBF to return to baseline. There was a washout period of 60 min between all stimuli.

Measurement of FBF using strain-gauge plethysmography

FBF was measured by using a mercury-filled silastic strain-gauge plethysmograph as previously described (15, 26, 31). Briefly, a strain gauge was attached to the upper part of both arms and connected to the plethysmography instrument and was supported above the level of the right atrium. A wrist cuff was inflated immediately before each FBF recording to a pressure of 50 mm Hg above the systolic pressure to exclude the hand circulation from the measurement. A second cuff was positioned at the upper part of each arm and inflated to 50 mm Hg for 10 sec in each 15-sec cycle to occlude venous outflow from the arm using a rapid cuff inflator (EC-20; D. E. Hokanson). Changes in flow hemodynamics were calculated as the mean of the final five measurements of each recording period for each dose. FBF was expressed as milliliters per 100 g of forearm tissue per minute (31).

Statistical analysis

Data are presented as means ± SE. The data after exercise training were compared with pretraining data using ANOVA or one- or two-tailed Student's paired *t* tests, as appropriate. Statistical tests were performed using StatView version 5.0 (SAS Institute Inc., Cary, NC). The significance level was set at ≤ 0.05.

TABLE 1. Subject characteristics

	Nondiabetic subjects		Diabetic patients
	Lean	Overweight	
Gender (male/female)	2/3	4/5	7/7
Age (yr)	32 ± 2	37 ± 2	43 ± 2[a]
BMI (kg/m^2)	23 ± 1.3	29 ± 0.9[a]	31 ± 0.8[a]
Body weight (kg)	62 ± 7.2	78 ± .3.3[c]	89 ± 4[c]
Fat mass (kg)	19 ± 4	29 ± 2.0[a]	33 ± 2.0[a]
FPG (mmol/liter)	5.1 ± 0.1	5.4 ± 0.1	8.7 ± 0.6[a,b]
FPI (pmol/liter)	15 ± 2.1	42 ± 11[a]	76 ± 14[a,b]
HbA_{1c} (%)	5.0 ± 0.1	5.3 ± 0.1	8.1 ± 0.5[c]
Total cholesterol (mmol/liter)	3.4 ± 0.3	5.2 ± 0.6[a]	5.2 ± 0.2[a]
Triglycerides (mmol/liter)	1.6 ± 0.3	2.2 ± 0.3	4 ± 0.8
HDL-cholesterol (mmol/liter)	1.4 ± 0.07	1.2 ± 0.1[a]	1.1 ± 0.1[c]
LDL-cholesterol (mmol/liter)	1.7 ± 0.3	3.7 ± 0.6[c]	3.4 ± 0.3[c]
SBP (mm Hg)	104 ± 6.5	118 ± 3.7	126 ± 4.6
DBP (mm Hg)	59 ± 3	63 ± 3	73 ± 3.7
Resting heart rate (bpm)	63 ± 2	71 ± 4	74 ± 2

Subject characteristics are baseline values expressed as mean ± SE. DBP, Diastolic blood pressure; FPG, fasting plasma glucose; FPI, fasting plasma insulin; SBP, systolic blood pressure; bpm, beats per minute.

[a] $P < 0.05$ *vs.* lean controls.

[b] $P < 0.05$ *vs.* overweight nondiabetic.

[c] $P < 0.01$ *vs.* lean controls.

Results

Subjects

The characteristics of the subjects are given in Table 1. Patients with type 2 diabetes had elevated glycosylated hemoglobin (HbA_{1c}), fasting plasma glucose, and insulin levels compared with nondiabetic subjects. There was no statistically significant difference in fat mass between the overweight nondiabetic and diabetic groups. The lean control group had a significantly lower body mass index (BMI), body weight, and fat mass. After 8 wk of exercise, BMI, total body weight, fat mass, and HbA_{1c} were unchanged in overweight and diabetic subjects (Table 2). This was expected, because subjects were instructed not to change their diet. As shown in Table 2, the lipid profile was improved in both groups after training. In particular, total and low-density lipoprotein (LDL)-cholesterol significantly decreased after training. High-density lipoprotein (HDL) was unchanged by exercise training. However, as LDL-cholesterol decreased, HDL increased as a percentage of total cholesterol ($P < 0.05$, Table 2).

Exercise training improves aerobic capacity and insulin sensitivity

There were no differences in pretraining values for either aerobic capacity or peak heart rate (Table 3). Exercise training significantly improved peak aerobic capacity in both nondiabetic and diabetic subjects ($P < 0.05$). In addition, oxygen consumption at a fixed work load (60 W) improved in overweight and diabetic subjects ($P < 0.05$, Table 3), especially in light of a small (nonsignificant) decrease in heart rate at this work rate. When expressed as a ratio, training improved VO_2 per heart beat at a work rate of 60 W ($P < 0.01$, Table 3).

In the postabsorptive state, endogenous glucose production (EGP) was similar in lean and overweight nondiabetic controls and type 2 diabetic patients. Basal EGP did not change after 8 wk of exercise training in either group (Table 4). During the euglycemic clamp, insulin infusion completely suppressed EGP in the lean nondiabetic group and nearly completely suppressed EGP in the obese nondiabetic subjects. In contrast, diabetic patients had impaired insulin suppression of EGP that was not improved by exercise training. During the clamp, insulin-stimulated glucose disposal was greatest in lean controls, intermediate in obese nondiabetic, and lowest in patients with type 2 diabetes (Table 4). ANOVA showed that 8 wk of exercise training increased glucose disposal modestly in both the overweight nondiabetic subjects and the diabetic patients ($P < 0.05$, Table 4). These two groups did not differ in their relative response to exercise training.

Exercise training improves brachial artery endothelial function

Vasodilatory function was assessed as changes in FBF using a mercury-filled silastic strain-gauge plethysmograph in response to infusion of ACh and SNP in the brachial artery of the nondominant (trained) arm. The effects of obesity and type 2 diabetes (before training) on ACh- and SNP-mediated vasodilatation are illustrated in Fig. 1, A and B, respectively. Basal FBF did not differ among the three groups of subjects.

TABLE 2. Effect of exercise training on clinical characteristics

	Overweight nondiabetic		Diabetic	
	Before training	After training	Before training	After training
BMI (kg/m^2)	29 ± 0.9	29 ± 0.9	31 ± 0.8	31 ± 0.6
Body weight (kg)	78 ± 3.3	78 ± 3.1	89 ± 4	88.5 ± 3.5
Fat mass (kg)	29 ± 2.0	29 ± 2.1	33 ± 2.0	33 ± 2.3
HbA_{1c} (%)	5.3 ± 0.1	5.3 ± 0.1	8.1 ± 0.5	8 ± 0.5
FPG (mmol/liter)	5.4 ± 0.1	5.3 ± 0.2	8.7 ± 0.6	9.4 ± 1
FPI (pmol/liter)	42 ± 11	42 ± 7	76 ± 14	76 ± 14
Total cholesterol (mmol/liter)	5.2 ± 0.6	4.5 ± 0.2[a]	5.2 ± 0.2	4.8 ± 0.2[a]
Triglycerides (mmol/liter)	2.2 ± 0.3	2 ± 0.2	4 ± 0.8	3.4 ± 0.4
HDL-cholesterol (mmol/liter)	1.2 ± 0.1	1.2 ± 0.08	1.1 ± 0.1	1.1 ± 0.1
LDL-cholesterol (mmol/liter)	3.7 ± 0.6	3 ± 0.2[a]	3.4 ± 0.3	3 ± 0.2[a]
HDL/total cholesterol (%)	23 ± 2	27 ± 2[a]	21 ± 2	23 ± 2[a]

Clinical subjects' characteristics are shown at baseline and after 8-wk exercise training. Values are expressed as mean ± SE. FPG, Fasting plasma glucose; FPI, fasting plasma insulin.

[a] $P < 0.05$ *vs.* pretraining by ANOVA analysis.

TABLE 3. Effect of aerobic exercise training on exercise capacity

	Overweight nondiabetic subjects		Diabetic patients	
	Before training	After training	After training	Before training
VO_2 peak (ml/kg FFM·min)	29 ± 1	37 ± 4[a]	33 ± 2	42 ± 3[a]
HR peak (bpm)	148 ± 5	154 ± 4	135 ± 5	140 ± 5
VO_2 (60 W) (ml/kg FFM·min)	16 ± 1.2	18 ± 0.9[a]	16 ± 0.84	18 ± 1[a]
HR (60 W) (bpm)	119 ± 3	116 ± 12	114 ± 4	110 ± 4
VO_2 (60 W)/HR (60 W) [(ml/kg FFM·min)/bpm]	0.14 ± 0.001	0.16 ± 0.007[b]	0.14 ± 0.01	0.16 ± 0.01[b]

Peak measurements are maximum values obtained during VO_2 peak test. HR peak is the mean heart rate during the last minute of exercise during the VO_2 peak test. VO_2 (60 W) represents O_2 consumption at a fixed work load of 60 W. HR (60 W) is heart rate achieved at a fixed work load of 60 W. Data are given in mean ± SE. bpm, Beats per minute; FFM, fat-free mass.

[a] $P < 0.05$ *vs.* before training.

[b] $P < 0.01$ *vs.* before training.

ACh increased FBF in a dose-responsive manner in all three groups (Fig. 1A); however, ACh-induced vasodilation was significantly decreased in both the overweight nondiabetic and type 2 diabetic patients compared with lean healthy subjects. Similarly, the hyperemia response to arterial occlusion was significantly greater in lean controls than in either overweight nondiabetic or diabetic subjects (Fig. 1B). SNP also increased FBF in all three groups and at the highest infusion rate was significantly reduced in overweight and diabetic subjects compared with lean controls ($P < 0.05$, Fig. 1C). Forearm vasodilatory responses were assessed in the overweight and diabetic subjects again after 8 wk of exercise training. Exercise training increased the forearm vasodilatory response to ACh and arterial occlusion ($P < 0.05$, Fig. 2, A and B), although the responses in both groups remained lower than the values in lean untrained control subjects. In contrast, the ability of the forearm vasculature to respond to SNP was unaltered by exercise training. In the dominant arm (not cannulated and not trained) of both groups, blood flow did not differ before and after training regardless of which substance was infused in the opposite arm (Fig. 2, A and B). Despite the improvements in both ACh- and hyperemia-induced vasodilation, there was no correlation between vasodilatory function and improvement in glucose disposal after training ($r = 0.22$; P value not significant).

Discussion

Physical activity improves insulin sensitivity and fuel metabolism, but the mechanisms responsible for these improvements are imperfectly understood. The known effects of exercise training on insulin action in skeletal muscle cannot completely explain the improvement in insulin sensitivity, so the present study was undertaken to determine whether improved vascular function accompanies exercise-induced enhancement of insulin sensitivity in insulin-resistant patients independent of type 2 diabetes. Insulin-resistant obese and type 2 diabetic subjects were studied before and after an 8-wk period of exercise training that we previously showed to improve insulin-stimulated glucose disposal in similar subjects (9). Similar to that study, in the present study, exercise training increased VO_2 peak by approximately 27% and insulin-stimulated glucose disposal by about 11%. This period of exercise training also was sufficient to improve the cholesterol profile of these subjects, decreasing total cholesterol by decreasing LDL-cholesterol, thereby increasing the proportion of total cholesterol present as HDL-cholesterol. Therefore, this period of exercise training was sufficient in intensity and duration to produce beneficial effects for the subjects.

The main purpose of the present study was to determine whether an improvement in vasodilatory function accompanies the exercise-induced improvement in insulin sensitivity in insulin-resistant individuals. Before training, compared with lean, insulin-sensitive control subjects who also had not undertaken any regular exercise, overweight and type 2 diabetic subjects had reduced vasodilation in response to both ACh and SNP. Under these conditions, it is not possible to distinguish between defects in endothelial-dependent and -independent vasodilation, because the decreased response to ACh could be at least partially a decrease in response of the vascular smooth muscle cells to nitric oxide (NO). Because SNP is a NO donor, in the experiments using

TABLE 4. Rates of glucose metabolism during euglycemic-hyperinsulinemic clamp

	Lean nondiabetic, untrained	Overweight nondiabetic		Diabetic	
		Before training	After training	Before training	After training
EGP (mg/kg FFM·min)					
Basal	3.4 ± 0.4	3.5 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2
Insulin	0	0.5 ± 0.3	0.1 ± 0.07	1.5 ± 0.3[a]	1.2 ± 0.4
Glucose disposal (mg/kg FFM·min)					
Basal	3.4 ± 0.4	3.5 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2
Insulin	7.9 ± 0.9	6.5 ± 0.5	7.2 ± 0.4[d]	3.8 ± 0.3[b,c]	4.2 ± 0.3[b,c,d]

EGP was calculated during the euglycemic-hyperinsulinemic clamp as described in *Subjects and Methods.* Data are expressed as mean ± SE. FFM, Fat-free mass.

[a] $P < 0.05$ *vs.* lean controls.

[b] $P < 0.01$ *vs.* lean controls.

[c] $P < 0.01$ *vs.* obese nondiabetic.

[d] $P < 0.05$ *vs.* before training.

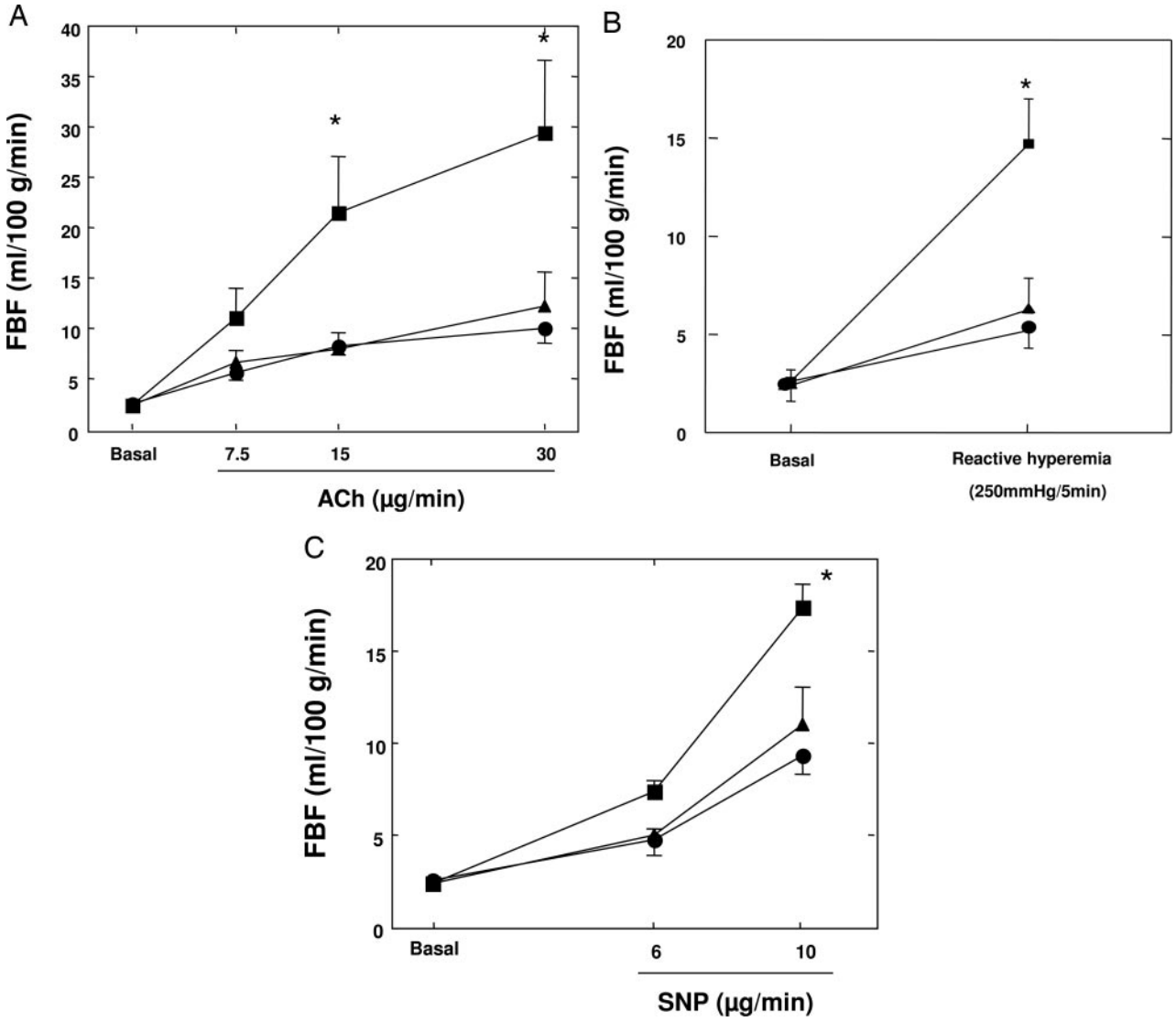

FIG. 1. FBF before training. A–C, FBF response to ACh (A), arterial occlusion (reactive hyperemia) (B), and SNP (C) in lean nondiabetic (■, n = 5), overweight nondiabetics (▲, n = 7), or type 2 diabetic patients (●, n = 13). FBF in the contralateral arm did not change during infusion of ACh or SNP (data not shown). FBF is expressed as ml/100 g tissue·min. *, $P < 0.05$ *vs.* lean controls. Data are means ± SE.

this compound, the endothelium does not contribute to the release of NO. Thus SNP is used to assay what has been termed endothelial-independent vasodilation. On the other hand, ACh triggers the release of NO via activation of endothelial NO synthase in the endothelium and is used to measure endothelial-dependent vasodilation. The hyperemic response to arterial occlusion was also assessed as a reflection of endothelial-dependent vasodilation (31). Some (16, 32), but not all (15, 26, 33), previous studies also have found such defects in vasodilation in response to a NO donor in insulin-resistant subjects. Possible explanations for these differences may include the severity of insulin resistance and vascular dysfunction as well as the presence or absence of a healthy control group for comparison. These findings point out the importance of including a truly lean, healthy control group in such studies. The presence of the defect in the response to a NO donor in both insulin-resistant groups indicates that this abnormality is not restricted to patients with diabetes but likely is a manifestation of insulin resistance itself. These results indicate that in severely insulin-resistant subjects, vascular dysfunction affects not only the endothelium but most likely smooth muscle cells as well. By comparison with the lean control group, the present results point out the magnitude of severity of vasodilatory dysfunction in profoundly insulin-resistant subjects such as those who participated in this study. It should be noted that although the lean control (32 yr) and obese subjects (37 yr) were somewhat younger than the diabetic (43 yr) patients, all groups fall within the age range showing similar vasodilatory function in a previous study (34), so it is unlikely that age differences play a major role in these findings.

After training, the vasodilatory response to ACh was improved in the obese and diabetic subjects, without a concomitant improvement in dilation in response to the NO donor SNP. Therefore, regardless of the abnormalities that are present before training, these data can be interpreted to

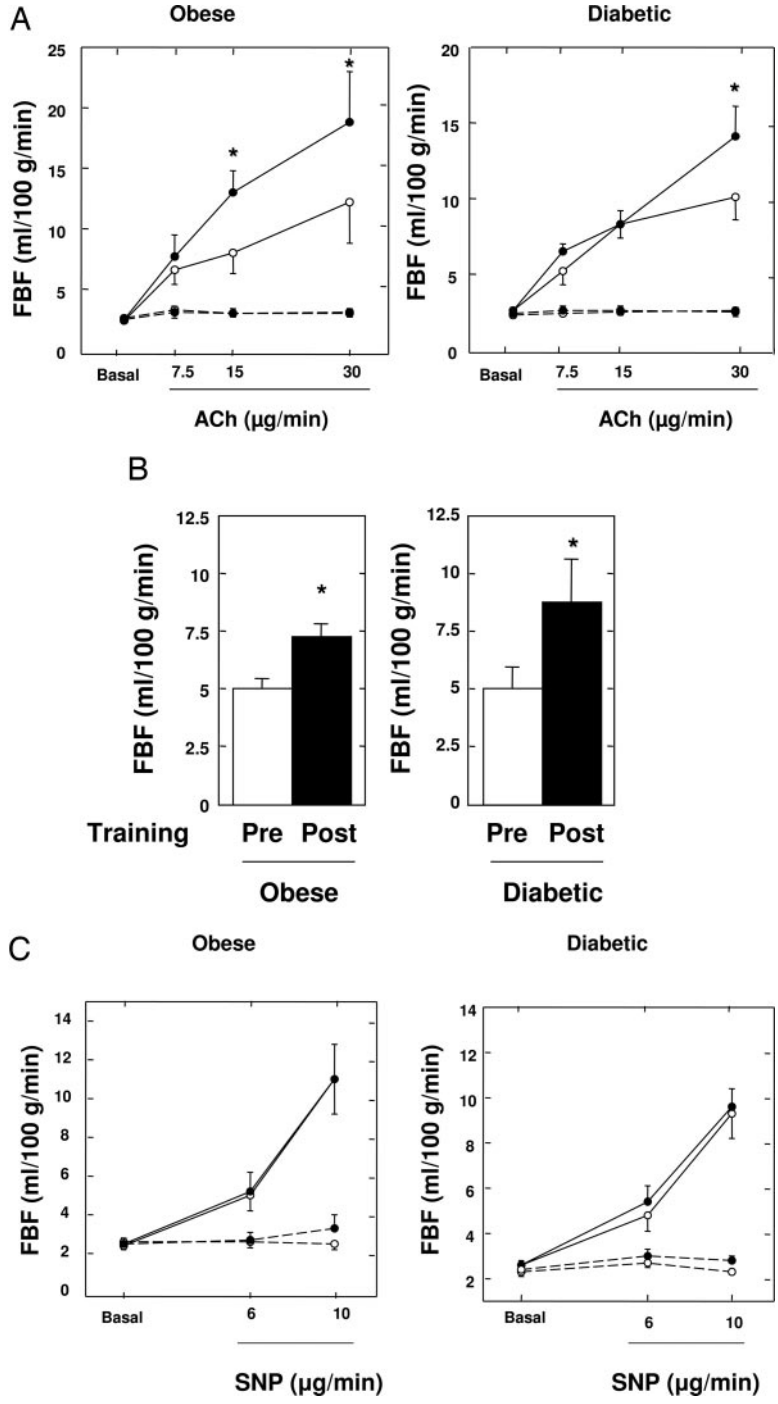

FIG. 2. Effect of exercise training on FBF. A, FBF response to ACh in overweight nondiabetics (n = 7, *left*) and type 2 diabetics (n = 13, *right*) (○, pretraining values; ●, posttraining values); B, arterial occlusion (reactive hyperemia) after 8-wk exercise in overweight nondiabetics (n = 5, *left*) and in type 2 diabetics (n = 10, *right*); C, FBF response to SNP in overweight nondiabetics (n = 7, *left*) and type 2 diabetics (n = 13, *right*) (○, pretraining values; ●, posttraining values). Blood flow in the contralateral arm not infused with ACh or SNP is shown by *dashed lines*. Data are means ± SE. *, $P < 0.05$ *vs.* pretraining.

mean that exercise improves the ability of the endothelium to muster a NO response. There was no improvement in the response of vascular smooth muscle to a given level of NO, however, as indicated by the SNP experiments. Taken together, these results suggest that there was endothelial dysfunction before exercise training in the insulin-resistant subjects, and the function of the endothelium improved after the training period. This occurred simultaneously with the improvement in insulin sensitivity, suggesting that the improvement in endothelial function is consistent with a role in improved insulin sensitivity in response to training. However, the extent of improvement in response to ACh and the increase in insulin sensitivity were not correlated. It is possible that the defect in smooth muscle may mask this relationship, but it is likely that the lack of correlation indicates the complexity of this relationship. The exercise-induced increase in endothelial-dependent vasodilation without an improvement in endothelial-independent vasodilation is consistent with previous results in patients with cardiovascular disease or type 2 diabetes (26, 35). Kingwell *et al.* (36) showed that 4 wk of exercise training positively improved endothelial function in obese, sedentary men. To the best of our

knowledge, the present study is the first to describe a simultaneous and similar improvement in endothelium function and insulin sensitivity in overweight nondiabetic, nonhypercholesterolemic, and type 2 diabetic subjects who underwent the same training program. Recently, Green *et al.* (37) published a study in which 8 wk of combined aerobic and resistance exercise was applied to overweight hypercholesterolemic subjects, patients with coronary artery disease, and patients with type 2 diabetes. After exercise training, all subjects had an enhanced response to ACh infusion in forearm resistance and conductance vessels, with no significant increase in response to SNP. Our results from the trained diabetic group also are in accord with other data (26, 35). A novel aspect of the present study is that the volunteers had no clinical evidence of cardiovascular disease, nor were they overtly dyslipidemic. Therefore, the present findings extend previous results to show that exercise training can produce improvement in endothelial function in insulin-resistant subjects before the onset of clinical manifestations of vascular disease. Unfortunately, in human subjects, it is not possible to measure NO production by the endothelium directly or even to determine whether endothelial NO synthase activity or expression increased. Nevertheless, results from cell experiments using increased shear stress (38, 39) or from studies in exercise animals (40–42) suggest that this mechanism is likely to be responsible for the exercise-training effects on endothelial function observed in the current study.

The modest (albeit statistically significant) improvement in insulin-stimulated glucose disposal compared with a larger improvement in VO_2 peak and vasodilatory function requires explanation. Performing these studies 48 h after the last training bout was done to wash out any acute exercise effects and study only the effect of training. However, it is possible that the acute effect of exercise on glucose disposal, VO_2 peak, and vasodilatory function decays at different rates.

So, although the acute effect of exercise on glucose disposal had likely vanished by 48 h (43), acute effects on the other parameters may not have disappeared completely. This may help to explain the lack of correlation between improvements in vasodilatory function and glucose disposal. Similarly, there may be a different dose-response relationship between training, insulin action, aerobic capacity, and vasodilatory function.

In summary, the results of this study show that severely insulin-resistant individuals, regardless of their state of glucose tolerance, are characterized by a decreased ability of the vascular smooth muscle cells to respond to NO and, most likely, decreased release of NO by the endothelium. Eight weeks of exercise training, which were sufficient to increase aerobic capacity (VO_2 peak) and improve insulin sensitivity and plasma lipids, also improved the ability of the endothelium to release NO. However, the severe defect in the ability of smooth muscle to respond to NO was not improved by exercise. We conclude that improved vasodilatory function accompanies increased insulin sensitivity after training. However, 8 wk of exercise does not normalize vasodilatory function or insulin sensitivity.

Acknowledgments

The expert technical assistance of Richard Castillo, Phyllis Eagan, and Sheila Taylor is gratefully acknowledged, as is the nursing assistance of Magda Ortiz and Janet Shapiro. We are grateful to Drs. Miyazaki Yoshinori, Eugenio Cersosimo, and Ralph DeFronzo for help and advice throughout.

Received May 26, 2006. Accepted September 22, 2006.

Address all correspondence and requests for reprints to: Lawrence J. Mandarino, Ph.D., Center for Metabolic Biology, Arizona State University, 350 East Orange Street, P.O. Box 873704, Tempe, Arizona 85287-3704. E-mail: Lawrence.Mandarino@asu.edu.

The studies were supported by National Institutes of Health Grants R01DK47936 and DK66483 (to L.J.M.).

Disclosure statement: The authors have nothing to disclose.

References

1. **Dohm GL** 2002 Exercise effects on muscle insulin signaling and action: invited review: regulation of skeletal muscle GLUT-4 expression by exercise. J Appl Physiol 93:782–787
2. **Christ-Roberts CY, Mandarino LJ** 2004 Glycogen synthase: key effect of exercise on insulin action. Exerc Sport Sci Rev 32:90–94
3. **Brozinick Jr JT, Etgen Jr GJ, Yaspelkis 3rd BB, Ivy JL** 1994 The effects of muscle contraction and insulin on glucose-transporter translocation in rat skeletal muscle. Biochem J 297:539–545
4. **Brozinick Jr JT, Etgen Jr GJ, Yaspelkis 3rd BB, Kang HY, Ivy JL** 1993 Effects of exercise training on muscle GLUT-4 protein content and translocation in obese Zucker rats. Am J Physiol Endocrinol Metab 265:E419–E427
5. **Frosig C, Jorgensen SB, Hardie DG, Richter EA, Wojtaszewski JFP** 2004 5′-AMP-activated protein kinase activity and protein expression are regulated by endurance training in human skeletal muscle. Am J Physiol Endocrinol Metab 286:E411–E417
6. **Chen ZP, Stephens TJ, Murthy S, Canny BJ, Hargreaves M, Witters LA, Kemp BE, McConnel GK** 2003 Effect of exercise intensity on skeletal muscle AMPK signaling in humans. Diabetes 52:2205–2212
7. **Nielsen JN, Mustard KJW, Graham DA, Yu H, MacDonald CS, Pilegaard H, Goodyear LJ, Hardie DG, Richter EA, Wojtaszewski JF** 2003 5′-AMP-activated protein kinase activity and subunit expression in exercise-trained human skeletal muscle. J Appl Physiol 94:631–641
8. **Clark SA, Chen ZP, Murphy KT, Aughey RJ, McKenna MJ, Kemp BE, Hawley JA** 2004 Intensified exercise training does not alter AMPK signaling in human skeletal muscle. Am J Physiol Endocrinol Metab 286:E737–743
9. **Christ-Roberts CY, Pratipanawatr T, Pratipanawatr W, Berria R, Belfort R, Kashyap S, Mandarino LJ** 2004 Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. Metabolism 53:1233–1242
10. **Tanner CJ, Koves TR, Cortright RL, Pories WJ, Kim YB, Kahn BB, Dohn GL, Houmard JA** 2002 Effect of short-term exercise training on insulin-stimulated PI 3-kinase activity in middle-aged men. Am J Physiol Endocrinol Metab 282:E147–E153
11. **Caballero AE** 2005 Metabolic and vascular abnormalities in subjects at risk for type 2 diabetes: the early start of a dangerous situation. Arch Med Res 36: 241–249
12. **Caballero AE** 2003 Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obes Res 11:1278–1289
13. **Haffner SM** 2003 Pre-diabetes, insulin resistance, inflammation and CVD risk. Diabetes Res Clin Pract 61:S9–S18
14. **Caballero A, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, King GL, LoGerfo FW, Horton ES, Veves A** 1999 Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. Diabetes 48:1856–1862
15. **Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD** 1996 Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 97:2601–2610
16. **Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA** 1996 Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 27:567–574
17. **Baron A** 1996 Insulin and vasculature: old actors, new roles. J Invest Med 44:406–412
18. **Davignon J, Ganz P** 2004 Role of endothelial dysfunction in atherosclerosis. Circulation 109 (Suppl 1):III27-III33
19. **Landmesser U, Homig B, Drexler H** 2004 Endothelial function: a critical determinant in atherosclerosis? Circulation 109(Suppl 1):II27–II33
20. **Green DJ, Maiorana A, O'Driscoll G, Taylor R** 2004 Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol (Lond) 561:1–25
21. **Walsh JH, Bilsborough W, Maiorana A, Best M, O'Driscoll GJ, Taylor RR,**

Green DJ 2003 Exercise training improves conduit vessel function in patients with coronary artery disease. J Appl Physiol 95:20–25
22. **Hakim AA, Curb JD, Petrovitch H, Rodriquez BL, Yano K, Ross GW, White LR, Abbott RD** 1999 Effects of walking on coronary heart disease in elderly men: the Honolulu Heart Program. Circulation 100:9–13
23. **Sesso HD, Paffenbarger Jr RS, Lee IM** 2000 Physical activity and coronary heart disease in men: the Harvard Alumni Health Study. Circulation 102:975–980
24. **Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE** 2002 Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 346:793–801
25. **Jolliffe JA, Rees K, Taylor RS, Thompson D, Oldridge N, Ebrahim S** 2001 Exercise-based rehabilitation for coronary heart disease. Cochrane Database Syst Rev 1:CD001800
26. **Maiorana A, O'Driscoll G, Cheetham C, Demdo C, Stanton K, Goodman C, Taylor R, Green D** 2001 The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. J Am Coll Cardiol 38:860–866
27. **Taylor JA, Hand G, Johnson DG, Seals DR** 1992 Augmented forearm vasoconstriction during dynamic exercise in healthy older men. Circulation 86: 1789–1799
28. **Brozek JGF, Anderson JT** 1962 Densitometric analysis of body composition: revision of some quantitative assumptions. Ann NY Acad Sci 110:113–140
29. **Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ** 2000 Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 105:311–320
30. **DeFronzo RA, Tobin JD, Reubin A** 1979 Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 6:E214–E223
31. **Higashi Y, Yoshizumi M** 2003 New methods to evaluate endothelial function: method for assesing endothelial function in humans using a strain-gauge plethysmography: nitric oxide-dependent and -independent vasodilation. J Pharmacol Sci 2003:399–404
32. **McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR** 1992 Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 35:771–776
33. **Higashi Y, Sasaki S, Nakagawa K, Kimura M, Nona K, Sasaki S, Hara K, Matsuura H, Goto C, Oshima T Chayamo K, Yoshizumi M** 2003 Low body mass index is a risk factor for impaired endothelium-dependent vasodilation in humans: role of nitric oxide and oxidative stress. J Am Coll Cardiol 42: 256–263
34. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Fasolo CB, Sudano I, Salvetti A** 1997 Hypertension causes premature aging of endothelial function in humans. Hypertension 29:736–743
35. **Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielsen S, Thiele H, Gumert JF, Mohr FW, Schuler G** 2003 Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. Circulation 107:3152–3158
36. **Kingwell BA, Sherrard B, Jennings GL, Dart AM** 1997 Four weeks of cycle training increases basal production of nitric oxide from the forearm. Am J Physiol Heart Circ Physiol 272:H1070–H1077
37. **Green DJ, Walsh JH, Maiorana A, Burke V, Taylor RR, O'Driscoll JG** 2004 Comparison of resistance and conduit vessel nitric oxide-mediated vascular function in vivo: effects of exercise training. J Appl Physiol 97:749–755
38. **Fisslthaler B, Dimmeler S, Hermann C, Busse R, Fleming I** 2000 Phosphorylation and activation of the endothelial nitric oxide synthase by fluid shear stress. Acta Physiol Scand 168:81–88
39. **Malek AM, Izumo S, Alper SL** 1999 Modulation by pathophysiological stimuli of the shear stress-induced up-regulation of endothelial nitric oxide synthase expression in endothelial cells. Neurosurgery 45:334–345
40. **Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH** 1994 Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. Circ Res 74:349–353
41. **Johnson LR, Rush JWE, Turk JR, Price EM, Laughlin MH** 2001 Short-term exercise training increases ACh-induced relaxation and eNOS protein in porcine pulmonary arteries. J Appl Physiol 90:1102–1110
42. **Laughlin MH, Pollock JS, Amann JF, Hollis ML, Woodman CR, Price EM** 2001 Training induces nonuniform increases in eNOS content along the coronary arterial tree. J Appl Physiol 90:501–510
43. **Thompson PD, Crouse S, Goodpaster B, Kelley D, Moyna N, Pescatello L** 2001 The acute versus the chronic response to exercise. Med Sci Sports Exerc 33:S438–S445

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.