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Skeletal muscle neuronal nitric oxide synthase μ protein is reduced in people with impaired glucose homeostasis and is not normalized by exercise training

Scott J. Bradley, Bronwyn A. Kingwell, Benedict J. Canny, Glenn K. McConell*

Department of Physiology, Monash University, Clayton, Victoria 3168, Australia
Alfred and Baker Medical Unit, Baker Heart Research Institute, Prahran, Victoria 3181, Australia
Department of Physiology, The University of Melbourne, Parkville, Victoria 3010, Australia

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Abstract

Skeletal muscle inducible nitric oxide synthase (NOS) protein is greatly elevated in people with type 2 diabetes mellitus, whereas endothelial NOS is at normal levels. Diabetic rat studies suggest that skeletal muscle neuronal NOS (nNOS) μ protein expression may be reduced in human insulin resistance. The aim of this study was to determine whether skeletal muscle nNOSμ protein expression is reduced in people with impaired glucose homeostasis and whether exercise training increases nNOSμ protein expression in these individuals because exercise training increases skeletal muscle nNOSμ protein in rats. Seven people with type 2 diabetes mellitus or prediabetes (impaired fasting glucose and/or impaired glucose tolerance) and 7 matched (sex, age, fitness, body mass index, blood pressure, lipid profile) healthy controls aged 36 to 60 years participated in this study. Vastus lateralis muscle biopsies for nNOSμ protein determination were obtained, aerobic fitness was measured (peak pulmonary oxygen uptake [V_{O_2} peak]), and glucose tolerance and insulin homeostasis were assessed before and after 1 and 4 weeks of cycling exercise training (60% V_{O_2} peak, 50 minutes × 5 d wk^{-1}). Skeletal muscle nNOSμ protein was significantly lower (by 32%) in subjects with type 2 diabetes mellitus or prediabetes compared with that in controls before training (17.7 ± 1.2 vs 26.2 ± 3.4 arbitrary units, $P < .05$). The V_{O_2} peak and indicators of insulin sensitivity improved with exercise training in both groups ($P < .05$), but there was no effect of exercise training on skeletal muscle nNOSμ protein in either group. In conclusion, individuals with impaired glucose homeostasis have reduced skeletal muscle nNOSμ protein content. However, because exercise training improves insulin sensitivity without influencing skeletal muscle nNOSμ protein expression, it seems that changes in skeletal muscle nNOSμ protein are not central to the control of insulin sensitivity in humans and therefore may be a consequence rather than a cause of diabetes.

1. Introduction

Exercise training is a well-recognized means of improving glucose tolerance in humans [1]. This effect is consistent with the fact that skeletal muscle is the major site of insulin-stimulated glucose disposal in the body [2]. The 2 main mechanisms controlling glucose uptake into skeletal muscle are insulin and muscle contraction. Exercise training may exert its beneficial effects on glucose tolerance via both mechanisms; however, the precise signaling pathways involved are unclear. The nitric oxide (NO)/cyclic guanosine monophosphate pathway has been implicated in muscle contraction–mediated glucose uptake in humans [3,4]. Nitric oxide also plays a role in insulin-stimulated muscle glucose uptake, although its effects are mainly hemodynamic [5-7] rather than directly on skeletal muscle glucose transport [8]. Nitric oxide is produced from L-arginine by the nitric oxide synthases (NOSs). Neuronal NOS (nNOS) μ is highly expressed in human skeletal muscle [9,10]. Endothelial NOS (eNOS) protein is expressed to a lesser extent than nNOSμ in human skeletal muscle [9] and is found predominately in the endothelium [10]. There is very little inducible NOS (iNOS) protein expression in skeletal muscle of normal, healthy humans [9,11].

* Corresponding author. Department of Physiology, The University of Melbourne, Parkville, Victoria 3010, Australia.
E-mail address: mcconell@unimelb.edu.au (G.K. McConell).

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It was recently shown that skeletal muscle iNOS protein was 4-fold higher in people with type 2 diabetes mellitus compared with healthy controls, whereas there was no difference in skeletal muscle eNOS protein levels [11]. No study has examined the level of skeletal muscle nNOSμ protein expression in people with impaired glucose tolerance or diabetes. Interestingly, mice lacking nNOSμ demonstrate peripheral (predominantly skeletal muscle) insulin resistance [12]. The purpose of the current study was to examine whether skeletal muscle nNOSμ protein expression is lower in individuals with impaired glucose homeostasis (prediabetes/type 2 diabetes mellitus) compared with healthy controls.

Intense short-term exercise training increases skeletal muscle nNOSμ protein expression in normal rats [13-15]. Although a human study found that skeletal muscle nNOSμ protein expression was not increased after 6 weeks of exercise training [16], we have found that skeletal muscle nNOSμ protein expression is significantly higher (approximately 60%) in endurance-trained compared with untrained individuals [17]. In the present study, we investigated whether an exercise training program that improves insulin sensitivity/glucose tolerance in people with impaired glucose homeostasis also increases skeletal muscle nNOSμ protein expression. We hypothesized that skeletal muscle nNOSμ protein would be lower in people with impaired glucose homeostasis than in healthy controls and that exercise training would increase skeletal muscle nNOSμ protein expression as well as insulin sensitivity/glucose tolerance.

2. Materials and methods

2.1. Participants

Fourteen volunteers provided written informed consent for the study, which was approved by the Alfred Hospital and Monash University Ethics Committees and conducted in accordance with the Declaration of Helsinki of the World Medical Association. No subject was taking medication; and all were nonsmokers with a body mass index (BMI) <35 kg m^{-2}, blood pressure ≤150/90 mm Hg, total cholesterol <6.5 mmol L^{-1}, and triglycerides <4.0 mmol L^{-1}. Fasting plasma glucose in conjunction with a 2-hour oral glucose tolerance test (OGTT; 75 g glucose) was used to ascertain whether sedentary individuals (aged 36 to 60 years) had normal glucose homeostasis (CON, n = 7), had impaired glucose tolerance (IGT)/impaired fasting glucose (IFG), or had type 2 diabetes mellitus (DIAB, n = 7; 3 with IGT/IFG and 4 with type 2 diabetes mellitus) (Table 1). *IGT* and *IFG* refer to a metabolic stage intermediate between normal glucose homeostasis and diabetes, now referred to as *prediabetes* [18]. The groups were matched for all parameters except fasting glucose and glucose tolerance, with 4 women and 3 men in each group (Table 1). Control participants had fasting plasma glucose <6.1 mmol L^{-1} and post–2-hour OGTT venous plasma glucose levels <7.8 mmol L^{-1} (prediabetes: fasting, 6.1-7.0 mmol L^{-1} and/or 2-hour OGTT, 7.8-11.0 mmol L^{-1}; type 2 diabetes mellitus: fasting, ≥7.0 mmol L^{-1} and/or 2-hour OGTT, ≥11.1 mmol L^{-1}) [18,19].

Table 1
Subject characteristics (pretraining)

	CON	DIAB	*P*
n	7	7	
Sex (M/F)	3:4	3:4	
Age (y)	49 ± 2	50 ± 4	.70
Height (m)	1.74 ± 0.03	1.69 ± 0.03	.31
Weight (kg)	86.6 ± 6.3	82.5 ± 4.6	.61
BMI (kg m^{-2})	28.3 ± 1.7	28.7 ± 1.4	.85
Systolic blood pressure (mm Hg)	126 ± 4	132 ± 4	.33
Diastolic blood pressure (mm Hg)	81 ± 2	85 ± 2	.23
Total cholesterol (mmol L^{-1})	5.5 ± 0.3	5.2 ± 0.2	.45
Triglycerides (mmol L^{-1})	1.2 ± 0.2	1.4 ± 0.3	.73
V_{O_2} peak (L min^{-1})	2.43 ± 0.25	2.03 ± 0.19	.22
V_{O_2} peak (mL kg^{-1} min^{-1})	28.2 ± 2.2	24.5 ± 1.9	.23

Pretraining subject characteristics in the CON and DIAB groups. Values are expressed as means ± SEM.

2.2. Experimental procedures

2.2.1. Study overview

After an overnight fast, participants attended the laboratory for a screening session involving measurement of fasting plasma glucose, triglycerides and cholesterol, and blood pressure, then an OGTT followed by an exercise stress test/peak pulmonary oxygen uptake (V_{O_2} peak) test. The following week, each subject presented for a vastus lateralis muscle biopsy (overnight fasted). They then undertook 4 weeks of exercise training. On days 6 and 27 of exercise training (ie, weeks 1 and 4 of training), V_{O_2} peak was reassessed. On days 7 and 28 of training (ie, weeks 1 and 4 of training), another muscle biopsy was obtained, followed by an OGTT (overnight fasted).

2.2.2. Glucose and insulin homeostasis

Each subject consumed 75 g of glucose to determine glucose tolerance (OGTT). Blood samples were drawn before the OGTT and every 30 minutes for 120 minutes after ingestion. Blood was placed on ice and centrifuged, and the plasma was frozen at −80°C. The homeostasis model assessment (HOMA) {[fasting insulin (in microunits per milliliter) × fasting glucose (in millimoles per liter)]/22.5} was used as a method to assess insulin resistance [20-24]. A high HOMA score indicates high insulin resistance and therefore low insulin sensitivity [25]. The HOMA and insulin sensitivity have been found to relate closely (correlation coefficients, −0.82 to −0.92) [20,22].

We also used the sum of the product of plasma glucose and plasma insulin concentrations at each time point during the OGTT as a further index of whole-body insulin resistance [23,26,27]. A high plasma insulin concentration in the presence of normal or increased plasma glucose concentration indicates insulin resistance (low insulin

sensitivity) [23]. Matsuda and De Fronzo [23] demonstrated that the product of the plasma glucose and insulin concentrations during an OGTT provides a better index of insulin sensitivity, as assessed by a euglycemic, hyperinsulinemic clamp, than the glucose-insulin ratio. As an indicator of insulin secretion, the insulinogenic (IG) index was calculated from the ratio of the 30-minute increment in insulin concentration to the 30-minute increment in glucose concentration after the oral glucose load [24,28].

2.2.3. V_{O_2} peak test

The initial screening stress test/V_{O_2} peak test included a 12-lead electrocardiogram. Participants performed continuous incremental cycling to volitional exhaustion on an electronically braked ergometer (Ergo-metrics 900 ergometer; Ergoline, Bitz, Germany). Expired air was analyzed for oxygen, carbon dioxide, and volume (Cosmed, Rome, Italy). All participants were sedentary at the time of recruitment as confirmed by their V_{O_2} peak values (lower 50th percentile for their age and sex [29]).

2.2.4. Muscle sampling

Skeletal muscle was obtained from the vastus lateralis using the percutaneous needle biopsy technique [30] under local anesthetic (1% lignocaine without epinephrine). The muscle was immediately frozen in liquid nitrogen and stored at −80°C for later analysis. At weeks 1 and 4 of exercise training, the skeletal muscle biopsies were obtained before commencing the OGTTs to avoid the possibility that skeletal muscle nNOSμ protein was acutely affected by ingestion of a large glucose load. The muscle biopsies were performed on alternate legs, with care taken with the third biopsy to avoid sampling too close to the original biopsy site.

2.2.5. Exercise training

Each participant was instructed to exercise train 5 days per week for 4 weeks on a magnetically braked cycle ergometer (Biogear 956; Greenmaster Industrial, Taiping City, Taiwan). Except for the initial exercise training session and the V_{O_2} peak sessions, all trainings were performed at home and monitored using heart rate recordings and weekly visits and/or phone calls. Home exercise sessions involved a 5-minute warm-up, 50 minutes at approximately 60% V_{O_2} peak (determined from the initial V_{O_2} peak test and confirmed by expired air analysis during the first training session), and then a 5-minute cooldown. The heart rate at 25 minutes of the first exercise training session was used to "prescribe" exercise for the subsequent training bouts. The subject completed all home exercise training bouts at the prescribed heart rate (equivalent to 60% V_{O_2} peak). The training, therefore, involved progressive overload because improved fitness necessitated an increase in the training workload to maintain the prescribed heart rate. Exercise time, heart rate, and average power (in watts) were recorded by the participants during all training sessions.

2.3. Analytical techniques

2.3.1. Blood analysis

Plasma insulin concentration was measured by radioimmunoassay (Linco Research, St Louis, MO), plasma glucose concentration was measured by an automated analyzer (YSI 2300 STAT glucose/L-lactate analyzer; YSI, Yellow Springs, OH), and plasma cholesterol and triglycerides were measured by an automated analyzer (Cholestech LDX System; Cholestech, Hayward, CA).

2.3.2. Muscle nNOSμ analysis

Thirty to forty milligrams of muscle was homogenized in buffer A (50 mmol L^{-1} Tris-HCl [pH 7.5], 1 mmol L^{-1} ethylene diamine-tetra-acetic acid [EDTA], 1 mmol L^{-1} ethylene-glycol-bis-(beta-amino-ethyl-ether)N,N,N′,N′-tetra-acetic acid [EGTA], 1 mmol L^{-1} dithiothreitol, 50 mmol L^{-1} sodium fluoride, 5 mmol L^{-1} sodium pyrophosphate, 10% glycerol, 1% Triton X-100, 10 μg mL^{-1} trypsin inhibitor, 2 μg mL^{-1} aprotinin, 1 mmol L^{-1} benzamidine, and 1 mmol L^{-1} phenylmethylsulfonyl fluoride). Protein concentration was determined in triplicate using a detergent compatible protein assay (DC Protein Assay; Bio-Rad Laboratories, New South Wales, Australia). Neuronal NOSμ was affinity purified using 2′,5′-ADP–Sepharose beads (Amersham Pharmacia Biotech, Uppsala, Sweden) [31]. The fractions were then subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis, and nNOSμ was detected by immunoblotting with a murine monoclonal primary antibody against human nNOS (N31020; Transduction Laboratory, Lexington, KY), a goat anti–mouse secondary antibody (1:2000) (Dako, Glostrup, Denmark), and an enhanced horseradish peroxidase-luminol chemiluminescence detection. Five microliters of recombinant human nNOS was used as the positive control standard, with the same batch of recombinant nNOS used for all the gels. The bands were quantitated (ImageQuant, GE Healthcare, New Jersey, USA) as the product of band intensity and area divided by background, standardized to the nNOS positive control so that the different gels could be compared (same batch of recombinant nNOS used for all gels). Each sample was measured in duplicate on independent gels, and the results were averaged. Skeletal muscle eNOS protein content was not examined because we [32] and others [9] have found that there is extremely low eNOS protein expression in human skeletal muscle. We also did not measure phosphorylated nNOSμ (at serine 1451) because we have previously shown that there is very little phosphorylation of this site in human skeletal muscle at rest [33-35].

2.4. Statistics

Baseline parameters were compared between groups using unpaired t tests. Measurements made across the training period were compared using analysis of variance (ANOVA) for repeated measures to determine the effects of time and treatment (group). If the ANOVA was significant, specific differences were determined using the least

significant difference test. The significance level was set at $P < .05$. All results are expressed as means ± SEM.

3. Results

3.1. Participants

There was no significant difference in age, sex balance, body mass, BMI, blood pressure, or lipid profile between the CON and DIAB groups (Table 1). Fasting plasma glucose, post-OGTT glucose and insulin (data not shown), and the HOMA insulin resistance index were all higher before training in DIAB than CON ($P < .05$, Table 2).

3.2. Exercise training

The CON group completed 19.1 ± 0.3 training sessions, whereas the DIAB group completed 19.3 ± 0.3 sessions. There was no significant difference in exercise training power output between the 2 groups. At the conclusion of the training period, both groups had increased their absolute training intensity compared with the start of the exercise training (DIAB, 20% ± 4%: from 78 ± 9 W to 93 ± 9 W; CON, 14% ± 3%: from 98 ± 12 W to 111 ± 11 W; $P < .05$). Exercise training had no significant effect on body mass in either group (end of training: CON, 85.9 ± 6.7 kg; DIAB, 82.4 ± 4.3 kg).

3.3. $\dot{V}O_2$ peak tests

There was no significant difference in $\dot{V}O_2$ peak, maximum power achieved, or exercise time to exhaustion between the 2 groups before exercise training, although all tended to be higher in CON than in DIAB (Tables 1 and 3). All these parameters increased with training in both groups, with no significant difference between groups (Table 3).

Table 2
Glucose and insulin homeostasis assessed before training (pretraining), after 1 week of training, and after 4 weeks of exercise training in the CON and DIAB groups

	Pretraining	Training	
		Wk 1	Wk 4
Fasting plasma glucose (mmol L^{-1})			
CON	5.0 ± 0.2	4.9 ± 0.1	5.0 ± 0.1
DIAB	8.1 ± 1.6 **	7.5 ± 1.5 **	6.8 ± 1.0 **
2-h OGTT plasma glucose (mmol L^{-1})			
CON	5.9 ± 0.4	5.1 ± 0.2	5.0 ± 0.2 *
DIAB	12.9 ± 2.2 **	11.6 ± 1.8 **	10.7 ± 1.6 *, **
G × I			
CON ***	2598 ± 494	2315 ± 372	1830 ± 348
DIAB ***	4202 ± 824	3360 ± 705	3082 ± 706
HOMA			
CON	2.8 ± 0.2	2.5 ± 0.2	3.0 ± 0.2
DIAB	6.8 ± 1.9 **	5.6 ± 1.5 **	4.9 ± 1.4 *, **
IG index			
CON	148 ± 46	128 ± 28	144 ± 34
DIAB	69 ± 23	70 ± 28	87 ± 29

Values are expressed as means ± SEM. G × I indicates the product of plasma glucose and insulin during the OGTT.
* $P < .05$ vs pretraining.
** $P < .05$ vs CON group.
*** $P < .05$ for time effect.

Table 3
The $\dot{V}O_2$ peak tests

	Pretraining	Training		% increase
		Wk 1	Wk 4	
$\dot{V}O_2$ peak (L min^{-1})				
CON	2.43 ± 0.25	2.49 ± 0.24	2.65 ± 0.22 *	11 ± 4
DIAB	2.03 ± 0.19	2.19 ± 0.19	2.43 ± 0.18 *	22 ± 6
$\dot{V}O_2$ peak (mL kg^{-1} min^{-1})				
CON	28.2 ± 2.2	29.0 ± 2.1	31.2 ± 2.2 *	12 ± 5
DIAB	24.5 ± 1.9	26.4 ± 1.8	29.5 ± 1.5 *	23 ± 6
Exercise time (s)				
CON	619 ± 45	648 ± 43	705 ± 47 *	14 ± 3
DIAB	513 ± 28	574 ± 23	626 ± 25 *	23 ± 7
Maximum power (W)				
CON	184 ± 22	192 ± 22	208 ± 24 *	14 ± 3
DIAB	151 ± 14	167 ± 12	182 ± 13 *	24 ± 7

Values are expressed as means ± SEM. $\dot{V}O_2$ peak (absolute and relative), exercise time to volitional exhaustion during $\dot{V}O_2$ peak test, and maximum power during $\dot{V}O_2$ peak test before training (pretraining), after 1 week of training, and after 4 weeks of training in the CON and DIAB groups. % increase (percentage increase) indicates the 4-week value compared with the pretraining value.
* $P < .05$ vs pretraining.

3.4. Glucose and insulin homeostasis

Fasting plasma glucose was unaffected by exercise training in CON but tended to be lower after training in DIAB (Table 2). In both groups, plasma glucose at 2 hours of the OGTT was lower ($P < .05$) after exercise training (Table 2; one subject with type 2 diabetes mellitus had improved to IGT and one subject with IGT had improved to "normal"). Fasting plasma insulin concentration was unaffected by training in CON (pretraining, 75 ± 5; 1 week, 69 ± 6; 4 weeks, 81 ± 6 pmol L^{-1}) but significantly reduced in DIAB (pretraining, 109 ± 23; 1 week, 98 ± 20; 4 weeks, 96 ± 24 pmol L^{-1}; $P < .05$; ANOVA). In both groups, the plasma insulin concentration 2 hours after the OGTT was approximately 20% lower after training; but this reduction was not statistically significant (CON: pretraining, 257 ± 37; 4 weeks, 196 ± 39 pmol L^{-1}; DIAB: pretraining, 508 ± 115; 4 weeks, 423 ± 87 pmol L^{-1}). Fasting plasma glucose, 2-hour post-OGTT plasma glucose, 2-hour OGTT plasma insulin, and calculated insulin resistance (HOMA) were higher in DIAB compared with CON at each time point ($P < .05$). Although the product of glucose and insulin during the OGTT tended to be higher in DIAB than CON at each time point, this was not significant ($P = .14$, ANOVA) (Table 2). The product of glucose and insulin decreased with exercise training in both groups, suggesting improved insulin sensitivity (Table 2). Exercise training also decreased HOMA in DIAB ($P < .05$) but had little effect in CON (Table 2). There was no significant effect of exercise training on calculated insulin secretion in response to the glucose load in either group as assessed by the IG index (at 30 minutes) (Table 2).

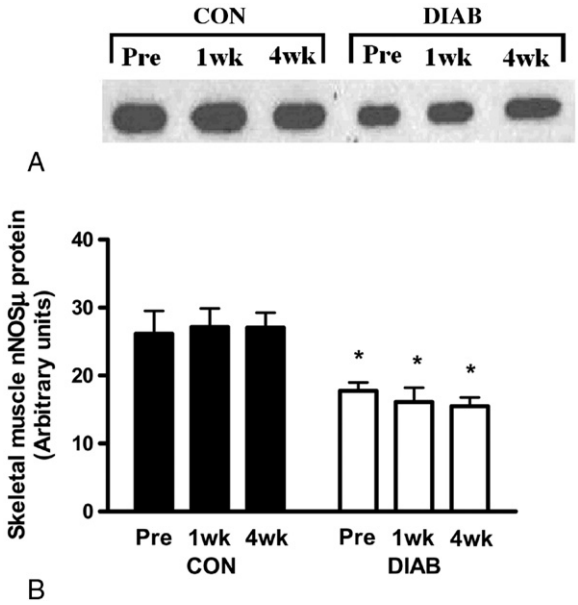

Fig. 1. A, Representative nNOSμ Western blot in vastus lateralis muscle before training, after 1 week of training, and after 4 weeks of training in one CON and one DIAB subject. B, Neuronal NOSμ protein expression in vastus lateralis muscle before training, after 1 week of training, and after 4 weeks of training in the CON and DIAB groups. The bands were quantitated as the product of band intensity and area divided by background, standardized to the nNOS positive control so that the different gels could be compared. Each sample was measured in duplicate on separate gels, and the results were averaged. Values are mean ± SEM; n = 7 per group, except at 4 weeks in CON, n = 6. *$P < .05$ vs CON group.

3.5. Skeletal muscle nNOSμ protein

A satisfactory muscle sample was not obtained for one control subject at 4 weeks of training. Based on our previous findings [36] and those of others [37], all skeletal muscle nNOS detected in this study was considered to be the splice variant nNOSμ. Indeed, nNOSμ migration on sodium dodecyl sulphate polyacrylamide gel electrophoresis occurred as a single band. Before training, nNOSμ protein was 32% lower in DIAB compared with CON (17.7 ± 1.2 vs 26.2 ± 3.4 arbitrary units, $P < .05$) (Fig. 1B). Within DIAB, there was no significant difference ($P > .05$) in nNOSμ protein between the 4 individuals with type 2 diabetes mellitus (18.4 ± 2.1 arbitrary units) and the 3 individuals with prediabetes (17.3 ± 1.7 arbitrary units). There was no significant effect ($P > .05$) of exercise training on skeletal muscle nNOSμ protein expression in either group (Fig. 1B). Average nNOSμ protein expression across all time points (pretraining, 1 week of training, and 4 weeks of training) was 39% lower in DIAB compared with CON (16.4 ± 0.9 vs 26.8 ± 1.4 arbitrary units, $P < .05$) (Fig. 1B).

4. Discussion

This study demonstrated that patients with prediabetes/type 2 diabetes mellitus have 35% to 40% lower skeletal muscle nNOSμ protein expression than matched, healthy control individuals. Four weeks of exercise training improved glucose tolerance and indicators of insulin sensitivity but did not affect skeletal muscle nNOSμ protein expression in either the DIAB group or in the matched healthy control group. It has recently been shown that skeletal muscle iNOS protein content is elevated in people with type 2 diabetes mellitus, whereas there is no difference in skeletal muscle eNOS protein levels compared with control participants. Because exercise training improves insulin sensitivity without influencing skeletal muscle nNOSμ protein expression, it seems that changes in skeletal muscle nNOSμ protein are not central to the control of insulin sensitivity in humans and therefore may be a consequence rather than a cause of diabetes. In addition, increases in skeletal muscle nNOSμ protein were not a requirement for exercise training–induced improvements in glucose tolerance and insulin sensitivity.

The current study found that exercise training reduced insulin resistance in the DIAB patients (therefore improved insulin sensitivity) as assessed by the HOMA and the product of glucose and insulin during the OGTT. Although the euglycemic, hyperinsulinemic clamp procedure is the criterion standard for determination of insulin sensitivity, both HOMA [23,25] and the product of glucose and insulin during OGTT [23,26,27] have been shown to correlate with insulin resistance. In addition, the fact that the same participants were measured on 3 occasions with each subject acting as their own control means that concerns about insulin-independent glucose kinetics such as gastric emptying and absorption differences between participants are unlikely to be relevant in this study. Importantly, we found that there was no increase in insulin secretion with exercise training as calculated by the IG index [24,28]. Therefore, it is unlikely that the calculated improved glucose tolerance and reduced insulin resistance after training were due to increases in insulin secretion.

It is possible that the lower skeletal muscle nNOSμ protein expression contributes to impaired glucose homeostasis in individuals with type 2 diabetes mellitus. Indeed, nNOSμ knockout mice demonstrate peripheral (predominantly skeletal muscle) insulin resistance [12]. However, because skeletal muscle iNOS protein has recently been shown to be elevated in people with type 2 diabetes mellitus [11], it would seem more likely that the increased NO production from iNOS [11], which produces NO at a very high level compared with nNOS, results in a compensatory down-regulation of skeletal muscle nNOS. Indeed, administration of lipopolysaccharide increases skeletal muscle iNOS expression and decreases nNOS protein expression in guinea pigs [38]. It seems that the inflammatory process of type 2 diabetes mellitus is responsible for the increase in skeletal muscle iNOS protein levels [11]. In rats, inflammatory cytokines increase skeletal muscle iNOS protein expression; and iNOS expression is associated with insulin resistance [39]. In addition, high-fat diet–induced insulin

resistance is prevented in iNOS knockout mice, indicating that skeletal muscle iNOS plays a fundamental role in the etiology of diabetes [40]. Future studies should examine whether exercise training decreases and tends to normalize the elevated skeletal muscle iNOS expression in type 2 diabetes mellitus.

In rats, several studies have found that short-term exercise training increases skeletal muscle nNOSμ protein levels. Balon and Nadler [13] reported that 8 weeks of treadmill running in rats resulted in a 4-fold increase in soleus muscle nNOSμ protein expression [13]. Furthermore, 4 weeks of treadmill running increased skeletal muscle nNOS protein and NOS activity in rat gastrocnemius muscle [15]. By contrast, in healthy, young humans, Frandsen et al [16] found no effect of 6 weeks of exercise training on skeletal muscle nNOSμ protein expression or NOS activity. Our study is the first to examine the effect of exercise training on skeletal muscle nNOSμ protein levels in patients with impaired glucose homeostasis. These 2 human studies therefore indicate that, unlike rodents, short-term exercise training (4-6 weeks) has no effect on skeletal muscle nNOSμ protein levels in human skeletal muscle.

There are at least 3 possible explanations for the discrepancy between the rat and human studies examining the effect of short-term exercise training on skeletal muscle nNOSμ protein expression. Firstly, species differences in the response of skeletal muscle nNOSμ to exercise training may mean that skeletal muscle nNOSμ is up-regulated by short-term exercise training in rats but not in humans. Secondly, because nNOSμ protein expression is lower in type 1 muscle fibers than in type 2 muscle fibers in the rat [41], while in humans nNOSμ protein expression is higher in type 1 than in type 2 muscle fibers [10], it is probable that endurance exercise training, which predominately targets type 1 muscle fibers, exerts more of an effect on rat muscle than human muscle nNOSμ protein. Thirdly, the exercise stimulus used may have been insufficient to increase skeletal muscle nNOSμ in the human studies. Each exercise session was very prolonged and/or intense in the rat exercise training studies that have found an increase in skeletal muscle nNOSμ protein expression [13-15], so it is possible that the milder exercise stimulus in the present study and in the study by Frandsen et al [16] was insufficient to bring about an increase in skeletal muscle nNOSμ protein expression. Indeed, we have found that well-trained endurance athletes have nearly double the skeletal muscle nNOSμ protein expression than untrained participants and also 10 days of very intense, prolonged exercise training increases skeletal muscle nNOSμ protein expression in previously sedentary humans [17].

In conclusion, we found that skeletal muscle nNOSμ protein expression was substantially lower in individuals with impaired glucose homoeostasis compared with well-matched healthy controls. Four weeks of exercise training improved glucose tolerance and reduced calculated insulin resistance but had no effect on skeletal muscle nNOSμ protein expression in people with or without prediabetes/type 2 diabetes mellitus. Taken with recent findings by others of elevated skeletal muscle iNOS protein in people with diabetes, these results suggest that changes in skeletal muscle nNOSμ protein are not central to the control of insulin sensitivity in humans and therefore may be a consequence rather than a cause of diabetes. In addition, exercise training does not improve glucose homeostasis by increasing skeletal muscle nNOSμ protein expression.

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