

Reduced Skeletal Muscle Inhibitor of κ B β Content Is Associated With Insulin Resistance in Subjects With Type 2 Diabetes

Reversal by Exercise Training

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Skeletal muscle insulin resistance plays a key role in the pathogenesis of type 2 diabetes. It recently has been hypothesized that excessive activity of the inhibitor of κ B (I κ B)/nuclear factor κ B (NF κ B) inflammatory pathway is a mechanism underlying skeletal muscle insulin resistance. However, it is not known whether I κ B/NF κ B signaling in muscle from subjects with type 2 diabetes is abnormal. We studied I κ B/NF κ B signaling in vastus lateralis muscle from six subjects with type 2 diabetes and eight matched control subjects. Muscle from type 2 diabetic subjects was characterized by a 60% decrease in I κ B β protein abundance, an indicator of increased activation of the I κ B/NF κ B pathway. I κ B β abundance directly correlated with insulin-mediated glucose disposal (R_d) during a hyperinsulinemic (40 mU \cdot m⁻² \cdot min⁻¹)-euglycemic clamp ($r = 0.63$, $P = 0.01$), indicating that increased I κ B/NF κ B pathway activity is associated with muscle insulin resistance. We also investigated whether reversal of this abnormality could be a mechanism by which training improves insulin sensitivity. In control subjects, 8 weeks of aerobic exercise training caused a 50% increase in both I κ B α and I κ B β protein. In subjects with type 2 diabetes, training increased I κ B α and I κ B β protein to levels comparable with that of control subjects, and these increments were accompanied by a 40% decrease in tumor necrosis factor α muscle content and a 37% increase in insulin-stimulated glucose disposal. In summary, subjects with type 2 diabetes have reduced I κ B protein abundance in muscle, suggesting excessive activity of the I κ B/NF κ B pathway. Moreover, this abnormality is reversed by exercise training. *Diabetes* 55:760–767, 2006

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AMPK, AMP-activated protein kinase; FFA, free fatty acid; GCRC, General Clinical Research Center; I κ B, inhibitor of κ B; IKK, I κ B kinase; IL, interleukin; NF κ B, nuclear factor κ B; TNF α , tumor necrosis factor α .

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Evidence has accumulated in recent years indicating that type 2 diabetes and other insulin-resistant disorders are characterized by chronic inflammation (1,2). Specifically, it has been postulated that excessive activity of the inhibitor κ B (I κ B)/nuclear factor κ B (NF κ B) inflammatory pathway may be an important molecular mechanism responsible for skeletal muscle insulin resistance (1,3–7). NF κ B is a family of transcription factors that regulate the expression of proinflammatory genes. In unstimulated cells, NF κ B is predominantly localized in the cytoplasm, associated with an inhibitory protein, I κ B. Several stimuli, including cytokines (1), reactive oxygen species, hyperglycemia, and free fatty acids (FFAs) (5), activate I κ B kinase (IKK), the upstream kinase of I κ B. Upon activation by inflammatory factors, IKK phosphorylates I κ B, causing rapid I κ B polyubiquitination and degradation by proteosomes. Following release from I κ B, NF κ B translocates from the cytoplasm to the nucleus, where it binds to target genes to stimulate transcription of inflammatory mediators such as tumor necrosis factor α (TNF α), interleukin (IL)-1 β , and IL-6 (1,8,9).

I κ Bs are members of a gene family that contain seven mammalian members, including I κ B α , I κ B β , I κ B ϵ , I κ B γ , Bcl-3, and the precursor Rel proteins p100 and p105. I κ B α and I κ B β share common properties to interact with NF κ B and inhibit DNA binding. NF κ B proteins consist of five members, including p65, p50, p52, RelB, and c-Rel. Dimerization of two NF κ B family members is necessary for their DNA-binding properties. The predominant activating NF κ B dimer in skeletal muscle is the p50-p65 heterodimer (10). NF κ B p65 contains a COOH-terminal transcriptional domain that is crucial for its ability to activate inflammatory gene expression (9).

Interventions aimed at blocking the I κ B/NF κ B pathway, such as genetic deletion of IKK β in mice (4) and the administration of salicylates, which inhibit IKK β , to subjects with type 2 diabetes (11) significantly improve peripheral insulin sensitivity. These findings strongly suggest that activation of the I κ B/NF κ B pathway plays an important role in the pathogenesis of insulin resistance. However, it is not known whether the I κ B/NF κ B pathway is excessively active in skeletal muscle from subjects with type 2 diabetes. The goal of the present study was to

TABLE 1
Clinical and laboratory characteristics, glucose metabolism, and exercise capacity

	Control		Type 2 diabetes	
	Pretraining	Posttraining	Pretraining	Posttraining
<i>n</i>		8		6
Sex (male/female)		2/6		4/2
Age (years)		36 ± 3		45 ± 3
Body weight (kg)	77 ± 7	77 ± 7	79 ± 5	80 ± 5
BMI (kg/m ²)	27 ± 1	27 ± 1	27 ± 2	27 ± 2
Fasting plasma glucose (mmol/l)	4.9 ± 0.1	4.9 ± 0.1	7.5 ± 0.9*	8.2 ± 1.0*
A1C (%)	4.9 ± 0.1	4.7 ± 0.2	8.1 ± 1.1*	6.6 ± 0.6*
Fasting plasma insulin (pmol/l)	23.8 ± 9.3	24.9 ± 10.6	60.1 ± 24.5*	44.0 ± 17.9*
Basal endogenous glucose production (mg · kg FFM ⁻¹ · min ⁻¹)	3.0 ± 0.2	2.7 ± 0.2	2.8 ± 0.2	2.6 ± 0.4
<i>R</i> _d (mg · kg FFM ⁻¹ · min ⁻¹)	7.8 ± 0.7	8.5 ± 1.0	4.0 ± 0.6*	5.5 ± 0.6*†
<i>V</i> _{O_{2peak}} (mg · kg FFM ⁻¹ · min ⁻¹)	36 ± 2	42 ± 2†	26 ± 3*	34 ± 3*†

Data are means ± SE, unless otherwise indicated. **P* < 0.05 vs. control subjects within each condition. †*P* < 0.05 vs. pretraining. FFM, fat free mass.

evaluate whether the activity of the IκB/NFκB pathway is increased in skeletal muscle from subjects with type 2 diabetes. We also examined whether an intervention known to improve insulin sensitivity, such as exercise training, could reverse abnormalities in the IκB/NFκB inflammatory pathway in skeletal muscle from subjects with type 2 diabetes. We hypothesized that inhibition of IκB/NFκB signaling in muscle is a mechanism by which training improves insulin sensitivity.

RESEARCH DESIGN AND METHODS

Six subjects with type 2 diabetes and eight healthy control subjects participated in the study. The metabolic data from these subjects have been previously reported (12). Each subject underwent a complete history, physical examination, screening laboratory tests, and a 75-g oral glucose tolerance test to determine the presence or absence of diabetes using established American Diabetes Association criteria. Three of six type 2 diabetic subjects were taking glyburide, which was withdrawn 3 days before the oral glucose tolerance test and insulin clamp studies. The remaining three type 2 diabetic subjects were treated with diet. The control subjects did not have a family history of type 2 diabetes and had normal glucose tolerance. Other than glyburide, no subject was taking any medication known to affect glucose metabolism. The study was approved by the institutional review board of the University of Texas Health Science Center at San Antonio, and all subjects gave written consent.

Following a 10- to 12-h overnight fast, subjects came to the General Clinical Research Center (GCRC) at the South Texas Veterans Health Care Hospital for a screening visit and glucose tolerance test. Within 3–7 days, subjects returned to the GCRC for determination of their peak aerobic capacity (*V*_{O_{2peak}}) (12). Within 3–7 days after the baseline *V*_{O_{2peak}} measurement, subjects returned to the GCRC at 8 A.M. after an overnight fast for a vastus lateralis muscle biopsy (13), followed by a 120-min euglycemic-hyperinsulinemic (40 mU · m⁻² · min⁻¹) insulin clamp study, which was performed with infusion of 3-³H-glucose (prime = 25 μCi, continuous infusion = 0.25 μCi/min) to determine rates of glucose appearance and disposal, as previously described (12). In control subjects, the 3-³H-glucose glucose was given as a prime (25 μCi)-continuous (0.25 μCi/min) infusion starting 120 min before insulin. In type 2 diabetic subjects, the prime was increased (25 μCi × fasting plasma glucose/100) and began 180 min before insulin. In diabetic subjects, following the start of insulin, the plasma glucose concentration was allowed to decrease to 100 mg/dl, at which level it was maintained. One week after the insulin clamp study, the subjects began an 8-week aerobic exercise training program on a stationary bicycle. The exercise intensity, duration, and frequency were progressively increased to 70% of *V*_{O_{2peak}} for 45 min four times per week, during the 8-week course of the exercise program. Once the training program was completed, the subjects returned to the GCRC for a repeat determination of *V*_{O_{2peak}}. A posttraining muscle biopsy and insulin clamp study was performed 24–36 h after the last exercise bout.

Laboratory analyses. Plasma insulin concentrations were measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA), glucose was measured using the oxidase method and a Beckman analyzer, and HbA_{1c} (A1C) was measured using a DCA 2000 analyzer (Bayer, Tarrytown, NY). FFA concentrations were determined by an enzymatic colorimetric method (Wako Chemicals,

Nuess, Germany), and plasma IL-6 and TNFα concentrations were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). **Muscle processing.** Muscle samples were homogenized as previously described (12). Briefly, muscle samples were weighted while still frozen and were homogenized in ice-cold lysis buffer (1:10, wt/vol) containing 50 mmol/l HEPES (pH 7.6), 150 mmol/l NaCl, 20 mmol/l sodium pyrophosphate, 20 mmol/l β-glycerophosphate, 10 mmol/l sodium fluoride, 2 mmol/l sodium orthovanadate (Na₃VO₄), 2 mmol/l EDTA (pH 8.0), 1% Nonidet P-40, 10% glycerol, 1 mmol/l phenylmethylsulfonylfluoride, 1 mmol/l MgCl₂, 1 mmol/l CaCl₂, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. Homogenates were incubated on ice for 20 min and then centrifuged at 15,000g for 20 min at 4°C. The supernatants were collected, and protein concentrations were measured by the Lowry method. Supernatants were stored at -80°C until used.

Western blotting. Muscle proteins (40 μg) were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes, except for TNFα, for which 150 μg protein were used. After blocking in Tris-buffered saline with 5% nonfat dry milk, the membranes were incubated overnight at 4°C with antibodies against IκBα (Cell Signaling, Beverly, MA), IκBβ (Santa Cruz Biotechnology, Santa Cruz, CA), NFκB p50 (Cell Signaling), NFκB p65 (Santa Cruz Biotechnology), IKKβ (Cell Signaling), phospho-IKKβ (Ser^{177/181}) (Cell Signaling), and TNFα (Santa Cruz Biotechnology) (14,15). For all these proteins, a 0.45-μm nitrocellulose membrane was used, except for TNFα, for which a 0.2-μm membrane was used. Bound antibodies were detected with anti-rabbit immunoglobulin horseradish peroxidase-linked whole antibody and by using enhanced chemiluminescent reagents (PerkinElmer Life Science, Boston, MA). The membranes were exposed to film, and band intensity was quantified using Image Tool Software (University of Texas Health Science Center at San Antonio, San Antonio, TX).

Calculations. During the postabsorptive state, steady-state conditions prevail and the rate of endogenous glucose appearance, which equals the rate of total body glucose uptake, is calculated as the 3-³H-glucose infusion rate (disintegrations per minute) divided by the steady-state plasma 3-³H-glucose specific activity (disintegrations per minute per milligram). During the insulin clamp, non-steady-state conditions prevail, and the rate of glucose production (*R*_a) is calculated using Steele's equation (16). The rate of endogenous glucose production during the insulin clamp equals the tracer-derived *R*_a minus the exogenous rate of glucose infusion. The rate of whole-body glucose disposal (*R*_d) equals the rate of residual endogenous glucose production plus the exogenous glucose infusion rate adjusting to maintain euglycemia (90- to 120-min time period).

Statistical analysis. All data are expressed as means ± SE. Differences between the control and diabetic groups were determined using the unpaired Student's *t* test. Differences within a group, pre- versus posttraining, were determined using the paired *t* test. Correlation analysis was performed by the Pearson product-moment method. For all analyses, a *P* < 0.05 was considered to be statistically significant.

RESULTS

Table 1 summarizes the subject's clinical and laboratory characteristics. There was no significant difference in age, body weight, and BMI between the type 2 diabetic and control subjects. Subjects with type 2 diabetes had higher

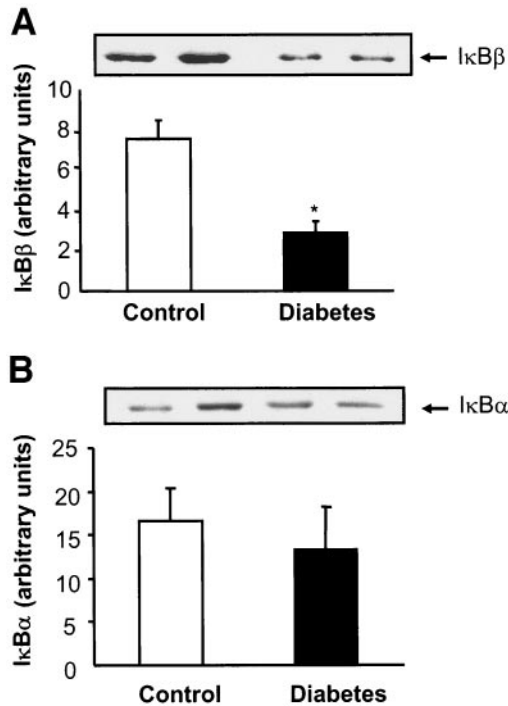


FIG. 1. I κ B protein content in skeletal muscle. I κ B β (A) and I κ B α (B) content (resting state) were measured as described in RESEARCH DESIGN AND METHODS. Graphical data are means \pm SE in eight control and six type 2 diabetic subjects. * $P < 0.05$. Representative blots also are shown for two subjects in each group.

fasting plasma glucose and insulin concentrations ($P < 0.05$), higher A1C ($P < 0.05$), and lower aerobic capacity (VO_{2peak}) ($P < 0.05$). There were no differences in FFA (690 ± 100 vs. 660 ± 100 μ mol/l), IL-6 (2.3 ± 0.5 vs. 2.3 ± 0.6 pg/ml), and TNF α (1.0 ± 0.1 vs. 1.1 ± 0.2 pg/ml) plasma concentrations between the control and type 2 diabetic groups.

Exercise training. Eight weeks of exercise training had no effect on body weight, BMI, or fasting plasma glucose and insulin concentrations in either group (Table 1). Plasma FFA, IL-6, and TNF α concentrations did not change with training. However, physical training significantly increased the VO_{2peak} by 17% ($P < 0.05$) and 31% ($P < 0.05$) in the control and diabetic subjects, respectively. In the diabetic subjects, the increase in VO_{2peak} after training also was accompanied by a tendency for a 19% decrease in A1C ($P = 0.05$).

Euglycemic-hyperinsulinemic clamp studies and insulin sensitivity. Basal endogenous glucose production was similar between groups, whereas insulin-mediated glucose disposal (R_d) was 95% higher in the control group ($P < 0.05$) (Table 1). Exercise training significantly increased R_d in the type 2 diabetic subjects by 37% ($P < 0.05$). In the control subjects, R_d tended to increase after training ($P = 0.1$). The smaller effect of training in control subjects is in part explained by a higher baseline R_d , resulting in a modest effect of training. Differences in sex distribution between groups did not explain the lesser effect of training on R_d observed in the control subjects (not shown).

I κ B/NF κ B signaling. To determine whether skeletal muscle from subjects with type 2 diabetes has excessive activity of I κ B/NF κ B pathway, we measured I κ B α and I κ B β protein abundance. Because I κ B sequesters NF κ B in

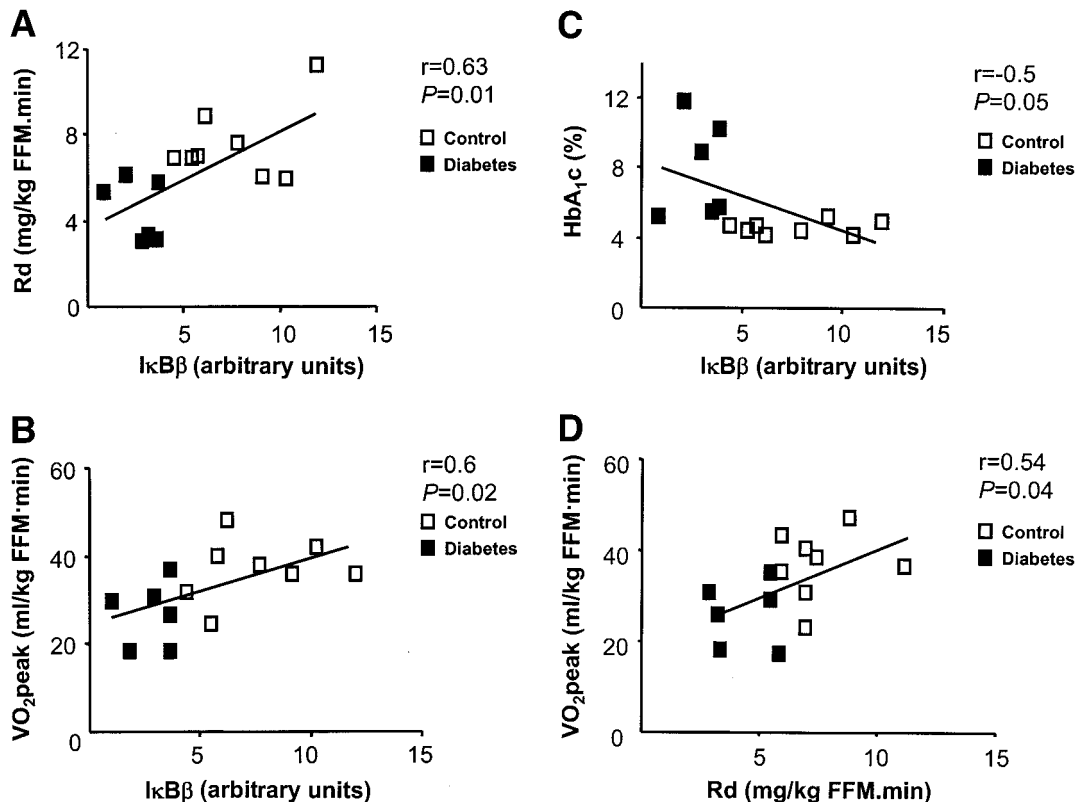


FIG. 2. Correlation between I κ B β content and insulin-mediated glucose disposal (R_d) (A), VO_{2peak} (B), and A1C (C) and between R_d and VO_{2peak} (D).

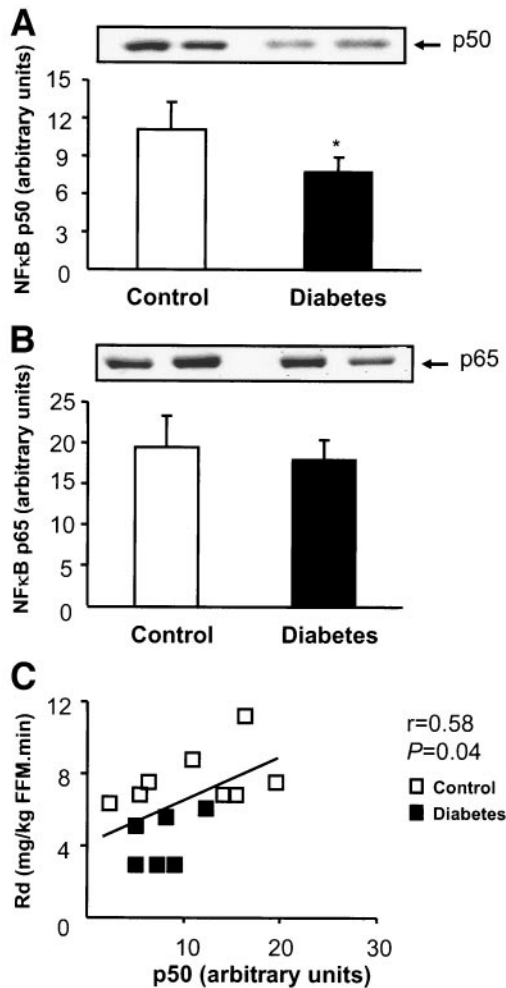


FIG. 3. NFκB protein content in skeletal muscle. NFκB p50 (A) and NFκB p65 (B) content (resting state) were measured as described in RESEARCH DESIGN AND METHODS. Graphical data are means \pm SE in eight control and six type 2 diabetic subjects. * $P < 0.05$. Representative blots also are shown for two subjects in each group. Figure 2C shows the correlation between NFκB p50 and insulin-mediated glucose disposal (R_d).

the cytoplasm and increases in IκB abundance inversely correlate with NFκB DNA binding in human skeletal muscle (17), a reduction in IκB abundance is considered to indicate activation of the IκB/NFκB pathway. In skeletal muscle from type 2 diabetic subjects, IκBβ was reduced by 60% compared with control subjects ($P < 0.05$) (Fig. 1A). IκBα was not significantly different in type 2 diabetic subjects (Fig. 1B). This reduction in IκB suggests that there is excessive activity of the IκB/NFκB axis in skeletal muscle from type 2 diabetic subjects. Importantly, there was a positive correlation between IκBβ and the rate of insulin-mediated glucose disposal ($r = 0.63$, $P = 0.01$) (Fig. 2A) and with the Vo_{2peak} ($r = 0.6$, $P = 0.02$) (Fig. 2B). Consistent with these findings, there was a negative correlation between IκBβ and the A1C concentration ($r = -0.5$, $P = 0.05$) (Fig. 2C). There was also a positive correlation between insulin-mediated glucose disposal and the Vo_{2peak} ($r = 0.54$, $P = 0.04$) (Fig. 2D). The predominant activating NFκB dimer in muscle is p50-p65 (9,10). We measured NFκB p50 and p65 protein in skeletal muscle and found that NFκB p50 protein was decreased by 30% in type 2 diabetic versus control subjects ($P < 0.05$) (Fig. 3A). There were no differences in p65 protein between groups

(Fig. 3B). In addition, there was a positive correlation between NFκB p50 protein content and insulin-mediated glucose disposal ($r = 0.58$, $P = 0.04$) (Fig. 3C).

Effect of training on the IκB/NFκB pathway. Because the IκB/NFκB pathway has been implicated in the cellular mechanisms responsible for skeletal muscle insulin resistance, we hypothesized that exercise training in patients with type 2 diabetes would reverse abnormalities in IκB/NFκB signaling and that this is a mechanism by which training improves insulin sensitivity. In control subjects, training caused a 50% increase in both IκBα ($P < 0.05$) and IκBβ ($P < 0.05$) protein content (Fig. 4A and B). After training, NFκB p50 protein tended to increase by 100% ($P = 0.05$) (Fig. 4C) in the control subjects but had no effect on NFκB p65 (Fig. 4D). Moreover, in the muscle from type 2 diabetic subjects, physical training increased IκBα and IκBβ protein by 98% ($P < 0.05$) and 185% ($P < 0.05$), respectively (Fig. 5A and B). Exercise training also increased NFκB p50 in subjects with type 2 diabetes by 140% ($P < 0.05$) (Fig. 5C), but similar to the control subjects, exercise had no effect on NFκB p65 (Fig. 5D). Furthermore, these increments in IκB and NFκB p50 protein were accompanied by a 37% increase in insulin-mediated glucose disposal (Table 1). These findings indicate that training can restore abnormalities in IκB and NFκB p50 content in muscle from subjects with type 2 diabetes.

IKKβ phosphorylation. We measured IKKβ phosphorylation to further examine the mechanism responsible for the reduction in IκB in the type 2 diabetic group. There was no difference in IKKβ phosphorylation between the groups, and training had no effect on IKKβ phosphorylation (Fig. 6) or protein content (not shown).

TNFα muscle content. It has been shown that type 2 diabetes is associated with higher muscle expression of TNFα, an NFκB-regulated gene (18). Accordingly, in the type 2 diabetic subjects the reduction in IκBβ was accompanied by a tendency for higher basal TNFα protein content by 25% compared with the control subjects ($P = NS$) (Fig. 7), although we could only measure TNFα muscle content in five control and four diabetic subjects because a large amount of muscle protein is required to measure TNFα muscle content by Western blotting and muscle tissue was no longer available for mRNA expression analysis. It is possible that this trend would have reached statistical significance with a higher number of samples. Consistent with previous reports (19), the increases in IκB and NFκB p50 caused by training were associated with a reduction in TNFα muscle content in both groups ($P < 0.05$) (Fig. 7).

DISCUSSION

In this study, we found that type 2 diabetic subjects have decreased IκBβ muscle content, suggesting enhanced IκB/NFκB signaling. Importantly, there was a positive correlation between IκBβ protein and insulin-mediated glucose disposal in type 2 diabetic and control subjects. These findings support the hypothesis that enhanced activation of the IκB/NFκB pathway is associated with insulin resistance in human skeletal muscle.

The mechanism responsible for the apparent increase in IκB/NFκB signaling in muscle from the type 2 diabetic subjects is not clear. One hypothesis involves lipid-induced activation of IκB/NFκB signaling. Intramyocellular lipid metabolites, particularly long-chain fatty acyl CoAs,

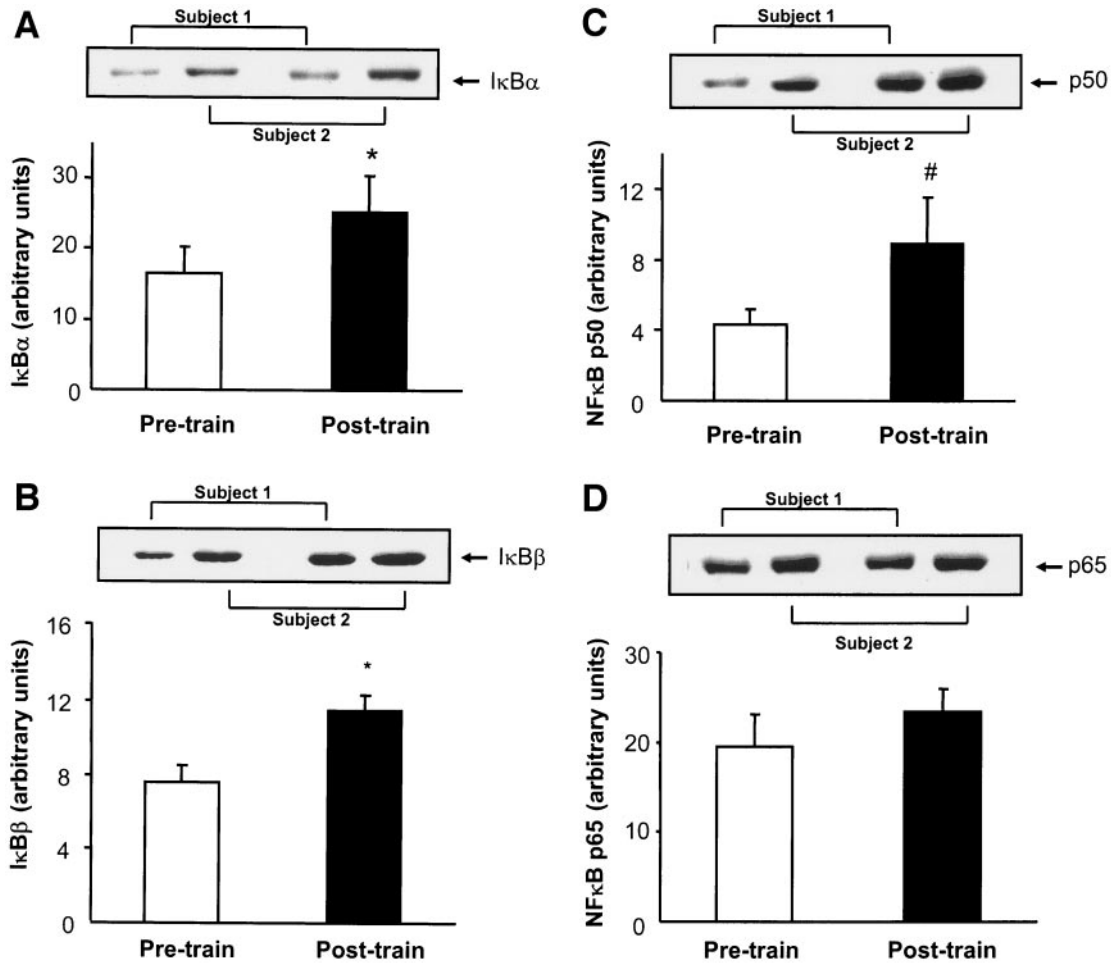


FIG. 4. Effect of exercise training on I κ B and NF κ B protein content in skeletal muscle from control subjects. I κ B α (A), I κ B β (B), NF κ B p50 (C), and NF κ B p65 (D) content were measured as described in RESEARCH DESIGN AND METHODS. Graphical data are means \pm SE in eight control subjects. * $P < 0.05$, # $P = 0.05$. Representative blots also are shown for two subjects before and after training.

diacylglycerol, and ceramides, have been shown to induce insulin resistance (20–25). Moreover, skeletal muscle of insulin-resistant subjects is characterized by increases in fatty acyl CoA (26) and ceramides (27). Recently, it was shown that lipid-induced insulin resistance in L6 myotubes (28) and muscle from rodents (3,4,29) and humans (5) is associated with activation of the I κ B/NF κ B pathway. A role for this pathway in mediating insulin resistance is further strengthened by the findings that inhibition of I κ B/NF κ B signaling improves insulin sensitivity (4,11). In support of our findings, Bhatt et al. (29) recently reported that diet-induced obesity in rats, an intervention that causes insulin resistance, lead to a decrease in muscle I κ B content. Interestingly, this group found that differences in I κ B muscle content between muscle fiber types were not explained by muscle triglyceride content (29), suggesting that intramyocellular lipids were not responsible for the reduction in I κ B. However, metabolites of triglycerides and fatty acids (i.e., fatty acyl CoAs, diacylglycerol, and ceramides), and not triglycerides per se, are believed to be responsible for the insulin resistance. We were unable to measure fatty acyl CoA, ceramide, or diacylglycerol muscle content because of the limited amount of tissue that can be obtained in humans using the percutaneous biopsy technique. Infusion of FFAs to humans (5) and rats (29) has been associated with activation of the I κ B/NF κ B pathway. In the present study, fasting plasma FFA concen-

trations were similar between groups. It should be noted, however, that fasting FFA levels do not reflect FFA turnover and day-long plasma FFA concentrations. Reaven et al. (30) studied type 2 diabetic subjects with similar blood glucose concentrations compared with the subjects included in the present study and found that the type 2 diabetic subjects had similar fasting FFA plasma levels compared with the nondiabetic control subjects, yet mean 24-h plasma FFA concentrations were significantly elevated.

Type 2 diabetes is characterized by low-grade chronic inflammation (1,31,32), and cytokines, including TNF α and IL-6, have been shown to activate the I κ B/NF κ B pathway. TNF α and IL-6 plasma concentrations were similar between groups. Therefore, it seems unlikely that these cytokines were responsible for the decrease in muscle I κ B. Nonetheless, there are many other exogenous inflammatory stimuli that were not measured in this study and could have caused I κ B/NF κ B axis activation. Reactive oxygen species are also known to simulate the I κ B/NF κ B pathway (33–35), and hyperglycemia-induced reactive oxygen species generation could have contributed to the lower I κ B seen in the type 2 diabetic group. Consistent with our findings, Bandyopadhyay et al. (36) did not find increased IKK β phosphorylation in muscle from insulin-resistant subjects. Because I κ B phosphorylation by IKK leads to I κ B ubiquitination and subsequent degradation, the decrease in I κ B content would then

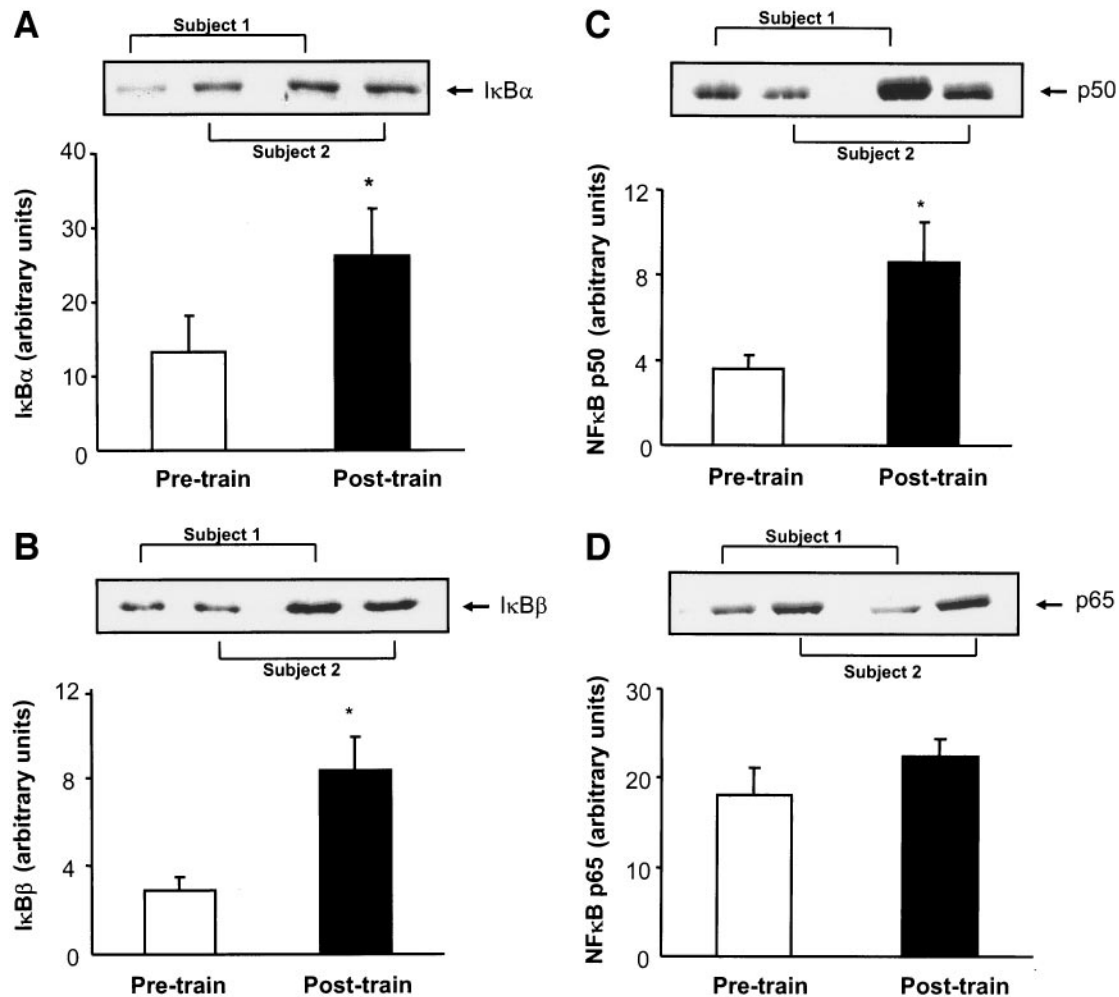


FIG. 5. Effect of exercise training on IκB and NFκB protein content in skeletal muscle from type 2 diabetic subjects. IκBα (A), IκBβ (B), NFκB p50 (C), and NFκB p65 (D) content were measured as described in RESEARCH DESIGN AND METHODS. Graphical data are means \pm SE in six type 2 diabetic subjects. * $P < 0.05$. Representative blots also are shown for two subjects before and after training.

have to be explained by IKK-independent phosphorylation. Protein kinase C and eIF2 kinase can also associate with and phosphorylate IκB (37).

How excessive activity of the IκB/NFκB pathway leads to muscle insulin resistance has not been fully elucidated. Insulin infusion increased insulin receptor substrate-1-associated phosphoinositide 3-kinase activity by 33% ($P < 0.05$) in control subjects but not in type 2 diabetic subjects (12). One potential mechanism for these decreases in insulin-stimulated glucose disposal and phosphoinositide 3-kinase activity in the diabetic group involves insulin receptor substrate-1 serine phosphorylation by IKKβ (38–40), but, as mentioned above, IKKβ phosphorylation was unchanged. Moreover, training-induced improvements in insulin sensitivity were not explained by changes in IKKβ phosphorylation or phosphoinositide 3-kinase activity. In insulin-resistant (lipid-treated) L6 muscle cells, inhibition of NFκB using a selective inhibitory peptide improved insulin-stimulated glucose transport (28). This indicates that NFκB, and genes that this transcription factor controls, may directly influence insulin sensitivity. Yet, over-activation of NFκB in transgenic mice was not associated with insulin resistance (41). Because the phenotype of these animals was characterized by severe muscle wasting, it is unclear how the muscle wasting influenced insulin sensitivity measurements. It is also possible that lifelong

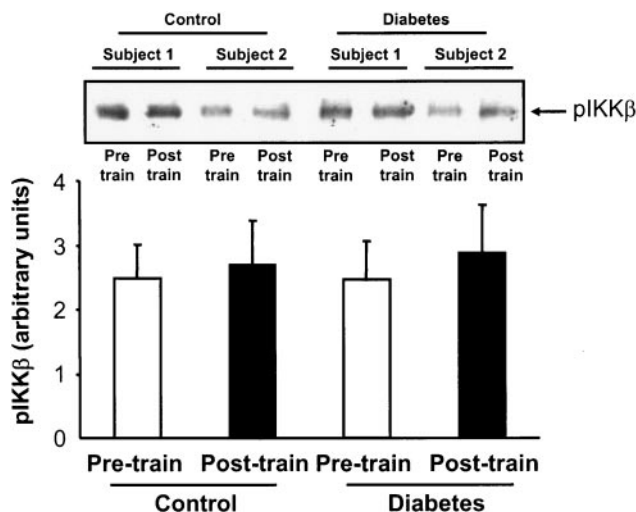


FIG. 6. IKKβ phosphorylation. IKKβ Ser^{177/181} phosphorylation was measured as described in RESEARCH DESIGN AND METHODS. Graphical data are means \pm SE in eight control and six type 2 diabetic subjects. Representative blots also are shown for two subjects in each group.

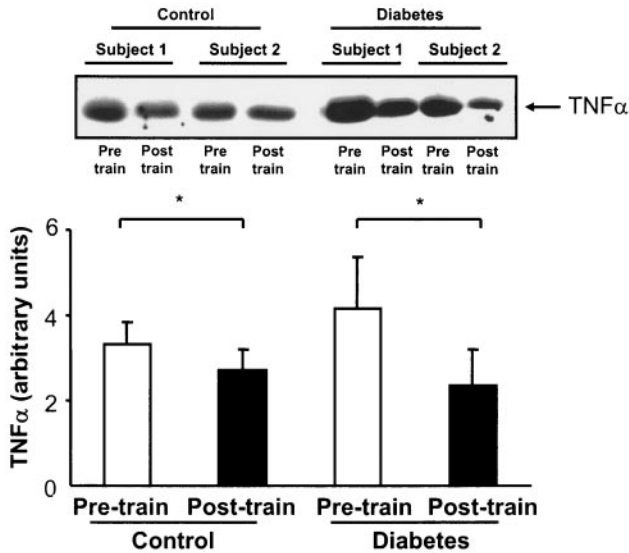


FIG. 7. TNF α muscle content. TNF α protein content was measured as described in RESEARCH DESIGN AND METHODS. Graphical data are means \pm SE in five control and four type 2 diabetic subjects. * $P < 0.05$. The mean \pm SE of the difference between pre- versus posttraining was 0.6 ± 0.19 and 1.8 ± 0.4 AU in the control and diabetic groups, respectively. Representative blots also are shown for two subjects in each group.

overactivation of NF κ B could lead to compensatory mechanisms aimed at normalizing insulin sensitivity. Further studies will help clarify how the I κ B/NF κ B pathway modulates insulin sensitivity in humans.

Few studies have examined the effect of exercise on the I κ B/NF κ B pathway. In rats, acute exercise activates I κ B/NF κ B signaling in muscle (42,43), while acute fatiguing exercise in humans reduces NF κ B activity (44). In the present study, we found that training increased I κ B and reduced TNF α muscle content, suggesting decreased I κ B/NF κ B signaling. This increase in I κ B was not associated with changes in plasma TNF α , IL-6, adiponectin (not shown), or FFA concentrations. Stimuli intrinsic to muscle could also be responsible for the training-induced changes in I κ B and TNF α . Training significantly increased AMP-activated protein kinase (AMPK) phosphorylation in both control and type 2 diabetic groups (N.M., L.J.M., unpublished observations). In endothelial cells, chemical AMPK activation with 5-aminoimidazole-4-carboxamide ribonucleoside has been shown to inhibit NF κ B activity (45). These results suggest that exercise-stimulated AMPK activation may be responsible for the inhibition of the I κ B/NF κ B axis. In contrast, AMPK activation with 5-aminoimidazole-4-carboxamide ribonucleoside had no effect on NF κ B activity in rat skeletal muscle (43), arguing against a role for AMPK as a regulator of I κ B/NF κ B signaling. A reduction in intramyocellular lipid content caused by training could help explain the changes in I κ B and TNF α . However, several studies have shown that trained athletes have increased triglyceride muscle content (46,47). The greater triglyceride storage in the trained athlete represents an adaptive response to training and provides a readily available source of energy for the contracting muscle. In contrast, elevated lipid stores in type 2 diabetes result from an imbalance between plasma FFA availability and oxidation and have been implicated in the development of insulin resistance. Future studies will be needed to examine whether elevated levels of fatty acyl CoA, diacylglycerol, and ceramides are related with abnormali-

ties in I κ B/NF κ B signaling and to assess the effect of training on the content of these metabolites.

Type 2 diabetic subjects had a more pronounced reduction in I κ B β content compared with I κ B α . While the I κ B α and I κ B β isoforms share many similarities, they also display differences. The I κ B α gene has a κ B recognition sequence in its promoter region (48). Thus, activation of NF κ B can induce resynthesis of I κ B α . In contrast, NF κ B does not induce I κ B β synthesis (8,49). Since the muscle of type 2 diabetic subjects appears to have excessive NF κ B activity, this could lead to selective resynthesis of I κ B α but not of I κ B β . Although it is not known whether I κ B β remains suppressed upon long-term NF κ B activation, in Jurkat cells cytokine-induced I κ B α degradation is rapidly restored within 2 h, whereas I κ B β remains low for at least 24 h (50). Whether this differential regulation between I κ B α and I κ B β occurs in other chronic diseases remains to be determined.

We found that NF κ B p50 subunit content was reduced in muscle of type 2 diabetic subjects, and this decrease correlated with reduced insulin sensitivity. This might appear counterintuitive since NF κ B stimulates transcription of inflammatory genes. While the p50-p65 heterodimer is the predominant activating NF κ B dimer, the p50 subunit also can dimerize with another p50 subunit to form p50-p50 homodimers. While the p50-p65 heterodimer activates transcription of inflammatory genes, the p50-p50 homodimer inhibits gene transcription (9,51-53). In view of our findings of reduced p50, one might speculate that type 2 diabetic subjects could have decreased abundance of the p50-p50 repressor homodimers, resulting in excessive NF κ B p50-p65 activity, and that training inhibited NF κ B activity by increasing p50-p50 homodimers. Future investigations are needed to determine whether NF κ B p50-p50 DNA binding is indeed reduced in muscle from type 2 diabetic subjects.

The present study shows an important association between insulin sensitivity and the content of I κ B β and NF κ B p50 in human muscle. Yet, these results do not prove causality. Studies using genetic and pharmacologic approaches to manipulate I κ B/NF κ B signaling will help establish the role of this pathway in insulin sensitivity regulation.

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