Original Article

Reduced Skeletal Muscle Inhibitor of κBβ 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 has recently been hypothesized that excessive activity of the inhibitor of κB (IkB)/nuclear factor κB (NFκB) inflammatory pathway is a mechanism underlying skeletal muscle insulin resistance. However, it is not known whether IkB/NFκB signaling in muscle from subjects with type 2 diabetes is abnormal. We studied IkB/NFκB 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 IkBβ protein abundance, an indicator of increased activation of the IkB/NFκB pathway. IkBβ abundance directly correlated with insulin-mediated glucose disposal (Rd) during a hyperinsulinemic (40 mU·m⁻²·min⁻¹)-euglycemic clamp (r = 0.63, P = 0.01), indicating that increased IkB/NFκB 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 IkBα and IkBβ protein. In subjects with type 2 diabetes, training increased IkBα and IkBβ 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 IkB protein abundance in muscle, suggesting excessive activity of the IkB/NFκB pathway. Moreover, this abnormality is reversed by exercise training. Diabetes 55:760–767, 2006

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 κB (IkB)/nuclear factor κB (NFκB) inflammatory pathway may be an important molecular mechanism responsible for skeletal muscle insulin resistance (1,3–7). NFκB is a family of transcription factors that regulate the expression of proinflammatory genes. In unstimulated cells, NFκB is predominantly localized in the cytoplasm, associated with an inhibitory protein, IkB. Several stimuli, including cytokines (1), reactive oxygen species, hyperglycemia, and free fatty acids (FFAs) (5), activate IkB kinase (IKK), the upstream kinase of IkB. Upon activation by inflammatory factors, IKK phosphorylates IkB, causing rapid IkB polyubiquitination and degradation by proteosomes. Following release from IkB, NFκB 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 (TNFα), interleukin (IL)-1β, and IL-6 (1,8,9).

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

Interventions aimed at blocking the IkB/NFκB pathway, such as genetic deletion of IKKβ in mice (4) and the administration of salicylates, which inhibit IKKβ, to subjects with type 2 diabetes (11) significantly improve peripheral insulin sensitivity. These findings strongly suggest that activation of the IkB/NFκB pathway plays an important role in the pathogenesis of insulin resistance. However, it is not known whether the IkB/NFκB pathway is excessively active in skeletal muscle from subjects with type 2 diabetes. The goal of the present study was to...
evaluate whether the activity of the IkB/NFkB 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 IkB/NFkB inflammatory pathway in skeletal muscle from subjects with type 2 diabetes. We hypothesized that inhibition of IkB/NFkB 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 glipizide, 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. We also examined whether an intervention known to improve insulin sensitivity, such as exercise training, could reverse abnormalities in the IkB/NFkB inflammatory pathway in skeletal muscle from subjects with type 2 diabetes. We hypothesized that inhibition of IkB/NFkB signaling in muscle is a mechanism by which training improves insulin sensitivity.

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

<table>
<thead>
<tr>
<th>Control</th>
<th>Posttraining</th>
<th>Type 2 diabetes</th>
<th>Posttraining</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Pretraining</td>
<td></td>
<td>Pretraining</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>8/2</td>
<td></td>
<td>6/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 ± 3</td>
<td></td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>77 ± 7</td>
<td>77 ± 7</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27 ± 1</td>
<td>27 ± 1</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Fastiging plasma glucose (mmol/l)</td>
<td>4.9 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>7.5 ± 0.9*</td>
</tr>
<tr>
<td>AIC (%)</td>
<td>4.9 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>8.1 ± 1.1*</td>
</tr>
<tr>
<td>Fastiging fasting insulin (pmol/l)</td>
<td>23.8 ± 9.3</td>
<td>24.9 ± 10.6</td>
<td>60.1 ± 24.5*</td>
</tr>
<tr>
<td>Basal endogenous glucose production (mg · kg FFM⁻¹ · min⁻¹)</td>
<td>3.0 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Rₐ (mg · kg FFM⁻¹ · min⁻¹)</td>
<td>7.8 ± 0.7</td>
<td>8.5 ± 1.0</td>
<td>4.0 ± 0.6*</td>
</tr>
<tr>
<td>V₀₂peak (mg · kg FFM⁻¹ · min⁻¹)</td>
<td>36 ± 2</td>
<td>42 ± 2*</td>
<td>26 ± 3*</td>
</tr>
</tbody>
</table>

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.

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
fasting plasma glucose and insulin concentrations \((P < 0.05)\), higher A1C \((P < 0.05)\), and lower aerobic capacity \((V_o2_{peak})\) \((P < 0.05)\). There were no differences in FFA \((690 ± 100 \text{ vs. } 660 ± 100 \mu \text{mol/l})\), IL-6 \((2.3 ± 0.5 \text{ vs. } 2.3 ± 0.6 \text{ pg/ml})\), and TNF\(\alpha\) \((1.0 ± 0.1 \text{ vs. } 1.1 ± 0.2 \text{ pg/ml})\) 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\(\alpha\) concentrations did not change with training. However, physical training significantly increased the \(V_o2_{peak}\) by 17% \((P < 0.05)\) and 31% \((P < 0.05)\) in the control and diabetic subjects, respectively. In the diabetic subjects, the increase in \(V_o2_{peak}\) 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\(\kappa\)B/NF\(\kappa\)B signaling.** To determine whether skeletal muscle from subjects with type 2 diabetes has excessive activity of I\(\kappa\)B/NF\(\kappa\)B pathway, we measured I\(\kappa\)B\(\alpha\) and I\(\kappa\)B\(\beta\) protein abundance. Because I\(\kappa\)B sequesters NF\(\kappa\)B in

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

**FIG. 2.** Correlation between I\(\kappa\)B\(\beta\) content and insulin-mediated glucose disposal \((R_d)\) \((A)\), \(V_o2_{peak}\) \((B)\), and A1C \((C)\) and between \(R_d\) and \(V_o2_{peak}\) \((D)\).
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 Vo2peak (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 Vo2peak (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 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,
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 IκB/NFκB pathway. A role for this pathway in mediating insulin resistance is further strengthened by the findings that inhibition of IκB/NFκB 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 IκB content. Interestingly, this group found that differences in IκB 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 IκB. 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 IκB/NFκB pathway. In the present study, fasting plasma FFA concentrations 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 TNFα and IL-6, have been shown to activate the IκB/NFκB pathway. TNFα and IL-6 plasma concentrations were similar between groups. Therefore, it seems unlikely that these cytokines were responsible for the decrease in muscle IκB. Nonetheless, there are many other exogenous inflammatory stimuli that were not measured in this study and could have caused IκB/NFκB axis activation. Reactive oxygen species are also known to simulate the IκB/NFκB pathway (33–35), and hyperglycemia-induced reactive oxygen species generation could have contributed to the lower IκB seen in the type 2 diabetic group. Consistent with our findings, Bandyopadhyay et al. (36) did not find increased IKKβ phosphorylation in muscle from insulin-resistant subjects. Because IKK phosphorylation by IKK leads to IκB ubiquination and subsequent degradation, the decrease in IκB content would then

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**FIG. 4.** Effect of exercise training on IκB and NFκB protein content in skeletal muscle from control 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 ± SE in eight control subjects. *P < 0.05, *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 IKK (38–40), but, as mentioned above, IKK phosphorylation was unchanged. Moreover, training-induced improvements in insulin sensitivity were not explained by changes in IKK 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, overactivation 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. 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 ± SE in six type 2 diabetic subjects. *P < 0.05. Representative blots also are shown for two subjects before and after training.

FIG. 6. IKK phosphorylation. IKK Ser177/181 phosphorylation was measured as described in RESEARCH DESIGN AND METHODS. Graphical data are means ± SE in eight control and six type 2 diabetic subjects. Representative blots also are shown for two subjects in each group.
overactivation of NFκB could lead to compensatory mechanisms aimed at normalizing insulin sensitivity. Further studies will help clarify how the IkB/NFκB pathway modulates insulin sensitivity in humans.

Few studies have examined the effect of exercise on the IkB/NFκB pathway. In rats, acute exercise activates IkB/NFκB signaling in muscle (42,43), while acute fatiguing exercise in humans reduces NFκB activity (44). In the present study, we found that training increased IkB and reduced TNFα muscle content, suggesting decreased IkB/NFκB signaling. This increase in IkB was not associated with changes in plasma TNFα, IL-6, adiponectin (not shown), or FFA concentrations. Stimuli intrinsic to muscle could also be responsible for the training-induced changes in IkB and TNFα. 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-aminomimidazole-4-carboxamide ribonucleoside has been shown to inhibit NFκB activity (45). These results suggest that exercise-stimulated AMPK activation may be responsible for the inhibition of the IkB/NFκB axis. In contrast, AMPK activation with 5-aminomimidazole-4-carboxamide ribonucleoside had no effect on NFκB activity in rat skeletal muscle (43), arguing against a role for AMPK as a regulator of IkB/NFκB signaling. A reduction in intramyocellular lipid content caused by training could help explain the changes in IkB and TNFα. 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 abnormal-

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

We found that NFκB 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 NFκB stimulates transcription of inflammatory genes. While the p50-p65 heterodimer is the predominant activating NFκB 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 NFκB p50-p50 activity, and that training inhibited NFκB activity by increasing p50-p50 homodimers. Future investigations are needed to determine whether NFκB 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 IkBβ and NFκB p50 in human muscle. Yet, these results do not prove causality. Studies using genetic and pharmacologic approaches to manipulate IkB/NFκB signaling will help establish the role of this pathway in insulin sensitivity regulation.

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