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Exercise normalises overexpression of TNF-α in knockout mice

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Abstract

TNF-α is linked with insulin resistance, as greater amounts of TNF are detected in muscle and adipose tissue in glycemically challenged people and TNF-α inhibits insulin receptor signalling. However, what modulates this overexpression of TNF-α is currently unknown. We examined the effect of 1 h exercise on overexpression of the TNF-α gene in TNF receptor 1 and 2 knockout mice. IL-6 knockout mice were included to elucidate the importance of IL-6 in regulating TNF-α in response to exercise. TNF-α gene expression was over-expressed in muscle in both TNFR knockout models. TNF-α overexpression returned to normal levels after exercise in the TNF-α receptor knockout models. In IL-6 knockout mice, a modest decrease in TNF-α was also observed. These data suggest that TNF-α-induced insulin resistance can be regulated by a single exercise bout by normalising TNF-α expression. This exercise effect can be mediated via IL-6, but also an IL-6 independent mechanism seems to exist.

Keywords: TNF-α; IL-6; Exercise; Type II diabetes

Exercise increases insulin sensitivity and decreases the risk of developing type II diabetes [1–3], however, the mechanisms whereby this happens are not fully understood. The role of cytokines in metabolism has gained interest in the last few years and may be regarded as exercise-mediated regulators of insulin sensitivity. Especially TNF-α, an abundantly expressed cytokine, seems to play a major role in metabolism and in relation to obesity and type II diabetes. TNF-α is linked with obesity and insulin resistance as increased levels of TNF-α mRNA are found in both muscle [4] and adipose tissue [5] as well as plasma protein [6] of patients with type II diabetes. Also several animal models of obesity and insulin resistance investigated so far demonstrate high TNF-α mRNA levels compared with controls [7–11]. A polymorphism in the TNF-α promoter region at position −308, which results in increased transcription of the TNF-α gene, is linked with decreased insulin sensitivity and increased percent body fat [12]. Furthermore, mice lacking TNF-α function are protected from obesity-induced insulin resistance [13], and TNF-α levels can be reduced by weight loss or treatment with the insulin-sensitiser pioglitazone [9], indicating an important role of TNF-α in insulin signalling.

TNF-α signals via two transmembrane receptors, TNF-α receptor 1 (p55) and TNF-α receptor 2 (p75), which contain different intracellular domains and subsequent signalling pathways [14]. Both receptors seem to be involved in glucose homeostasis regulation, as TNF-α can impair insulin-mediated glucose uptake predominantly via the receptor 1 (R1) [15], whereas the TNF-α receptor 2 (R2) is linked with obesity, insulin resistance, and type II diabetes [16,17]. Lack of both receptors or knockout of the TNF-α gene has been shown to lead to incomplete, but improved, insulin sensitivity, further indicating a major role of TNF-α as an inducer of insulin resistance [18]. TNF-α induces insulin

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resistance by inhibiting the insulin receptor signalling pathway via the IRS-1 activation and GLUT-4 translocation [19], and may thus provide an important link to the pathogenesis of obesity and type II diabetes.

An endotoxin challenge in humans results in an increase in plasma TNF-α, which can be blunted by exercise. This blunting is completely mimicked by infusion of rhIL-6, a cytokine known to rise up to 100-fold in plasma in response to exercise, in doses similar to that obtained during exercise, prior to the endotoxin infusion [20]. This study designated IL-6 as a key mediator in inhibiting TNF-α during an exercise bout. However, whether IL-6 is the only regulator of TNF-α in response to an exercise bout is unknown.

Pilot studies from our laboratory have demonstrated increased expression of TNF-α in TNF-α R1 and R2 KO mice. We used these mice as a TNF-α overexpression model, to investigate the effects of exercise on dysregulated TNF-α levels, and whether a possible effect of exercise on TNF-α levels would be present in IL-6 KO mice, to investigate whether this cytokine is unique in negatively regulating TNF-α in response to exercise.

Materials and methods

Mice. Ninety-two male and female mice of 3 months of age were obtained for the study. Of these, 34 were TNF-α receptor 1 KOs, 20 were TNF-α receptor 2 KOs, 24 were IL-6 KOs, and 36 were littermate C57 control mice. The mice were maintained in a 12:12 h light–dark cycle and were allowed water and food ad libitum. Mice were divided into 2 groups; 1 group undergoing 1 h of swimming exercise (water temperature: 36.5 °C) followed by immediate sacrificing of the mice. The other group served as resting controls, sacrificed at time points corresponding to the exercising mice, in order to avoid circadian variations in cytokine gene expression. Soleus muscle and gastrocnemius muscle were dissected from the mice and analysed by real-time PCR for TNF-α gene expression levels.

RNA extraction. RNA was extracted using TriZol (Life Technologies) according to the manufacturer's instructions. In short, 1 ml of Trizol was added to the sample, homogenised using a Brinkman Polytron (version PT 2100) on setting 26 for 15–20 s, and placed on ice. One hundred microliters of chloroform:isoamyl alcohol (24:1) was added and samples were thoroughly shaken followed by centrifugation at 12,000*g* for 15 min at 4 °C. The upper aqueous layer was transferred to a fresh tube and the same volume of ice-cold isopropanol was added. The samples were subsequently stored at −20 °C for 1 h and spun at 12,000*g* for 15 min at 4 °C. The resulting pellet was washed with 0.5 ml of 75% ethanol in DEPC-treated water and centrifuged at 6000*g* for 10 min. Pellets were re-dissolved in 15 μl DEPC-treated water and allowed to dissolve on ice, after which samples were ready for reverse transcription.

Reverse transcription. Two micrograms of total RNA was reverse transcribed using the Applied Biosystems (Denmark) Taqman RT-Kit according to the manufacturer's directions. The cDNA was prepared using random hexamers.

PCR. TNF-α gene expression was measured using real-time PCR with 18S RNA as the reference gene. Each sample was run in triplicate as singleplex in a reaction volume of 10 μl on a Taqman (version 7900) for 50 cycles under standard real-time PCR conditions. For TNF amplification a reagent mixture of 35 μl was made up with 50 ng sample, 1× of TNF-α pre-developed assay reagents, and 1× of mastermix and made up to 35 μl with water. For 18s, 1× of 18s, 1× of mastermix, and 10 ng of sample were mixed and made up to a total of 35 μl with water. All assay reagents were obtained from Applied Biosystems.

Data were analysed using the standard curve method and subsequent semiquantitative analysis comparing all samples to the resting controls (for review, see [21]).

Statistics. All data were normally distributed after log-transformation. Thus, all data are presented as geometric means ± geometric SEM. An un-paired *t* test was used to evaluate any differences over time and between mice strains using SigmaStat 2.03. Results were considered significant at $P < 0.05$.

Results

TNF-α gene expression in soleus muscle was elevated at rest in both TNFR1 (3.57-fold +2.60/−0.66) and TNFR2 KO (2.62-fold +0.36/−0.23) compared to controls (Fig. 1). Following exercise, no change was observed in controls, whereas TNF-α mRNA levels decreased to levels comparable to controls.

TNF-α gene expression did not differ between mice strains in gastrocnemius muscle and was not affected by exercise (Fig. 2).

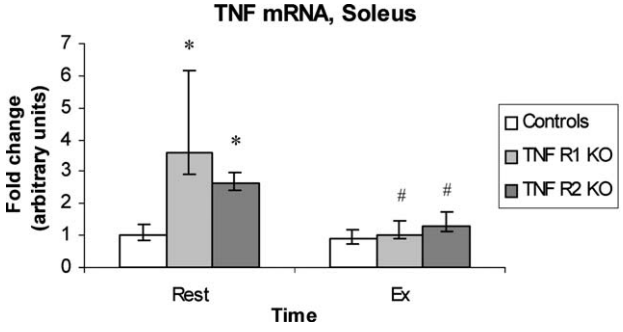

Fig. 1. TNF mRNA expression in soleus muscle at rest and after 1 h of exercise. Both resting TNF R1 KO mice and TNF R2 KO mice demonstrated increased TNF-α mRNA levels compared to resting controls. Upon exercise TNF mRNA was decreased to levels comparable to controls. No change in TNF mRNA was observed in controls upon exercise. *Denotes significant difference from resting controls. #Denotes significant difference from respective resting mouse strain.

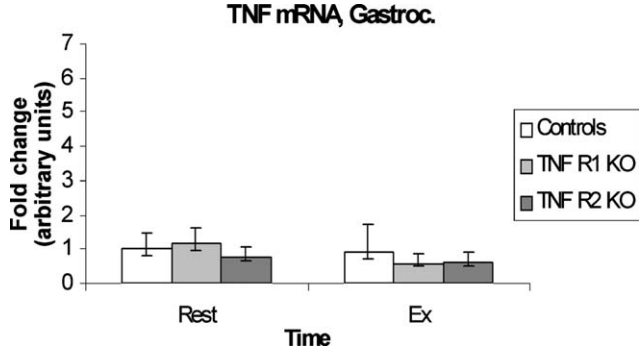

Fig. 2. TNF mRNA levels in gastrocnemius muscle at rest and after 1 h of exercise. TNF-α mRNA levels in gastrocnemius did not differ between mice strains nor with exercise.

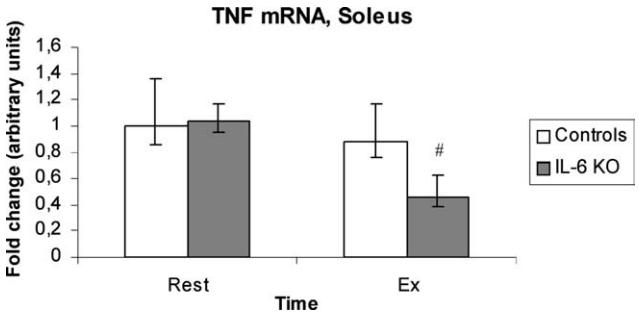

Fig. 3. TNF-α mRNA in soleus muscle of IL-6 KO mice at rest and after 1 h of exercise. Resting IL-6 KO demonstrated normal TNF-α mRNA levels compared to resting controls. Upon exercise TNF mRNA was decreased in IL-6 KO mice. #Denotes significant difference from respective resting mouse strain.

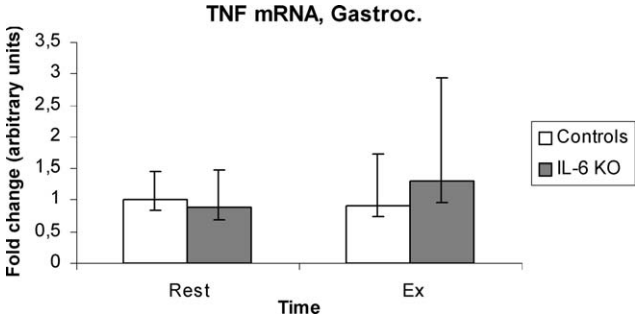

Fig. 4. TNF-α mRNA in gastrocnemius muscle of IL-6 KO mice at rest and after 1 h of exercise. TNF-α mRNA did not differ in gastrocnemius between mice strains nor with exercise.

In IL-6 KO mice, TNF-α gene expression in soleus muscle was normal at rest, but still elicited a significant decrease upon exercise (1.04 +0.13/−0.09-fold at rest vs. 0.46 +0.17/−0.08-fold after exercise) (Fig. 3).

TNF-α mRNA levels were unaffected in IL-6 KO gastrocnemius muscle at rest and upon exercise (Fig. 4).

No differences were observed between male and female mice.

Discussion

At rest, both TNF-α receptor 1 and 2 KO mice demonstrated increased TNF-α gene expression levels in soleus muscle as previously indicated in adipose tissue [22]. This increased TNF-α expression in the TNF-α receptor KO models could be due to a compensatory effect for the lack of signalling through either of the receptors, thus leading to overexpression of TNF-α.

In response to exercise, TNF-α gene expression levels decreased in both of the TNF receptor knockout models, resulting in expression levels comparable to those observed in the controls. It is well known that exercise enhances insulin sensitivity, but the mechanisms whereby this occurs are not fully understood. Elevated levels of TNF-α in adipose tissue and muscle are observed in several studies of patients with type II diabetes and animal models hereof [4,5,9]. Normalisation of dysregulated TNF-α levels could be one mechanism whereby exercise increases insulin sensitivity. Accordingly, TNF-α inhibits the insulin receptor signalling pathway, which makes this protein a likely candidate for the induction of insulin resistance. We therefore suggest that one mechanism whereby exercise increases insulin sensitivity in patients with type II diabetes is by normalising dysregulated TNF-α gene expression.

Infusion of IL-6 previous to an endotoxin challenge has demonstrated a regulatory effect of IL-6 on TNF-α [20]. In the IL-6 deficient mice, exercise still evoked a modest reduction in TNF-α gene expression, suggesting that factors other than IL-6 can take part in regulating TNF-α in response to exercise. Whether this effect is modulated via other cytokines increased in response to exercise remains to be determined. However, one alternative pathway for this regulation could be via PPAR-γ, a transcription factor involved in several metabolic processes in response to exercise. PPAR-γ is expressed in skeletal muscle and is known to decrease TNF-α mediated insulin resistance [23]. Thus, PPAR-γ could provide another mechanism whereby TNF-α is normalised by exercise.

Elevated TNF-α gene expression and protein levels are closely linked to obesity and insulin resistance in humans [4,5]. TNF-α induces insulin resistance by inhibiting the insulin-signalling pathway and may be an initiator of obesity and insulin resistance. Thus, regulation of TNF-α gene expression could prove extremely important in the protection against type II diabetes. The findings from the present study demonstrate that just one bout of exercise normalises the dysregulated TNF-α gene expression levels in the TNFR KO mice. This is also observed in mice lacking the IL-6 protein, suggesting that mechanisms other than those involving IL-6 can regulate TNF-α in response to exercise. These data suggest that in people with dysregulated TNF-α gene expression, exercise would inhibit the overproduction of TNF-α mRNA, especially in the insulin-sensitive type I fibres. What the alternative mechanism to IL-6 is remains to be elucidated.

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