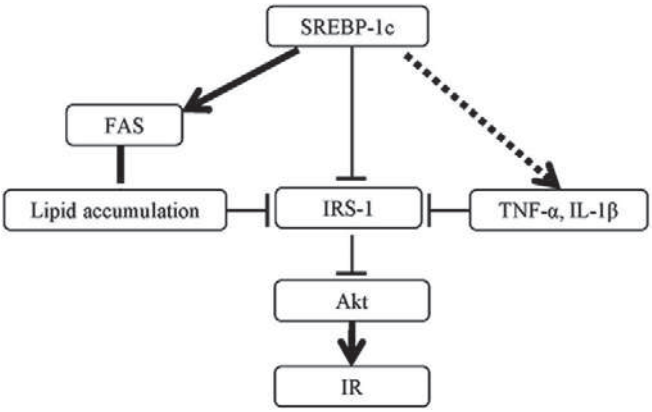
the regulatory effect and molecular mechanisms of SREBP-1c on IRS-1-associated insulin signaling in myotubes.
Materials and methods: Adenovirus vectors expressing SREBP-1c were tranfected into rat primary and L6 myotubes to study the regulatory effects of SREBP-1c activation on transduction of IRS-1-associated insulin signaling and expressions of inflammatory cytokines. Luciferase reporter analysis, electrophoretic mobility shift assay, and chromatin immunoprecipitation assay were used to detect the direct binding site of SREBP to the IRS-1 promoter.
Results: We found that accumulation of SREBP-1c decreased gene and protein levels of IRS-1 and suppressed insulin-stimulated p-IRS-1(Tyr608) and p-Akt (Ser473) activation in a dose dependent manner. Concomitantly, SREBP-1c induced gene expressions of fatty acid synthase (FAS) and promoted expressions of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) with increasing titers of transfection. *In vitro* and *in vivo* interaction assays showed that SREBP-1c directly inhibited IRS-1 transcription activity through non-SRE domain at a location of -200 to +50bp of the promoter.
Conclusion: In this study, we report a new mechanism for insulin resistance in lipotoxicity that a high level of SREBP-1c, a pivotal transcription factor regulating de novo lipogenesis, can result in muscular insulin resistance through a direct effect of suppressing IRS-1 transcription, as well as an indirect effect of promoting the release of inflammatory cytokines. Our study highlights reciprocal relationships between metabolic pathways and suggests that SREBP-1c could be one pathogenesis involved in skeletal muscle insulin resistance.



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177

Potent anti-inflammatory properties of adiponectin on the skeletal muscle
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Background and aims: Adiponectin (ApN) is an adipokine, which is decreased in the metabolic syndrome, thereby playing a key role in the pathogenesis of these disorders. We have previously shown that ApN exerts important anti-inflammatory effects on skeletal muscle in mice exposed to acute inflammation (LPS injection) or metabolic stress (obesogenic diet). The aim of this work is to test the potency of the anti-inflammatory properties of ApN by using a model with severe and sustained inflammation.
Materials and methods: Mdx mice (a mouse model of Duchenne muscular dystrophy, where persistent inflammation worsens the consequences of the genetic deficiency) were crossed with transgenic mice that over-express adiponectin (Tg-ApN mice) in order to generate mdx-Tg-ApN mice. Different markers of inflammation/oxidative stress (TNF-α, IL-1β, NF-κB, CD68, CD3, peroxiredoxin 3/5 (PRDX3/5), 4-hydroxynonenal (HNE)) were studied and quantified by immunohistochemistry, ELISA and western blot. In vivo functional tests were also carried out to determine the global force of mice. Finally, the extent of muscle damage was quantified by Evans Blue Dye (EBD) injection following an eccentric exercise.
Results: Compared to WT mice, muscles of mdx mice presented significant increases (+ 10 to 15 fold; p<0.001) in the expression of pro-inflammatory factors (TNF-α, IL-1β, NF-κB) and oxidative stress markers (PRDX3/5, HNE) as well as a massive infiltration of macrophages and T lymphocytes, as shown by CD68 and CD3 immunolabeling respectively. All of these abnormalities were drastically reduced in mdx-Tg-ApN mice (- 60 to 75 % vs mdx; p<0.001). In addition, mdx-Tg-ApN mice exhibited higher global muscular force (+ 50 % vs mdx; p<0.01) along with a significant decrease in EBD content in their muscle fibers (- 50 to 60 % vs mdx; p<0.001).
Conclusion: Adiponectin proves to be an extremely potent anti-inflammatory agent that protects muscle against injury. These anti-inflammatory properties of ApN are of interest in the metabolic syndrome as well as in other diseases where inflammation plays a triggering or worsening pathogenic role.
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178

Fractalkine is a novel myokine which protects myotubes from TNF-alpha induced insulin resistance
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Background and aims: Fractalkine has been shown to be secreted by adipose tissue and associated with type 2 diabetes. We have previously demonstrated that its mRNA expression was increased in skeletal muscle cells treated with TNF-alpha. Nevertheless, fractalkine secretion, regulation and action on skeletal muscle remain to be studied. The aims of our study were to identify the physiological source of fractalkine and to evaluate the impact of fractalkine on human skeletal muscle cells with or without induction of insulin resistance by TNF-alpha.
Materials and methods: Fractalkine levels were measured in plasma from 5 healthy males performing 3h of bicycling exercise at 50% VO2 max. and in skeletal muscle biopsies for mRNA and protein. In 9 healthy subjects performing 2 h of one legged knee extensor exercise, muscle biopsies were obtained at 0, 2, 5 and 24h from both resting and exercising leg. Plasma fractalkine was also measured in 10 healthy males who had received 4h TNF-alpha infusion or placebo on 2 different days. Human skeletal muscle biopsies were collected from abdominal muscle and cells were cultured in order to measure the impact of fractalkine on 3H-2-deoxyglucose uptake (60 min) and insulin signalling with or without induction of insulin resistance by TNF-alpha (20 ng/ml for 1 or 24h).
Results: Plasma fractalkine increased rapidly after the beginning of exercise, reaching a maximum after 3h of bicycling (1.4+/-0.4 µg/l; p<0.01) and returning to basal levels by 24h. Using a model of one legged knee extensor exercise, fractalkine mRNA in the exercising leg muscle was increased 6.6+/-1.8-fold (p<0.01) and protein expression 4.2+/-0.8-fold (p<0.01) when compared to the resting leg. Moreover, human volunteers who received TNF-alpha infusion showed an increase of plasma fractalkine after 2h, with a maximum after 4h (8.2+/-0.8 µg/l; p<0.001). In order to explore autocrine action, human primary myotubes were treated with recombinant fractalkine for 24h and then with TNF-alpha to induce insulin resistance. Myotubes pretreated with fractalkine showed an increase of basal glucose uptake (120+/-18 vs. 190+/-8 cpm/mg protein/min; p<0.05). Nevertheless, fractalkine protected myotubes from the impact of TNF-alpha on glucose uptake after insulin stimulation (140+/-31 for 1h TNF-alpha vs. 220+/-12 fractalkine + TNF-alpha and 146 +/-25 for 24h TNF-alpha vs 245 +/-9 for Fractakine + TNF-alpha; p<0.05). Fractalkine pretreatment (25 ng/ml for 24h) also protected against the negative impact of TNF-alpha on AS160, Akt, and IRS-1 phosphorylation after insulin stimulation (10 min, 100 nmol/l). Moreover, TNF-alpha (24h) induced phosphorylation of ERK, mTOR and NFkB was prevented in human skeletal muscle cells pretreated with 25 ng/ml of fractalkine for 48h.
Conclusion: Our study using human subjects shows that fractalkine is expressed by skeletal muscle and regulated by exercise and TNF-alpha infusion. We further show that fractalkine protects human skeletal muscle cells from TNF-alpha induction of insulin resistance, indicating a possible autocrine role in the exercising state in addition to the previously suggested endocrine role following release from adipose tissue.

179

G-CSF, a novel fatty acid-dependent myokine
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Background and aims: We recently identified *CSF3* as a palmitate-responsive myocyte gene encoding G-CSF. Yamada et al. and others showed elevated