Increased irisin abundance in muscle and blood circulation after treadmill exercise in mice

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INTRODUCTION

Irisin, the exercise induced, secreted cleavage product of fibronectin type III domain-containing protein 5 (Fndc5), is a potential mediator of positive metabolic effects of exercise. It was demonstrated that recombinant Fndc5 induced a thermostogenic program in white adipose tissue thus indirectly linking exercise with browning of adipose tissue (Bostroem et al., 2012). Cleavage and modification of Fndc5 was proposed as prerequisite for this action. The study investigated the effect of exercise on Fndc5/irisin and peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC1-α) in mice.

MATERIAL AND METHODS

Animals and samples:
• 70 days old, male DkHTp mice (Dummerstorf high treadmill performance, selected over 117 generations)
• 3 groups, one bout of treadmill exercise at 70 days of age, voluntary wheel running for 3 weeks (49th to 70th day of age) or sedentary control, n = 11 to 12 in each group
• Blood and leg muscles (femoral and crus)

mRNA expression:
• RNA extraction with Qiazol Lysis Reagent (Qiagen)
• cDNA synthesized with iScript cDNA Synthesis Kit (BioRad)
• RNA extraction with Qiazol Lysis Reagent (Qiagen)
• mRNA expression:
  • Blood and leg muscles (femoral and crus)
  • 3 groups, one bout of treadmill exercise at 70 days of age, voluntary wheel running for 3 weeks (49th to 70th day of age) or sedentary control, n = 11 to 12 in each group
  • Blood and leg muscles (femoral and crus)

Protein analyses:
• Western Blot of 20 µg muscle protein or 30 µg serum protein
• Detection with antibodies against full-length Fndc5 at ~25 kDa (sc-13067, Santa Cruz Biotechnology), and HRP conjugated goat anti rabbit IgG secondary antibody (New England Biolabs)

Immunohistochemistry:
• Cryosections of femoris muscle, antibodies like for WB
  • Detection with antibodies against full-length Fndc5 at ~25 kDa
  • Western Blot of 20 µg muscle protein or 30 µg serum protein

Table 1. Sequences of primer sets used for amplification of specific cDNA

<table>
<thead>
<tr>
<th>Primer</th>
<th>bp</th>
<th>Sequence 5'-3'</th>
<th>Acc. No.</th>
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<td>CCTGCTCTTGTCCTGCTTG</td>
<td>NM_000975</td>
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<td>Hprt1</td>
<td>90</td>
<td>GCCCTCTGAGACGCCTTTT</td>
<td>NM_013568</td>
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<td>CAAGCGAGGCTAAGGACA</td>
<td>NM_027402</td>
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<td>PGC1-α</td>
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<td>PGC1-α</td>
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<td>CTTGAAGAGGCTGACGAAGG</td>
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<tr>
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<td>reverse</td>
<td>TCACAGAAACCACGGGAAAG</td>
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</tr>
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</table>

RESULTS

Running performance:
- Mice of the RW group, with free access to the running wheel, ran 4.7 ± 2.6 km per day during 3 weeks of voluntary endurance exercise.
- Mice subjected to a single submaximal test on a treadmill (TM group) ran 5.1 ± 1.6 km.

Irisin abundance:
- Irisin (~12 kDa) is abundant in murine muscle and circulates in serum.
- Irisin abundance in serum and femoral muscle increased by acute exercise (Fig. 1 A, B).

Table 2. Gene expression fold change of control

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
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<th>Treadmill</th>
<th>P</th>
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<tr>
<td>PGC1-α</td>
<td>0.78</td>
<td>1.25</td>
<td><strong>0.52</strong></td>
<td>&lt;0.05</td>
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<td>Fndc5</td>
<td>0.81</td>
<td>1.26</td>
<td><strong>0.52</strong></td>
<td>&lt;0.05</td>
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</table>

Table 3. Irisin protein abundance in % of control

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>Running wheel</th>
<th>Treadmill</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irisin</td>
<td>100</td>
<td>105</td>
<td><strong>113</strong></td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Figure 1: Protein abundance of irisin in (A) serum and (B) muscle tissue of mice after 3 weeks of voluntary exercise in a running wheel or one bout of treadmill exercise relative to a sedentary control. Representative parts of respective western blots are shown above. *** indicate significant difference to control (P < 0.001).

Fndc5 mRNA and protein abundance:
- Fndc5 mRNA and protein abundance did not respond to exercise (Fig. 2 A, B).

Figure 2: Fndc5 mRNA (A) and protein (B) abundance in muscle tissue of mice. (A) mRNA abundance was normalized to B2m and Hprt1. Bars represent means of fold changes compared to control group with 95% confidence intervals marked as vertical lines. (B) Protein abundance in % of control. Representative parts of western blots are shown above.

Localization of Fndc5 and Irisin in muscle tissue:
- Fndc5 was localized at the cell membrane and Irisin in the intercellular space between muscle fibers (Fig. 3 A, B).

Figure 3. Cryosections of rectus femoris of a TM group mouse immunostained with anti-Fndc5 (A) or irisin (B) primary antibodies and MRNF48 anti-rabbit IgG secondary antibody. Fndc5 was detected at the muscle fiber membrane (A, arrowheads) and in the cytoplasm, as well as in additional cells in the connective tissue (A, arrows). Irisin was mainly located in the intercellular space (B, arrows).

PGC1-α mRNA and protein abundance:
- Abundance of transcript 4 was 9- and 6-fold higher in TM compared to CO mice in femoral and crus muscles, respectively, and transcript 3 was 33- and 10-fold higher (Fig. 4 A, B).
- The protein abundance was not affected by exercise.

Figure 4: Expression of PGC1-α transcripts 1, 3, and 4 mRNA in femoral (A) and crus (B) muscle tissue of mice, normalized to B2m and Hprt1. Bars represent means of fold changes compared to control with 95 % confidence intervals. * indicate significant differences to control (P < 0.05, **P < 0.01). (B) Relationship between PGC1-α mRNA abundance, normalized to Hprt1 (2-ΔΔCT), in femoral (FM) and crus (CM) muscles and running distance during one bout of treadmill exercise.

CONCLUSION

Our results indicate that irisin exists in skeletal muscle and serum of mice and increases immediately in response to acute but not to repeated voluntary exercise. Since this increase was not paralleled by an induction of PGC1-α protein and Fndc5 mRNA and protein it is likely that the acute irisin response is mediated by additional, unknown factors. The elevated mRNA abundance of different PGC1-α transcripts after acute exercise however, may indicate that PGC1-α induces recovery of muscular Fndc5 and irisin.