Effect of exercise and insulin on SREBP-1c expression in human skeletal muscle: potential roles for the ERK1/2 and Akt signalling pathways

T. Boonsong1, L. Norton, K. Chokkalingam, K. Jewell, I. Macdonald, A. Bennett and K. Tsintzas
Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, Medical School, Queen’s Medical Centre, Nottingham NG7 2UH, U.K.

Abstract
SREBP-1c (sterol-regulatory-element-binding protein 1c) is a transcription factor that regulates genes associated with glucose and fatty acid metabolism and exhibits responsiveness to insulin and exercise. We have examined the effects of exercise on basal and insulin-mediated changes in the activation (phosphorylation) of the signalling molecules involved in the regulation of SREBP-1c and related them to changes in the expression of SREBP-1c in human skeletal muscle. Eight healthy men performed one-legged cycling for 90 min; 24 h later a hyperinsulinaemic euglycaemic clamp for 4 h was performed. Muscle biopsies were obtained from the rested (control) leg and the exercised leg immediately after exercise and before and after the insulin clamp. Immediately after exercise, phosphorylation of ERK (extracellular-signal-regulated kinase) 1, ERK2 and Akt (protein kinase B) was higher in the exercised than the control leg. SREBP-1c mRNA content was not affected by exercise, whereas its protein level was lower in the exercised than the control leg and returned to pre-exercise levels 24 h later. Similarly, SREBP-1c mRNA content was ~1.5-fold higher in the exercised than the control leg 24 h after exercise. Insulin infusion up-regulated SREBP-1c mRNA level ~2-fold, but did not affect its protein level. Phosphorylation of Akt also in increased in response to insulin clamp, whereas phospho-ERK1 and -ERK2 levels were unchanged. Neither exercise nor insulin affected STAT3 (signal transducer and activator of transcription 3) or p38 MAPK (mitogen-activated protein kinase) phosphorylation. These findings suggest that exercise-induced changes in muscle SREBP-1c expression might be mediated by the activation of the ERK1/2 pathway, whereas Akt might be a positive regulator of SREBP-1c in human skeletal muscle under insulin-stimulated conditions.

Introduction
SREBP-1c (sterol-regulatory-element-binding protein 1c) is a transcription factor that mediates the transcriptional effects of insulin on glycolytic and lipogenic genes [i.e. HKII (hexokinase II), ACC (acyetyl-CoA carboxylase) and FAS (fatty acid synthase)] in skeletal muscle [1-3]. Animal [2-4] and human [1] studies have shown that fasting decreases and refeeding increases SREBP-1c expression in skeletal muscle. Several studies have also shown that muscle SREBP-1c exhibits responsiveness to acute exercise and training [5-7]. However, the signalling pathways that regulate SREBP-1c and its transcriptional targets in human skeletal muscle are not clear. It has been suggested that Akt [protein kinase B (PKB)] [8] and p38 MAPK [mitogen-activated protein kinase] [9,10] are positive regulators, whereas STAT3 (signal transducer and activator of transcription 3) [11], a known mediator of IL-6 (interleukin-6) [12], and ERK (extracellular-signal-regulated kinase) 1/2 [13] are negative regulators of SREBP-1c expression. Cell culture experiments from our laboratory have demonstrated that IL-6-mediated STAT3 activation causes a decrease in SREBP-1c promoter activity (T. Boonsong, I. Macdonald, K. Tsintzas and A. Bennett, unpublished work). We have examined the effects of exercise on basal and insulin-mediated changes in the activation (phosphorylation) of the signalling molecules involved in the regulation of SREBP-1c and related them to changes in SREBP-1c expression in human skeletal muscle.

Effect of exercise and insulin on SREBP-1c regulation
Eight healthy men (aged 24 ± 2 years, body mass index 24 ± 1 kg/m²; means ± S.E.M.) underwent a 4 h hyperinsulinaemic euglycaemic clamp 24 h after completing 90 min of one-legged cycling at moderate intensity (60% of maximal oxygen uptake), while keeping the other leg sedentary (control). Muscle biopsies were taken from both legs immediately after exercise, and before and after 4 h of insulin infusion for the measurement of mRNA and protein contents using quantitative RT (reverse transcription)–PCR and Western blotting respectively. Blood samples were obtained before, during and after exercise, and before and after the insulin clamp for the determination of blood metabolites and IL-6 concentrations. Results are shown in Table 1. Exercise decreased serum insulin and increased plasma NEFA (non-esterified fatty acid)
levels, and their values returned to pre-exercise levels before the insulin clamp. During the insulin clamp, serum insulin was maintained at ~80 m-units/l, and plasma NEFA concentrations were suppressed. There were no significant changes in plasma IL-6 in response to either exercise or insulin infusion.

SREBP-1c mRNA levels were similar in both legs, whereas its protein levels decreased ~1.7-fold (*P < 0.05) in the exercised leg immediately after exercise and returned to normal levels 24 h later. Similarly, SREBP-1c mRNA content was ~1.5-fold higher (*P < 0.01) in the exercised than the control leg 24 h after exercise. In response to the insulin clamp, SREBP-1c mRNA levels increased ~2-fold (*P < 0.001) in both legs, but did not affect its protein levels. Immediately after exercise, phosphorylation of ERK1 and ERK2 was ~1.3- * (P < 0.05) and ~2.0-fold * (P < 0.05) higher in the exercised than the control leg respectively. There was no difference in the phosphorylation of ERK1/2 between legs 24 h after exercise. Furthermore, there were no insulin-induced changes in both pERK (phospho-ERK) 1 and pERK2. Phosphorylation of Akt was ~1.2-fold * (P < 0.05) higher in the exercised than the control leg immediately after exercise. Insulin infusion significantly increased pAkt (phospho-Akt) levels in the control leg ~1.5-fold * (P < 0.05) and tended to increase it in the exercised leg (P = 0.06). There were no statistically significant changes in STAT3 and p38 MAPK phosphorylation in response to either exercise or insulin infusion in both legs.

**Conclusion**
The exercise-induced reduction in skeletal muscle SREBP-1c expression might be mediated by activation (phosphorylation) of the pERK1/2 pathway, whereas the up-regulation of SREBP-1c expression in response to insulin stimulation might be mediated by activation of Akt. The increase in SREBP-1c expression during the recovery period may be due to changes in circulating levels of insulin and NEFAs, but not IL-6, which remained unaffected by treatment.

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**Table 1** | Protein and mRNA levels of SREBP-1c and phosphorylation state of key signalling molecules in non-exercised leg (control) and exercised leg after 90 min of one-legged cycling (post-exercise), and before (pre-clamp) and after (post-clamp) 4 h of insulin infusion in human skeletal muscle.

Values are means ± S.E.M. (arbitrary units), p-STAT3, phospho-STAT3; p-p38 MAPK, phospho-p38.

<table>
<thead>
<tr>
<th>Levels (arbitrary units)</th>
<th>Control leg</th>
<th>Exercised leg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-exercise</td>
<td>Pre-clamp</td>
</tr>
<tr>
<td><strong>SREBP-1c</strong></td>
<td></td>
<td></td>
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<tr>
<td>mRNA/actin</td>
<td>0.49 ± 0.03</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>Protein/actin</td>
<td>3.58 ± 0.99</td>
<td>2.63 ± 1.07</td>
</tr>
<tr>
<td>pERK1/actin</td>
<td>0.32 ± 0.05</td>
<td>0.53 ± 0.11</td>
</tr>
<tr>
<td>pERK2/actin</td>
<td>0.26 ± 0.05</td>
<td>0.49 ± 0.11</td>
</tr>
<tr>
<td>pAkt/total Akt</td>
<td>0.63 ± 0.06</td>
<td>0.66 ± 0.06</td>
</tr>
<tr>
<td>pSTAT3/STAT3</td>
<td>0.38 ± 0.05</td>
<td>0.68 ± 0.09</td>
</tr>
<tr>
<td>p-p38 MAPK/total p38 MAPK</td>
<td>1.34 ± 0.08</td>
<td>1.52 ± 0.20</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with control post-exercise; †P < 0.05; ††P < 0.01 and †††P < 0.001 compared with control pre-clamp; ‡P < 0.001 compared with exercised pre-clamp.

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**References**

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