



### Regular insulin administration increases muscle mass and exercise performance in otherwise healthy Sprague Dawley rats

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Insulin is a major metabolic regulator and is known to support healthy muscle growth through increased glucose uptake and stimulation of protein synthesis. These metabolic actions suggest insulin doping has the potential to improve muscle performance during exercise, but whether this occurs has not been investigated. Therefore, the primary aim of this study was to determine whether exogenous insulin administration alters muscle function and exercise performance in otherwise healthy rats.

Male Sprague Dawley rats weighing approximately 200g were entered into a four-week experiment. One group of rats was provided with access to running wheels overnight three times a week for the duration of the study as a means of regular exercise. The morning following each exercise bout (~8am), rats were injected intraperitoneally with either saline (0.9% NaCl; n=4), glucose (0.2g/kg; n=4), or insulin plus glucose (1U insulin + 0.2g/kg glucose; n=4). A second group of rats were housed in normal cages (no running wheels) and injected with saline (n=5), glucose (n=4) or insulin plus glucose (n=4) as above. At least 48 hours after the final exercise bout, field stimulation of the hindleg was performed to assess changes in hindleg muscle function/strength. To do this, rats were anaesthetised with pentobarbitone (84mg/kg) and one hindlimb was stimulated for 30 minutes, with contractions lasting 100ms at 2Hz and 30 volts. Force development was measured, and force output was recorded every five minutes for 30 minutes to assess muscle fatigue. At the end of the field stimulation, rats were euthanised, tissue were collected, weighed, and stored for further analysis.

In rats given access to exercise, running distance in saline injected rats increased from day 1 to day 28, but this was not statistically significant ( $2.9 \pm 0.5$ km/day vs  $11.2 \pm 4.3$ km/day,  $p = 0.097$ ). In rats given exogenous insulin injections, running distance also increased from day 1 to day 28 ( $5.0 \pm 2.4$ km/day vs.  $22.5 \pm 12.2$ km/day,  $p < 0.001$ ) and was higher than saline injected rats ( $p = 0.136$ ). In field stimulation experiments, peak force development was higher in insulin injected rats with access to running wheels than both sedentary rats injected with insulin ( $1461 \pm 6$ g vs.  $1529 \pm 9$ g,  $p < 0.001$ ) and saline injected rats with access to running wheels ( $1336 \pm 6$ g vs  $1529 \pm 9$ g,  $p < 0.001$ ). Calf muscle mass was assessed in all rats at the end of the intervention and expressed as percent of total body weight. Muscle mass in insulin injected rats that had access to running wheels increased slightly compared with saline rats that exercised ( $0.66 \pm 0.03$  vs.  $0.69 \pm 0.03$ ,  $p = 0.561$ ) and increased significantly compared to insulin injected rats that did not exercise ( $0.57 \pm 0.03$  vs.  $0.69 \pm 0.03$ ,  $p < 0.001$ ).

In summary, our data suggest that insulin has the potential to improve muscle mass and exercise performance in rats given access to running wheels regularly over four weeks. The mechanisms responsible for this increase in muscle mass and performance and whether these translate to humans remain to be determined.