Digoxin and exercise effects on Na^+,K^+-pump activity, content, isoform gene and protein expression in human skeletal muscle

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Digoxin is a specific inhibitor of the Na^+,K^+-pump and is used to treat patients with severe heart failure. In these patients, digoxin binds and blocks ~13% of Na^+,K^+-pumps in skeletal muscle and exacerbates muscle K^+ loss during exercise. Furthermore in heart failure patients there is no compensatory upregulation of Na^+,K^+-pump with chronic digitalisation. We have shown that exercise impairs Na^+,K^+-pump activity, whilst in isolated rat muscles, Na^+,K^+-pump inhibition leads to early muscle fatigue (Clausen, 2003). Hence, Na^+,K^+-pump function is likely to be important for skeletal muscle performance. However, the effects of digoxin on Na^+,K^+-pump content, activity, protein abundance or isoform expression in skeletal muscle of healthy individuals are unknown and were investigated here.

Ten active, but not well-trained healthy volunteers (9 M, 1 F) gave written informed consent. Exercise tests were performed after taking digoxin (DIG, 0.25 $mg.d^{-1}$) or a placebo (CON) for 14 d, in a randomised, counterbalanced, cross-over, double blind design, with trials separated by 4 weeks. Subjects performed incremental cycle ergometer exercise to measure VO_{2peak} and to determine 33, 67 and 90% VO_{2peak} work rates. On d 14 subjects completed 10 min cycling at each of 33% and 67% VO_{2peak}, then to fatigue at 90% VO_{2peak}. Muscle biopsies taken at rest, after 67%, 90%VO_2 peak and 3 h recovery were analysed for Na^+,K^+-pump content (^{3}H-ouabain binding site), maximal activity (3-*O*-methyfluorescein phosphatase, 3-*O*-MFPase), isoform protein abundance and mRNA expression. The Na^+, K^+-pump isoform (α_1-α_3, β_1-β_3) protein contents were measured on muscle extracts, using specific antibodies and western blotting, with isoform mRNA expression determined with real-time RT-PCR analysis.

Serum digoxin was 0.7±0.2 nM at d 13 and 0.8±0.2 nM at d 14 (Mean±SD). Despite this, muscle maximal Na^+,K^+-pump activity was unchanged by digoxin. However, Na^+,K^+-pump activity was decreased after exercise, by 13% and 11% at fatigue and 3 h post-exercise, compared to rest, respectively (P<0.05). Furthermore, there was no change in the Na^+,K^+-pump content with either digoxin or exercise (Rest Digoxin 373±95, Rest Placebo 368±75 pmol.g wet $weight^{-1}$). No significant change occurred with digoxin for mRNA expression of any of the α_1, α_2, α_3, β_1, or β_3 isoforms. However, digoxin increased the mRNA expression of the total α mRNA (sum of α_1, α_2, α_3) and the total β mRNA (sum of β_1 and β_3) at rest by 1.9- and 1.8-fold, respectively (P<0.05), suggesting an effect of digoxin on Na^+,K^+-pump gene expression. An exercise effect was observed on α_3 mRNA expression, being 2.1-and 2.4-fold higher at 3 h post-exercise than during exercise at 67% VO_2peak and fatigue, respectively (P<0.05). Similarly, β_3 mRNA expression at 3 h post-exercise was increased by 1.8-, 1.4- and 1.6-fold, compared to rest, 67% VO_2peak exercise and fatigue, respectively (P<0.05). Digoxin did not alter the protein abundance of any isoform in resting muscle. However, at 3 h post-exercise, the protein abundance was greater with digoxin than in placebo for both α_2 and β_3 (P<0.05). The β_1 protein expression was increased at 3 h post-exercise by 2.2-and 1.5-fold compared to during exercise at 67% VO_2peak and fatigue, respectively (P<0.05). Similarly, β_3 protein expression was increased at 67% VO_2peak and 3 h post-exercise compared to rest, by 1.5-and 1.6-fold, respectively (P<0.05).

In summary, digoxin treatment had only minimal effects on muscle Na^+,K^+-pumps in healthy individuals. Whilst Na^+,K^+-pump content, activity or isoform protein expression at rest were unchanged, the subunit total mRNA expression was increased with digoxin and a greater post-exercise protein abundance was found with digoxin for α_2 and β_3. The lack of reduction in pumps with digitalisation in healthy muscles suggests either that pumps were upregulated and/or that digoxin dissociation was increased.

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