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The role of an endogenous regulator of calcineurin in the regulation of glucose homeostasis

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We have studied the role in glucose homeostasis of a protein which acts as an endogenous inhibitor of the phosphatase calcineurin (CaN), which is important in the regulation of transcription and in protein phosphorylation associated with cell signalling. Treatment of post-operative transplant patients with immunosuppressants such as cyclosporin A and FK 506, which are CaN inhibitors, can induce diabetes (Weir & Fink, 1999). This occurs due to the inhibition of the CaN/NFAT transcription pathway which regulates pancreatic β-cell function including growth, proliferation and insulin secretion (Heit *et al.*, 2006). We are investigating the effect of increased expression of this protein and its role in the pathogenesis of diabetes. Transgenic mice were generated to overexpress this gene using human cDNA. Mouse pancreatic islets were isolated from dead mice by perfusion of the pancreas with *Collagenase P* following ligation of the bile duct at the entrance to the duodenum. mRNA expression levels of genes of interest were examined using quantitative real-time RT-PCR. Pancreatic islets were exposed *in vitro* to 16.7 mM glucose for 6 days and expression of our gene was found to increase 2.5 fold ($p < 0.05$). Using pancreatic islets from our transgenic mice, we are currently investigating the expression of genes regulated by CaN in β-cells such as those mutated in hereditary forms of monogenic type 2 diabetes (MODY) and other genes important in β-cell survival, proliferation and insulin production. Using an ACCU-CHEK® Performa glucometer we find that our transgenic mice develop age-dependent diabetes characterized by increased fasting blood glucose values of 5.8 ± 0.34 mmol/L ($n = 9$) at 60 days old compared to 4.2 ± 0.21 mmol/L ($n = 9$) in age and sex-matched wild-type mice ($p < 0.05$). Differences in fasting blood glucose values progressively increase with age, and body weight analysis confirms that these changes are not due to differences in body weight between the two genotypes. Glucose tolerance, measured by injecting 2 mg glucose/g body weight, is also reduced in our transgenic mice, with glucose values reaching peak levels of 27.5 ± 1.44 mmol/L ($n = 5$) after 60 minutes compared to 19 ± 1.27 mmol/L ($n = 5$) in wild-type mice ($p < 0.01$). This is not due to increased insulin resistance in our transgenic mice. These findings highlight a novel role of this gene in regulating glucose homeostasis, and its upregulation in hyperglycemia many be a causative link to the β-cell failure and hypoinsulinemia that occurs in the later stages of type 2 diabetes.

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Acute stimulation of fatty acid oxidation does not alter energy expenditure

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There is great interest in developing drugs that stimulate fat oxidation pathways as potential treatments for obesity and insulin resistance. However, it is unclear whether elevating fat oxidation will result in weight loss, unless there is also an associated increase in energy expenditure. To investigate this we examined whole-body energy metabolism in rats treated with compounds that stimulate fat oxidation *via* activation of the AMP-activated protein kinase pathway.

Energy expenditure (VO_2) and substrate oxidation (indicated by the respiratory exchange ratio [RER]) were measured using an Oxymax indirect calorimetry system. Rats were acclimatised overnight and at 10 am dosed with either vehicle (saline, $n = 12$), metformin (500 mg/kg, $n = 10$), AICAR (250 mg/kg, $n = 11$) or the mitochondrial uncoupler, dinitrophenol (DNP; 30 mg/kg, $n = 10$). In the 8 hour period following dosing, animals treated with metformin, AICAR and DNP all displayed increased fat oxidation compared with vehicle-treated animals, as evidenced by a reduction in RER (0.87-0.89 *vs* 0.92, $p < 0.001$). However during this time only DNP increased VO_2 (1957 ± 22 ml/kg/h, $p < 0.001$) compared with vehicle-treated animals (1633 ± 17 ml/kg/h), with no difference observed for metformin (1682 ± 18 ml/kg/h) or AICAR (1627 ± 16 ml/kg/h). As there was no change in energy expenditure with metformin and AICAR, our results suggested that the increase in fat oxidation was associated with a decrease in the oxidation of other substrates. To test this possibility, EDL muscles isolated from rats (anaesthetised with 60 mg/kg sodium pentobarbital i.p.) were treated with either AICAR (2 mM) or DNP (0.5 mM) and *ex vivo* palmitate and glucose oxidation was measured. Consistent with its effect to elevate energy expenditure, DNP treatment caused a significant increase in the oxidation of both palmitate (+41%, $p < 0.01$) and glucose (+77%, $p < 0.01$). In contrast, AICAR robustly increased palmitate oxidation (+45%, $p < 0.001$), but decreased glucose oxidation by 28% ($p < 0.01$).

These results suggest that in the absence of any change in energy demand, acute increases in fat oxidation are accompanied by a concomitant decrease in the oxidation of other substrates, and subsequently no change in energy expenditure.

Glucose and insulin modulate the expression and activity of the thioredoxin antioxidant system in cultured human skeletal muscle fibres

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The thioredoxin antioxidant system is composed of thioredoxin-1 and -2, the endogenous inhibitor thioredoxin binding protein-2 (TPB-2), and thioredoxin reductase. It is ubiquitously expressed and regulates redox-sensitive signalling pathways and cellular redox balance. Reactive oxygen species (ROS), glucose and insulin modulate the thioredoxin antioxidant system. Glucose increases TBP-2 expression in a dose dependent manner and TBP-2 mRNA transcripts are elevated in *vastus lateralis* muscles of prediabetics and diabetics. *In vivo* and *in vitro*, TPB-2 expression is inversely correlated to glucose uptake in peripheral tissues. Stressed cells secrete thioredoxin and thioredoxin plasma levels are increased in patients with glucose intolerance. Regulation of the thioredoxin antioxidant system is complex and has not been well studied in skeletal muscle. A time course study was completed to investigate how glucose and insulin affect the gene and protein expression and cellular localisation of the thioredoxin-1 and -2, TBP-2 and thioredoxin reductase in cultured skeletal muscle fibres. The effects of glucose and insulin treatment on muscle ROS production and deoxy-glucose uptake were also evaluated.

Primary skeletal muscle cell cultures were established from *vastus lateralis* skeletal muscle biopsies from healthy elderly donors (N = 6, 69 ± 3 years) and grown in 5% oxygen (normoxia for muscle tissue) and 5% carbon dioxide. At passage 4, muscle fibres at 4 days post-differentiation were treated with 5.5 mM (normal-glycaemia) or 15.5 mM (hyperglycaemia) glucose in the presence of 100 pM insulin for 2 h, 6 h, and 48 h for experimental analysis. Gene expression of thioredoxin-1 and TBP-2, but not thioredoxin-2, was increased following 48 h of treatment with 5.5 mM or 15.5 mM glucose plus insulin compared with 2 h and 6 h ($p < 0.05$). At 48 h, the increase in TBP-2 mRNA transcripts tended to be greater in cells exposed to hyperglycemia, whereas the increase in thioredoxin-1 gene expression was unaffected by glucose concentration. Similar to gene expression, at 48 h of treatment with 5.5 mM or 15.5 mM glucose plus insulin TBP-2 protein levels were significantly increased in the nucleus and cytoplasm compared to 2 h and 6 h ($p < 0.05$). At 48 h the increase in TBP-2 protein expression in the nucleus was independent of glucose concentration, whereas in the cytoplasm TBP-2 protein levels tended to be 2.5-fold higher in response to hyperglycemia. Cytoplasmic and nuclear thioredoxin-1 protein levels were similar following 2 h, 6 h, and 48 h of treatment. Neither glucose nor insulin affected cytoplasmic thioredoxin reductase protein levels following up to 48 h of treatment. Since hyperglycemia is associated with increased thioredoxin secretion, an ELISA assay was used to measure secreted thioredoxin in the media following 24 h and 48 h of treatment with 5.5 mM or 15.5 mM glucose plus 100 pM insulin. Secreted thioredoxin was higher following 48 h than 24 h of treatment ($p = 0.05$), which helps explain the possible discrepancy between thioredoxin-1 gene and protein expression. Media thioredoxin content was increased by ~10% in muscle fibres treated with 5.5 mM glucose plus insulin and by ~40% in muscle fibres treated with 15.5 mM glucose plus insulin. Thus, up to 48 h of chronic exposure to 100 pM insulin and 5.5 mM or 15.5 mM of glucose are needed to affect thioredoxin-1 or TBP-2 gene and/or protein expression and localisation in cultured skeletal muscle fibres. The increase in TBP-2 and thioredoxin-1 gene expression in response to chronic insulin exposure may be mediated by increased ROS levels. Muscle fibres were treated for 48 h with 5.5 mM or 15.5 mM glucose with or without 100 pM insulin and ROS production was assessed by monitoring the oxidation of 2′,7′-dichlorodihydrofluorescein-diacetate using a scanning fluorometer. In muscle fibres treated with 5.5 mM glucose, insulin increased ROS levels by ~30% ($p = 0.06$) and in cells treated with 15.5 mM glucose, insulin increased ROS levels by ~45% ($p < 0.05$). Given the effects of glucose and insulin on ROS levels and thioredoxin antioxidant system components, basal and insulin stimulated deoxy-glucose uptake was assessed following in 48 h of treatment with 5.5 mM or 15.5 mM glucose plus 100 pM insulin. Hyperglycemia reduced basal ($p < 0.05$) and insulin stimulated (100 nM; $p = 0.06$) glucose uptake. In conclusion, chronic rather than acute exposure to insulin and hyperglycaemia increased ROS levels in cultured muscle fibres and this was associated with altered thioredoxin signalling and reduced glucose uptake.

Role of nitric oxide in mitochondrial biogenesis in L6 myocytes

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Background and Aim. Skeletal muscle mitochondrial biogenesis is reduced in people with type 2 diabetes and exercise increases mitochondrial biogenesis. The signalling mechanisms associated with this increase in mitochondrial biogenesis by exercise are not fully understood. Contraction is associated with increases in calcium levels, adenosine monophosphate activated protein kinase (AMPK) activity and nitric oxide (NO) production, and there is evidence that all three may play a role in skeletal muscle mitochondrial biogenesis (Ojuka, 2004; Nisoli *et al.*, 2004). For instance, caffeine, which causes sarcoplasmic reticulum calcium release, and 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), an AMPK activator, both increase mitochondrial biogenesis in L6 myotubes (Ojuka, 2004). Neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS) are expressed in muscle, and it has been shown that NO donors increase mitochondrial biogenesis in L6 myotubes (Nisoli *et al.*, 2004). Since nNOS and eNOS are calcium dependent enzymes, and AMPK phosphorylates nNOS and eNOS, it is possible that AICAR and caffeine increase mitochondrial biogenesis, at least in part, *via* NO. Our aim was to determine whether caffeine and AICAR increase mitochondrial biogenesis in L6 myotubes, at least in part, *via* NOS, by using the NOS inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME).

Methods. L6 rat myotubes cells were cultured and differentiated in DMEM media with 2% horse serum (HS), 1% penicillin and streptomycin, on a 6-well plate coated with type I collagen at 37°C. 8 different treatments were presented: No treatment; 100μM L-NAME; 100μM S-nitroso-N-acetylpenicillamine (SNAP, NO donor) ± 100μM L-NAME; 2mM AICAR ± 100μM L-NAME; or 5mM caffeine ± 100μM L-NAME. All treatments were applied for 5 hours per day over 5 days in a DMEM media containing 10% HS, 5% FBS, 1% BSA, 1% Penicillin and Streptomycin, 1mM L-carnitine and 0.5mM oleic acid. Total protein was extracted 24 hours following the final day of treatment. Protein abundance of PGC-1α, COX I and COX IV relative to β-actin was determined *via* immunoblotting using commercially available antibodies and fluorescence. The experiment was repeated 3 times on 3 different occasions ending up with n=8 for each treatment.

Results. AICAR, caffeine and SNAP treatments increased PGC-1α protein expression by approximately 1.9-fold ($p < 0.01$), 1.8-fold ($p < 0.01$) and 1.9-fold ($p < 0.01$) compared to control cells respectively. COX IV protein expression was also significantly upregulated in all treatments with a 3-fold ($p < 0.05$), a 2.5-fold ($p < 0.05$) and a 3.3-fold ($p < 0.05$) increase following AICAR, caffeine and SNAP treatments, respectively. Furthermore, COX I protein significantly increased with AICAR and SNAP treatments but not with caffeine treatment. As expected, the increase in PGC-1α, COX I and COX IV protein in response to SNAP were not significantly reduced by NOS inhibition. However, NOS inhibition caused a small attenuation of the increase in PGC-1α, COX I and COX IV protein expression in response to AICAR and caffeine such that the increases were no longer significant.

Conclusion. Our data suggest that NO induces the expression of the proteins PGC-1α, COX I and COX IV in L6 myocytes, consistent with previously demonstrated activation of mitochondrial biogenesis in these cells (Nilsoli *et al.*, 2003). We found some evidence that AICAR and caffeine may both be activating mitochondrial biogenesis, at least in part, *via* NO. However, further investigations are required to fully understand these cellular interactions.

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Effects of simvastatin on skeletal muscle mitochondrial biogenesis and mitochondrial enzyme activities

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Cardiovascular Disease affects 3.5 million Australians, with elevated levels of low density lipoprotein (LDL) cholesterol being a major risk factor. Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, inhibit the rate limiting step in the mevalonate pathway and thus reduce cholesterol synthesis. Statins are the most prescribed drug in the world, with over 100 million prescriptions filled in 2004, in USA alone (Dirks *et al*., 2005). Although generally well tolerated, statins, particularly at high doses, can result in a skeletal muscle myopathy that ranges from muscle pain, stiffness, cramps and fatigue to rare cases of severe rhabdomyolysis. These events may affect up to 11% of patients (Bruckert *et al*., 2005). Preliminary evidence suggests that statin treatment in humans results in decreased skeletal muscle mitochondrial enzyme activity (Paiva *et al.*, 2005). Moreover, recent studies suggest that statins appear to preferentially affect fast twitch skeletal muscle fibre types (Westwood *et al*., 2005). Our aim was to investigate whether high dose statin administration would lead to an increase in mitochondrial biogenesis as a compensatory effect against decreased mitochondrial enzyme activity.

Male *Sprague Dawley* rats (5-6 weeks) were assigned to control (n=8), simvastatin 60 $mg \cdot kg^{-1} \cdot day^{-1}$ (n=8) and simvastatin 80 $mg \cdot kg^{-1} \cdot day^{-1}$ (n=8) treatment groups. 60 $mg \cdot kg^{-1} \cdot day^{-1}$ and 80 $mg \cdot kg^{-1} \cdot day^{-1}$ dosage was chosen as a previous study on rats was successful at inducing a statin associated myopathy after 10 days (Westwood *et al*., 2005). Rats were orally gavaged daily for 14 days with vehicle (5% methylcellulose) or vehicle + simvastatin (5 ml/kg). On day 15, rats were killed with an overdose of pentobarbitone (0.7ml pentobarbital sodium-325$mg \cdot ml^{-1}$) and the soleus, EDL and plantaris muscles were rapidly excised. Muscles were examined for activities of citrate synthase (CS) (citric acid cycle) and beta hydroxyacyl-coenzyme A dehydrogenase (β-HAD) (fatty acid beta oxidation pathway). A range of histological stains were utilised on soleus and plantaris (8μm sections) for examining general muscle morphology by haematoxylin + eosin (H+E). Stains indicating mitochondrial function including, succinate dehydrogenase (SDH), cytochrome c oxidase (COX), nicotinamide adenine dinucleotide tetrazolium reductase (NADH) and a combined stain of COX/ SDH were also utilised. Western blotting analysis of soleus, plantaris and EDL muscles were conducted to investigate mitochondrial biogenesis markers (PGC-1α, NRF-1 and T-fam). Upstream signalling pathways associated with mitochondrial biogenesis were also investigated (P-CREB, P-Akt, mTOR, P-AMPK, NOS, P-ACC).

The administration of simvastatin for 14 days did not alter *ad libitum* food consumption in either the 60 $mg \cdot kg^{-1} \cdot day^{-1}$ or 80 $mg \cdot kg^{-1} \cdot day^{-1}$ group ($p > 0.05$) compared to controls, however, normalised (to starting weight) body weights indicated that the 80 $mg \cdot kg^{-1} \cdot day^{-1}$ and 60 $mg \cdot kg^{-1} \cdot day^{-1}$ groups had 20.5% and 10.2% less weight gain, respectively, compared to control group ($p < 0.05$). Plantaris ($p < 0.05$) and EDL ($p < 0.01$) β-HAD activity was reduced in the 80 $mg \cdot kg^{-1} \cdot day^{-1}$ group. In addition, EDL β-HAD activity was reduced in the 60 $mg \cdot kg^{-1} \cdot day^{-1}$ group ($p < 0.05$). CS activity was significantly increased in plantaris 60 $mg \cdot kg^{-1} \cdot day^{-1}$($p < 0.05$) and 80 $mg \cdot kg^{-1} \cdot day^{-1}$ ($p < 0.01$) treatment groups. There was no change in β-HAD activity in soleus and no change in CS activity in soleus and EDL. Western blot analysis revealed no change in any mitochondrial biogenesis markers, or upstream protein phosphorylation involved in pathways responsible for the activation of mitochondrial biogenesis. Histological stains did not indicate altered mitochondrial SDH, NADH and COX and H+E did not reveal any abnormal morphology.

In conclusion, high dose statins induced a significant reduction in fatty acid beta oxidation enzyme function in the fast-twitch muscles but not in the slow-twitch soleus. This suggests fast-twitch muscles are more sensitive to statin administration compared to slow-twitch skeletal muscle. The administration of simvastatin had no effect on mitochondrial biogenesis markers or muscle morphology. These data show that although there was altered beta oxidation enzyme activity, it was not sufficient to cause an up regulation of mitochondrial biogenesis or alter muscle structure and function over a period of two weeks.

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Ingestion of a protein hydrolysate is accompanied by an accelerated *in vivo* digestion and absorption rate when compared with its intact protein

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Ageing is associated with a gradual loss of skeletal muscle mass, often referred to as sarcopenia. These age-related changes in skeletal muscle mass are attributed to a disruption in the regulation of skeletal muscle protein turnover. Recently published data suggests that the muscle protein synthetic response to food intake is reduced in the elderly (Koopman *et al.*, 2006). The latter is thought to represent a key factor responsible for the age-related decline in muscle mass in the elderly. Reductions in the rate and efficiency of protein digestion and subsequent absorption of the amino acids in the elderly could be responsible for the blunted muscle protein synthetic response to food intake (Boirie *et al.*, 1997). As a result, is has been suggested that the ingestion of a protein hydrolysate, as opposed to its intact protein, might accelerate protein digestion and absorption, increase plasma amino acid availability and, as such, augment the postprandial muscle protein synthetic response. However, evidence to support the proposed differences in digestion and absorption characteristics of a protein hydrolysate compared to its intact protein *in vivo* in humans remains lacking. In the present study, we tested the hypothesis whether the ingestion of a protein hydrolysate would accelerate protein digestion and absorption rates, resulting in a greater increase in plasma amino acid availability and muscle protein synthesis rate when compared with the ingestion of its intact protein.

Elderly men (64 ± 1 y, n = 10) were randomly assigned into 2 cross-over experiments in which they consumed a single bolus of 35 g specifically produced intrinsically L-[1-^{13}C]phenylalanine labeled intact (CAS) or hydrolyzed (CASH, PeptoPro®) casein. Furthermore, primed continuous infusions with L-[ring-$^{2}H_5$]phenylalanine, L-[1-^{13}C]leucine and L-[ring-$^{2}H_2$]tyrosine were applied, and blood and muscle tissue samples were collected to assess the appearance rate of dietary protein derived phenylalanine in the circulation, and the subsequent muscle protein fractional synthetic rate (FSR) over a 6 h post-prandial period.

Exogenous phenylalanine appearance rate was greater following ingestion of CASH when compared with CAS (p < 0.001). Splanchnic extraction was significantly lower in CASH *vs* CAS treatment (p < 0.01), and resulted in a 27 ± 6% higher exogenous phenylalanine appearance rate following CASH ingestion (p < 0.001). In accordance, plasma AA concentrations increased to a greater extent following the ingestion of CASH, with ~25-50% higher peak AA concentrations in the CASH *vs* CAS treatment (p < 0.01). Muscle protein synthesis rates, calculated from the oral tracer, averaged 0.054 ± 0.0004 and 0.068 ± 0.0006 %·h^{-1} in the CAS and CASH treatment, respectively (p = 0.10).

This study clearly shows that the ingestion of a protein hydrolysate, as opposed to its intact protein, accelerates protein digestion and absorption from the intestine and lowers splanchnic amino acid extraction. As a result, ingestion of a protein hydrolysate is an effective strategy to augment post-prandial plasma amino acid availability in vivo in healthy, elderly men, which may increase muscle protein synthesis.

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Local insulin-like growth factor binding proteins are essential for successful skeletal muscle regeneration

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Increasing insulin-like growth factor-I (IGF-I) levels in skeletal muscle hastens muscle fibre regeneration after injury (Schertzer & Lynch, 2006). The actions of IGF-I are modulated strongly by six IGF binding proteins (IGFBPs) at both the systemic and local tissue level (Duan & Xu, 2005). Treating mice with an IGF aptamer that inhibits the systemic actions of the IGFBPs and elevates 'free' endogenous IGF-I can similarly hasten muscle fibre regeneration (Schertzer *et al*., 2007). Since IGFBPs also have effects on skeletal muscle independent of IGF-I, we tested the hypothesis that local IGFBPs are required for successful skeletal muscle repair after injury, and that muscle-specific inhibition of IGFBPs would compromise muscle fibre regeneration.

Twelve-week old C57BL/6 mice were anaesthetised (100 mg/kg ketamine/ 10 mg/kg xylazine), and the tibialis anterior (TA) muscle of the right hindlimb injected with the myotoxin, Notexin, to cause complete degeneration of all fibres and initiate spontaneous muscle fibre regeneration. Muscles were excised at different times post-injury to examine transcript expression of the IGFBPs. In separate groups of mice, the TA muscles were injected with an IGF aptamer (100 μg in DMSO, NBI-31772; Calbiochem), an IGFBP-2 antibody (200 μg/ml; R & D Systems), or their appropriate vehicle control at 3 days post-injury. Muscle function and histology were evaluated at 10, 14 and 21 days post-injury using methods described in detail previously (Schertzer *et al*., 2007). The effect of the IGF aptamer on myoblast proliferation *in vitro* was also determined. C2C12 cells were plated in 6-well plates at a density of 2.5×10^4 cells/ml in growth medium (DMEM, 10% FBS, 1% antibiotics) supplemented with varying concentrations of IGF aptamer (0.1-10 μM). After 48 hrs cells were trypsinised and counted using a haemocytometer.

The various IGFBP transcripts were differentially expressed during muscle regeneration, suggesting that IGFBPs play different roles during the various phases of regeneration. Inhibiting all six IGFBPs with the IGF aptamer suppressed functional recovery (P_o), and reduced the proportion of muscle fibres at 10 and 14 days post-injury ($p < 0.05$). The IGF aptamer also dose-dependently inhibited the proliferation of C2C12 cells *in vitro*. Specific inhibition of IGFBP-2 with a blocking/neutralising antibody during the early stages of regeneration also affected regeneration with structural and functional recovery being compromised ($p < 0.05$, main effect). These data indicate that the other IGFBPs were unable to compensate for the lack of IGFBP-2, highlighting the importance of this IGF binding protein in successful muscle repair after injury.

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Human sarcopenia reveals an increase in SOCS-3 and myostatin and a reduced efficiency of Akt phosphorylation

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Sarcopenia is the general term for a reduction in muscle quality and function due to aging. This is evidenced by muscle atrophy, particularly in fast type II fibres, which is related to the reduction of maximal voluntary strength (Deschenes, 2004). Sarcopenia has important socio-economic consequences since falls are a major source of morbidity and mortality in the increasing population of older adults (Mahoney *et al*., 1994). We tested the hypothesis that sarcopenia in humans may be linked to increased levels of TNFα and SOCS3; the latter causing perturbations in growth hormone (GH) signalling and increasing myostatin (Liu *et al*. 2003). Consequently, this would result in a reduced phosphorylation and activation of Akt signalling (Morissette *et al*., 2006) and therefore inhibit muscle hypertrophy and activate signalling cascades associated with muscle atrophy. The aims of the present study were to determine whether age-related sarcopenia in humans was linked with perturbations in TNFα, SOCS3, GH, STAT5 and IGF levels as well decreases in the Akt/GSK/mTOR and increases in the Akt/FKHR/atrogene signalling pathways.

This study investigated the regulation of several genes and proteins involved in the activation of key signalling pathways promoting muscle hypertrophy including GH/STAT5, IGF-1/Akt/GSK-3β/4E-BP1 and muscle atrophy including TNFα/SOCS3 and Akt/FKHR/atrogene, in muscle biopsies from 13 young (20 + 0.2 years) and 16 old (age, 70 + 0.3 years) males.

In old, when compared with the young subjects, type II muscle fibre cross sectional area was reduced by 40-45% and TNFα and SOCS-3 were increased by 2.8- and 1.5-fold, respectively. Growth hormone receptor (GHR) protein and IGF-1 mRNA were decreased by 45%. Total Akt, but not phosphorylated Akt, was increased by 2.5-fold and this corresponded to a 30% reduction in the efficiency of Akt phosphorylation in the older subjects. Phosphorylated and total GSK-3β was increased by 1.5- and 1.8-fold respectively, while 4E-BP1 levels were unchanged. Nuclear FKHR and FKHRL1 were decreased by 73% and 50%, respectively, with no changes in the atrophy target genes, atrogin-1 and MuRF1. Myostatin mRNA and protein levels were elevated by 2- and 1.4-fold, respectively.

This is the first study to compare the regulation of several key signalling pathways known to control skeletal muscle hypertrophy, including GH/STAT5, IGF-1/Akt/GSK/4E-BP1, and skeletal muscle atrophy, including TNFα/SOCS3 and Akt/FKHR/atrogene, in muscle biopsies from young and old men. Sarcopenia appears to be associated with an increase in TNFα and SOCS-3 which may result in a reduction of GHR levels or sensitivity. The increase in total Akt protein, but not in Akt phosphorylation, in muscle from the older subjects, suggests an inefficiency in Akt activation, and by analogy, reduced protein synthesis. The increase in myostatin mRNA and protein levels in the older subjects suggests that myostatin is a prime candidate inhibiting Akt phosphorylation in the elderly.

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