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Muscle insulin resistance and sarcopenia

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Insulin resistance and sarcopenia: mechanistic links between common co-morbidities

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Abstract

Insulin resistance (IR) in skeletal muscle is a key defect mediating the link between obesity and type 2 diabetes, a disease that typically affects people in later life. Sarcopenia (age-related loss of muscle mass and quality) is a risk factor for a number of frailty-related conditions that occur in the elderly. In addition, a syndrome of 'sarcopenic obesity' (SO) is now increasingly recognised, which is common in older people and is applied to individuals that simultaneously show obesity, IR and sarcopenia. Such individuals are at an increased risk of adverse health events compared with those who are obese or sarcopenic alone. However, there are no licenced treatments for sarcopenia or SO, the syndrome is poorly defined clinically and the mechanisms that might explain a common aetiology are not yet well characterised. In this review, we detail the nature and extent of the clinical syndrome, highlight some of the key physiological processes that are dysregulated and discuss some candidate molecular pathways that could be implicated in both metabolic and anabolic defects in skeletal muscle, with an eye towards future therapeutic options. In particular, the potential roles of AKT/mammalian target of rapamycin signalling, AMP-activated protein kinase, myostatin, urocortins and vitamin D are discussed.

Associations between obesity, diabetes and skeletal muscle ageing

The International Diabetes Federation has estimated that there were 382 million people living with diabetes in 2013, with this number predicted to rise to 592 million by 2035, of which the significant majority would be of >40 years old (IDF 2013). Of these, 90% suffer from type 2 diabetes (T2D), which is characterised by both β-cell failure and resistance to the actions of insulin at the tissue level (insulin resistance, IR). As skeletal muscle is responsible for the majority of the body's postprandial glucose disposal, IR in this tissue results in substantial whole-body metabolic disturbances. However, it is likely that the metabolic disturbances associated with T2D are further exacerbated by the marked loss of skeletal muscle mass that can also be associated with these conditions (Park *et al.* 2009, Kim *et al.* 2010). Specifically, loss of muscle mass induces a 2–3% decline in basal metabolic rate per decade after the age of 20 years and 4% per decade after the age of 50 years, resulting from concomitant loss of mitochondrial volume density and oxidative capacity (Conley *et al.* 2000).

This loss of muscle mass in the elderly is also the principal factor responsible for 'frailty', a syndrome that has been clinically defined as the possession of three of: unintentional weight loss (10 pounds (~4.5 kg) in the past year), self-reported exhaustion, weakness (poor grip strength), slow walking speed and low physical activity (Fried *et al.* 2001). Loss of muscle mass (atrophy) is an inevitable, although somewhat modifiable, process that occurs with ageing (Sayer *et al.* 2008), when it is referred to as primary sarcopenia. In contrast, secondary sarcopenia can result from reduced physical activity or pathological causes, such as cachexia associated with malnutrition, organ failure, inflammatory disease, malignancy or endocrine disease (Cruz-Jentoft *et al.* 2010). Sarcopenia has been implicated as a risk factor for numerous adverse health outcomes associated with frailty, including weakness, falls and fractures, immobility, functional decline, disability and loss of independence in the elderly (Cruz-Jentoft *et al.* 2010, Batsis *et al.* 2014). It has also been associated with increased mortality in some prospective studies (Landi *et al.* 2013) but not others (Cesari *et al.* 2009).

The concept of 'sarcopenic obesity' (SO) was introduced to highlight a syndrome present in a group of older patients in whom obesity is accompanied by sarcopenia and IR (Baumgartner 2000). The prevalence of SO in a recent study of adults in the USA was estimated to be 18% of women and 42% of men with a mean age of ~70 years, with increased risks of mortality being demonstrated for either obese or sarcopenic women (Batsis *et al.* 2014). However, the significance of concurrent obesity and sarcopenia was really emphasised by a separate study of older people, which demonstrated a 2–3 times higher risk of developing disability associated with reduced activities of daily living in individuals with SO vs others with sarcopenia or obesity alone (Baumgartner *et al.* 2004). Currently, the characterisation of SO is as yet confined to a group of clinical and epidemiological observations, rather than being underpinned by defined common mechanisms (Bollheimer *et al.* 2012). Nevertheless, the apparently high prevalence of SO and its profound consequences for healthcare provision mandates that additional research is carried out into the mechanisms underpinning the syndrome, in order to establish whether the muscle loss and IR associated with SO are indeed inevitable co-morbidities and to identify more effective therapies. The recommended therapeutic interventions are confined to lifestyle changes and are of limited effect, as there are no currently licenced medications for the treatment of sarcopenia (Bouchonville & Villareal 2013).

In this review, we intend to highlight potential mechanisms and pathways that might underpin both sarcopenia and IR in ageing muscle, which may, in the future, be of interest as therapeutic targets for SO.

Clinical and functional delineation of sarcopenia and SO

The study of sarcopenia is still hampered by a lack of consensus regarding both definitions and techniques for assessment. Various diagnostic criteria have been used in studies to date; however, these have frequently been established purely on Gaussian distributions of measurements made in the test populations (Baumgartner *et al.* 1998, Janssen *et al.* 2002, Newman *et al.* 2003). More recently, two consensus statements have been issued aiming at defining sarcopenia objectively. The European Working Group on Sarcopenia in Older People stipulated that low muscle mass and either low muscle strength or physical performance should be present for a positive diagnosis to be made (Cruz-Jentoft *et al.* 2010), whereas the Society of Sarcopenia, Cachexia and Wasting Disorders defined 'sarcopenia with limited mobility' as lean appendicular mass/height^2 of two SDs or more below the mean for 20–30 year olds, with a walking speed of ≤1 m/s (Morley *et al.* 2011).

In addition to the challenges of defining SO, its assessment may be confounded by unchanging or increasing body mass index in older individuals due to increased adiposity, as this will mask any coincident loss of skeletal muscle mass. Therefore, evaluation of SO necessitates the careful assessment of body composition by other methods (Muller *et al.* 2012). For example, in a recent cross-sectional survey that considered risk factors for and associations with SO in Korean people >65 years, sarcopenia was defined as weight-adjusted dual-X-ray absorptiometry-determined appendicular skeletal muscle mass <2 standard deviations below the mean for healthy young adults (Ryu *et al.* 2013). In the separate longitudinal Korean Sarcopenic Obesity Study, the extent of visceral obesity at the start of the study was shown to correlate with the extent of loss of appendicular muscle over ~2 years of follow-up, indicating that there may be a causal component to this association. However, baseline muscle mass was unable to predict the development of obesity (Kim *et al.* 2014).

A further challenge to the definition and assessment of SO is that loss of muscle strength with age is substantially more pronounced than loss of mass, suggesting that the
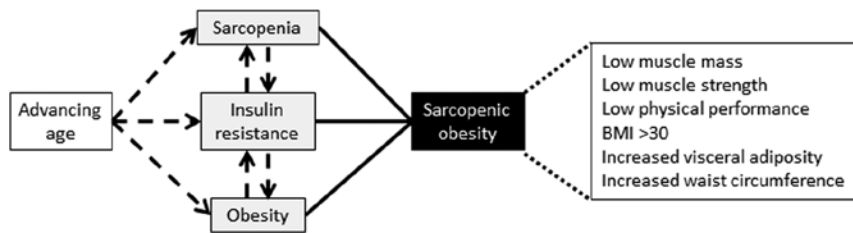
Figure 1
Clinical characterisation of sarcopenic obesity (SO). Sarcopenia, obesity and insulin resistance (IR) increase in prevalence with advancing age. When individuals display several of the clinical signs listed, they may be defined as showing SO. Dotted arrows indicate likely causative relationships and suggest that IR may be central to the syndrome.

close relationship between muscle cross-sectional area and mass in younger people is not maintained in sarcopenia (Klein *et al.* 2002). Moreover, this notion asserts that the loss of skeletal muscle quality is a significant contributor to age-related frailty (Goodpaster *et al.* 2006). Therefore, the term 'dynapenia' has been proposed as a more clinically relevant alternative to sarcopenia, to reflect the fact that loss of muscle function and mass is not reciprocally related and that the former is more relevant to an increased risk of adverse events, such as falls (reviewed in Manini & Clark 2012). Indeed, using a tertile-based classification of both muscle strength and adiposity in a small study population, it was shown that the presence of 'dynapenic obesity', but not SO, was predictive of increased risk of falls (Scott *et al.* 2014). However, as sarcopenia and SO are the terms that are best established in clinical use (Cruz-Jentoft *et al.* 2010), we have used these terms in this review. A general summary of the factors involved in SO is presented as Fig. 1.

Although numerous animal models have been established to study muscle atrophy associated with disuse (Bodine *et al.* 2001*a*), denervation (Muller *et al.* 2007), sepsis (Breuille *et al.* 1998), cancer cachexia (Temparis *et al.* 1994) and glucocorticoid administration (Gardiner *et al.* 1980), it seems that sarcopenia associated with ageing is mechanistically distinct from acute atrophy induced by such disease processes (Edstrom *et al.* 2006). Furthermore, the study of *bona fide* sarcopenia in animal models is hampered by the length of time animals must be housed in order to reach an age at which it is detectable (20–24 months for rodents) (Muller *et al.* 2007, Bollheimer *et al.* 2012, Bernet *et al.* 2014, Tardif *et al.* 2014, Fry *et al.* 2015). In addition, studies of animal models of SO demonstrating pathophysiological or molecular mechanisms pertinent to the development of the syndrome in humans have rarely been reported. However, some researchers have studied aged rats with diet-induced obesity (Bollheimer *et al.* 2012, Tardif *et al.* 2014), whereas obese Zucker rats are characterised by marked obesity, IR and generalised muscle atrophy (Nilsson *et al.* 2013), and thus may be useful for the study of SO at a younger age.

IR with respect to skeletal muscle glucose, lipid and protein metabolism

Peripheral glucose utilisation is reduced as part of the IR that develops with age (Gumbiner *et al.* 1992) and is substantially impaired in T2D (Cusi *et al.* 2000); however, protein turnover is also dysregulated. Skeletal muscle accounts for 40–50% of lean body mass in an adult human and therefore for the majority of whole-body insulin-stimulated glucose disposal (Baron *et al.* 1988, DeFronzo & Tripathy 2009). Thus, muscle mass is an important determinant of glucose and energy homeostasis (Wolfe 2006) and is determined by the balance between protein synthesis and breakdown in the tissue. An abundant supply of essential amino acids both inhibits proteolysis and stimulates protein synthesis (Castellino *et al.* 1987, Giordano *et al.* 1996, Cuthbertson *et al.* 2005), whereas at least in younger people, insulin has a predominant effect to inhibit protein catabolism in muscle (Fukagawa *et al.* 1985, Gelfand & Barrett 1987, Abdulla *et al.* 2016).

Insulin-mediated accretion of muscle mass has been ascribed to activation of p38 MAPK and mammalian target of rapamycin (mTOR)/p70S6 kinase, and thus stimulation of mRNA translation (Kimball *et al.* 1998, Guillet *et al.* 2004*a*, Fujita *et al.* 2007). In humans, it is most likely that these effects are mediated through enhanced amino acid availability or delivery through increased perfusion (Fujita *et al.* 2006, Timmerman *et al.* 2010), all of which have been reported to be impaired in aged muscle (Bell *et al.* 2005, Cuthbertson *et al.* 2005, Rasmussen *et al.* 2006, Groen *et al.* 2014). Thus, the concept of age-related 'anabolic resistance' has been proposed to describe the reduced muscle protein synthesis that occurs in response to nutrients (Cuthbertson *et al.* 2005) or insulin (Rasmussen *et al.* 2006, Fujita *et al.* 2009) and the reduced insulin-mediated suppression of proteolysis (Guillet *et al.* 2004*b*, Wilkes *et al.* 2009) that is associated with sarcopenia.

Interestingly, resistance to the anabolic action of insulin has been demonstrated in older individuals of normal muscle mass and may therefore precede the physical manifestations of sarcopenia (Rasmussen *et al.* 2006). Indeed,
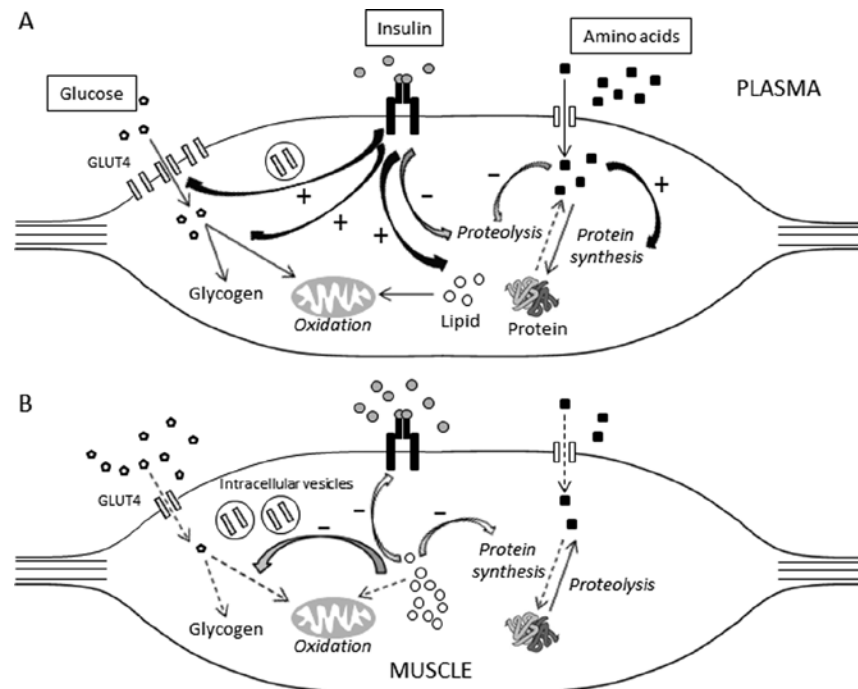
Figure 2
Insulin resistance and 'Anabolic resistance' in skeletal muscle and the role of intramyocellular lipid (IMCL) deposition. (A) Normal muscle of young adult. Protein synthesis predominates over proteolysis under stimulation by supply of essential amino acids and insulin. Optimal insulin sensitivity favours glucose disposal and oxidation of lipids. (B) Muscle of aged adult with sarcopenic obesity. Obesity-associated increases in intramyocellular lipid deposition, among other factors, causes impaired insulin signalling, protein synthesis and glucose metabolism. There is also a reduced anabolic response to exercise, amino acids and insulin. However, the extent of this resistance to insulin on protein, lipid and glucose metabolism varies between individuals. Straight arrows: metabolite flux. Broken straight arrows: reduced metabolite flux. Filled curved arrows: stimulatory effect. Open curved arrows: inhibitory effect.

it seems that differential IR with respect to glucose, protein and lipid metabolism can develop with ageing, IR and SO. For example, many older individuals are sensitive to insulin with regard to glucose metabolism, but not protein synthesis (Fujita *et al.* 2006). However, insulin, essential amino acids and resistance exercise are all less effective at inducing increases in muscle protein synthesis with increasing adiposity (Guillet *et al.* 2009, Nilsson *et al.* 2013, Murton *et al.* 2015). Metabolite fluxes within young, normal muscle and in muscle from older SO patients are shown in Fig. 2.

Adding further complexity, muscles of differing fibre type composition show contrasting sensitivity of both glucose and protein metabolism to insulin (Lillioja *et al.* 1987, Baillie and Garlick 1991). T2D is characterised by reduced numbers of predominantly oxidative type I fibres and increased numbers of predominantly glycolytic type II fibres (Oberbach *et al.* 2006), with the proportion of type I fibres correlating positively with insulin sensitivity (Stuart *et al.* 2013). Ageing also results in a preferential reduction in the size of type II fibres (Lexell 1995), and the net result is that

reduced mitochondrial activity (Johannsen *et al.* 2012) and IR (Groen *et al.* 2014, Tardif *et al.* 2014) may also be evident in muscle. In summary, it appears that IR, loss of muscle mass and changes in muscle fibre type all have the potential to independently or additively alter whole-body glucose homeostasis with ageing.

Clearly, defects that impair insulin-stimulated glucose disposal into muscle and thus negatively impact on whole-body glucose homeostasis will likely be compounded by concurrent sarcopenia, as in SO. It is known that interventions aimed at increasing muscle mass counter the development of IR (Dela *et al.* 1996); however, it is still not fully appreciated whether this is merely due to a proportionate increase in capacity for glucose disposal, or whether metabolic adaptation works synergistically with an increase in muscle mass. Recent studies in our laboratories have illustrated the potential for a dual effect, as manipulating bioavailability of single proteins in individual muscles, for example by inhibition of myostatin (MSTN; Cleasby *et al.* 2014), can result in enhanced glucose disposal on a per unit mass basis in

addition to increased muscle mass and therefore an enhancement in the total capacity for glucose disposal into the tissue.

Possible mechanisms: accumulation of intramyocellular lipid and intermuscular adipocytes

Both aberrant adipogenesis in muscles and excess intracellular lipid deposition have been associated with impaired muscle mass and insulin sensitivity. Increased adipocyte infiltration between muscle fascicles has been associated with both impaired gait (Scott et al. 2015) and IR (Albu et al. 2005). Furthermore, a longitudinal study demonstrated that progressive loss of muscle mass/quality was associated with increasing intermuscular fat in both ageing humans (Delmonico et al. 2009) and rats (Tardif et al. 2014), whereas another recent paper has shown that cultured intermuscular adipocytes produce pro-diabetic substances, providing evidence of a causal relationship (Laurens et al. 2016). Additionally, a mechanistic link between expansion of visceral adipose tissue and muscle atrophy has been suggested by the observation of reduced expression of contractile proteins in human myotubes co-cultured with visceral adipocytes from obese subjects (Pellegrinelli et al. 2015).

The impact of accumulation of intramyocellular lipid (IMCL) has been thoroughly studied, and there is a well-established association between IMCL and muscle IR and T2D. However, triacylglycerol, the main storage form of lipid, is not considered to be mechanistically linked with the development of IR (reviewed in Turner et al. 2014). Instead, the more bioactive derivatives ceramide and diacylglycerol have direct inhibitory effects on insulin signalling and metabolism (Chibalin et al. 2008, Ussher et al. 2010). Increased IMCL has also been associated with impaired muscle function in a number of studies. Lipid infusion results in reduced protein synthesis in response to both amino acids and insulin in healthy human volunteers (Stephens et al. 2015), whereas diet-induced obesity and ectopic deposition of lipid in muscle rather than adipose tissue are also associated with impaired protein synthesis in rodents (Anderson et al. 2008, Masgrau et al. 2012, Tardif et al. 2014). This is associated with increased phosphorylation of elongation factor 2B, a key mediator of ribosomal protein synthesis, in rodent muscle and a saturated fatty acid (SFA)/ceramide-induced increase in elongation factor 2α activation in cultured muscle cells (Tardif et al. 2014). However, the nature of the lipids is important because diets enriched in the SFAs impair muscle protein synthesis in rats than those based on unsaturated fatty acids (Tardif et al. 2011), in addition to their increased tendency to cause IR (Budohoski et al. 1993). The effect of increased IMCL on metabolite fluxes in the muscle of sarcopenic obese patients is shown in Fig. 2.

Inflammation in obesity and in muscle

Obesity is now recognised to be a subclinical inflammatory state characterised by increased infiltration of adipose tissue with pro-inflammatory cell types, most notably macrophages (Lumeng et al. 2007). Macrophage infiltration has also been demonstrated by a number of groups (Hevener et al. 2007, Fink et al. 2014), but not all (Tam et al. 2012), to be a feature of obesity-associated IR in skeletal muscle, and a synergistic interaction between macrophages and fatty acids that leads to impaired muscle insulin action has been reported (Varma et al. 2009). However, an alternative proposal is that dyslipidaemia associated with obesity activates cellular stress signalling pathways and thereby apoptosis and atrophy in skeletal muscle (Sishi et al. 2011). In particular, SFA can specifically induce pro-inflammatory macrophage activation and consequent p38 MAPK-mediated IR in cultured myotubes, an effect that is ameliorated by the UFA palmitoleate (Talbot et al. 2014). This role of p38 MAPK contrasts with its positive involvement in normal insulin-stimulated glucose disposal into muscle (Kimball et al. 1998), while in addition, loss of skeletal muscle satellite cell self-renewal is associated with impaired p38 MAPK α/β activation in aged muscle (Bernet et al. 2014), implying that non-specific inhibition of this kinase is unlikely to yield overall beneficial effects in vivo. The explanation for these apparently disparate roles of p38 MAPK may be distinct functional specificities of the four identified isoforms of the kinase (Brault et al. 2013), a possibility that has not yet been fully investigated.

Further evidence implicates the balance between M1- and M2-type macrophage levels in muscle function. Obesity is characterised by the accumulation of M1-type macrophages, at the relative expense of the M2 subtype (Lumeng et al. 2007). However, muscle expression of M1-related cytokines correlates positively with muscle mass and strength (Beenakker et al. 2013), whereas M2a-type macrophages accumulate in ageing muscle (Wang et al. 2015). Thus, the shift in macrophage phenotype with ageing may be in the opposite direction to that in insulin-resistant muscle.
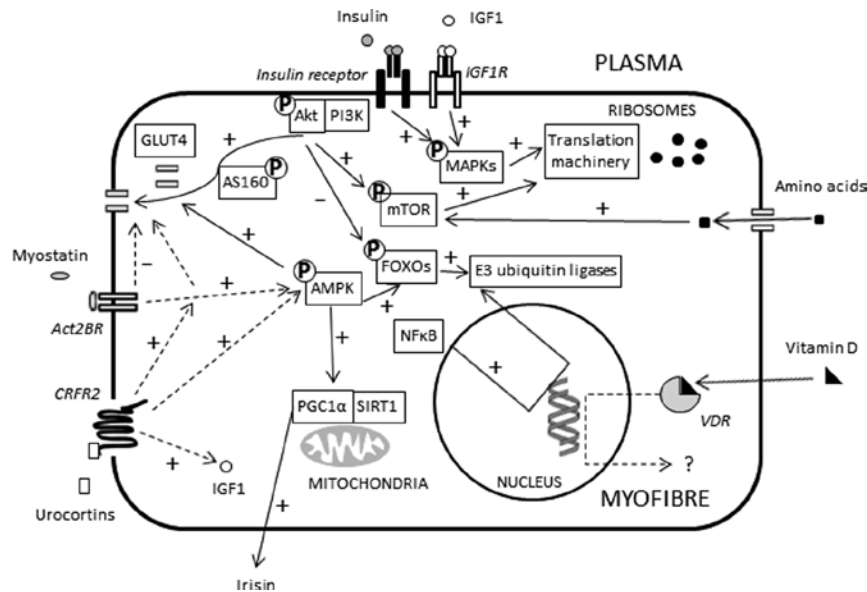
Figure 3

Roles of selected candidate molecular mediators in skeletal muscle glucose and protein metabolism. Published effects of insulin, insulin-like growth factor 1 (IGF1), amino acids, myostatin, urocortins and vitamin D on signalling pathways and effector machinery (glucose transporters, mitochondrial function, translation and activation of E3 ubiquitin ligases) relating to glucose and protein metabolism, as discussed in the text. Unmarked arrow: movement of molecules. Arrow with '+': direct stimulatory effect on expression or activity. Arrow with '−': direct inhibitory effect on expression or activity. Broken arrow: indirect effect. P indicates phosphorylation. ACT2BR, activin 2B receptor; AMPK, AMP-activated protein kinase; AS160, AKT substrate of 160 kDa; CRFR2, corticotrophin-releasing factor receptor 2; FOXO, forkhead transcription factor; GLUT4, glucose transporter 4; IGF1R, IGF1 receptor; NFκB, nuclear factor κB; PGC1α, peroxisome proliferator-activated receptor coactivator 1α; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositol 3-kinase; SIRT1, sirtuin 1; VDR, vitamin D receptor.

Skeletal muscle inflammation is also characterised by activation of the classical signalling pathway to the transcription factor nuclear factor κB (NF-κB). Chronic activation of this pathway causes profound atrophy in mouse muscle (Cai et al. 2004), while correspondingly it is activated by immobilisation of muscle (Bar-Shai et al. 2005), and targeted ablation of the NF-κB activating enzyme inhibitor κB kinase 2 (IκK2) improves skeletal muscle strength, maintains mass and promotes regeneration (Mourkioti et al. 2006). However, short-term muscle fibre-specific overexpression of IκK2 or the p65 subunit of NF-κB, sufficient to cause atrophy, does not impair insulin-stimulated glucose disposal (Polkinghorne et al. 2008), providing further evidence that these two phenotypes are not inextricably linked as part of a pro-inflammatory phenotype.

Other molecular pathways potentially mediating the development of both sarcopenia and IR

A summary of the roles of the molecules and pathways in glucose and protein metabolism discussed here is shown in Fig. 3.

Phosphatidylinositol 3-kinase/AKT and mTOR signalling

Insulin and insulin-like growth factor-1 (IGF1) have predominant metabolic and anabolic effects on muscle respectively. However, upon binding to their cognate receptors, both exert their effects by recruitment of intracellular adaptor proteins, including insulin receptor substrate 1, to the receptor complex and activation of phosphatidylinositol 3-kinase (PI3K). The resulting phosphoinositol triphosphate promotes phosphorylation of protein kinase B/AKT, which then phosphorylates substrates that orchestrate the various physiological effects of the two hormones. Increased glucose disposal is mediated predominantly through phosphorylation of AKT substrate of 160 kDa (TBC1D4) and TBC1D1, and thus movement of GLUT4-containing vesicles to the plasma membrane (Cartee & Funai 2009), as well as disinhibition of glycogen synthesis by phosphorylation of glycogen synthase kinase 3. AKT mediated activation of mTOR, and thus p70S6 kinase and eukaryotic translation initiation factor 4E-binding protein 1, is responsible for protein synthesis, and indeed amino acid-stimulated protein synthesis is also mediated through activation of mTOR. In parallel, AKT mediated inhibition of forkhead transcription factor (FOXO) activity reduces

expression of the E3 ubiquitin ligases that are principally responsible for mediating atrophy (atrogin-1/muscle atrophy F-box and muscle RING finger 1; Schiaffino et al. 2013).

Consequently, activation of proximal PI3K pathways would be expected to have dual positive effects on muscle size and metabolism. This is clearly illustrated by muscle overexpression of *Akt* in rodents, which causes both muscle hypertrophy and increased glucose disposal per unit muscle mass, with the predominant effects determined by the isoform used (AKT 1 vs AKT 2-predominant effects, respectively; Bodine et al. 2001b, Cleasby et al. 2007). Furthermore, defects in both components of the pathway were identified in leptin receptor-null (db/db) mice and obese Zucker rats. Treatment with the insulin-sensitising thiazolidinedione drug rosiglitazone also led to an improvement in muscle mass, leading to the suggestion that IR *per se* could cause muscle wasting through suppression of PI3K/AKT signalling (Wang et al. 2006, Katta et al. 2010). However, it is equally plausible that activation of the AKT-mTOR cellular signalling pathways following peroxisome proliferator-activated receptor (PPAR)γ stimulation by rosiglitazone impacts positively on both IR and muscle mass. The physiological relevance of this is unclear, as impairment of the AKT-mTOR pathway in muscle does not seem to occur naturally in ageing humans or mice (Sandri et al. 2013); however, it may yet represent a therapeutic target.

AMP-activated protein kinase (AMPK)

AMPK is a cellular energy sensor that is activated by an increased AMP:ATP ratio, leading to increased glucose and fatty acid uptake and oxidation in skeletal muscle (Koh et al. 2008). It plays a major role in coordinating energy use during exercise in muscle, but also mediates the long-term effects of exercise, through mitochondrial biogenesis. This process is initiated by AMPK-mediated activation of silent mating-type information regulator 2 homolog 1 (SIRT1) and PPAR coactivator-1α (PGC1α) (Mounier et al. 2015). AMPK has been extensively studied as a potential molecular target for the development of novel therapies for T2D (Coughlan et al. 2014), and recent work has identified an additional role for AMPK in muscle turnover/plasticity. It can protect against age-related functional and mitochondrial impairment by promoting myocyte macroautophagy, an essential process for cellular maintenance (Bujak et al. 2015). AMPK likely mediates the effects of adiponectin to promote macroautophagy (Liu et al. 2015), which partly mediates this adipokine's insulin-sensitising effect in muscle

(Patel et al. 2012). However, the effects of AMPK on muscle mass appear less favourable. A study of ageing rodents showed an inverse relationship between activating AMPK phosphorylation and load-induced hypertrophy (Thomson & Gordon 2005). Furthermore, AMPK stimulates myofibrillar protein degradation through increased FOXO expression (Nakashima & Yakabe 2007) and causes down-regulation of the mTOR pathway, thus restricting protein synthesis (Bolster et al. 2002). In addition, liver kinase b1, one of AMPK's upstream kinases, has been shown to limit differentiation of satellite cells (stem cells present in adult skeletal muscle) through the same mechanism (Shan et al. 2014). Thus, further studies are necessary to ascertain whether AMPK activation would have a net beneficial effect in individuals with both IR and sarcopenia.

Myostatin

MSTN is now well established as a central determinant of muscle size and mass, as demonstrated by the pronounced increases in muscle mass caused by gene-inactivating mutations in mice (McPherron et al. 1997) and by naturally occurring genetic loss-of-function variants in several domestic species (Hill et al. 2010). Consistent with this, its expression has also been shown to be increased in sarcopenia in some studies (Leger et al. 2008); however, this has not been a universal finding (Ratkevicius et al. 2011).

However, in addition to its effects on muscle mass, MSTN deficiency has more recently been shown to have beneficial effects on metabolism, adiposity and insulin sensitivity. Both *Mstn*-null mice (Guo et al. 2009) and mice treated with either soluble MSTN receptor activin receptor IIb (Akpan et al. 2009), which sequesters the mature peptide in the plasma, or the natural inhibitor follistatin-like 3 (Brandt et al. 2015) show increased muscle glucose utilisation and insulin sensitivity, associated with increased lean mass and decreased fat mass.

Genetic or pharmacological inactivation of MSTN increases activation of AMPK (Zhang et al. 2011), increases lipolysis and fatty acid oxidation in peripheral tissues, and also increases the expression of brown adipocyte markers in white adipose tissue (Zhang et al. 2012), providing a number of potential mechanisms for its metabolic activity. Importantly, we have also recently shown that short-term local impairment of MSTN action in rats by overexpression of the MSTN propeptide and sequestration of the active peptide enhances skeletal muscle glucose disposal to a greater extent than would be expected due to increased muscle mass alone, implying that additive or synergistic mechanisms are in operation.

The associated increase in glucose transporter (GLUT1 and GLUT4) protein levels may underpin the metabolic effects observed (Cleasby *et al.* 2014).

A number of modalities utilising inhibition of MSTN activity as a therapeutic approach have not yet borne fruit, although antisense-mediated destructive exon skipping is currently being evaluated. This has shown some promise in preserving muscle mass in a mouse model of Duchenne muscular dystrophy (Lu-Nguyen *et al.* 2015), and its metabolic effects are currently under investigation.

Urocortins

Urocortins (Ucns) are neuropeptide ligands for the corticotrophin-releasing factor receptor 2 (CRFR2) that are expressed not only in the central nervous system but also in peripheral metabolic tissues. There are particularly high levels of UCN2 and CRFR2 in skeletal muscle (Chen *et al.* 2006), implying that these 'stress regulators' play a role in muscle physiology. Furthermore, *Crfr2* expression was reduced on an average by 71 and 92% in the soleus and tibialis cranialis muscles, respectively, of aged mice 24 vs 3 month old; $n=6$, $P<0.001$).

Global knockout of either *Ucn2* or *Crfr2* produced mice that were resistant to diet-induced obesity and IR (Bale *et al.* 2003, Chen *et al.* 2006), the former also demonstrating increased muscle mass. Interestingly, however, global overexpression of *Ucn3* also resulted in mice with marked muscular hypertrophy. These mice had increased *Igf1* expression in muscle and also resisted the increased adiposity and metabolic abnormalities associated with feeding a high-fat diet, despite the lack of endogenous muscle *Ucn3* expression (Jamieson *et al.* 2011). In order to dissect the muscle-autonomous component of this phenotype further and to indicate whether CRFR2 might have the potential as a therapeutic target, we performed short-term overexpression of *Ucn3* in rat muscle and showed increased glucose disposal, associated with elevated levels of glucose transporter expression, and phosphorylation of both AMPK and insulin signalling intermediates, before any increased muscle mass was detectable (Roustit *et al.* 2014). Thus, a strategy to target CRFR2 may also have the potential to improve muscle mass and metabolism additively.

Vitamin D

There has recently been renewed interest in potential novel roles for vitamin D, including in the maintenance of muscle mass and insulin sensitivity, which has been provoked in part by the identification of a high prevalence of vitamin D deficiency among adults (Bates *et al.* 2011). Profound dietary insufficiency leads to impaired muscle strength as a result of hypophosphataemia in rats (Schubert & DeLuca 2010). However, epidemiological and intervention studies in humans have yielded contradictory results with regard to the role of vitamin D in muscle mass/function and metabolic endpoints. For example, insulin sensitivity has been reported to be either improved or unaffected by vitamin D supplementation (Talaei *et al.* 2013, Wongwiwatthananukit *et al.* 2013). Vitamin D supplementation was reported to increase muscle fibre size in immobile older women (Ceglia *et al.* 2013); however, a recent systematic review of studies showed a benefit of supplementation for individuals with vitamin D deficiency at the start of the trial in terms of improved muscle strength, but not in muscle mass or power (maximum force generated in minimum time; Beaudart *et al.* 2014). These contradictory findings might be a result of insufficient study power and/or imprecise subject selection in many instances.

Attempts to explain a hypothesised role for vitamin D in muscle on a molecular level have been few to date; however, knockout of the vitamin D receptor (*Vdr*) in mice resulted in reduced muscle size, impaired motor activity (Burne *et al.* 2006) and abnormal muscle development (Endo *et al.* 2003), In addition, *Vdr*-null mice are leaner (Narvaez *et al.* 2009), possibly due to increased uncoupling protein expression (Wong *et al.* 2009), but conversely have recently been shown to be insulin resistant, a phenotype that was shown to be mediated through increased muscle FOXO1 activation (Chen *et al.* 2016). Further work is needed to define the mechanistic links between vitamin D, the VDR and ageing-related phenotypes.

Additional therapeutic perspectives

Sarcopenia (Baumgartner *et al.* 1999, Raguso *et al.* 2006, Lee *et al.* 2007, Park *et al.* 2010, Genton *et al.* 2011, Scott *et al.* 2011, Szulc *et al.* 2004) and SO (Ryu *et al.* 2013) have been associated with low levels of physical activity in both cross-sectional and longitudinal studies, whereas exercise-based interventions are well established to improve both muscle mass and performance (Skelton *et al.* 1995, Vincent *et al.* 2002) and insulin sensitivity (Fujita *et al.* 2007) in aged individuals. However, it is clear that these interventions are of more use in the prevention, rather than treatment, of sarcopenia or SO and metabolic

dysfunction, as elderly individuals are often too frail to undertake the degree of exercise required to achieve a beneficial effect (Wolfe 2006), while they may also suffer from anabolic resistance.

In addition, it is clear that a profound reduction in dietary energy intake can have a remarkable effect to alleviate IR and T2D (Steven & Taylor 2015). However, the inevitable lean tissue mass that is lost using this approach alone renders it undesirable in the already sarcopenic elderly, unless concurrent exercise or appropriate nutritional supplementation is undertaken (Yoshimura et al. 2014, Verreijen et al. 2015). Although a comprehensive assessment of dietary approaches is out with the scope of this review, it is clear that motivation and compliance can frequently be a major limiting factor in the success of such lifestyle interventions (Evangelista et al. 2003).

In terms of current pharmacotherapy, androgen replacement in hypogonadal men is effective in increasing muscle mass; however, its use is as yet unproven in normal ageing individuals and is accompanied by undesirable side effects (Giannoulis et al. 2012). Nevertheless, androgen use may also be associated with an improvement in insulin sensitivity (Traish et al. 2009). The development of selective androgen receptor-modulating therapies may help mitigate many of these side effects. Preclinical and phase II trials of candidate drugs have demonstrated beneficial effects on insulin sensitivity as well as on muscle mass and strength (Gao et al. 2005, Dalton et al. 2011, Min et al. 2009).

One possible novel therapeutic approach might be to stimulate satellite cell activity and thus myofibre regeneration or replacement (Bernet et al. 2014), with the intention not only of improving muscle strength but also the capacity for glucose disposal. However, satellite cell ablation in adult mice did not affect age-related sarcopenia in a recent study (Fry et al. 2015), implying that strategies aimed at stimulating their fusion or proliferation may not be effective. Furthermore, chronic activation of pathways triggering muscle growth, such as the IGF1AKT pathway (Bellacosa et al. 2005), involves the activation of known oncogenes, and thus the risk of tumour development.

PGC1α is another molecular target that might be a promising candidate for alleviation of both metabolic inefficiency and sarcopenia. This molecule is regarded as a key mediator of the beneficial effects of endurance exercise. Increased expression of PGC1α in muscle improves metabolic fitness and prevents sarcopenia in ageing mice (Wenz et al. 2009), although it is unclear whether it promotes muscle strength in addition. Activation of PGC1α has been shown to result in increased secretion of a novel hormone, irisin, which alleviates IR in mice (Bostrom et al. 2012), although the significance of this finding for human biology has been questioned (Timmons et al. 2012, Raschke et al. 2013). Nevertheless, there is much interest in designing an 'exercise mimetic' drug, based on such a molecular target, which would improve both muscle mass/function and metabolism, to tackle obesity-related metabolic disorders. However, it would seem unlikely that an approach aimed at targeting a single mediator would be successful in human trials.

Conclusions and challenges for the future

This review has discussed current knowledge of the physiological and molecular mechanisms that govern both atrophy/sarcopenia and IR in skeletal muscle. We have aimed to highlight potential common ground between these mechanisms that could point to future development of novel therapies for SO in the elderly.

A number of challenges remain to address the deficiencies in our knowledge of this syndrome:
(i)   To establish a robust clinical definition of SO to enhance study design and thus permit improved comparability between clinical studies.
(ii) To establish whether sarcopenia and muscle IR are in fact inevitable co-morbidities, given the substantial overlap in the molecular pathways that are dysregulated in each.
(iii) To develop a more suitable animal model for SO to permit more practical mechanistic studies and preclinical therapeutic trials.
(iv) To further elucidate the key molecular pathways mediating both pathologies, permitting identification of molecular targets suitable for the development of combined therapies.

Addressing these priorities will hopefully provide a sounder footing to attempt more rational treatment of this common and debilitating condition.

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