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Myokines in skeletal muscle physiology and metabolism: Recent advances and future perspectives

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Abstract

Myokines are molecules produced and secreted by skeletal muscle to act in an auto-, para- and endocrine manner to alter physiological function of target tissues. The growing number of effects of myokines on metabolism of distant tissues provides a compelling case for crosstalk between skeletal muscle and other tissues and organs to regulate metabolic homoeostasis. In this review, we summarize and discuss the current knowledge regarding the impact on metabolism of several canonical and recently identified myokines. We focus specifically on myostatin, β-aminoisobutyric acid, interleukin-15, meteorin-like and myonectin, and discuss how these myokines are induced and regulated as well as their overall function. We also review how these myokines may serve as potential prognostic biomarkers that reflect whole-body metabolism and how they may be attractive therapeutic targets for treating muscle and metabolic diseases.

KEYWORDS

interleukin-15, metabolism, meteorin-like, myonectin, myostatin, β-aminoisobutyric acid

1 | INTRODUCTION

It was long thought that the function of skeletal muscle was propulsion and posture and that the sole interaction between muscle and bone was through mechanical loading for the support of body posture and to facilitate various movements.[1,2] For example, skeletal muscle possesses a remarkable ability to adapt to various physiologic conditions such as chronic disuse or exercise and bone mass follows muscle mass with unloading conditions such as bed rest, spaceflight or exercise.[3,4] However, over the past decade, there has been growing evidence of the regulation of muscle mass and the metabolism of distant tissues by release from skeletal muscle of soluble factors known as myokines.[1,4-6] Both high-intensity exercise and disuse can promote numerous physiological adaptations by increasing or decreasing the pool of available myokines.[1,4-6] A multitude of studies have demonstrated that myokines can regulate physiological and metabolic pathways in other tissues.[1,4-6]

The myokines that have been described to date are comprised of peptides, growth factors, cytokines and small organic acids. These molecules exert an effect, positive or negative, on muscle function and overall metabolism and include: myostatin (MSTN), the small organic acid β-aminoisobutyric acid (BAIBA), meteorin-like (METRNL), myonectin, various interleukins (IL-6, IL-8, IL-7 and IL-15), irisin, fibroblast growth factor 21 (FGF21), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), leukaemia inhibitory factor (LIF) and follistatin-like protein-1 (FSTL-1).[1,2,5,7,8] The diverse and growing repertoire of myokines provides the basis for crosstalk between skeletal muscle and other organs, such as adipose tissue, bone, liver, pancreas, kidney and brain.[1,4-6]

Skeletal muscle is the most abundant tissue in healthy individuals, constituting nearly 40% of body mass in young adults, and any endocrine activity would be expected to have a robust effect on physiology of the organism.[2,3] Muscle atrophy is linked with metabolic abnormalities associated with a variety of clinical conditions, including disuse, diabetes, obesity, renal failure, chronic obstructive pulmonary disease and cancer.[1,4-6] For example, individuals with spinal cord injury (SCI) experience extreme immobilization as a result of paralysis below the spinal lesion.[9] Because of the rapid loss in muscle, reduced basal metabolic rate and low physical activity, they are highly predisposed to obesity, insulin resistance and diabetes mellitus.[10-12] Obesity, defined as excess body fat, is associated with a wide range of diseases and metabolic abnormalities such as insulin resistance, systemic inflammation, and mitochondrial oxidative stress and dysfunction.[10,13-15] It is believed that individuals with SCI are at risk for developing a parallel cascade of deleterious changes.[10,13-15] The muscle atrophy seen in these conditions may lead to changes in the myokine profile and signalling via other soluble factors secreted by fat, bone or immune cells which, collectively, may provide an additional disruption of metabolism.[10,13-15]

Several studies have shown that in the context of obesity, the development of insulin resistance is influenced by inflammation of adipose tissue.[16-18] Adipose tissue, which is a potent endocrine organ, is mainly composed of three types of fat: brown adipose tissue (BAT), white adipose tissue (WAT) and "beige/brite" (brown in white) fat formed from WAT.[16-18] BAT activity has gained attention recently for it has been shown to be associated with increased energy expenditure, improved glucose and lipid homoeostasis and reduced body weight and fat mass.[16-18] Moreover, skeletal muscle and beige fat are functionally linked, as physical activity releases myokines which increase beige fat and insulin signalling.[16-18] Increasing skeletal muscle mass and activity are ways to increase energy expenditure and decrease adiposity with associated improvements in insulin resistance.[16-18]

The rising prevalence of insulin resistance in the general population highlights the need for a better understanding of its pathogenesis. Skeletal muscle is one of the main targets of insulin because it is responsible for more than 60% of insulin-stimulated clearance of glucose from the circulation; of note, muscle is one of the first tissues to develop insulin resistance and importantly, insulin action is greatly influenced by physical activity.[19-22] While the muscle responds directly to insulin by translocation of GLUT4 to the sarcoplasmic membrane, myokines likewise influence whole-body metabolism of glucose and lipids, as they have been repeatedly shown to act on adipose, liver, pancreas and intestine tissues.[19-24]

As described in more detail below, over 100 myokines have been identified by proteomics approaches and more than 250 putative myokines were reported based on GWAS and transcriptomics analyses. Several recent reviews have summarized knowledge regarding the more extensively studied myokines including MSTN, IL-6 and irisin.[2,3,21,25-27] One of the most intriguing biological activities of myokines is their ability to modulate insulin action and metabolism of specific cells which is almost certainly of significant biomedical importance. In this review, we summarize advances in our understanding of the several newly discovered myokines (BAIBA, IL-15, METRNL and myonectin) regulated by physical activity and their potential to modulate cellular metabolism; we also review relationships of MSTN to metabolism as MSTN is the most extensively studied of the myokines that modulate metabolism and insulin action. We also discuss the possibility that this selected group of myokines may serve as potential prognostic biomarkers which reflect changes in whole-body metabolism and are attractive therapeutic targets for strategies for treating muscle and metabolic diseases. The biomedical applications of these myokines will also be briefly discussed.[1,2,5,7,8]

2 | MYOSTATIN

Myostatin (MSTN), also known as growth and differentiation factor 8 (GDF8), belongs to the transforming growth factor β (TGF-β) superfamily. MSTN expression primarily occurs in skeletal muscle, with low levels of mRNA reported in adipose and cardiac tissues.[22,28-30] MSTN acts as a negative regulator of skeletal muscle growth and development by suppressing satellite cell activation, myoblast proliferation and myofibre hypertrophy.[22,28-30] MSTN's expression has been suggested to participate in muscle wasting during spinal cord injury (SCI), ageing, liver disease, COPD and cancer.[22,28-30]

The various components of the MSTN signalling pathway have been defined.[31-35] In brief, the activated MSTN peptide has been shown to bind to one of the two activin type II receptors (ActRIIB), which recruit, phosphorylate and thereby activate the activin type I receptors (ALK4 and ALK5), leading

to the phosphorylation by Alk4/5 and activation of SMAD2 and SMAD3. SMAD2/3 function as the final intracellular mediators for MSTN as they translocate into the nucleus and regulate the transcription of target genes such as FoxO, Bix3 and Runx1.[36] MSTN signalling is inhibited by SMAD7[37,38] which upon stimulation of cells by MSTN, interferes with the formation of the SMAD2/3 complex thereby preventing SMAD2/3 nuclear translocation.[37,38]

The regulation of MSTN and its function in the control of muscle mass have been studied in detail.[22,30,39,40] MSTN was implicated as a key regulator of muscle health after SCI using rat and mouse models.[10] In a rat model of complete SCI, expression of MSTN mRNA was not increased in whole muscle homogenate, but there was elevated ActRIIB mRNA expression and increased nuclear localization of the SMAD2/3, suggesting greater sensitivity to MSTN after SCI.[41] In a separate study, the miRNA profile of whole gastrocnemius homogenates after SCI-induced muscle paralysis was associated with TGFβ family signalling by bioinformatics analysis as dysregulated miRNAs target multiple components of myostatin signalling.[42] Another study investigated the effects of inhibition of MSTN with a soluble ActRIIB receptor after a complete SCI in mice.[43] The MSTN inhibitor reduced the amount of body mass lost compared to vehicle-treated animals over 8 weeks post-SCI.[43] The mice given the MSTN inhibitor had greater upper limb muscle masses but, interestingly, paralysed muscle mass was unaffected by the MSTN inhibitor.[43] These findings suggest the inhibition of MSTN may need to be used in conjunction with inhibitors of other signals of atrophy to prevent the loss of skeletal muscle which occurs with SCI-induced muscle paralysis.

Another form of skeletal muscle wasting is sarcopenia, the age-related decline in skeletal muscle mass and function.[19,29,44] There is disagreement in the literature regarding changes in MSTN expression during sarcopenia.[19,29,44] One proposals is that the effect of MSTN during ageing may be limited to specific stages of sarcopenia.[19,29,44] Further systematic studies are required to better understand the roles of MSTN in the progression of sarcopenia.

Our understanding of how MSTN may regulate tissues other than muscle is steadily increasing. Recent studies suggest that MSTN is a key regulator of whole-body metabolism, and consequently, is associated with the development of obesity, insulin resistance and type 2 diabetes mellitus (T2DM).[2,5,22,28,45,46] MSTN mRNA and protein levels are reported to be increased in muscle and adipose tissues of leptin-deficient obese mice as well as in wild-type mice fed a high-fat diet (HFD).[45] These observations are further supported by studies showing higher mRNA levels of MSTN in muscle from type 2 diabetics as well as in non-obese insulin resistant subjects.[47] MSTN knockout mice have demonstrated resistance to dietary-induced obesity and have improved metabolic phenotypes because of increased muscle mass and increased thermogenesis through beiging of white adipose tissue (WAT).[30,45,48] Moreover, inhibition of MSTN activity prevents the development of insulin resistance after chronic feeding of a high-fat diet in mice.[49,50] Furthermore, it was reported that the addition of exogenous MSTN elevates insulin resistance in both muscle and liver of mice and that the levels of MSTN decreased with aerobic exercise associated with an improvement in insulin sensitivity in humans.[51] Collectively, these data suggest that MSTN affects whole-body metabolism and MSTN levels correlate with obesity; the possibility that MSTN could initiate the development of insulin resistance and type 2 diabetes should also be considered.

The molecular mechanisms responsible for MSTN-mediated regulation of whole-body metabolism have not been well delineated. A clinically relevant target for understanding the molecular mechanisms of MSTN in insulin resistance and T2DM is glucose transporter 4 protein (GLUT4).[24,52-54] GLUT4 expression mediates insulin-dependent glucose uptake, is responsible for the majority of glucose transport into muscle and adipose cells in response to insulin, and is reduced during pre-diabetes and diabetes.[24,55] MSTN inhibits GLUT4 mRNA and protein expression and glucose uptake in C2C12 cells and that these actions are dependent on SMAD2/3 signalling.[56] Also, tissue-specific reductions in GLUT4 have potent metabolic effects on other tissues. For example, mice with muscle-specific GLUT4 deficiency display decreased insulin responsiveness in adipose tissue and liver, whereas those with adipose-specific GLUT4 reduction display muscle and liver insulin resistance.[23,54,57] Strikingly, overexpression of GLUT4 in the adipose tissue of muscle-specific GLUT4 deficient mice reversed glucose intolerance and diabetes.[23,55] Another study identified MSTN as an inducer of phosphotyrosine interaction domain containing 1 (PID1) protein in human muscle cells.[58-60] PID1 is well known for its role in the development of insulin resistance in both white adipocytes and muscle cells and MSTN activation of PID1 leads to the diminished insulin-stimulated tyrosine phosphorylation of IRS1.[58-60] As IRS1 is a critical mediator in the insulin-signalling pathway; the degradation of IRS1 is linked with impairment in further activation of IRS1/PI3K/AKT/GLUT4 signalling resulting in reduced glucose uptake by muscle cells.[58-60]

Because of the well-established impact of MSTN signalling on muscle mass, coupled with the fact that MSTN is produced almost exclusively by skeletal muscle and that its presence is not essential for life, pharmacological interventions that target the MSTN signalling pathway are relevant as potential therapies for muscle-wasting diseases and muscle metabolic diseases.[61] The majority of anti-MSTN drug candidates block the interaction between mature MSTN and its receptors through antibodies, ligand traps or overexpression of natural inhibitors such as follistatin.[61-63] Yet, because the receptor recognition surfaces of mature MSTN and other

TGFβ family members share a high degree of similarity, many of these MSTN-targeted biologics may cross-react with other members of the TGFβ family, typically GDF11 or Activin A.[61-64] However, TGFβ family prodomains share much less sequence conservation than the mature ligands. One group has developed human monoclonal antibodies that selectively bind the MSTN and GDF11 precursor forms; a subset of these antibodies inhibits MSTN proteolytic activation and prevents muscle atrophy, but does not bind the mature forms of myostatin, BMP9, BMP10 or TGFB1, or any form of GDF11 or Activin A in *vivo*.[64] One variant, muSRK-015p effectively increases muscle mass and function in mouse models of spinal muscular atrophy (SMA).[64] Furthermore, muSRK-015P treatment improves the cortical and trabecular bone phenotypes in these mice, making it an intriguing therapy for chronic muscle disuse as a way to protect muscle and bone health.[64]

3 | B-AMINOISOBUTYRIC ACID (BAIBA)

β-aminoisobutyric acid (BAIBA) is a recently discovered small molecule myokine produced by skeletal muscle during exercise.[7,25,65] BAIBA consists of two enantiomers, L-BAIBA and D-BAIBA. The production and degradation of L-BAIBA is catalysed by 4-aminobutyrate aminotransaminase (ABAT), a mitochondrial enzyme which is expressed mainly in the brain, kidney, liver and muscles.[66] D-BAIBA is produced in cytosol as an intermediate product of thymine degradation, and its degradation also occurs in mitochondria through the action of an enzyme found in liver and kidneys, alanine:glyoxylate aminotransferase 2 (AGXT2).[66] L-BAIBA (hereafter referred to as BAIBA) being the biologically active form, is produced by contracting muscle regardless of fibre type, gender and age.[65]

β-aminoisobutyric acid is under the control of the transcriptional coactivator peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α).[7,25,65] The increase in PGC-1α observed during physical exercise results in a concomitant increase in BAIBA serum levels.[7,25,65] The identification of BAIBA as a PGC-1α-mediated and exercise-induced signal has significant implications for our understanding of muscle biology and its protective role against the development of metabolic diseases. Increases in BAIBA have been demonstrated to have many beneficial effects which include protection against bone loss and diet-induced obesity, increased expression of brown adipocyte-specific genes in white adipocytes, increased mitochondrial biogenesis and fatty acid β-oxidation, and reduced insulin resistance and inflammation in skeletal muscle; L-BAIBA also reduced hepatic endoplasmic reticulum (ER) stress and glucose/lipid metabolic disturbance in mice with type 2 diabetes.[7,25,65,67,68]

Emerging evidence links BAIBA to osteocyte function. Osteocytes are cells which derive from a subpopulation of osteoblasts that are encased within the mineralized bone matrix and orchestrate bone remodelling thus serving as a critical determinant of bone mass.[10,12,69] In patients with SCI, rapid and extensive sublesional bone loss develops because of an uncoupling of bone remodelling in favour of bone resorption.[12,69] The bone-derived protein, osteocalcin, produced by osteoblasts, is typically reduced chronically after SCI and thought to play an integral role in the link between bone and metabolic health.[70] Therefore, osteocyte viability is crucial to bone health, since the loss of osteocytes directly impairs the capacity for bone to respond to changes in mechanical loading.[10,12,69] The discovery of novel mechanisms that mediate organ crosstalk, such as muscle-derived BAIBA, is a significant step toward understanding humoral interactions between these tissues.[33,65,71]

Kitase et al have elegantly described the physiological role of BAIBA on osteoblasts as precursors of osteocytes using the osteoblast cell model, MC3T3-E1.[65] They have shown BAIBA to function as an osteocyte protective factor against reactive oxygen species (ROS) by blocking mitochondrial fission and preserving mitochondrial integrity upon exposure to reactive oxygen species. In these studies, BAIBA was able to prevent apoptosis and loss of bone following hindlimb unloading.[65] Several membrane receptors for BAIBA were proposed, including G protein-coupled receptors (GPRs), such as Mas-related GPR type D (MRGPRD). For example, Kitase et al discovered that BAIBA signals through the MRGPRD.[65] Furthermore, these authors hypothesized that the relative decline in MRGPRD expression in osteocytes with age predicts that the capacity of BAIBA to promote osteocyte survival would also decline with age. Consistent with this prediction, MRGPRD is highly expressed in osteocytes from young but not older mice.[65] Thus, the protective effect of BAIBA was lost with age, not because of loss of the muscle capacity to produce BAIBA but rather to the downregulation of MRGRPD expression in osteocytes with ageing.[65] These findings have implications for understanding and correcting the attenuated effect of exercise on bone with ageing.[25,65] Moreover, further investigation is warranted into the molecular mechanisms by which BAIBA influences bone and bone-derived factors, such as osteocalcin, to influence metabolic health.

4 | INTERLEUKIN-15

Interleukin-15 (IL-15) is a member of the IL-2 superfamily and has attracted considerable attention as a myokine whose expression contributes to the beneficial impact of physical exercise on muscle energy metabolism. High concentrations of serum IL-15 show potential to regulate mitochondrial function, lipid deposition and mobilization, myofibre

composition, BAT function, obesity, insulin sensitivity and other metabolic disorders.[72,73]

Physical activity is known to increase mitochondrial content in skeletal muscle, counteracting age-related decline in muscle function and protecting against metabolic and cardiovascular complications.[72,73] Acute IL-15 treatment of L6 myotubes mimicking circulating (1-10 pg/ml) and muscle interstitial (100 pg/ml-20ng/ml) IL-15 expression levels, enhances muscle glucose uptake and mitochondrial oxidative functions in association with activation of the AMPK pathway, a nutrient and exercise related pathway.[74] However, chronic IL-15 exposure resulted in a U-shaped effect on mitochondrial oxidative functions; electron transport chain (ETC) formation was increased with low IL-15 levels but decreased at higher IL-15 concentrations.[74] It was also shown that IL-15 exerts protective effects against H_2O_2-mediated oxidative stress and enhances mitochondrial activity through a PPARδ-dependent mechanism in skeletal muscle cells.[75,76] While the acute role of IL-15 seems beneficial, further investigations on the effects of IL-15 should be performed to understand if sustained elevation of IL-15 levels results in adverse effects, such as generating additional systemic inflammation. A similar U-shaped profile of activity has been demonstrated with IL-6; IL-6 expression is increased within an acute timeframe after exercise which is beneficial, while chronic elevations of IL-6 are most likely detrimental.[2] Taken together, these results suggest that when released during muscle contraction, IL-15 may act in an autocrine/paracrine fashion that contributes to the beneficial effects of exercise on skeletal muscle.

Several lines of evidence suggest that IL-15 is capable of blocking HFD-induced obesity, glucose intolerance and insulin resistance.[72,73,77,78] It is well known that excessive lipid deposition in the liver is one of the primary physiological alterations related to obesity.[72,73,77,78] IL-15 downregulates the accumulation of lipids in preadipocytes and reduces WAT mass partly through stimulation of adiponectin secretion without compromising food intake in rodents. These outcomes suggest that IL-15 may be a potent mediator of exercise-induced muscle-fat crosstalk.[78,79] Intriguingly, IL-15 treatment is capable of preventing HFD-stimulated fatty liver.[80-82] Hydrodynamic delivery of a plasmid expressing IL-15 to HFD-fed mice reduced liver weights and decreased liver triacylglycerol (TAG) levels compared to HFD-fed control.[80-82] The beneficial role of IL-15 on the prevention of obesity may also include its effects on glucose homoeostasis and insulin resistance.[80-82] In HFD-mice, hydrodynamic delivery of a plasmid expressing IL-15 resulted in improved insulin sensitivity, as evidenced by reduced fasting glucose levels and decreased blood insulin levels.[83,84] Importantly, obese subjects have lower circulating IL-15 relative to lean individuals and circulating IL-15 is inversely related to trunk fat mass and per cent body fat; these findings support the possibility that there exists a skeletal muscle–blood–adipose IL-15 axis in humans associated with obesity.[85] In addition, it has been demonstrated that the production of IL-15 induced by physical activity may help to attenuate or even suppress the negative effects of tumor necrosis factor-alpha (TNF-α) in obese and diabetic subjects, in whom a low-grade systemic inflammatory state exists, by regulating blood glucose and lipid levels and diminishing proteolysis in striated muscle.[86-88] Such "low grade systemic inflammation" is characterized by a two- to threefold increase in the systemic concentrations of cytokines such as TNF-α, an inflammatory marker noted as critical in the development of insulin resistance and muscle wasting in diabetic patients and decreased production of adiponectin.[86-88] In contrast, overexpression of IL-15 stimulates adiponectin secretion, lowers the concentration of circulating leptin with a concerted loss of intra-abdominal fat mass and counteracts TNF-α action on muscle protein degradation.[86-88] It is important to note that while these findings support a role for IL-15 in determining insulin sensitivity and control of obesity, the molecular mechanism(s) by which Il-15 ameliorates the detrimental effects of TNF-α have not been fully established.

Collectively, the data indicate that increasing IL-15, either exogenously or endogenously by exercise, may be a promising approach in the prevention and treatment of obesity and T2DM; however, the underlying molecular mechanisms need to be more clearly defined. In C2C12 myotubes, IL-15 increases basal and insulin-stimulated glucose uptake, GLUT4 translocation and GLUT4 mRNA levels.[74,89] It has been reported that the signalling pathway for IL-15, induces hypoxia-inducible factor-1 α (HIF-1α) expression via JAK3/STAT3 activation. The activation of HIF-1α expression leads to an increase in GLUT4 translocation from the cytoplasm to the plasma membrane, accounting for the increase in glucose uptake in cultured myocytes in vitro and in isolated skeletal muscle ex vivo.[73,74,76,89] Although this line of evidence provides some insight into the molecular mechanism behind the stimulatory effect of IL-15 on insulin action, further studies are essential to firmly establish the unknown factors involved in the IL-15/JAK3/STAT3 signalling axis. Moreover, IL-15 has obvious relevance to understanding of, and to the potential treatment and/or prevention of, metabolic disorders, such as insulin resistance or diabetes.[73,74,76,89]

5 | METEORIN-LIKE (METRNL)

Meteorin-like (METRNL; also known as subfactin) is a recently identified myokine induced upon exercise and cold exposure. METRNL is known to increase systemic energy expenditure, induce white adipocyte beiging, improve

glucose tolerance and insulin sensitivity, and promote anti-inflammatory gene programs in monocytes, adipocytes and skeletal muscle.[90-92]

It has been reported that mRNA expression and release of METRNL increases in white adipose tissue during acute cold exposure and in muscle after acute bouts of exercise. Moreover, an increase in PGC-1α-mediated signalling stimulates increased METRNL expression.[91] In a muscle-specific PGC-1α (isoform PGC-1α4) transgenic mouse, METRNL mRNA and protein were upregulated by about four- and eightfold, respectively.[91] PGC-1α is known to be associated with mitochondrial biogenesis and resistance to muscle atrophy, and downstream expression of METRNL is likely to be associated with the beneficial outcomes associated with upregulation of PGC-1α4.[91] The role of METRNL in alleviating insulin resistance and lipid-mediated inflammation in skeletal muscle via AMP-activated protein kinase (AMPK) or peroxisome proliferator-activated receptor δ (PPARδ) has recently been investigated.[92] METRNL treatment increased AMPK phosphorylation and PPARδ expression both in mouse skeletal muscle and 2 week post-induction of differentiation in C2C12 cells.[92] Furthermore, METRNL administration rescued glucose intolerance and reduced HFD-induced body weight gain in mice without affecting caloric intake.[92] These results suggest that body weight loss may be caused by ß-oxidation in adipose tissue and beiging of white adipose tissue through PGC-1α4.[92] Supporting evidence has shown METRNL mRNA expression was increased in the resting state after 20 days of short-term high-volume high-intensity interval training (HIIT), and HIIT resulted in the elevated levels of potential fat browning myokines in skeletal muscle.[93] Taken together, these studies suggest that METRNL may offer exercise-mediated protection against metabolic disorders.

Outside of muscle cells, adipocyte-specific overexpression of METRNL attenuates insulin resistance whereas adipocyte-specific reduction of METRNL expression exacerbates insulin resistance in HFD-fed mice.[94,95] Moreover, it has been reported that the circulating levels of METRNL are lower in obese patients.[96] A study by Lee et al demonstrated lower levels of MERNTL in newly diagnosed patients with T2DM and pre-diabetes subjects in comparison to controls.[97] A similar observation was reported in patients with T2DM as well as its association with the parameters of glucose and lipid metabolism and markers of the endothelial function.[96]

However, there are some conflicting data regarding the expression levels of METRNL in those with T2DM and obesity. One group has reported elevated levels of METRNL in individuals with T2DM compared to non-diabetic controls while another group has shown that METRNL expression in the adipose tissue is higher in obese children in comparison to lean children.[98,99] Further studies are required to address these discrepancies.

6 | MYONECTIN

Myonectin, also known as C1q tumour necrosis factor-α-related protein isoform 15 (CTRP15), is a myokine homologous to adiponectin with respect to domain structure, and it is expressed and secreted predominantly by skeletal muscle.[8] Whether there is a linkage between myonectin expression and exercise remains unclear. Increased myonectin expression was observed in skeletal muscle and in circulation in mice given access to a running wheel for 2 weeks as compared to mice with access to a locked wheel.[100] It remains uncertain whether increased expression was because of acute exercise or to the increased food consumption following the acute bout of exercise, which would mimic a "re-feeding" state that elevates myonectin expression.[8]

Several studies do indicate that myonectin expression and levels are regulated by glucose and fats and that one function of myonectin is to regulate hepatic fatty acid uptake and oxidation.[8] Myonectin expression and circulating myonectin levels were increased 2 hours after overnight-fasted mice were given a bolus of glucose or emulsified Intralipid® (an IV fat emulsion).[8] In a separate cohort of mice, the administration of recombinant myonectin lowered circulating levels of free fatty acids (FFAs) by promoting glucose and fatty acid uptake and oxidation in both the liver and adipose tissue.[8] Furthermore, in obese and diabetic animals, circulating myonectin levels increased significantly.[50,101] Based on these findings, it has been proposed that myonectin may function as a nutrient-sensing myokine that simultaneously determines responses to and is regulated by nutritional state.[8,50,82] Moreover, these studies suggest that myonectin may be a potential candidate for a role in the pathogenesis of insulin resistance and T2DM. In support of these proposals, higher plasma myonectin levels were seen in individuals with impaired glucose tolerance (IGT) and in T2DM patients compared with normal subjects in cross-sectional and interventional studies.[50] Additionally, circulating myonectin was higher in T2DM patients than in pre-diabetic subjects, indicating a progressive increase in myonectin levels from a pre-diabetic to diabetic state, and providing additional evidence that myonectin is elevated in obese individuals.[50] The gradual elevation in myonectin with glucose intolerance and the consistency in elevated myonectin levels with obesity makes myonectin an intriguing prospect as a circulating biomarker of adiposity and obesity-related metabolic diseases.[50] In a separate study, young individuals were given an oral glucose tolerance test to assess the effects of rapidly increasing glucose and insulin levels on circulating myonectin at rest as well as during a euglycemic-hyperinsulinemic state and after a 45 minutes bout of treadmill exercise at 60% of VO_2 peak.[50] Interestingly, circulating

myonectin levels were not affected by any of the experimental conditions in healthy individuals.[50]

Collectively, the data suggest that elevated levels of myonectin were observed in individuals with glucose intolerance as well as those with obesity.[8,50] Greater levels of myonectin may be a useful biomarker for the onset of diabetes as well as a potent target for studies that focus on identifying future therapies to mitigate insulin resistance.[8,50,101] However, further studies are needed to clarify the molecular mechanisms involved in myonectin regulation.

7 | MYOKINES AS BIOMARKERS

The continued search for biomarkers for diagnosis, prediction of prognosis, and therapeutic targets for muscle atrophy and metabolic diseases has produced a list of potential candidate genes for further biological validation. For example, more than 100 myokines have been identified by different proteomics approaches, but these techniques may not detect all myokines. mRNA sequencing has been employed as an alternative, unbiased approach to identify and study gene expression of secreted myokines and is a useful approach by which to expand our knowledge of the skeletal muscle secretome.[102-104] One study used global mRNA sequencing to detect genes that were upregulated in human skeletal muscle as an outcome of acute and/or long-term exercise.[103] In total, the group detected almost 250 genes encoding putative myokines that were upregulated after acute and/or long-term training.[103] Some transcripts encode well-known myokines, such as irisin, BDNF and IL-6, which have been described extensively and are reviewed elsewhere, [2,3,5,102-104]; others have not been studied previously in skeletal muscle and may encode novel myokines.[102,103]

In addition, Genome-wide association studies (GWAS) have been instrumental in the identification of a multitude of pleiotropic candidate single-nucleotide polymorphisms (SNPs)/loci associated with traits related to skeletal muscle physiology and metabolism, with multiple loci overlapping between the traits.[102,105] For example, some studies have examined relationships between SNPs and muscle strength in GWAS studies. Muscle strength, measured by isometric hand grip strength, is a widely used proxy of muscular fitness, an established marker of frailty, and predictor of physical decline and functional impairments. Higher grip strength has been found to be prognostic of recovery after hip fracture surgery while lower grip strength is associated with the reduced quality of life in the ageing population.[102,105] To investigate the genetic determinants of variation in grip strength, two grip-strength GWAS have been reported to date, yielding 64 muscle strength-related loci identified within the UK Biobank.[102,105] It was

TABLE 1 Summary table of myokine regulation of skeletal muscle physiology and metabolism

Myokines	Alias	Production site	Functions in muscle biology and metabolism
Myostatin	MSTN; GDF8; MSLHP	Almost exclusively in the skeletal muscle; Some expression in adipose, and cardiac tissues	Negative regulator of muscle mass; Skeletal muscle wasting; Development of obesity, insulin resistance and T2DM
β-aminoisobutyric acid	BAIBA (L-BAIBA and D-BAIBA)	Skeletal muscle	Exercise factor; Increases expression of brown adipocyte-specific genes in white adipocytes; Protects against bone loss, obesity, insulin resistance; Increases mitochondrial biogenesis and fatty acid β-oxidation; Improves inflammation in skeletal muscle and reduces hepatic endoplasmic reticulum (ER) stress
Interleukin-15	IL-15	Skeletal muscle	Exercise factor; Increases hypertrophy; Enhances muscle glucose uptake and mitochondrial oxidative function; Increases insulin sensitivity and reduces obesity; Promotes anti-inflammatory gene programs
Meteorin-like	METRNL; Subfactin; Cometin; Glial Cell Differentiation Regulator	Skeletal muscle; Adipose tissue	Exercise factor; Increases expression of brown adipocyte-specific genes in white adipocytes, Improves glucose tolerance; Promotes anti-inflammatory gene programs in obese/diabetic mice
Myonectin	C1q tumour necrosis factor-α-related protein isoform 15 (CTRP15); C1QTNF5	Skeletal muscle	Negative regulator of muscle mass; nutrient-sensing myokine Increases glucose intolerance Circulating biomarker of adiposity and obesity-related metabolic diseases

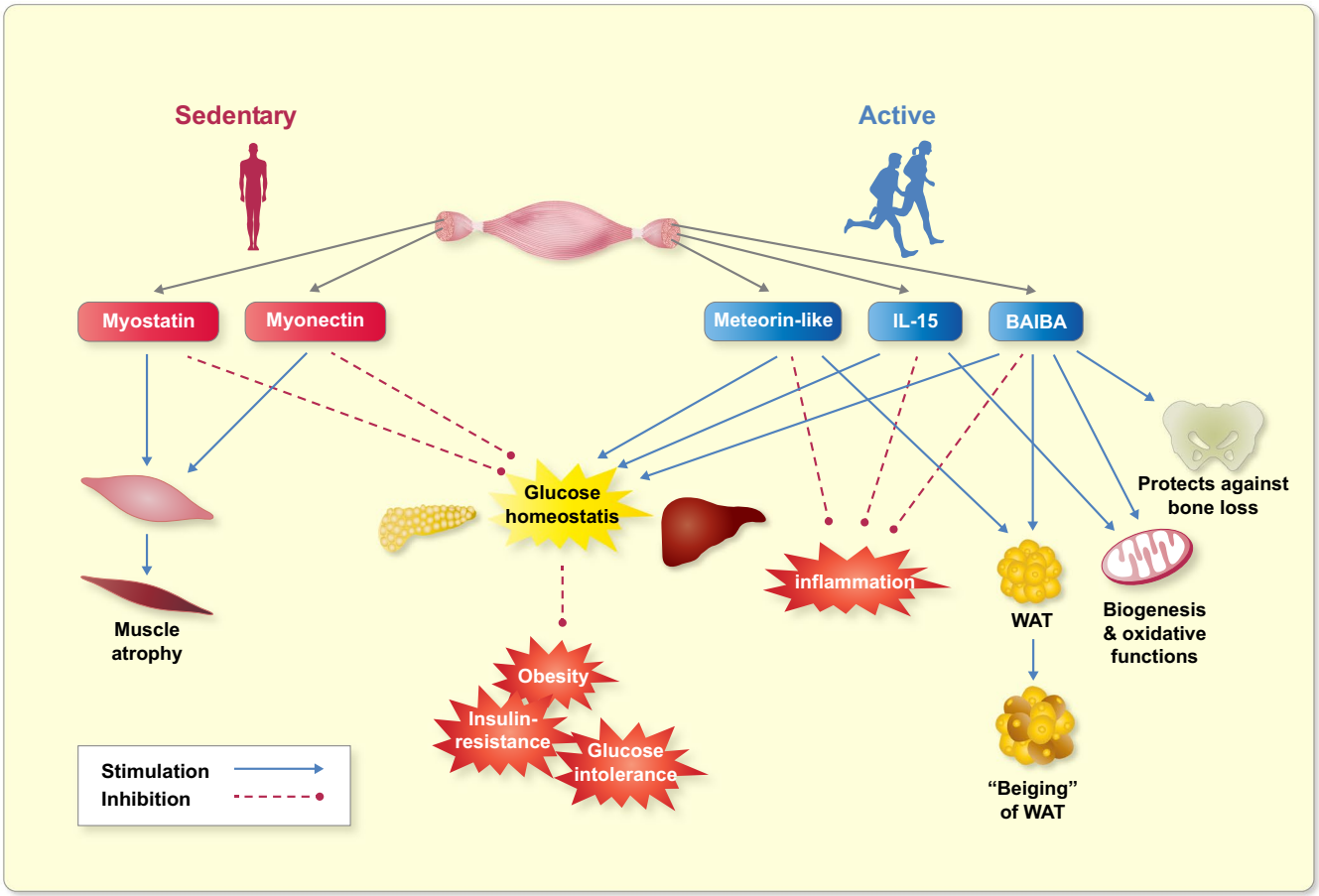

FIGURE 1 Myokine regulation of skeletal muscle physiology and metabolism. Summary of selected key functions of myokines produced and secreted by skeletal muscle which exert an effect, positive or negative, on muscle function and overall metabolism. They include myostatin (MSTN), the small organic acid β-aminoisobutyric acid (BAIBA), meteorin-like (METRNL), interleukin IL-15 (IL-15) and myonectin. These diverse myokines provide the basis for crosstalk between skeletal muscle and other organs, such as adipose tissue, bone and liver. Exercise-induced release of myokines provides a conceptual basis for our understanding of how exercise training may impact metabolic diseases

discovered that a number of these loci were located within or close to genes implicated in structure and function of skeletal muscle fibres (ACTG1), involvement in the regulation of neurotransmission (PEX14, TGFA and SYT1), excitation-contraction coupling (SLC8A1) or monogenic syndromes with involvement of psychomotor impairment (PEX14, LRPPRC and KANSL1).[102,105] For example, ACTG1 knockout mice demonstrate muscle weakness as well as whole-body functional deficits. STY1 has been associated to synaptic defects at the neuromuscular junctions in spinal muscular atrophy, while the overexpression of SLC8A1 in muscle produces muscular changes like those of muscular dystrophy.[102,105] Moreover, three lead SNPs (rs10186876, rs6687430 and rs754512) for grip strength were located near genes implicated in monogenic syndromes characterized by psychomotor and/or neurological impairment.[102,105] The characterization of these loci via genetic and functional work will help elucidate new pathways involved in the regulation of muscle strength and suggest new strategies to tackle muscle wasting.[102,105] Continuation of this line of research is likely to provide additional information as to expression how different myokines is regulated, and how expression levels correlate with muscle physiology and metabolism.

8 | CONCLUSION

In this review, the current understanding of the impact of myokines on muscle biology and metabolism were discussed with a focus on newly characterized myokines with known or suspected roles in regulating metabolism (Table 1, Figure 1). Myokines provide a mechanism for crosstalk between skeletal muscle and other tissues and organs and set a new paradigm in exercise biology and metabolic homoeostasis (Figure 1).

Before broad conclusions can be made regarding the role or impact of a particular myokine, it is critical that the findings from pre-clinical models are reproduced in man. Expression profiles of myokines could provide the information necessary to maximize the health-promoting benefits of

exercise. It may also be possible to develop small compounds derived from myokines to provide treatment of metabolic diseases. Knowledge regarding effects of different forms of exercise, such as endurance, isometric, eccentric and resistance exercise, remain to be fully elucidated and may be important in fully understanding the potential of these myokines to regulate cellular and whole-body metabolism. An area not discussed in this review is the effect of myokines with growth factors, adipokines or hepatokines. Interactions between MSTN and insulin or IGF-I signalling have been investigated; MSTN reduces signal transduction downstream of IGF-1 or insulin receptors at multiple levels from IRS-1 to AKT and mTORC1.[56] It now appears that adipokines, and possibly other hormones, mitigate these deleterious effects of MSTN.[106]

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

DKD and CPC were involved in drafting of the article. DKD, ZAG and CPC were involved in critical revision of the article for important intellectual content and gave the final approval of the article.

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REFERENCES

1. Pedersen BK. Muscle as a secretory organ. *Compr Physiol*. 2013;3:1337-1362. https://doi.org/10.1002/cphy.c120033.
2. Cardozo CP, Graham ZA. Muscle–bone interactions: Movement in the field of mechano–humoral coupling of muscle and bone. *Ann N Y Acad Sci*. 2017;1402:10-17. https://doi.org/10.1111/nyas.13411.
3. Karstoft K, Pedersen BK. Skeletal muscle as a gene regulatory endocrine organ. *Curr Opin Clin Nutr Metab Care*. 2016;19:270-275. https://doi.org/10.1097/MCO.0000000000000283.
4. Pedersen BK, Akerström T, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Physiol*. 2007;103:1093-1098. https://doi.org/10.1152/japplphysiol.00080.2007.
5. Huh JY. The role of exercise-induced myokines in regulating metabolism. *Arch Pharm Res*. 2018;41:14-29. https://doi.org/10.1007/s12272-017-0994-y.
6. Peterson JM, Mart R, Bond CE. Effect of obesity and exercise on the expression of the novel myokines, Myonectin and Fibronectin type III domain containing 5. *PeerJ*. 2014;2:e605. https://doi.org/10.7717/peerj.605.
7. Roberts L, Boström P, O'Sullivan J, et al. β-Aminoisobutyric acid induces browning of white fat and hepatic β-oxidation and is inversely correlated with cardiometabolic risk factors. *Cell Metab*. 2014;19:96-108. https://doi.org/10.1016/j.cmet.2013.12.003.
8. Seldin MM, Peterson JM, Byerly MS, Wei Z, Wong GW. Myonectin (CTRP15), a novel myokine that links skeletal muscle to systemic lipid homeostasis. *J Biol Chem*. 2012;287:11968-11980. https://doi.org/10.1074/jbc.M111.336834.
9. Thuret S, Moon L, Gage FH. Therapeutic interventions after spinal cord injury. *Nat Rev Neurosci*. 2006;7:628-643. https://doi.org/10.1038/nrn1955.
10. Bauman WA, Cardozo CP. Spinal cord injury: Skeletal pathophysiology and clinical issues. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*: Eighth Edition. Oxford, UK: Wiley-Blackwell; 2013. doi:10.1002/9781118453926.ch122.
11. Frontera WR, Ochala J. Skeletal muscle: A brief review of structure and function. *Behav Genet*. 2015;96:183-195. https://doi.org/10.1007/s00223-014-9915-y.
12. Qin W, Bauman WA, Cardozo C. Bone and muscle loss after spinal cord injury: Organ interactions. *Ann N Y Acad Sci*. 2010;1211:66-84. https://doi.org/10.1111/j.1749-6632.2010.05806.x.
13. Ellies LG, Johnson A, Olefsky JM. Obesity, inflammation, and insulin resistance. In: Dannenberg AJ, Berger NA, eds. *Obesity, Inflammation and Cancer*. Switerland: Springer Nature; 2013. doi:10.1007/978-1-4614-6819-6_1.
14. Tian X, Yan C, Liu M, et al. CREG1 heterozygous mice are susceptible to high fat diet-induced obesity and insulin resistance. *PLoS ONE*. 2017;12:e0176873. https://doi.org/10.1371/journal.pone.0176873.
15. Chen Y, Henson S, Jackson AB, Richards JS. Obesity intervention in persons with spinal cord injury. *Spinal Cord*. 2006;44:82-91. https://doi.org/10.1038/sj.sc.3101818.
16. Ye J. Beneficial metabolic activities of inflammatory cytokine interleukin 15 in obesity and type 2 diabetes. *Front Med*. 2015;9:139-145. https://doi.org/10.1007/s11684-015-0377-z.
17. Luo L, Liu M. Adipose tissue in control of metabolism. *J Endocrinol*. 2016;231:R77-R99. https://doi.org/10.1530/JOE-16-0211.
18. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose tissue remodeling: Its role in energy metabolism and metabolic disorders. *Front Endocrinol (Lausanne)*. 2016;7:30. https://doi.org/10.3389/fendo.2016.00030
19. Evans WJ. Skeletal muscle loss: Cachexia, sarcopenia, and inactivity. *Am J Clin Nutr*. 2010;91:1123S-1127S. https://doi.org/10.3945/ajcn.2010.28608A.
20. Rivas DA, Fielding RA. Skeletal muscle. In: Caballero B, ed. *Encyclopedia of Human Nutrition*. Oxford, UK: Academic Press; 2012. doi:10.1016/B978-0-12-375083-9.00188-4.
21. Eckardt K, Görgens SW, Raschke S, Eckel J. Myokines in insulin resistance and type 2 diabetes. *Diabetologia*. 2014;57:1087-1099. https://doi.org/10.1007/s00125-014-3224-x.

22. Sharma M, McFarlane C, Kambadur R, Kukreti H, Bonala S, Srinivasan S. Myostatin: Expanding horizons. *IUBMB Life*. 2015;67:589-600. https://doi.org/10.1002/iub.1392.
23. Huang S, Czech MP. The GLUT4 Glucose Transporter. *Cell Metab*. 2007;5:237-252. https://doi.org/10.1016/j.cmet.2007.03.006.
24. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev*. 2013;93:993-1017. https://doi.org/10.1152/physrev.00038.2012.
25. Kammoun HL, Febbraio MA. Come on BAIBA light my fire. *Cell Metab*. 2014;19:1-2. https://doi.org/10.1016/j.cmet.2013.12.007.
26. Lightfoot AP, Cooper RG. The role of myokines in muscle health and disease. *Curr Opin Rheumatol*. 2016;28:661-666. https://doi.org/10.1097/BOR.0000000000000337.
27. Leal LG, Lopes MA, Batista ML. Physical exercise-induced myokines and muscle-adipose tissue crosstalk: A review of current knowledge and the implications for health and metabolic diseases. *Front Physiol*. 2018;9. https://doi.org/10.3389/fphys.2018.01307.
28. Gonzalez-Cadavid NF, Bhasin S. Role of myostatin in metabolism. *Curr Opin Clin Nutr Metab Care*. 2004;7:451-457. https://doi.org/10.1097/01.mco.0000134365.99523.7f.
29. Elkina Y, von Haehling S, Anker SD, Springer J. The role of myostatin in muscle wasting: An overview. *J Cachexia Sarcopenia Muscle*. 2011;2:143-151. https://doi.org/10.1007/s13539-011-0035-5.
30. Lee S-J, McPherron AC. Regulation of myostatin activity and muscle growth. *Proc Natl Acad Sci*. 2002;98:9306-9311. https://doi.org/10.1073/pnas.151270098.
31. Lee S-J, Reed LA, Davies MV, et al. Regulation of muscle growth by multiple ligands signaling through activin type II receptors. *Proc Natl Acad Sci*. 2005;102:18117-18122. https://doi.org/10.1073/pnas.0505996102.
32. Sartori R, Milan G, Patron M, et al. Smad2 and 3 transcription factors control muscle mass in adulthood. *Am J Physiol Physiol*. 2009;296:C1248-C1257. https://doi.org/10.1152/ajpcell.00104.2009.
33. Elkasrawy MN, Hamrick MW. Myostatin (GDF-8) as a key factor linking muscle mass and bone structure. *J Musculoskelet Neuronal Interact*. 2010;10:56-63.
34. Han HQ, Zhou X, Mitch WE, Goldberg AL. Myostatin/activin pathway antagonism: Molecular basis and therapeutic potential. *Int J Biochem Cell Biol*. 2013;45:2333-2347. https://doi.org/10.1016/j.biocel.2013.05.019.
35. Kollias HD, McDermott JC. Transforming growth factor-β and myostatin signaling in skeletal muscle. *J Appl Physiol*. 2007;104:579-587. https://doi.org/10.1152/japplphysiol.01091.2007.
36. Ross S, Hill CS. How the Smads regulate transcription. *Int J Biochem Cell Biol*. 2008;40:383-408. https://doi.org/10.1016/j.biocel.2007.09.006.
37. Forbes D, Jackman M, Bishop A, Thomas M, Kambadur R, Sharma M. Myostatin auto-regulates its expression by feedback loop through Smad7 dependent mechanism. *J Cell Physiol*. 2006;206:264-272. https://doi.org/10.1002/jcp.20477.
38. Zhu X, Topouzis S, Liang LF, Stotish RL. Myostatin signaling through Smad2, Smad3 and Smad4 is regulated by the inhibitory Smad7 by a negative feedback mechanism. *Cytokine*. 2004;26:262-272. https://doi.org/10.1016/j.cyto.2004.03.007.
39. Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: Molecular mechanisms and promising therapies. *Nat Rev Drug Discov*. 2014;14:58-74. https://doi.org/10.1038/nrd4467.
40. Elliott B, Renshaw D, Getting S, Mackenzie R. The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta Physiol*. 2012;205:324-340. https://doi.org/10.1111/j.1748-1716.2012.02423.x.
41. Zhao W, Wu Y, Zhao J, Pan J, Bauman WA, Cardozo CP. Nandrolone Normalizes determinants of muscle mass and fiber type after spinal cord injury. *J Neurotrauma*. 2011;29:1663-1675. https://doi.org/10.1089/neu.2011.2203.
42. De Gasperi R, Graham ZA, Harlow LM, Bauman WA, Qin W, Cardozo CP. The signature of MicroRNA dysregulation in muscle paralyzed by spinal cord injury includes downregulation of MicroRNAs that target myostatin signaling. *PLoS ONE*. 2016;11:e0166189. https://doi.org/10.1371/journal.pone.0166189.
43. Graham ZA, Collier L, Peng Y, et al. A soluble activin receptor IIB fails to prevent muscle atrophy in a mouse model of spinal cord injury. *J Neurotrauma*. 2015;33:1128-1135. https://doi.org/10.1089/neu.2015.4058.
44. Narici MV, Maffulli N. Sarcopenia: Characteristics, mechanisms and functional significance. *Br Med Bull*. 2010;95:139-159. https://doi.org/10.1093/bmb/ldq008.
45. Allen DL, Hittel DS, McPherron AC. Expression and function of myostatin in obesity, diabetes, and exercise adaptation. *Med Sci Sports Exerc*. 2011;43:1828-1835. https://doi.org/10.1249/MSS.0b013e3182178bb4.
46. Huang Z, Chen X, Chen D. Myostatin: A novel insight into its role in metabolism, signal pathways, and expression regulation. *Cell Signal*. 2011;23:1441-1446. https://doi.org/10.1016/j.cellsig.2011.05.003.
47. Palsgaard J, Brøns C, Friedrichsen M, et al. Gene expression in skeletal muscle biopsies from people with type 2 diabetes and relatives: Differential regulation of insulin signaling pathways. *PLoS ONE*. 2009;4:e6575. https://doi.org/10.1371/journal.pone.0006575.
48. Shan T, Liang X, Bi P, Kuang S. Myostatin knockout drives browning of white adipose tissue through activating the AMPK-PGC1-Fndc5 pathway in muscle. *FASEB J*. 2013;27:1981-1989. https://doi.org/10.1096/fj.12-225755.
49. Zhang C, McFarlane C, Lokireddy S, et al. Inhibition of myostatin protects against diet-induced obesity by enhancing fatty acid oxidation and promoting a brown adipose phenotype in mice. *Diabetologia*. 2012;55:183-193. https://doi.org/10.1007/s00125-011-2304-4.
50. Li K, Liao X, Wang K, et al. Myonectin predicts the development of type 2 diabetes. *J Clin Endocrinol Metab*. 2018;103:139-147. https://doi.org/10.1210/jc.2017-01604.
51. Hittel DS, Axelson M, Sarna N, Shearer J, Huffman KM, Kraus WE. Myostatin decreases with aerobic exercise and associates with insulin resistance. *Med Sci Sports Exerc*. 2010;42:2023-2029. https://doi.org/10.1249/MSS.0b013e3181e0b9a8.
52. Coleman SK, Rebalka IA, D'Souza DM, Deodhare N, Desjardins EM, Hawke TJ. Myostatin inhibition therapy for insulin-deficient type 1 diabetes. *Sci Rep*. 2016;6. https://doi.org/10.1038/srep32495.
53. Leto D, Saltiel AR. Regulation of glucose transport by insulin: Traffic control of GLUT4. *Nat Rev Mol Cell Biol*. 2012;13:383-396. https://doi.org/10.1038/nrm3351.
54. Abel ED, Peroni O, Kim JK, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature*. 2001;409:729-733. https://doi.org/10.1038/35055575.

55. Carvalho E, Kotani K, Peroni OD, Kahn BB. Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. *Am J Physiol Metab*. 2005;289:E551-E561. https://doi.org/10.1152/ajpendo.00116.2005.
56. Liu XH, Bauman WA, Cardozo CP. Myostatin inhibits glucose uptake via suppression of insulin-dependent and -independent signaling pathways in myoblasts. *Physiol Rep*. 2018;6:e13837. https://doi.org/10.14814/phy2.13837.
57. Zisman A, Peroni OD, Abel ED, et al. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med*. 2000;6:924-928. https://doi.org/10.1038/78693.
58. Wu WL, Gan WH, Tong ML, et al. Over-expression of NYGGF4 (PID1) inhibits glucose transport in skeletal myotubes by blocking the IRS1/PI3K/AKT insulin pathway. *Mol Genet Metab*. 2011;102:374-377. https://doi.org/10.1016/j.ymgme.2010.11.165.
59. Shi C-M, Xu G-F, Yang L, et al. Overexpression of TFAM protects 3T3-L1 adipocytes from NYGGF4 (PID1) overexpression-induced insulin resistance and mitochondrial dysfunction. *Cell Biochem Biophys*. 2013;66:489-497. https://doi.org/10.1007/s12013-012-9496-1.
60. Chen L, Wang X-Y, Zhu J-G, et al. PID1 in adipocytes modulates whole-body glucose homeostasis. *Biochim Biophys Acta - Gene Regul Mech*. 2018;1861:125-132. https://doi.org/10.1016/j.bbagrm.2018.01.001.
61. Smith RC, Lin BK. Myostatin inhibitors as therapies for muscle wasting associated with cancer and other disorders. *Curr Opin Support Palliat Care*. 2013;7:352-360. https://doi.org/10.1097/SPC.0000000000000013.
62. Cash JN, Rejon CA, McPherron AC, Bernard DJ, Thompson TB. The structure of myostatin:follistatin 288: Insights into receptor utilization and heparin binding. *EMBO J*. 2009;28:2662-2676. https://doi.org/10.1038/emboj.2009.205.
63. FedorukMN,RupertJL.Myostatininhibition:Apotentialperformance enhancement strategy? *Scand J Med Sci Sport*. 2008;18:123-131. https://doi.org/10.1111/j.1600-0838.2007.00759.x.
64. Long KK, O'Shea KM, Khairallah RJ, et al. Specific inhibition of myostatin activation is beneficial in mouse models of SMA therapy. *Hum Mol Genet*. 2018;28:N:1–14. https://doi.org/10.1093/hmg/ddy382.
65. Kitase Y, Vallejo JA, Gutheil W, et al. β-aminoisobutyric Acid, l -BAIBA, is a muscle-derived osteocyte survival factor. *Cell Rep*. 2018;22:1531-1544. https://doi.org/10.1016/j.celrep.2018.01.041.
66. Tanianskii D, Jarzebska N, Birkenfeld A, O'Sullivan J, Rodionov R. Beta-aminoisobutyric acid as a novel regulator of carbohydrate and lipid metabolism. *Nutrients*. 2019;11:524. https://doi.org/10.3390/nu11030524.
67. Shi C-X, Zhao M-X, Shu X-D, et al. β-aminoisobutyric acid attenuates hepatic endoplasmic reticulum stress and glucose/lipid metabolic disturbance in mice with type 2 diabetes. *Sci Rep*. 2016;6:21924. https://doi.org/10.1038/srep21924.
68. Jung TW, Hwang HJ, Hong HC, Yoo HJ, Baik SH, Choi KM. BAIBA attenuates insulin resistance and inflammation induced by palmitate or a high fat diet via an AMPK–PPARδ-dependent pathway in mice. *Diabetologia*. 2015;58:2096-2105. https://doi.org/10.1007/s00125-015-3663-z.
69. Bauman WA, Cardozo CP. Osteoporosis in Individuals with Spinal Cord Injury. *PM R*. 2015;7:188-201. https://doi.org/10.1016/j.pmrj.2014.08.948.
70. Giudici KV, Kindler JM, Martin BR, et al. Associations among osteocalcin, leptin and metabolic health in children ages 9–13 years in the United States. *Nutr Metab*. 2017;14:9-13. https://doi.org/10.1186/s12986-017-0171-9.
71. Qin Y, Peng Y, Zhao W, et al. Myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal microRNA-218: A novel mechanism in muscle-bone communication. *J Biol Chem*. 2017;292:11021-11033. https://doi.org/10.1074/jbc.M116.770941.
72. Nielsen AR, Pedersen BK. The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. *Appl Physiol Nutr Metab*. 2007;32:833-839. https://doi.org/10.1139/H07-054.
73. Argilés JM, López-Soriano FJ, Busquets S. Therapeutic potential of interleukin-15: a myokine involved in muscle wasting and adiposity. *Drug Discov Today*. 2009;14:208-213. https://doi.org/10.1016/j.drudis.2008.10.010.
74. Nadeau L, Patten DA, Caron A, et al. IL-15 improves skeletal muscle oxidative metabolism and glucose uptake in association with increased respiratory chain supercomplex formation and AMPK pathway activation. *Biochim Biophys Acta - Gen Subj*. 2019;1863:395-407. https://doi.org/10.1016/j.bbagen.2018.10.021.
75. Li F, Li Y, Tang Y, et al. Protective effect of myokine IL-15 against H2O2-mediated oxidative stress in skeletal muscle cells. *Mol Biol Rep*. 2014;41:7715-7722. https://doi.org/10.1007/s11033-014-3665-9.
76. Thornton SM, Krolopp JE, Abbott MJ. IL-15 mediates mitochondrial activity through a PPAR δ -dependent-PPAR α -independent mechanism in skeletal muscle cells. *PPAR Res*. 2016; https://doi.org/10.1155/2016/5465804.
77. Quinn LS. Interleukin-15: a muscle-derived cytokine regulating fat-to-lean body composition. *J Anim Sci*. 2008;86:E75-83. https://doi.org/10.2527/jas.2007-0458.
78. Carbó N, López-Soriano Joaquín, Costelli P, et al. Interleukin-15 mediates reciprocal regulation of adipose and muscle mass: A potential role in body weight control. *Biochim Biophys Acta - Gen Subj*. 2001;1526:17-24. https://doi.org/10.1016/S0304-4165(00)00188-4.
79. Quinn LS, Strait-Bodey L, Stroud AM, Anderson BG, Argilés JM. Oversecretion of interleukin-15 from skeletal muscle reduces adiposity. *Am J Physiol Metab*. 2008;296:E191-E202. https://doi.org/10.1152/ajpendo.90506.2008.
80. Reyes JL, Vannan DT, Vo T, et al. Neutralization of IL-15 abrogates experimental immune-mediated cholangitis in diet-induced obese mice. *Sci Rep*. 2018;8:3127. https://doi.org/10.1038/s41598-018-21112-7.
81. Pistilli EE, Devaney JM, Gordish-Dressman H, et al. Interleukin-15 and interleukin-15Rα SNPs and associations with muscle, bone, and predictors of the metabolic syndrome. *Cytokine*. 2008;43:45-53. https://doi.org/10.1016/j.cyto.2008.04.008.
82. Raschke S, Eckel J. Adipo-Myokines: Two sides of the same coin - Mediators of inflammation and mediators of exercise. *Mediators Inflamm*. 2013;2013:1-16. https://doi.org/10.1155/2013/320724.
83. Sun H, Liu D. Hydrodynamic delivery of interleukin 15 gene promotes resistance to high fat diet-induced obesity, fatty liver and improves glucose homeostasis. *Gene Ther*. 2015;22:341-347. https://doi.org/10.1038/gt.2014.114.

84. Sun H, Ma Y, Gao M, Liu D. IL-15/sIL-15Rα gene transfer induces weight loss and improves glucose homeostasis in obese mice. *Gene Ther*. 2016;23:349-356. https://doi.org/10.1038/gt.2016.4.
85. Pierce JR, Maples JM, Hickner RC. IL-15 concentrations in skeletal muscle and subcutaneous adipose tissue in lean and obese humans: local effects of IL-15 on adipose tissue lipolysis. *Am J Physiol Metab*. 2015;308:E1131-E1139. https://doi.org/10.1152/ajpendo.00575.2014.
86. Calle MC, Fernandez ML. Effects of resistance training on the inflammatory response. *Nutr Res Pract*. 2010;4:259. https://doi.org/10.4162/nrp.2010.4.4.259.
87. Duan Y, Li F, Wang W, et al. Interleukin-15 in obesity and metabolic dysfunction: current understanding and future perspectives. *Obes Rev*. 2017;18:1147-1158. https://doi.org/10.1111/obr.12567.
88. Sánchez-Jiménez R, Alvarado-Vásquez N. IL-15 that a regulator of TNF-α in patients with diabetes mellitus type 2. *Med Hypotheses*. 2013;80:776-777. https://doi.org/10.1016/j.mehy.2013.03.009.
89. Krolopp JE, Thornton SM, Abbott MJ. IL-15 activates the Jak3/STAT3 signaling pathway to mediate glucose uptake in skeletal muscle cells. *Front Physiol*. 2016;7:626. https://doi.org/10.3389/fphys.2016.00626.
90. Ushach I, Burkhardt AM, Martinez C, et al. METEORIN-LIKE is a cytokine associated with barrier tissues and alternatively activated macrophages. *Clin Immunol*. 2015;156:119-127. https://doi.org/10.1016/j.clim.2014.11.006.
91. Rao R, Long J, White J, et al. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell*. 2014;157:1279-1291. https://doi.org/10.1016/j.cell.2014.03.065.
92. Jung TW, Lee SH, Kim H-C, et al. METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARδ-dependent pathways in skeletal muscle of mice. *Exp Mol Med*. 2018;50:122. https://doi.org/10.1038/s12276-018-0147-5.
93. Eaton M, Granata C, Barry J, Safdar A, Bishop D, Little JP. Impact of a single bout of high-intensity interval exercise and short-term interval training on interleukin-6, FNDC5, and METRNL mRNA expression in human skeletal muscle. *J Sport Heal Sci*. 2018;7:191-196. https://doi.org/10.1016/j.jshs.2017.01.003.
94. Zheng SL, Li ZY, Song J, Liu JM, Miao CY. Metrnl: A secreted protein with new emerging functions. *Acta Pharmacol Sin*. 2016;37:571-579. https://doi.org/10.1038/aps.2016.9.
95. Li Z-Y, Zheng S-L, Wang P, et al. Subfatin is a Novel Adipokine and Unlike Meteorin in Adipose and Brain Expression. *CNS Neurosci Ther*. 2014;20:344-354. https://doi.org/10.1111/cns.12219.
96. Dadmanesh M, Aghajani H, Fadaei R, Ghorban K. Lower serum levels of Meteorin-like/Subfatin in patients with coronary artery disease and type 2 diabetes mellitus are negatively associated with insulin resistance and inflammatory cytokines. *PLoS ONE*. 2018;13:e0204180. https://doi.org/10.1371/journal.pone.0204180.
97. Lee JH, Kang YE, Kim JM, et al. Serum Meteorin-like protein levels decreased in patients newly diagnosed with type 2 diabetes. *Diabetes Res Clin Pract*. 2018;135:7-10. https://doi.org/10.1016/j.diabres.2017.10.005.
98. Chung HS, Hwang SY, Choi JH, et al. Implications of circulating Meteorin-like (Metrnl) level in human subjects with type 2 diabetes. *Diabetes Res Clin Pract*. 2018;136:100-107. https://doi.org/10.1016/j.diabres.2017.11.031.
99. Löffler D, Landgraf K, Rockstroh D, et al. METRNL decreases during adipogenesis and inhibits adipocyte differentiation leading to adipocyte hypertrophy in humans. *Int J Obes*. 2017;41:112-119. https://doi.org/10.1038/ijo.2016.180.
100. Seldin MM, Wong GW. Regulation of tissue crosstalk by skeletal muscle-derived myonectin and other myokines. *Adipocyte*. 2012;1:200-202. https://doi.org/10.4161/adip.20877.
101. Gamas L, Matafome P, Seicą R. Irisin and myonectin regulation in the insulin resistant muscle: Implications to adipose tissue: Muscle crosstalk. *J Diabetes Res*. 2015;2015:1-8. https://doi.org/10.1155/2015/359159.
102. Trajanoska K, Rivadeneira F, Kiel DP, Karasik D. Genetics of bone and muscle interactions in humans. *Curr Osteoporos Rep*. 2019;17:86-95. https://doi.org/10.1007/s11914-019-00505-1.
103. Pourteymour S, Eckardt K, Holen T, et al. Global mRNA sequencing of human skeletal muscle: Search for novel exercise-regulated myokines. *Mol Metab*. 2017;6:352-365. https://doi.org/10.1016/j.molmet.2017.01.007.
104. Hao R-H, Yang T-L, Rong Y, et al. Gene expression profiles indicate tissue-specific obesity regulation changes and strong obesity relevant tissues. *Int J Obes*. 2018;42:363-369. https://doi.org/10.1038/ijo.2017.283.
105. Willems SM, Wright DJ, Day FR, et al. Large-scale GWAS identifies multiple loci for hand grip strength providing biological insights into muscular fitness. *Nat Commun*. 2017;8:16015. https://doi.org/10.1038/ncomms16015.
106. Liu X-H, Pan JP, Bauman WA, Cardozo CP. AdipoRon prevents myostatin-induced upregulation of fatty acid synthesis and downregulation of insulin activity in a mouse hepatocyte line. *Physiol Rep*. 2019;7:e14152. https://doi.org/10.14814/phy2.14152.

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