Editorial

The ups and downs of exercise and insulin sensitivity: a role for the myokine myostatin in glucose metabolism?

Hjorth et al. (2016) analyse the effects of acute and long-term exercise on myokine gene expression and insulin sensitivity in humans. The combination of exercise, metabolic analysis, deep gene expression analysis in both muscle and adipose tissue, and follow-up in vitro assays make this a particularly thorough study. This work will help investigators direct future studies regarding the role of the growth factor myostatin on metabolism in muscle and adipose tissue.

Exercise has known positive benefits for body composition, insulin sensitivity, cardiovascular health and cognition. Skeletal muscle has greatly fluctuating energy demands that need to be accommodated at rest, for quick bursts of activity or for lengthy trials of endurance. While we have a long history of research into the mechanisms of exercise adaptation, the effects of secreted peptides have received particular interest recently. Muscle can increase its energy supply for contraction by mobilizing its own stores of glycogen or lipid and by increasing translocation of glucose transporters to the cell surface. Muscle is itself incapable of synthesizing glucose, however, and must rely on uptake from circulating glucose when its glycogen stores are depleted. Lipolysis in adipose tissue is another source of energy for muscle. It seems plausible that muscle would exert endocrine effects on other tissues during exercise and with adaptation to chronic exercise to enlist their aid in meeting its varying demands of energy substrate types as well as levels. Pedersen and colleagues suggested the term ‘myokine’ based on their work demonstrating production and secretion of IL-6 by contracting muscle during exercise and the effects of IL-6 on promoting lipolysis and lipid oxidation (Pedersen et al. 2003). As originally conceived, a myokine was defined as an endocrine hormone that was produced by muscle and released to the circulation to then act on another tissue. In this manner, a myokine would regulate processes such as lipolysis, glycogen breakdown, endogenous glucose production, appetite or hormone secretion, in other tissues. Over time, the term became interpreted loosely to mean any secreted protein made by muscle whether or not it circulates or acts in an autocrine or paracrine manner. In addition to IL-6 and other interleukins, the growing list of myokines includes irisin, myonectin, members of the fibroblast growth factor family, members of the transforming growth factor family and secreted inhibitors, and others.

Hjorth, Norheim and colleagues have conducted a study with middle-aged sedentary men (the MyoGlu Intervention study) to examine changes in gene expression with exercise to find mediators of the metabolic effects of exercise. In this issue, they describe their search for exercise-responsive myokines (Hjorth et al. 2016). They flipped the typical question about exercise induction of myokines to ask what secreted factors produced in muscle are downregulated with exercise?

The subjects in the MyoGlu study were middle-aged sedentary men. Half had either elevated fasting glucose or an impaired oral glucose tolerance test but were otherwise healthy. All subjects underwent a 45-minute bicycling exercise bout, both at baseline and after 12 weeks of combination aerobic and strength training, and had biopsies taken of the vastus lateralis muscle and subcutaneous abdominal adipose tissue. Hyperinsulinaemic–euglycaemic clamp analysis, the gold standard for measuring insulin sensitivity, was performed at baseline and at the end of the 12 weeks of training. The researchers performed deep RNA sequencing of tissue biopsies and mined the data for changes in different categories of genes of interest.

Of the genes encoding secreted proteins that were downregulated by at least 50% in skeletal muscle with all subjects grouped together, the growth factor myostatin was the most reduced at the end of the 12 weeks of training as compared to baseline (Fig. 1). The myostatin gene is a negative regulator of skeletal muscle mass expressed predominantly in skeletal muscle with lower levels of expression in adipose tissue (McPherron 2010). Myostatin loss of function mice clearly have reduced adipose tissue and improved insulin sensitivity. Although there appear to be positive metabolic effects of any mouse with increased muscle mass, there are some indications for a role for myostatin in metabolism separated from that of regulating muscle size (McPherron 2010). Several studies have shown that myostatin gene expression in skeletal muscle is elevated with insulin resistance or obesity in mice or humans even when there is no difference in lean mass. For instance, one study found that expression of the myostatin gene in muscle was higher in type 2 diabetics and their first-degree relatives com-
pared to control subjects despite similar lean mass (Palsgaard et al. 2009). Similarly, muscle myostatin gene expression was roughly negatively correlated with higher glucose utilization in response to insulin in older adults (Ryan et al. 2013). In rodents, results of myostatin treatment effects on glucose metabolism are mixed. Rodent muscle mass is generally very sensitive to myostatin inhibitors making it difficult to separate the effect on hypertrophy from that of metabolism. Local treatment of mouse muscle with adenovirus expressing a myostatin inhibitor for 17 days increased basal and insulin-stimulated glucose uptake to a greater degree than it increased muscle mass (Cleasby et al. 2014). This could be interpreted as a myostatin effect on metabolism although not necessarily a direct effect. Overall, progress has been hampered by the use of questionable myostatin dosages and/or protein quality. Currently, clear evidence of a direct role for myostatin in metabolism separate from its role in regulating muscle size in humans is lacking.

The study reported here addresses some of these issues. Hjorth et al. found that myostatin gene expression in skeletal muscle is not different between controls and dysglycaemic subjects at baseline, unlike the results of Palsgaard et al. (2009) with diabetic subjects and their first-degree relatives. In both groups, myostatin expression decreases with an acute bout of bicycling exercise, although more so in the controls, and with 12 weeks of training (Fig. 1). This decrease in myostatin expression with exercise confirms most previous studies. It also provides more evidence that myostatin expression is not strictly tied to lean mass because the investigators found no change in fat-free mass with 12 weeks of combination training. Changes in expression of the genes for a myostatin receptor, secreted inhibitors and an intracellular inhibitor of myostatin signalling are also consistent with decreased myostatin signalling, particularly after acute exercise.

The authors looked for a relationship between myostatin gene expression and insulin sensitivity. Eighty per cent of insulin-stimulated glucose uptake during a hyperinsulinaemic–euglycaemic clamp is by skeletal muscle which makes the clamp test largely a measure of muscle insulin sensitivity (DeFronzo et al. 1979). Correlation analysis by Hjorth et al. showed that myostatin expression in muscle was negatively correlated with the glucose infusion rate during the clamp at baseline. In other words, subjects with higher myostatin expression needed less glucose to maintain normal glycaemia in the hyperinsulinaemic state and were thus less insulin sensitive than those with lower myostatin expression. This confirms a previous report of the negative association of myostatin expression with clamp glucose disposal in sedentary older subjects (Ryan et al. 2013).

The researchers go on to perform appropriate in vitro assays to look for direct effects of myostatin on glucose metabolism and insulin signalling. Comendably, the researchers use a high-quality source of myostatin and confirm protein activity on their cells in vitro at reasonable concentrations. They found that insulin-stimulated glucose uptake into myotubes differentiated from primary myoblasts from adult men was not inhibited by myostatin. Phosphorylation of Akt with or without insulin stimulation was not affected by myostatin treatment. This is a surprising result considering the previously reported ability of myostatin to reduce basal or insulin-like growth factor induced Akt phosphorylation (Morissette et al. 2009,
Trendelenburg et al. (2009). This suggests that the correlation between myostatin gene expression and insulin sensitivity in their subjects is not mediated by a direct interaction between the myostatin and insulin signalling pathways.

Hjorth et al. did, however, show that myostatin affected glucose metabolism. They found that myostatin increased basal glucose uptake beginning at 4 h after the addition of myostatin with the strongest increase after 24 h of myostatin treatment. This result and the lack of a response at the 2-h time point suggest that the effect on glucose uptake could have been via transcription and translation rather than a direct effect on translocation of glucose transporters. In addition, this increase in basal glucose uptake by myostatin was additive with that of insulin. This shows that myostatin increases glucose uptake by a signalling pathway separate from that of insulin. They also show that at least some of this extra glucose taken up by myotubes after myostatin treatment enters the glycolysis pathway and is oxidized. In contrast, even though muscle gene expression analysis showed a negative correlation of myostatin expression with genes in the oxidative phosphorylation, citric acid cycle and fatty acid metabolism pathways, they could find no effect of myostatin on oleic acid oxidation in myotubes in vitro. Taken together, these results show that myostatin increases net energy utilization in human myotubes by an effect on glucose uptake and oxidation.

If myostatin does not regulate insulin signalling and lean mass is unchanged, how is myostatin gene expression related to the glucose infusion rate in vivo? Myostatin gene expression was negatively correlated with expression of genes for the slow-type myosin heavy chain 7, oxidative phosphorylation, and fatty acid metabolism but positively correlated with expression of the fast-type myosin heavy chain 1 gene. This is consistent with the known expression of myostatin at greater levels in fast glycolytic muscle compared to slow oxidative muscle. Slow oxidative fibres are known to be more insulin sensitive, and diabetics have proportionally more fast glycolytic fibres than slow oxidative fibres (Sun et al. 2008). Perhaps the correlation of myostatin with the glucose infusion rate tells us more about the fibre-type distribution of the subjects than it does about the role of myostatin in insulin sensitivity. This could be a simple explanation for the negative association between myostatin gene expression and the glucose infusion rate both before and after training. Fewer fast glycolytic-type fibres and more slow oxidative fibres could explain both higher insulin sensitivity and lower myostatin gene expression. The same would hold true for post-training effects if there was a shift towards slower oxidative fibres. However, this explanation would not account for the acute reduction in myostatin expression in skeletal muscle found in this study.

Along these lines, judging from the increased expression of the slow myosin heavy chain 7 gene and the decrease in expression of myostatin with differentiation, the myotubes used in this study were of the slow oxidative type. Carrying out glucose uptake and oxidation experiments in vitro with both fast glycolytic and slow oxidative myotubes would be informative to see whether the effects of myostatin on glucose uptake vary with fibre type. However, increased basal glucose uptake with myostatin treatment has been reported using several cell lines of different tissue types. One reasonable hypothesis put forward by the authors is that myostatin may induce some process that requires more energy. The effect on basal glucose uptake may be an indirect effect of myostatin’s action protein degradation, for instance.

The role of myostatin in adipose tissue in humans has received relatively little attention so the simultaneous analysis of its effects in muscle and adipose tissue is welcomed. Hjorth et al. found quite different results with adipose tissue than with muscle. Myostatin expression in adipose tissue was slightly increased after training but only in control subjects (Fig. 1). Correlation analysis of myostatin expression in adipose tissue with metabolic parameters and gene expression pathways showed that myostatin was positively correlated with markers of insulin sensitivity, such as fasting insulin and HbA1c, and with expression of genes for insulin signalling. Unlike in skeletal muscle, myostatin in adipose tissue was only weakly correlated with the glucose infusion rate during the clamp perhaps reflecting the dominance of skeletal muscle on this parameter.

The role for myostatin in regulating the metabolism of adipocytes, however, remains unclear. They carried out in vitro experiments with Simpson–Golabi–Behmel syndrome cells, a human adipocyte cell line, and confirmed responsiveness of this line to myostatin by the induction of SMAD2 phosphorylation, an intracellular mediator of myostatin signalling. Still, the investigators could find no effect of myostatin protein on basal or insulin-stimulated glucose uptake or fatty acid uptake. Myostatin has been shown by many researchers to inhibit adipogenesis in 3T3-L1 pre-adipocytes, but in C3H10T1/2 multipotent mesenchymal cells, myostatin can substitute weakly for glucocorticoid in an adipogenic cocktail (Feldman et al. 2006). A transgenic mouse model overexpressing myostatin in adipocytes has smaller, less differentiated adipocytes that have increased glucose uptake and increased insulin sensitivity (Feldman et al. 2006) consistent with the expression pathway correlations found by Hjorth et al. Taken together, myostatin may regulate adipo-
genesis to maintain a portion of adipocytes at an early stage of differentiation that is highly insulin sensitive rather than directly regulating insulin sensitivity per se in adipocytes.

An often overlooked aspect of myostatin biology relevant to analysis of both in vitro and many in vivo experiments is the complex multi-step process beginning with production of the ligand through receptor binding. Full-length myostatin dimerizes and is cleaved at an internal processing site to produce an amino-terminal pro-region and a carboxy-terminal disulphide-linked dimer. This carboxy-terminal dimer is the mature active form of myostatin that can bind the receptor and is the form used for in vitro experiments as well as for some in vivo injection experiments. However, this is not the form of myostatin secreted from cells. Endogenously produced myostatin is secreted in a latent complex with the amino-terminal pro-region bound non-covalently to the mature carboxy-terminal dimer. This latent complex cannot bind to the receptor and must be activated by a second protease cleavage event within the pro-region to release the mature active dimer. Thus, there are many potential steps to regulate myostatin signalling, and analysing gene expression or even protein expression alone has the potential to mislead. In this study, serum myostatin protein changed very little – decreasing only ~7.5% with training and increasing slightly with acute exercise (Fig. 1). Presumably, most of this circulating myostatin protein is produced by muscle. Given that myostatin gene expression was reduced with exercise, these results suggest that myostatin levels may also be regulated at the level of translation or degradation. The increase in circulating myostatin despite a reduction in skeletal muscle gene expression suggests that there could also be regulation in how much is kept in the extracellular matrix versus secreted into the circulation.

On top of this, there is redundancy in the inhibitors, receptors and intracellular signalling mediators between myostatin and other related growth factors. It is possible that some in vitro or in vivo effects using the active form of myostatin are not normally those of myostatin in vivo but are instead mimicking those of a related factor that is not found in a latent complex, such as activin. Which tissues can actually activate a related factor that is not found in a latent complex with the amino-terminal propeptide increases glucose transport expression and enhances skeletal muscle glucose disposal. Am J Physiol Endocrinol Metab 306, E814–E823.

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pathway. Nevertheless, this does not rule out a role for myostatin in meeting contraction-induced energy demands through other local or endocrine mechanisms. This work further suggests that the metabolic effects of myostatin loss of function in rodents on insulin sensitivity may be misleading. The tremendous increase in muscle mass in these animals – for some muscles exceeding twice the mass of that of wild-type – and the corresponding energy demand may overpower our ability to discern more subtle metabolic mechanisms that are relevant to human physiology.

**Conflict of interest**

The author declares no conflict of interest.

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