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Abstract

Diabetes is a complex disease that affects many organs directly or indirectly. Type 2 diabetes mellitus is characterized by insulin resistance with a relative deficiency in insulin secretion. It has become apparent that inter-organ communication is of great importance in the pathophysiology of diabetes. Far from being an inert tissue in terms of inter-organ communication, it is now recognized that skeletal muscle can secrete so-called myokines that can impact on the function of distant organs/tissues both favourably and unfavourably. We have proposed that communication between insulin-resistant skeletal muscle and β-cells occurs in diabetes. This is a novel route of communication that we further suggest is modified by the prevailing degree of insulin resistance of skeletal muscle. This review focuses on the various myokines [interleukin-6 (IL-6), tumor necrosis factor-α, CXCL10, follistatin and IL-8] which have been identified either after different types of exercise or in the secretome from control and insulin-resistant human skeletal myotubes. We will also summarize studies on the impact of several myokines on [...]
Bimodal impact of skeletal muscle on pancreatic beta-cell function in health and disease

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SUMMARY

Diabetes is a complex disease that affects many organs directly or indirectly. Type 2 diabetes mellitus is characterized by insulin-resistance with a relative deficiency in insulin secretion. It has become apparent that inter-organ communication is of great importance in the pathophysiology of diabetes. Far from being an inert tissue in terms of inter-organ communication, it is now recognized that skeletal muscle can secrete so-called myokines that can impact on the function of distant organs/tissues both favorably and unfavorably. We have proposed that communication between insulin-resistant skeletal muscle and beta-cells occurs in diabetes. This is a novel route of communication that we further suggest is modified by the prevailing degree of insulin resistance of skeletal muscle. This review focuses on the various myokines (IL-6, TNF-alpha, CXCL10, follistatin and IL-8) which have been identified either after different types of exercise or in the secretome from control and insulin resistant human skeletal myotubes. We will also summarise studies on the impact of several myokines on pancreatic beta-cell proliferation, survival and function.
INTRODUCTION

Diabetes in its two major forms is characterised by absolute or relative insulin deficiency. It is thought that multiple signals lead to impaired beta-cell function and probably decreased beta-cell mass in type 2 diabetes (T2DM), with autoimmune beta cell destruction underlying type 1 diabetes (T1DM). Insulin resistance is obviously a key player in T2DM but it plays a larger role in the disease process of T1DM than commonly recognized. Concurrently with a rising incidence of T1DM, obesity, and physical inactivity have increased steadily in children and adolescents. The role of insulin resistance in T1DM has only recently been gaining acceptance.

Several studies have demonstrated that the physiological response to exercise induces an increase in the systemic levels of a number of cytokines with anti-inflammatory properties (1-3). Skeletal muscle has been identified as an endocrine organ, which produces and releases cytokines that were named myokines (4), acting both locally and on remote tissues. Several studies have thus demonstrated that contracting skeletal muscles release a select panel of myokines, which may work in a hormone-like fashion, exerting specific endocrine effects on visceral fat or mediating direct anti-inflammatory effects (5-6). Other myokines work locally within the muscle via paracrine mechanisms, exerting their effects on signaling pathways involved in fat metabolism (6).

Myokines secreted during physiological exercise have also been shown to improve insulin sensitivity in T2D patients (7). Skeletal muscle is the largest insulin sensitive organ in the body and plays a major role in whole body substrate homeostasis in the post-prandial state. Impaired insulin action in skeletal muscle may lead to the pathological condition of insulin resistance, whereby normal concentrations of insulin produce a subnormal biological response (8). Based on
these earlier observations and given that skeletal muscle is a major endocrine organ based on mass, we decided to explore the potential impact on beta-cell function of myokines secreted from skeletal muscle with different insulin sensitivity.

**Muscle as an endocrine organ:**

In the past, several studies have shown that adipose tissue can be seen as an endocrine organ, inspiring Dr. Bente Klarlund Pedersen to propose that skeletal muscle should also be considered as an endocrine organ. She was the first to suggest that cytokines and other peptides were produced, expressed, and released by muscle fibres, coining the term “Myokines” (9). This model provides a conceptual basis explaining the multiple negative consequences of a physically inactive lifestyle. Over the past year, Pedersen’s group has shown that if the endocrine and paracrine functions of the muscle are not stimulated, this will cause dysfunction of several organs and tissues as well as an increased risk of cardiovascular disease, cancer, and dementia (4-5, 10-12).

IL-6 is recognised as the predominant “exercise” cytokine (7, 13-14). It is released by the muscle in response to contractions, both in type I and type II muscle (4). While IL-6 secretion is AMPK dependent in type I muscle (15), the pathway involved in type II muscle remains unknown. Several studies have demonstrated that skeletal muscles have the capacity to secrete other myokines such as IL-8, IL-15 (16), Brain Derived Neurotrophic Factor (BDNF) (12), Leukemia Inhibitory factor (LIF) (17) and FGF21 (18) after exercise. Recently, myonectin has been identified as a myokine regulated by exercise, diet and metabolic rate (19), while Irisin, a newly discovered myokine, has been shown to be induced by exercise in rodents and humans (20). Irisin acts on white adipose cells in culture and *in-vivo* to stimulate UCP1 expression and a
broad program of brown-fat-like development (20), providing a novel axis for communication between muscle and this metabolically active tissue.

Interestingly, TNF-alpha expression in serum has been shown to be increased only after long training such as marathon (21). Moreover, TNF-alpha is believed to be a major cytokine involved in “conversation” between adipose tissue and muscle, with dramatically increased levels in obesity possibly leading to insulin-resistance in skeletal muscle (22-23).

Recently, using human primary skeletal muscle cells, we have shown that skeletal muscle cells can secrete myokines in-vitro with different profiles depending upon insulin sensitivity (24). Using a combination of oligonucleotide array membranes and quantitative RT-PCR we were able to show that the mRNA expression of CCL5; CX3CL1; CXCL10; CXCL2; IL-1beta; IL6; IL8; C3; CCL11; CCL2; CCL7; CXCL11; CXCL6; CXCL3; CXCL9; IL15; CXCL1; TNF-alpha; IL-1Ra was upregulated in human skeletal muscle cells treated with TNF-alpha in order to induce insulin resistance. Using antibody arrays we further discovered that CCL5, CXCL10, CXCL2, IL-6, IL-8, CCL2, CCL7, CXCL6, CXCL3, and CXCL1 were detectable and increased in the medium from TNF-alpha-treated (insulin-resistant) human myotubes when compared with untreated cells (24). Another study performed in L6 muscle cells has shown that TNF-alpha treatment induces a modification in the secretion of 28 different myokines: 10 were up regulated, whereas 18 were found down regulated (25).

The mechanisms involved in myokine secretion by skeletal muscle remain to be elucidated. Nevertheless, using transgenic and knockout mouse models to perturb AMPK signaling Dr. Juleen Zierath’s group has shown that AMPK-dependent pathways regulate IL-6 release from isolated oxidative skeletal muscle. Moreover, they showed that in-vitro exposure to a pharmacological activator of AMPK reduces IL-6 mRNA expression and protein release, at least in part via AMPK-independent pathways (15).
Impact of the human skeletal muscle cell secretome on pancreatic beta-cells:

Recently, we tested the hypothesis that skeletal muscle with different insulin sensitivity can impact differentially on pancreatic beta-cell function and survival (24). Human skeletal muscle cells (vastus lateralis) from 9 different healthy subjects patients were prepared and growth either in control condition or exposed to TNF-alpha for 24h to induce insulin resistance (26). The conditioned media were then collected and added to pancreatic beta-cells (Figure 1). We have found that the secretome from normally insulin sensitive muscle cells increased rat primary beta-cell proliferation as well as glucose induced insulin secretion (GSIS) in both rat and human primary beta-cells. By contrast, conditioned medium from insulin resistant skeletal (TNF-alpha treated) muscle cells increased apoptosis of both rat and human primary beta-cells, and decreased rat primary beta-cell proliferation. We also observed a decrease in GSIS from both species of primary beta-cells (24). It is important to note here TNF-alpha alone decreases GSIS (26) but has no effect on beta-cell apoptosis or proliferation; the decreased proliferation and increased apoptosis of beta-cells following treatment with medium conditioned by insulin-resistant muscle cells was thus due uniquely to secreted products rather than to the exogeous cytokine (24). Using this artificial in-vitro model (Figure 1) we were thus able to conclude that the secretome from skeletal muscle cells contains factors that can impact on beta-cells either positively (insulin sensitive muscle) or negatively (insulin resistant muscle).

The myokines that we have identified in our conditioned media include several cytokines and chemokines already tested independently for their impact on pancreatic beta-cells. Taking the example of IL-1beta, it was thus shown that this cytokine on its own can impact positively or negatively on the function, survival and proliferation of beta-cells, depending on concentration
and time of exposure (27). We have also documented positive effects on survival and proliferation of low levels of IL-1beta secreted from beta-cells themselves when plated on a particular extracellular matrix (28) and have identified the major signalling pathway underlying these positive effects on proliferation (29). However, the impact on beta cells of the combination of the dozens of myokines and other products including multiple metabolites that are secreted from muscle cells had never been investigated previously. It will require more detailed studies to identify the major players and the way they interact with each other leading to the biological endpoint observed in beta-cells.

**TNF-alpha impact on pancreatic beta-cells:**

As mentioned previously, TNF-alpha has been classified as myokine (21). We also find it in the secretome of human skeletal muscle cells in the control condition, albeit at low levels (24). Several groups, our own included, have studied the impact of very high concentrations of TNF-alpha on insulin secreting cell lines or primary beta-cells (30-33). Such treatment of transformed beta-cells induces insulin resistance, decreases glucose induce insulin secretion and stimulate apoptosis (31-33). Using human and rat primary sorted beta-cells, we have found that TNF-alpha treatment for 24h inhibits GSIS without impacting on apoptosis and proliferation (26). This is associated with a decrease in glucose-stimulated phosphorylation of key proteins in the insulin signalling pathway including Akt, AS160 and other Akt substrates, ERK as well as the insulin receptor itself (Figure 2). Strikingly, TNF-alpha treatment decreased IRS-2 protein levels, although mRNA expression was unchanged. While TNF-alpha treatment increased MAP4K4 mRNA, knockdown of MAP4K4 by siRNA protected beta-cells against the detrimental effects of TNF-alpha on both insulin secretion and signalling. We thus identified MAP4K4 as a key
upstream mediator of TNF-alpha action on the beta-cell (Figure 2) (26). Interestingly, although as mentioned TNF-alpha treatment failed to induce apoptosis in primary beta-cells, it was shown to stimulate apoptosis in an insulin-secreting cell line via the activation of JNK (33). Intriguingly, careful analysis of the data presented in our previous study indicates that the low levels of TNF-alpha secreted by insulin-sensitive (control) human myotubes promotes beta-cell proliferation together with a small improvement in GSIS, indicating bimodal effects for this cytokine reminiscent of those described above for IL-1beta.

**CXCL10 impact on pancreatic beta-cells:**

Interferon-gamma-inducible protein (IP-10), also named chemokine (C-X-C motif) ligand 10 (CXCL10) was found increased in the secretome of human skeletal muscle cells treated with TNF-alpha (24). CXCL10 levels have been shown to be increased also in the serum from Type 1 and Type 2 diabetic patients (34-35) leading several group to study the impact of this chemokine on the endocrine pancreas. Transgenic mice with over expression of CXCL10 in the pancreas show an increase of beta-cell apoptosis as well as impaired beta-cell function. The authors concluded in that particular study that CXCL10 expression in the pancreas accelerated the autoimmune process (36). The direct impact of CXCL10 on pancreatic beta-cell function and survival has been studied in-vitro using human islets exposed to different concentrations of recombinant CXCL10. It was concluded that 0.1 or 50 ng/mL of CXCL10 induces beta-cell apoptosis, impairs insulin secretion, and decreases insulin mRNA. Interestingly, CXCL10 at the higher but not lower concentration also stimulated beta-cell proliferation. The impact of CXCL10 on beta-cells was mediated by the activation of the signalling pathway TLR4/Akt/ JNK, and the cleavage of p21-activated protein kinase 2 (37).
**IL-6 impact on pancreatic beta-cells.**

The field of mykoine research started with the discovery that IL-6 increases in the circulation during an acute bout of exercise and that it originated from exercising leg (38). This finding was further supported by an increase in IL-6 expression in biopsies obtained from skeletal muscles repeatedly during an exercise bout (39). Several effects of exercise-released IL-6 have been reported. In relation to the inflammatory response, IL-6 was demonstrated to have anti-inflammatory properties: the exercise-induced plasma IL-6 increase could thus blunt the LPS mediated TNF-alpha response (40). Several studies have been performed trying to elucidate the metabolic properties of exercise-induced IL-6. Glucose and lipid metabolism have been investigated in healthy subjects undergoing recombinant IL-6 and stable isotope labeled tracer infusions. Acute elevation of IL-6 did not mobilize glucose release during fasting (41), with no effects on glucose oxidation. By contrast, acute systemic elevation of IL-6 in healthy subjects affected lipid metabolism. Using a similar model in healthy subjects, the authors have shown an increase of the lipolytic effect revealed by increases in plasma free fatty acids, an up-regulation in muscle fatty acid turnover and oxidation (42). Acute elevation of IL-6 markedly reduces plasma concentration of amino acids (43). Taken together, IL-6 has been demonstrated to have multiple physiological effects on metabolism in healthy humans, and modulates the inflammatory response. Studies of IL-6 effects on beta-cell function have shown diverse results, with some finding a negative effect (44) and others find an enhancing effect of IL-6 on insulin production (45-46). Recently, a seminal study published by Ellingsgaard et al, demonstrated a role for IL-6 in a muscle-entero-pancreatic loop. Here IL-6 in an exercise setting was found to release GLP-1 from L-cells in the intestine and to further improve beta-cell function via local islet GLP-1 production in alpha cells, leading to improved glycemic control (47). The perception of the
endocrine role of IL-6 in metabolism is that it is dual, as IL-6 is elevated acutely with exercise with beneficial effects, but a chronic elevation in plasma IL-6 is associated with negative clinical endpoints including a contribution to T2DM (48).

**Follistatin and impact on the endocrine pancreas.**

Plasma follistatin is rapidly elevated by exercise and peaks in recovery where it is elevated some hours (49). The origin of exercise-induced follistatin seems to be dependant of the type of exercise performed. During resistance exercise, follistatin mRNA expression increases in biopsies from skeletal muscle tissue from women in hormonal replacement therapy (50). However during endurance exercise, as bicycling, no changes could be detected in the skeletal muscle tissue of healthy young men, but mice submitted to swimming increased follistatin mRNA expression markedly in the liver (49). Taken together these studies suggest that resistance exercise induces follistatin expression in the skeletal muscle and endurance exercise mainly in the liver. Follistatin has no known receptor but binds and neutralises members of the TGF-beta family in plasma; in fact most of the circulating follistatin is bound to activin-A (51). Activins have important roles in pancreatic development (52) and have been implicated in the control of insulin secretion (53). Activins and their receptors are present in the developing pancreas and adult islet cells (54-55). Follistatin, a potent endogenous activin antagonist, is also produced in adult islets. During development of the pancreas, mesenchymal cells express follistatin, which seems to be important for the development of the exocrine part of the pancreas (54-55). In the mature pancreas follistatin is primarily expressed in the beta-cells (52). This beta-cell follistatin expression disappears 24 h after injection of streptozotocin to rats, suggesting that follistatin in the adult beta-cell might play a role in cell survival (56). Beta-cell function is dependent on the
balance of activin-A and follistatin. An increased expression of activin-A leads to impaired insulin secretion, which is fully reversible with exogenous follistatin (57). This finding has led us to hypothesise that exercise-induced follistatin may impact beta-cell function. Effects on alpha cells have also been described, where follistatin can blunt the suppressive effect of activin-A on glucagon expression (58). Finally, some positive effects of follistatin have been observed in relation to pancreatic fibrosis, where follistatin can inhibit pancreatic stellate cell production of collagen, giving follistatin a possible therapeutic role (59). Older evidence exists that exercise training reduces plasma insulin excursions in response not only to glucose but also to non-glucidic secretagogues (60); perhaps this is driven by the effects of exercise induced follistatin on the endocrine pancreas.

**IL-8 impact on pancreatic beta-cells.**

Plasma IL-8 is increased with the acute inflammatory response (61), insulin resistance (62), and obesity (63). However similarly to IL-6, plasma IL-8 also increases with an acute bout of exercise (64). In the endocrine pancreas one study has demonstrated that IL-8 is predominantly expressed by the beta-cells (65), whereas another study has demonstrated at the protein level that this cytokine is expressed in the alpha-cells and that blocking IL-8 leads to reduced migration of monocytes/macrophages (66). In contrast to IL-6, IL-8 has primarily been described in a pro-inflammatory setting and little evidence exists that it has beneficial properties in relation to the endocrine pancreas. Nevertheless more studies are needed to be able to conclude about a direct impact on beta or alpha-cells.
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Figure legends:

Figure 1: Protocol to study the impact of skeletal muscle secretome on pancreatic beta-cells:
Human skeletal muscle myotubes from control patients were prepared and exposed to TNF-alpha for 24h to induce insulin resistance. The conditioned media were then collected and added to pancreatic beta-cells for 24h. Note that there were two control conditions: 1. conditioned medium without TNF-alpha; 2. medium used to culture muscle cells without TNF-alpha for up to 24h, with the addition of TNF-alpha immediately before culture with beta-cells. The goal was to test the action of the secretome from control and insulin resistant myotubes on survival, proliferation and glucose stimulation of insulin secretion in sorted primary rat and human beta-cells plated on extra cellular matrix to facilitate their attachment and spreading. This protocol was used and data presented in a previous publication(24).

Figure 2: Schematic view of the impact of TNF-alpha on sorted primary beta-cells:
Rat and human primary beta-cells were sorted and treated with TNF-alpha for 24h (26). We showed in this study that the action of TNF-alpha on primary beta-cells was mediated by the activation of MAP4K4, JNK and p70S6kinase. This activation was associated with a decrease of IRS-2 expression as well as a decrease of glucose induces AKT and AS160 phosphorylation.
Liver, Pancreas, Adipose tissue

Skeletal muscle

IL-6
BDNF
CXCL10

IL-6
IL-8
IL-15
BDNF
LIF

IL-6
Irisin
BDNF
**Pancreatic Beta-Cells**

**Human Skeletal Muscle Cells**

- **Control Cond. Medium**
- **Control Cond. Medium + TNF-α at time of addition to beta-cells**
- **Insulin resistance Cond. Medium**

**CONDITIONED MEDIUM (24h)**

**Apoptosis**

**Proliferation**

**Insulin secretion**

**24 h TNFα**

**Insulin resistance**
- TNF-alpha
  - MAP4K4
    - p-JNK
      - IRS-2
        - p-Akt/p-AS160
          - Insulin Secretion
    - p70S6Kinase