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Potential role of skeletal muscle glucose metabolism on the regulation of insulin secretion

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Summary

Pancreatic beta cells sense glucose flux and release as much insulin as required in order to maintain glycaemia within a narrow range. Insulin secretion is regulated by many factors including glucose, incretins, and sympathetic and parasympathetic tones among other physiological factors. To identify the mechanisms linking obesity-related insulin resistance with impaired insulin secretion represents a central challenge. Recently, it has been argued that a crosstalk between skeletal muscle and the pancreas may regulate insulin secretion. Considering that skeletal muscle is the largest organ in non-obese subjects and a major site of insulin- and exercise-stimulated glucose disposal, it appears plausible that muscle might interact with the pancreas and modulate insulin secretion for appropriate peripheral intracellular glucose utilization. There is growing evidence that muscle can secrete so-called myokines that can have auto/para/endocrine actions. Although it is unclear in which direction they act, interleukin-6 seems to be a possible muscle-derived candidate protein mediating such inter-organ communication. We herein review some of the putative skeletal muscle-derived factors mediating this interaction. In addition, the evidence coming from *in vitro*, animal and human studies that support such inter-organ crosstalk is thoroughly discussed.

Keywords: Beta cell, glucose flux, insulin resistance, myokines.

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Introduction

Systemic glucose homeostasis is a complex process in which pancreatic beta cells sense glucose flux and release insulin to keep glycaemia within a narrow range. Optimal insulin secretion must be adjusted to insulin demand; otherwise insulin excess will cause hypoglycaemia and insufficient insulin secretion will lead to hyperglycaemia (1).

Pancreas size in an adult human represents about 0.1% of whole body mass (2), while islet mass is only 1–2% of the entire pancreas (3). Therefore, less than 0.001% (beta cells are about one-half of pancreatic islets) of whole body size is responsible for glucose homeostasis, particularly under postprandial conditions, on which maximal insulin secretion is required. It is fascinating how such a tiny part of the human body (and other mammals) can release a sufficient amount of insulin that allows an appropriate peripheral glucose disposal. Such observation may suggest the existence of a crosstalk between pancreas and peripheral tissues in order to better adjust insulin secretion to its demand.

In this scenario, insulin sensitivity has been strongly linked to some aspects of beta cell function, such as fasting and glucose-stimulated insulin secretion (4). This interaction is described by an inverse correlation, which usually follows a hyperbolic function (Fig. 1). Thus, how much insulin is released in response to a given glucose load appears to take into account the degree of insulin sensitivity. Such finding reinforces the notion that insulin-sensitive tissues may communicate with the pancreas regarding their

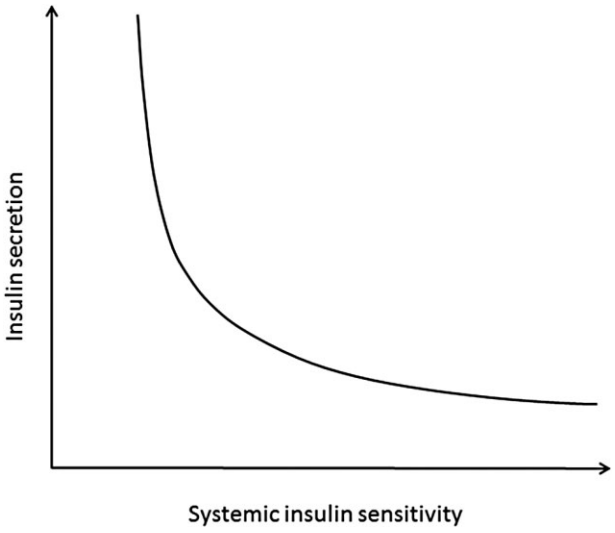

Figure 1 Hyperbolic relationship between peripheral insulin sensitivity and first-phase insulin secretion. Adapted from Weyer *et al.* (4).

insulin needs, particularly under postprandial conditions. Whether insulin-sensitive tissues play a role on short- (i.e. fasting to postprandial state) or long-term (i.e. chronic adaptation) regulation of insulin secretion is critical for understanding the nature of this potential inter-organ communication.

On one hand, a short-term inter–organ-dependent regulation should be able to quickly influence insulin secretion after a meal. Given that insulin sensitivity does not change acutely, one can speculate that an insulin–sensitive-based organ communication would not play a role on adjusting insulin secretion to rapid changes in nutrient availability. Alternatively, any eventual information coming from insulin-sensitive organs may hypothetically participate in the adaptation of insulin secretion to chronic states of insulin resistance including obesity, type 2 diabetes, pregnancy and puberty.

On the other hand, insulin secretion may still be responsive to acute changes in glucose flux (i.e. uptake, oxidation or storage) in insulin-sensitive tissues. Part of the rationale for this idea comes from the existence of a well-known interaction between brain and the pancreas for controlling insulin secretion (5,6). The brain is an important and exclusive glucose-consuming organ under physiological conditions [~100 g per day in adult humans (7,8)]. This organ is sensitive to low circulating glucose concentration, which finally determines a lower glucose flux to neurons. In order to ensure a constant glucose supply, hypoglycaemia triggers a brain-mediated counter-regulatory response that increases hepatic glucose production (possibly mediated through the vagus nerve) (9) and suppresses glucose-induced insulin secretion through sympathetic nerves (10). By analogy to the brain, skeletal muscle may influence insulin secretion to secure an appropriate glucose flux into major insulin-sensitive glucose-requiring organs. This will also prevent hyperglycaemia, as well as a glucose overflow into tissues during the transition from fasting to postprandial conditions.

We herein elaborate the argument that skeletal muscle, as the largest organ in non-obese subjects (11) and a major site of insulin- and exercise-stimulated glucose disposal (12), releases insulin sensitivity- and/or glucose flux-responsive mediators that adjust insulin secretion to the actual insulin need for appropriate peripheral intracellular glucose utilization. The release of these mediators could be chronically modulated by insulin resistance, and acutely influenced by meal- and exercise-induced changes in glucose flux (Fig. 2). Based on the classical inverse relationship between insulin sensitivity and secretion, one can hypothesize that these mediators will have a net suppressive effect on insulin secretion. We analysed *in vitro*, animal and human evidence that highlights a potential role of skeletal muscle on the regulation of insulin secretion.

Current understanding of the interaction between insulin secretion and insulin sensitivity

Kahn *et al.* (13) proposed few years ago that the inverse relationship between insulin secretion and insulin sensitivity (Fig. 1) may be determined by at least three conditions/mediators (i) increased beta cell glucose metabolism; (ii) high circulating free fatty acid (FFA) concentration and signalling and (iii) enhanced beta cell incretin sensitivity. Additionally, insulin itself also shows to increase insulin secretion (14,15). The rationale supporting these conditions/mediators is discussed later.

Beta cell glucose metabolism

In humans, glucose enters beta cells via facilitated diffusion through glucose transporter (GLUT) 1 and GLUT3 (3,16). Glucose is phosphorylated into glucose-6-phosphate in a reaction catalysed by hexokinase IV (17). Unlike other hexokinases, this enzyme is not inhibited by glucose-6-phosphate, which allows the glucose flux through this enzyme closely matching glucose utilization (18). Thus, in beta cells, the limiting step for glucose metabolism at normal glycaemia is hexokinase IV activity. In turn, in the mitochondrial matrix of beta cells, glycolytic-derived pyruvate is oxidized in the Krebs cycle after being converted into acetyl-coenzyme A. Nicotinamide adenine dinucleotide and flavin adenine dinucleotide derived from the Krebs cycle will feed the electron transport chain and enhance mitochondrial adenosine triphosphate (ATP) synthesis (19). The higher ATP-to-adenosine diphosphate ratio will close ATP-sensitive K^+ channels leading to beta cell

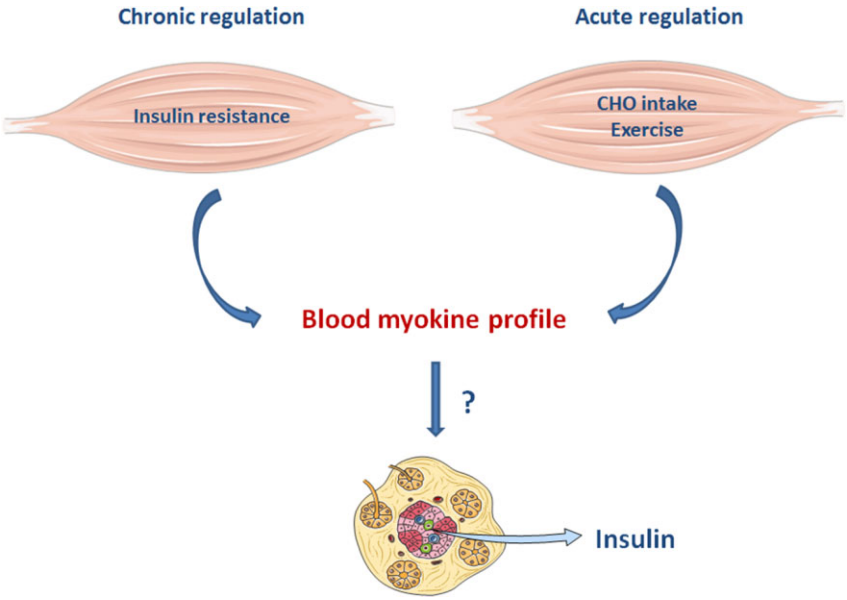

Figure 2 Hypothetical role of skeletal muscle insulin sensitivity and glucose flux as determinant factors of the myokinome profile and insulin secretion. The release of myokines into the bloodstream could be chronically modulated by skeletal muscle insulin resistance (obesity, type 2 diabetes, pregnancy or puberty), and acutely influenced by meal- and/or exercise-induced changes in glucose flux. The myokine balance would influence beta cell metabolism and differentially modulate insulin secretion. Thus, insulin-sensitive muscle might have a suppressive effect on insulin secretion, whereas insulin-resistant muscle would secrete an insulin secretion-stimulating myokine profile.

depolarization. Such an effect will open voltage-operated Ca^{2+} channels increasing cytosolic Ca^{2+} concentration that will finally lead to exocytosis of insulin granules (20,21). In this regard, beta cell mitochondrial function is expected to be highly regulated to allow proper insulin secretion (22).

Insulin resistance is characterized by structural pancreatic (increased islet and beta cell mass) and beta cell functional adaptationsthat enhance insulin secretion (23). Thus, increased glycaemia is not evident during the early stages of insulin resistance, particularly during the first 10 min after glucose load (first-phase insulin secretion). Then, insulin-resistant versus insulin-sensitive individuals should have similar beta cell glucose uptake and metabolism. In addition, beta cell glucose sensitivity, a specific beta cell function parameter defined as the slope of the insulin secretion versus glucose concentration dose–response curve, has shown to be unrelated with insulin resistance (24). Therefore, any eventual increase in beta cell glucose metabolism seems to be an unlikely factor driving increased insulin secretion.

Circulating FFA concentration and signalling

Long-chain fatty acids in the presence of high glucose concentrations can bind to G-protein-coupled receptor (GPR40), which induce intracellular calcium mobilization and subsequent stimulation of insulin secretion (25,26). Indeed, mice over-expressing GPR40 in pancreatic beta cells show increased glucose-stimulated insulin secretion (27). The role of FFA on the interaction between insulin sensitivity and secretion should consider the following arguments.

It has been well accepted that impaired adipose tissue insulin sensitivity can impair insulin-dependent inhibition of lipolysis leading to increased FFA efflux and high circulating FFA concentration, particularly in conditions of enlarged fat mass and insulin resistance (e.g. obesity). However, Karpe *et al.* (28) recently compiled a large set of studies and found no evidence to support the notion that obesity is accompanied by increased fasting blood FFA concentration, a physiological state of high lipolytic activity. On the other hand, obesity, insulin resistance and type 2 diabetes are also characterized by impaired suppression of insulin-mediated serum FFA concentration (29–31), as well as increased lipoprotein lipase-mediated fatty acid spillover (32). Such phenomena may elevate postprandial circulating FFA concentration and increase insulin secretion.

In any case, an eventual role of increased circulating FFA concentration mediating the relationship between insulin-sensitive tissues and the pancreas should consider that systemic insulin sensitivity under postprandial conditions is mostly driven by skeletal muscle insulin sensitivity (29).There is no proof that an inverse correlation also applies to describe the specific relationship between adipose tissue insulin sensitivity and insulin secretion.

Beta cell incretin sensitivity

Insulin secretion can also be stimulated by incretins being glucagon-like peptide 1 (GLP1) and glucose-dependent insulinotropic polypeptide important ones (33). These molecules are mostly released from the gastrointestinal tract, being the rate of glucose absorption across the intestinal cells a major determinant of their secretion. Incretins can enhance insulin secretion particularly in the presence of high glucose concentration (34), and are responsible for the larger insulin secretion after oral versus intravenous glucose administration (35).

One may speculate that insulin resistance might be accompanied by higher sensitivity to incretins and/or increased blood incretins concentration that will lead to enhanced insulin secretion. A specific study comparing the role of insulin resistance independent of obesity, glucose intolerance and type 2 diabetes on circulating incretin levels and action has not been conducted. Therefore, the role of incretins as direct mediators of the relationship between insulin sensitivity and insulin secretion remains undetermined.

Studies comparing circulating incretin levels across individuals of different body mass index, which often pose contrasting insulin sensitivity or glucose tolerance, have found similar (36,37) or lower (38) levels in obese versus non-obese individuals. With regards to incretin action, Muscelli *et al*. (38) assessed this aspect in individuals separated according tertiles of body mass index. As predicted, leaner participants (lowest tertile) had higher insulin sensitivity when compared with individuals from the second and third tertiles. Interestingly, the highest incretin effect was found in individuals from the first tertile. These findings are against the hypothetical role of incretin driving-augmented insulin secretion in insulin resistance.

Insulin-enhancing effect on insulin secretion

Insulin by itself also appears to promote its own secretion *in vivo* (39) as well as in isolated islets (40) and beta cells (41). One of the most striking evidence about the role of insulin on its secretion came from beta cell-specific insulin receptor knockout (BIRKO) mice. In this model, impaired glucose-stimulated insulin secretion and glucose intolerance are observed (42). In parallel, human pancreatic islets from patients with type 2 diabetes have reduced mRNA expression of the insulin receptor, insulin receptor substrate 2 and the protein kinase Akt2, which are surrogate markers of impaired insulin action (43). In addition, the effect of insulin on its own secretion is reduced in type 2 diabetic and glucose-intolerant versus healthy individuals (14) as well as in insulin-resistant versus insulin-sensitive volunteers (15). Thus, impaired beta cell insulin signalling and sensitivity may be part of the mechanism contributing to impaired glucose-stimulated insulin secretion in patients with type 2 diabetes (43).

Very recently, the physiological role of insulin as enhancer of its own secretion has been strongly criticized. Rhodes *et al*. (44) argued that prolonged exposure to insulin, and/or high concentrations of insulin in all cells that express the insulin receptor effectively desensitizes the insulin receptor signalling pathway. Furthermore, insulin when secreted, rapidly gains the venal islet microcirculation from where it is readily cleared from the islet milieu. Then, insulin concentration achieved around beta cells is minimal and ineffective to transduce insulin signalling. Alternatively, the *in vivo* role of insulin on its secretion (14,39) can be attributed to an insulin-mediated central nervous system rather an autocrine effect. Taken together, the physiological relevance of insulin regulating its secretion remains unclear.

Novel insights about the interaction between insulin secretion and insulin sensitivity

As discussed so far, none of the conditions/mediators described earlier can satisfactorily explain the relationship between insulin sensitivity and insulin secretion. We hereby propose that skeletal muscle may play a critical role in this metabolic interaction. This organ has shown to be an active endocrine tissue with action on adipose tissue, liver, brain, bones and the pancreas (45,46). Skeletal muscle might release insulin–sensitive- and/or glucose flux-dependent mediators able to influence insulin secretion. The rationale for this idea is presented later.

Rationale supporting an interaction between skeletal muscle and pancreas

In the last years, we have focused our interest in the study of glucose metabolism in insulin-sensitive and insulin-resistant individuals under fasting, postprandial and insulin-infused conditions (47–49). We observed that during a euglycaemic–hyperinsulinaemic clamp, both impaired intracellular oxidative and non-oxidative glucose disposal were mostly consequence of insulin resistance (48,50). Basically, the lower insulin-stimulated glucose disposal rate in insulin-resistant versus insulin-sensitive individuals determined a corresponding decrease in intracellular glucose utilization.

Although this explanation appears logical, our finding was against the notion that intrinsic (e.g. mitochondrial) cellular defects in glucose metabolism were present in obesity and type 2 diabetes (51). This controversial outcome led us to study insulin-stimulated glucose utilization under physiological conditions, i.e. in response to a 75-g oral glucose load.

When we compared the postprandial response between insulin-resistant (non-diabetic) and insulin-sensitive individuals (insulin sensitivity defined by a euglycaemic–hyperinsulinaemic clamp), we observed the characteristic hyperinsulinaemia in the former group. In addition, a modestly higher but significant glycaemia was also detected in the insulin-resistant versus insulinsensitive group. However, whole-body glycolytic and glucose oxidative rates were similar among groups (49) (Fig. 3). We concluded that postprandial hyperinsulinaemia and modest hyperglycaemia overcome insulin resistance by enhancing glucose uptake and utilization.

It was striking to note that intracellular glucose disposal (at glycolytic and oxidative levels) was finely matched

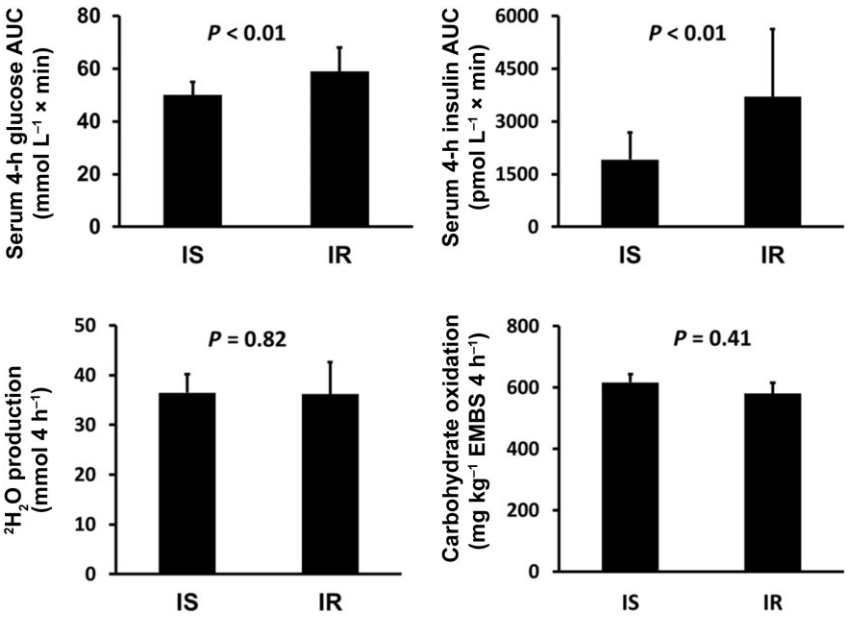

Figure 3 Glycaemia, insulinaemia and glucose metabolism (glycolytical and oxidative disposal) in insulin-sensitive and insulin-resistant individuals in response to a 75-g oral glucose dose. ^2H_2O, deuterated water; AUC, area under the curve; EMBS, estimated metabolic body size (fat-free mass [kg] + 17.7); IR, insulin-resistant; IS, insulin-sensitive.

between subjects of contrasting insulin sensitivity. Even when higher glycaemia could further increase insulin secretion in insulin-resistant subjects, somehow the body seems to acknowledge that intracellular glucose homeostasis is 'just fine'. Considering that whole-body glucose disposal after an oral glucose load as well as during physical exercise mostly occur in skeletal muscle (52), then, one can speculate that these tissues may be global sensors of peripheral glucose availability, and interact with other organs such as the pancreas in order to adjust insulin secretion to insulin needs. The discovery of hundreds of proteins secreted from skeletal muscle (53) reinforces the notion that these tissues may have an active role in regulating insulin secretion.

Skeletal muscle as a circulating factor-secreting organ

In the last decade, the notion that skeletal muscle can secrete multiple active factors (myokines) has gained support (45,46). Well-known skeletal muscle-secreted proteins are myostatin (54) and interleukin-6 (IL-6) (55). Myostatin circulates in the blood and is a negative regulator of muscle growth (54,56). In turn, IL-6 is mostly secreted in response to muscle contraction and plays a critical role in the metabolic adaptation to exercise (57). Later on in time, Bortoluzzi *et al.* (58) characterized the human skeletal muscle secretome (myokinome) using a computational approach to identify the putative myokinome. Over 300 proteins met the criteria for secreted proteins including 78 uncharacterized proteins. Furthermore, by analysing conditioned media from human and mice myotubes, multiple secreted proteins were recently identified (53,59,60).

Among the myokines that may mediate an interaction between insulin-sensitive tissues and the pancreas, IL-6 is highlighted although its role seems controversial. In this regard, IL-6 showed both positive (61) and negative (62) influence on insulin secretion. Additionally, plasma IL-6 concentration was directly related with acute glucose-stimulated insulin secretion in humans (63), whereas an acute increase in circulating IL-6 showed null effect on insulinaemia in either rodents or humans *in vivo* (64–66). Additional findings show that a whole-body IL-6 KO mouse model had normal insulin levels (67); however, skeletal muscle-specific IL-6 transgenic mice developed hyperinsulinaemia (68). The discrepancy might be due to differential IL-6 levels achieved among studies. Physiological concentrations of IL-6 (<100 pg mL^{-1}) show a stimulatory effect on glucose-stimulated insulin secretion. By contrast, neutral or inhibitory effects of IL-6 were reported at high concentrations of this cytokine (500–25,000 pg mL^{-1}) (69–71).

Alternative myokines that might participate in this muscle–pancreas crosstalk are IL-1β, chemokine C-C motif ligand 5, monocyte chemotactic protein 1, IL-8 and chemokine C-X-C motif ligand 10 (60). As further detailed later, these myokines were differentially found in conditioned media from tumour necrosis factor alpha (TNFα)-treated (insulin resistant) and non-treated human myotubes, which was accompanied by corresponding changes in beta cell insulin secretion.

Among the factors that stimulate muscle protein secretion, muscle contraction makes a distinction. In addition, the degree of muscle cell insulin sensitivity also seems to affect the muscle protein secretion profile. Meanwhile, it remains undetermined if muscle cell glucose flux may also

play a role. To identify the underlying mechanisms triggering the release of muscle-derived proteins and its influence on insulin secretion and glucose homeostasis deserve further research.

Muscle insulin sensitivity or glucose flux as putative determinant factors of muscle protein secretion pattern

As earlier mentioned, skeletal muscle insulin sensitivity and glucose flux may be independent factors involved in an inter-organ crosstalk under acute and chronic conditions. Insulin sensitivity is defined as the ability of insulin to exert its action. Such action is mostly restricted to glucose metabolism, although it is known that insulin has multiple actions. From a methodological perspective, insulin sensitivity is accurately determined by a non-physiological approach based on the extent at which glucose uptake is stimulated at a given insulin dose (i.e. euglycaemic–hyperinsulinaemic clamp) (72). Then, decreased insulin sensitivity is paralleled by impaired glucose uptake. However, under physiological conditions as observed upon oral glucose stimulation, contrasting degree of insulin sensitivity among individuals may well be accompanied by a similar glucose uptake rate (49,73). On the other hand, insulin sensitivity does not change in the transition from fasting to postprandial state; however, quantitatively important changes in glucose uptake during that period are expected (49,73). Therefore, it is relevant to differentiate the putative independent role of insulin sensitivity and glucose flux in determining a given muscle secretion profile.

This conceptual framework gives insight for better analysing some of the available evidence regarding the influence of insulin resistance on the myokinome. For instance, Bouzakri *et al.* (60) observed that specific proteins were differentially found in conditioned media collected from human insulin-sensitive versus insulin-resistant myotubes (insulin resistance induced by TNFα treatment over 24 h). Such finding suggests that insulin resistance might drive muscle secretion profile. Considering that muscle cells were maintained under basal conditions (no insulin), then, the classical feature of insulin resistance i.e. impaired insulin-stimulated glucose transport, will not be evident. Therefore, the role played by insulin resistance when no insulin is present in the muscle cell secretory profile is elusive. As a whole, insulin sensitivity and glucose flux may interact and be part of a complex interplay of short- and long-term relevance in the regulation of insulin secretion and glucose homeostasis. The available evidence from cellular, animal and human studies is further discussed later and main relevant findings are summarized in Table 1.

Evidence in cellular and animal models

One pioneer study describing the existence of a skeletal muscle–pancreas crosstalk came from a muscle-specific *PGC1α* (a master gene of mitochondrial biogenesis) KO mouse model (MKO) (62). This experiment was originally designed to test the hypothesis that a specific impairment in skeletal muscle mitochondrial capacity will lead to defective muscle insulin action. On the contrary, this MKO

Table 1 Evidence supporting a skeletal muscle–pancreas crosstalk in the regulation of insulin secretion

Study	Experimental design/model	Insulin secretion-related outcome*	Candidate myokines
Handschin *et al.* (62)	Skeletal muscle-specific *PGC1α* KO mice	Decreased *in vivo* GSIS and normal *in vitro* GSIS (mouse pancreatic islets)	IL-6
Ellingsgaard *et al.* (61)	Exercised mice (treadmill running)	Increased *in vivo* GSIS	IL-6
Hirner *et al.* (74)	Skeletal muscle-specific RF1 TG mice	Increased insulinaemia upon ipGTT	Unknown
Bouzakri *et al.* (60)	Conditioned media from TNFα-treated human myotubes	Decreased *in vitro* GSIS (human and rat beta cells)	IL-6/8, CCL-2/5/7, CXCL-1/2/3/6/10
Dela *et al.* (85)	3 months of training in T2DM subjects	Increased *in vivo* GSIS in T2DM with higher remaining pancreatic function	Not suggested
Malin *et al.* (86)	12 weeks of training in obese pre-diabetic subjects	Decreased insulinaemia upon OGTT	Not suggested
Tsuchiya *et al.* (78)	6, 9 and 12 weeks of training in rats	Increased *in vitro* GSIS upon 9 and 12 week of training (rat pancreatic islets)	Not suggested
Almeida *et al.* (81)	8 weeks of training in rats	Decreased *in vitro* GSIS upon 8 week of training (rat pancreatic islets)	Not suggested

*When compared with control group.
CCL, chemokine C-C motif ligand; CXCL, chemokine C-X-C motif ligand; GSIS, glucose-stimulated insulin secretion; IL-6, interleukin-6; ipGTT, intraperitoneal glucose tolerance test; KO, knockout; OGTT, oral glucose tolerance test; RF1, RING finger protein 1; T2DM, type 2 diabetes mellitus; TG, transgenic; TNFα, tumour necrosis factor alpha.

mouse presented similar or even enhanced insulin sensitivity when compared with wild-type mice.

However, MKO mice developed hyperglycaemia under fasting and fed conditions that was accompanied by hypoinsulinaemia and impaired *in vivo* glucose-stimulated insulin secretion. Additionally, isolated islets of this MKO model showed abnormal pancreatic islet morphology, grossly enlarged islets compared with control, but smaller individual beta cells, suggesting a higher number of beta cells in MKOs. Interestingly, when tested *in vitro* for their ability to secrete insulin in response to glucose, no differences in total insulin content and insulin secretion (at low and high glucose concentrations) were detected between control and MKO pancreatic islets. This finding prompted the idea that an extrinsic factor, presumably arising from muscle of the MKOs, was having a negative effect on pancreatic islets function under *in vivo* conditions.

In this regard, Handschin *et al*. (62) found that muscle tissue from MKOs showed increased expression of several inflammatory-related genes such as IL-6 and TNFα. Furthermore, circulating IL-6 concentration was increased in these MKO animals. They also demonstrated that isolated mouse pancreatic islets treated for 24 h with IL-6 manifested suppressed glucose-stimulated insulin secretion. Because no features of inflammation or apoptosis were detected in pancreas from MKO versus wild-type mice, IL-6 or other skeletal muscle-derived circulating factors appears to affect glucose-stimulated insulin secretion by an inflammatory-independent mechanism. Thus, IL-6 seems to be a candidate muscle-derived protein mediating a putative muscle–pancreas crosstalk.

The potential role of IL-6 mediating this inter-organ communication was also proposed by Ellingsgaard *et al*. (61); although in this study, IL-6 appeared to stimulate insulin secretion. This research demonstrated that IL-6 coming from contracting skeletal muscle or white adipose tissue promoted GLP1 secretion from L cells and pancreatic alpha cells leading to improved beta cell insulin secretion and glucose tolerance. Indeed, blocking IL-6 action with an antibody to this cytokine in *db/db* mice accelerated deterioration of glucose tolerance. *In vitro*, IL-6 increased GLP1 production from mice L cells and human α cells through increased proglucagon and prohormone convertase 1/3 expression. Thus, IL-6 appears to be part of a compensatory mechanism to enhance insulin secretion and preserve glucose homeostasis in states of insulin resistance.

Another study suggesting a potential muscle–pancreas crosstalk came from a muscle-specific transgenic mouse for RING finger protein 1, a protein responsible for proteasome-dependent protein degradation. Once again, the outcomes diverged from the original aim. Indeed, the authors hypothesized that MRF1 overexpression would lead to muscle wasting (74), but no features of increased muscle degradation were observed. However, these transgenic animals showed lower hepatic glycogen content and hyperinsulinaemia both at fast and fed conditions accompanied by delayed serum insulin peak. At the skeletal muscle level, reduced protein content of the α subunit of pyruvate dehydrogenase, its regulating kinase (PDK2), and glycogenin mRNA expression (critical enzyme for *de novo* glycogen synthesis) were detected. Taken together, this study may suggest that skeletal muscle alterations in glucose metabolism can trigger the release of a soluble factor able to affect hepatic and pancreatic function.

An animal model that deserves analysis corresponds to muscle-specific insulin receptor KO mice (MIRKO). As expected, these mice exhibit severe muscle insulin resistance [~75% decrease in glucose utilization under hyperinsulinaemic–euglycaemic clamp conditions (75) (76)]; however, glucose tolerance is essentially normal (76). Interestingly, adipose tissue from MIRKO mice shows a threefold increase in insulin-stimulated glucose uptake *in vivo* relative to controls (75). This increase in fat glucose uptake appears to be due to some unknown circulating factors released from the muscle of MIRKO mice. In fact, isolated adipocytes from MIRKO mice had similar *in vitro* insulin-stimulated glucose transport when compared with adipocytes from wild-type mice.

Consistent with this shift in the partitioning of glucose into adipose tissue, MIRKO mice show increased whole-body fat content, hypertriglyceridaemia and increased serum FFA concentration. Therefore, these results suggest that muscle, either through changes in substrate availability or by acting as an endocrine tissue communicates with and regulates insulin sensitivity in other tissues. As an extension of these findings, mice having a deletion of the insulin receptor in pancreatic beta and muscle cells (BIRKO/MIRKO) have better glucose-stimulated insulin secretion when compared with BIRKO mice (77). These findings highlight the fact that changes in muscle metabolism induced by the deletion of the insulin receptor gene may lead to a differential myokine profile that can rescue the abnormal glucose homeostasis in BIRKO mice.

Further information that supports the existence of a skeletal muscle–pancreas crosstalk came from the assessment of glucose-stimulated insulin secretion in beta cells treated with conditioned media from insulin-sensitive and insulin-resistant (induced by 24-h TNFα treatment) human myotubes (60). Multiple differences in the myotube-derived soluble protein profile were detected, which was accompanied by increased beta cell death, decreased cell proliferation and impaired glucose-stimulated insulin secretion when compared with incubated beta cells with conditioned media from non-TNFα-treated myotubes. As discussed earlier, the role of insulin resistance on this finding when no insulin was present is unclear.

An alternative strategy that suggests the endocrine role of muscle on insulin secretion regulation comes from studies

including exercise. In those studies, glucose-stimulated insulin secretion from isolated pancreatic islets differs between the control and exercised groups, although not always following a consistent pattern (78–82). For instance, Tsuchiya *et al*. (78) found enhanced glucose-stimulated insulin secretion in exercised rats, whereas, Almeida *et al*. (81) observed reduced glucose-stimulated insulin secretion in response to training.

Evidence in humans

Direct evidence supporting (or negating) an interaction between skeletal muscle and the pancreas has still not been provided. On the other hand, there is no study intended to specifically assess such muscle–pancreas crosstalk in humans. Meanwhile, some studies assessing the role of insulin on its own secretion might provide insight on the existence of such skeletal muscle–pancreas crosstalk in humans (8,14,39,83). We will only comment the study of Halperin *et al*., because it is the most informative and all of them showed similar outcomes (14).

Glucose-stimulated insulin secretion was measured in healthy, glucose-intolerant and type 2 diabetic subjects after a 4-h saline infusion (i.e. low insulinaemia and low glucose disposal rate) and few weeks later after a 4-h isoglycaemic–hyperinsulinaemic clamp (i.e. high insulinaemia and high glucose disposal rate). It was concluded that pre-exposure to insulin versus saline infusion enhanced glucose-stimulated insulin secretion. This finding was in line with *in vitro* evidence showing that insulin promotes its own secretion (41). Interestingly, this effect was impaired in individuals with altered glucose homeostasis, which suggests that an underlying defect in beta cell insulin signalling may be taking place.

Recently, Rhodes *et al*. (44) proposed another explanation, on which the increased insulin-dependent insulin secretion is mediated via alleviating the negative feedback of insulin through the central nervous system. It is unclear how the divergent effect between individuals with different glucose tolerance may be attributed to that mechanism.

In line with the concept that skeletal muscle glucose flux may be an important driving factor of insulin secretion, we herein propose an alternative view point for Halperin's findings (14). Based on the fact that peripheral (mostly in skeletal muscle) glucose flux at the level of its uptake (and presumably glucose oxidation and storage as well) also differed between the saline (no insulin) versus insulin infusion condition (14), one can also envision that peripheral glucose flux could influence the myokinome and mediate the change in insulin secretion in response to insulin versus saline infusion. Testing of this hypothesis will require isolating the role of blood insulin concentration, insulin resistance and glucose flux on insulin secretion.

In this regard, the independent role of hyperinsulinaemia and insulin-stimulated glucose disposal rate on insulin secretion was examined in a large-scale, multi-centre study (n = 1,314) (15). Using multiple linear regression analysis, this study confirmed that exposure to hyperinsulinaemia promoted insulin secretion (as assessed by C-peptide secretion) during a isoglycaemic–hyperinsulinaemic clamp. This finding was independent of changes in glycaemia, body mass index, age, sex and familial diabetes background. Interestingly, such response was modulated by the degree of insulin sensitivity. Indeed, at similar insulinaemia, insulin secretion was enhanced in insulin-sensitive when compared with insulin-resistant individuals. One can understand this finding as indicative of an interaction between muscle and the pancreas.

Alternatively, impaired insulin-stimulated insulin secretion in insulin-resistant individuals may be just another facet of systemic insulin resistance. Thus, impaired insulin action at peripheral level (liver, muscle, fat) may also occur in pancreatic beta cells as found in a rodent model of diet-induced insulin resistance (84). If insulin plays a meaningful physiological role regulating its own secretion, then impaired beta cell insulin signalling may lead to decreased insulin secretion. Future studies should explore key insulin signalling nodes across tissues and cells including beta cells from classical human/animal insulin-resistant models in order to prove this hypothesis.

Additional evidence suggesting an eventual skeletal muscle–pancreas crosstalk is observed in response to physical training. Dela *et al*. (85) found in subjects who have type 2 diabetes that a 3-month physical training programme enhance glucose- and arginine-stimulated insulin secretion, although this effect was only evident in individuals with preserved remaining secretory capacity. Additionally, Malin *et al*. (86) noted in pre-diabetic obese individuals that a 12-week exercise intervention also increased insulin secretion.

Concluding remarks

An interaction between skeletal muscle and pancreas appears to occur, although so far most of the evidence comes from genetically modified animal models. This evidence is also supported by *in vitro* findings showing that muscle-derived proteins can affect beta cell insulin secretion. Meanwhile, it is largely unknown whether this inter-organ communication proceeds under physiological conditions. Furthermore, it remains undetermined which are the triggering cellular and molecular factors that determine the release of muscle-derived proteins. Even more important, one can ask why an interaction between muscle and the pancreas may be needed.

In this regard, the human body has a well-organized system to prevent hypoglycaemia, which is mostly regulated

by the brain (87). This organ is a main and exclusive insulin-independent glucose consumer (7,8); thereby, the critical role of the brain on controlling the supply of such nutrient makes sense. Following this rationale, skeletal muscle as the main insulin-dependent glucose consumer could also exert a role regulating glucose tolerance. Skeletal muscle might release some kind of 'signal' to inhibit insulin secretion when intracellular glucose (or its metabolites) exceeds a given threshold. Additionally, skeletal muscle could also enhance insulin secretion and favour glucose disposal during a meal or in a subsequent one, the latter a phenomenon known as the second-meal effect (88). This action could prevent hyperglycaemia and promote adequate intracellular glucose disposal throughout the day.

Regarding the role of these myokineson insulin secretion, whether insulin synthesis, degradation or secretion, are specifically targeted by these molecules must be elucidated. Under the context of an endocrine interaction, these myokines might also influence peripheral insulin clearance, which add complexity to the regulation of blood insulin concentration and action.

It must also be mentioned that studies oriented to specifically isolate the role of small changes in serum glucose or post-meal FFA concentration from other more complex mechanisms is required. Such studies oriented to identify muscle-secreted proteins in response to specific manipulations of skeletal muscle glucose flux, and their impact on pancreatic insulin secretion at *in vitro* and *in vivo* level will provide critical insights.

Finally, we conclude that skeletal muscle as an endocrine organ could modulate its secretory profile to communicate with other organs. Such crosstalk with the pancreas might be of benefit for maintaining an optimal insulin secretion and glucose homeostasis. Chronic insulin resistance and the acute fasting-to-postprandial changes in glucose flux are among the conditions/factors that may play a role determining the myokine secretion pattern. Understanding these mechanisms may contribute to design better treatments for conditions of altered glucose homeostasis.

Conflict of interest statement

The authors declare that there is no duality of interest associated with this paper.

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References

1. Sachdeva MM, Stoffers DA. Minireview: meeting the demand for insulin: molecular mechanisms of adaptive postnatal beta-cell mass expansion. *Mol Endocrinol* 2009; **23**: 747–758.
2. Schaefer JH. The normal weight of the pancreas in the adult human being: a biometric study. *Anat Rec* 1926; **32**: 119–132.
3. Rorsman P, Braun M. Regulation of insulin secretion in human pancreatic islets. *Annu Rev Physiol* 2013; **75**: 155–179.
4. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 1999; **104**: 787–794.
5. Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. *Diabetes* 2004; **53**: 1714–1720.
6. Winzell MS, Ahrén B. Role of VIP and PACAP in islet function. *Peptides* 2007; **28**: 1805–1813.
7. Hatazawa J, Brooks RA, Di Chiro G, Bacharach SL. Glucose utilization rate versus brain size in humans. *Neurology* 1987; **37**: 583–588.
8. Kolb H, Mandrup-Poulsen T. An immune origin of type 2 diabetes? *Diabetologia* 2005; **48**: 1038–1050.
9. Lin HV, Accili D. Hormonal regulation of hepatic glucose production in health and disease. *Cell Metab* 2011; **14**: 9–19.
10. Porte D Jr. Sympathetic regulation of insulin secretion: its relation to diabetes mellitus. *Arch Intern Med* 1969; **123**: 252–260.
11. Kim J, Wang Z, Heymsfield SB, Baumgartner RN, Gallagher D. Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *Am J Clin Nutr* 2002; **76**: 378–383.
12. Baron AD, Brechtel G, Wallace P, Edelman SV. Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol* 1988; **255**: E769–E774.
13. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; **444**: 840–846.
14. Halperin F, Lopez X, Manning R, Kahn CR, Kulkarni RN, Goldfine AB. Insulin augmentation of glucose-stimulated insulin secretion is impaired in insulin-resistant humans. *Diabetes* 2012; **61**: 301–309.
15. Mari A, Tura A, Natali A *et al*. Influence of hyperinsulinemia and insulin resistance on *in vivo* beta-cell function: their role in human beta-cell dysfunction. *Diabetes* 2011; **60**: 3141–3147.
16. De Vos A, Heimberg H, Quartier E *et al*. Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. *J Clin Invest* 1995; **96**: 2489–2495.
17. Lynedjian PB, Mobius G, Seitz HJ, Wollheim CB, Renold AE. Tissue-specific expression of glucokinase: identification of the gene product in liver and pancreatic islets. *Proc Natl Acad Sci USA* 1986; **83**: 1998–2001.
18. Matschinsky FM, Glaser B, Magnuson MA. Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities. *Diabetes* 1998; **47**: 307–315.
19. Detimary P, Gilon P, Henquin JC. Interplay between cytoplasmic Ca2 + and the ATP/ADP ratio: a feedback control mechanism in mouse pancreatic islets. *Biochem J* 1998; **333**(Pt 2): 269–274.
20. Ashcroft FM, Harrison DE, Ashcroft SJ. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature* 1984; **312**: 446–448.
21. Cook DL, Hales CN. Intracellular ATP directly blocks K+ channels in pancreatic B-cells. *Nature* 1984; **311**: 271–273.
22. Deeney JT, Prentki M, Corkey BE. Metabolic control of β-cell function. *Semin Cell Dev Biol* 2000; **11**: 267–275.
23. Gonzalez A, Merino B, Marroquí L *et al*. Insulin hypersecretion in islets from diet-induced hyperinsulinemic obese female mice is associated with several functional adaptations in individual β-cells. *Endocrinology* 2013; **154**: 3515–3524.

24. Mari A, Ferrannini E. β-Cell function assessment from modelling of oral tests: an effective approach. *Diabetes Obes Metab* 2008; **10**: 77–87.
25. Itoh Y, Kawamata Y, Harada M *et al*. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* 2003; **422**: 173–176.
26. Stein DT, Stevenson BE, Chester MW *et al*. The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. *J Clin Invest* 1997; **100**: 398–403.
27. Nagasumi K, Esaki R, Iwachidow K *et al*. Overexpression of GPR40 in pancreatic beta-cells augments glucose-stimulated insulin secretion and improves glucose tolerance in normal and diabetic mice. *Diabetes* 2009; **58**: 1067–1076.
28. Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes* 2011; **60**: 2441–2449.
29. Kelley DE, Williams KV, Price JC, McKolanis TM, Goodpaster BH, Thaete FL. Plasma fatty acids, adiposity, and variance of skeletal muscle insulin resistance in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2001; **86**: 5412–5419.
30. Poynten AM, Gan SK, Kriketos AD, Campbell LV, Chisholm DJ. Circulating fatty acids, non-high density lipoprotein cholesterol, and insulin-infused fat oxidation acutely influence whole body insulin sensitivity in nondiabetic men. *The J Clin Endocrinol Metab* 2005; **90**: 1035–1040.
31. Galgani JE, Heilbronn LK, Azuma K *et al*. Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes* 2008; **57**: 841–845.
32. McQuaid SE, Hodson L, Neville MJ *et al*. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? *Diabetes* 2011; **60**: 47–55.
33. Drucker DJ. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. *Diabetes* 2013; **62**: 3316–3323.
34. Tillil H, Shapiro ET, Miller MA *et al*. Dose-dependent effects of oral and intravenous glucose on insulin secretion and clearance in normal humans. *Am J Physiol* 1988; **254**: E349–E357.
35. Mccullough AJ, Miller LJ, Service FJ, Go VLW. Effect of graded intraduodenal glucose infusions on the release and physiological action of gastric inhibitory polypeptide. *J Clin Endocrinol Metab* 1983; **56**: 234–241.
36. Verdich C, Toubro S, Buemann B, Lysgard Madsen J, Juul Holst J, Astrup A. The role of postprandial releases of insulin and incretin hormones in meal-induced satiety – effect of obesity and weight reduction. *Int J Obes Relat Metab Disord* 2001; **25**: 1206–1214.
37. Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 2011; **54**: 10–18.
38. Muscelli E, Mari A, Casolaro A *et al*. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes* 2008; **57**: 1340–1348.
39. Bouche C, Lopez X, Fleischman A *et al*. Insulin enhances glucose-stimulated insulin secretion in healthy humans. *Proc Natl Acad Sci USA* 2010; **107**: 4770–4775.
40. Borelli MI, Francini F, Gagliardino JJ. Autocrine regulation of glucose metabolism in pancreatic islets. *Am J Physiol Endocrinol Metab* 2003; **286**: E111–E115.
41. Aspinwall CA, Lakey JR, Kennedy RT. Insulin-stimulated insulin secretion in single pancreatic beta cells. *J Biol Chem* 1999; **274**: 6360–6365.
42. Kulkarni RN, Brüning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 1999; **96**: 329–339.
43. Gunton JE, Kulkarni RN, Yim S *et al*. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. *Cell* 2005; **122**: 337–349.
44. Rhodes CJ, White MF, Leahy JL, Kahn SE. Direct autocrine action of insulin on β-cells: does it make physiological sense? *Diabetes* 2013; **62**: 2157–2163.
45. Trayhurn P, Drevon CA, Eckel J. Secreted proteins from adipose tissue and skeletal muscle – adipokines, myokines and adipose/muscle cross-talk. *Arch Physiol Biochem* 2011; **117**: 47–56.
46. Pedersen BK. Muscle as a secretory organ. *Compr Physiol* 2013; **3**: 1337–1362.
47. Galgani JE, de Jonge L, Rood JC, Smith SR, Young AA, Ravussin E. Urinary C-peptide excretion: a novel alternate measure of insulin sensitivity in physiological conditions. *Obesity (Silver Spring)* 2010; **18**: 1852–1857.
48. Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. *Am J Physiol Endocrinol Metab* 2008; **295**: E1009–E1017.
49. Galgani JE, Ravussin E. Postprandial whole-body glycolysis is similar in insulin-resistant and insulin-sensitive non-diabetic humans. *Diabetologia* 2012; **55**: 737–742.
50. Stull AJ, Galgani JE, Johnson WD, Cefalu WT. The contribution of race and diabetes status to metabolic flexibility in humans. *Metabolism* 2010; **59**: 1358–1364.
51. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 2000; **49**: 677–683.
52. Ferrannini E, Bjorkman O, Reichard GA Jr *et al*. The disposal of an oral glucose load in healthy subjects. A quantitative study. *Diabetes* 1985; **34**: 580–588.
53. Hartwig S, Raschke S, Knebel B *et al*. Secretome profiling of primary human skeletal muscle cells. *Biochim Biophys Acta* 2013; doi: 10.1016/j.bbapap.2013.08.004.
54. Lee SJ. Regulation of muscle mass by myostatin. *Annu Rev Cell Dev Biol* 2004; **20**: 61–86.
55. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 2008; **88**: 1379–1406.
56. 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.
57. Carey AL, Steinberg GR, Macaulay SL *et al*. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation *in vitro* via AMP-activated protein kinase. *Diabetes* 2006; **55**: 2688–2697.
58. Bortoluzzi S, Scannapieco P, Cestaro A, Danieli GA, Schiaffino S. Computational reconstruction of the human skeletal muscle secretome. *Proteins* 2006; **62**: 776–792.
59. Bostrom P, Wu J, Jedrychowski MP *et al*. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; **481**: 463–468.
60. Bouzakri K, Plomgaard P, Berney T, Donath MY, Pedersen BK, Halban PA. Bimodal effect on pancreatic beta-cells of secretory products from normal or insulin-resistant human skeletal muscle. *Diabetes* 2011; **60**: 1111–1121.
61. Ellingsgaard H, Hauselmann I, Schuler B *et al*. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med* 2011; **17**: 1481–1489.

62. Handschin C, Choi CS, Chin S *et al*. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J Clin Invest* 2007; **117**: 3463–3474.
63. Andreozzi F, Laratta E, Cardellini M *et al*. Plasma interleukin-6 levels are independently associated with insulin secretion in a cohort of Italian-Caucasian nondiabetic subjects. *Diabetes* 2006; **55**: 2021–2024.
64. Kim HJ, Higashimori T, Park SY *et al*. Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action *in vivo*. *Diabetes* 2004; **53**: 1060–1067.
65. Tsigos C, Papanicolaou DA, Kyrou I, Defensor R, Mitsiadis CS, Chrousos GP. Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. *J Clin Endocrinol Metab* 1997; **82**: 4167–4170.
66. van Hall G, Steensberg A, Sacchetti M *et al*. Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab* 2003; **88**: 3005–3010.
67. Ellingsgaard H, Ehses JA, Hammar EB *et al*. Interleukin-6 regulates pancreatic alpha-cell mass expansion. *Proc Natl Acad Sci USA* 2008; **105**: 13163–13168.
68. Franckhauser S, Elias I, Rotter Sopasakis V *et al*. Overexpression of Il6 leads to hyperinsulinaemia, liver inflammation and reduced body weight in mice. *Diabetologia* 2008; **51**: 1306–1316.
69. Eizirik DL, Sandler S, Welsh N *et al*. Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. *J Clin Invest* 1994; **93**: 1968–1974.
70. Sandler S, Bendtzen K, Eizirik DL, Welsh M. Interleukin-6 affects insulin secretion and glucose metabolism of rat pancreatic islets *in vitro*. *Endocrinology* 1990; **126**: 1288–1294.
71. Southern C, Schulster D, Green IC. Inhibition of insulin secretion from rat islets of Langerhans by interleukin-6. An effect distinct from that of interleukin-1. *Biochem J* 1990; **272**: 243–245.
72. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: E214–E223.
73. Woerle HJ, Szoke E, Meyer C *et al*. Mechanisms for abnormal postprandial glucose metabolism in type 2 diabetes. *Am J Physiol Endocrinol Metab* 2005; **290**: E67–E77.
74. Hirner S, Krohne C, Schuster A *et al*. MuRF1-dependent regulation of systemic carbohydrate metabolism as revealed from transgenic mouse studies. *J Mol Biol* 2008; **379**: 666–677.
75. Kim JK, Michael MD, Previs SF *et al*. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. *J Clin Invest* 2000; **105**: 1791–1797.
76. Brüning JC, Michael MD, Winnay JN *et al*. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 1998; **2**: 559–569.
77. Mauvais-Jarvis F, Virkamaki A, Michael MD *et al*. A model to explore the interaction between muscle insulin resistance and beta-cell dysfunction in the development of type 2 diabetes. *Diabetes* 2000; **49**: 2126–2134.
78. Tsuchiya M, Manabe Y, Yamada K, Furuichi Y, Hosaka M, Fujii NL. Chronic exercise enhances insulin secretion ability of pancreatic islets without change in insulin content in non-diabetic rats. *Biochem Biophys Res Commun* 2013; **430**: 676–682.
79. de Carvalho Leite N, Ferreira TR, Rickli S *et al*. Glycolytic and mitochondrial metabolism in pancreatic islets from MSG-treated obese rats subjected to swimming training. *Cell Physiol Biochem* 2013; **31**: 242–256.
80. Gomes RM, Tófolo LP, Rinaldi W *et al*. Moderate exercise restores pancreatic beta-cell function and autonomic nervous system activity in obese rats induced by high-fat diet. *Cell Physiol Biochem* 2013; **32**: 310–321.
81. Almeida FN, Proença ARG, Chimin P, Marçal AC, Bessa-Lima F, Carvalho CRO. Physical exercise and pancreatic islets: acute and chronic actions on insulin secretion. *Islets* 2012; **4**: 296–301.
82. Oliveira CA, Paiva MF, Mota CA *et al*. Exercise at anaerobic threshold intensity and insulin secretion by isolated pancreatic islets of rats. *Islets* 2010; **2**: 240–246.
83. Anderwald C, Tura A, Grassi A *et al*. Insulin infusion during normoglycemia modulates insulin secretion according to whole-body insulin sensitivity. *Diabetes Care* 2011; **34**: 437–441.
84. Francini F, Gagliardino JJ, Borelli MI. Decreased islet sensitivity to insulin in hamsters with dietary-induced insulin resistance. *Life Sci* 2008; **82**: 817–822.
85. Dela F, von Linstow ME, Mikines KJ, Galbo H. Physical training may enhance β-cell function in type 2 diabetes. *Am J Physiol Endocrinol Metab* 2004; **287**: E1024–E1031.
86. Malin SK, Solomon TPJ, Blaszczak A, Finnegan S, Filion J, Kirwan JP. Pancreatic β-cell function increases in a linear dose-response manner following exercise training in adults with prediabetes. *Am J Physiol Endocrinol Metab* 2013; **305**: E1248–E1254.
87. Herman MA, Kahn BB. Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. *J Clin Invest* 2006; **116**: 1767–1775.
88. Bonuccelli S, Muscelli E, Gastaldelli A *et al*. Improved tolerance to sequential glucose loading (Staub–Traugott effect): size and mechanisms. *Am J Physiol Endocrinol Metab* 2009; **297**: E532–E537.