Bimodal Effect on Pancreatic \( \beta \)-Cells of Secretory Products From Normal or Insulin-Resistant Human Skeletal Muscle

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OBJECTIVE—Type 2 diabetes is characterized by insulin resistance with a relative deficiency in insulin secretion. This study explored the potential communication between insulin-resistant human skeletal muscle and primary (human and rat) \( \beta \)-cells.

RESEARCH DESIGN AND METHODS—Human skeletal muscle cells were cultured for up to 24 h with tumor necrosis factor (TNF)-\( \alpha \) to induce insulin resistance, and mRNA expression for cytokines was analyzed and compared with controls (without TNF-\( \alpha \)). Conditioned media were collected and candidate cytokines were measured by antibody array. Human and rat primary \( \beta \)-cells were used to explore the impact of exposure to conditioned media for 24 h on apoptosis, proliferation, short-term insulin secretion, and key signaling protein phosphorylation and expression.

RESULTS—Human myotubes express and release a different panel of myokines depending on their insulin sensitivity, with each panel exerting differential effects on \( \beta \)-cells. Conditioned medium from control myotubes increased proliferation and glucose-stimulated insulin secretion (GSIS) from primary \( \beta \)-cells, whereas conditioned medium from TNF-\( \alpha \)-treated insulin-resistant myotubes (TMs) exerted detrimental effects that were either independent (increased apoptosis and decreased proliferation) or dependent on the presence of TNF-\( \alpha \) in TM (blunted GSIS). Knockdown of \( \beta \)-cell mitogen-activated protein 4 kinase 4 prevented these effects. Glucagon-like peptide 1 protected \( \beta \)-cells against decreased proliferation and apoptosis evoked by TMs, while interleukin-1 receptor antagonist only prevented the latter.

CONCLUSIONS—Taken together, these data suggest a possible new route of communication between skeletal muscle and \( \beta \)-cells that is modulated by insulin resistance and could contribute to normal \( \beta \)-cell functional mass in healthy subjects, as well as the decrease seen in type 2 diabetes.

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Regulation of \( \beta \)-cell function and mass represent a critical issue for understanding diabetes, a disease characterized in its two major forms by a relative or absolute deficiency in the number of pancreatic \( \beta \)-cells and their function. Type 1 (insulin-dependent) diabetes thus results from autoimmune destruction of pancreatic \( \beta \)-cells (1), and it is now recognized that there is decreased \( \beta \)-cell mass and function in type 2 diabetes (2) but the precise underlying mechanism remains to be determined. Insulin resistance is obviously a key player in type 2 diabetes, but it also plays a larger role in the disease process of type 1 diabetes than is commonly recognized (3).

Far from being an inert tissue in terms of interorgan communication, it is now recognized that skeletal muscle can secrete so-called myokines, which can impact both favorably and unfavorably on the function of distant organs/tissues. Interleukin (IL)-6 is the prototype myokine and was identified as the first long-searched-for muscle contraction–induced factor (4). It has been suggested that IL-6 impacts on \( \beta \)-cell function, but more detailed studies are needed to clarify the direct impact of this cytokine on pancreatic \( \beta \)-cells in health and disease, especially because the \( \alpha \)-cell has been identified as a major IL-6 target in islets (5). Adiponectin, first identified as a secretory product of adipocytes, has also been shown to be secreted by skeletal muscles (6) and can impact positively on pancreatic \( \beta \)-cell function and survival (7).

More generally, studies performed in vitro have confirmed that various cytokines and chemokines can impact positively or negatively on the function, survival, and proliferation of \( \beta \)-cells (8), including IL-1\( \beta \) (9), IL-6 (5,10), adiponectin (7), and tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) (11) as well as chemokine (C-C motif) ligand 5 (CCL5), monocytic chemoattractant protein-1 (MCP-1), IL-8, and CXCl chemokine ligand (CXCL10) (12,13). This raises the possibility of beneficial or detrimental effects of cytokines on \( \beta \)-cells depending on concentration and biological context.

Skeletal muscle plays a major role in whole-body substrate homeostasis in the postprandial state, and impaired action on this tissue contributes to the pathological condition of insulin resistance (14). The recent identification of skeletal muscle as an endocrine organ that produces and releases myokines expands our knowledge of how the nervous, endocrine, and immune systems contribute to the maintenance of homeostasis, also when challenged by changing physiological demands (4). Given that skeletal muscle is the prevalent organ in the human body in terms of its mass, we decided to explore if skeletal muscle with...
distinct insulin sensitivity can differentially impact on β-cell function.

During the last years, multiple studies have been performed to understand the mechanism of insulin resistance (15). We showed previously that TNF-α can induce insulin resistance in human skeletal muscle by altering the insulin signaling pathway (16), providing a unique system for molecular dissection in vitro (17). The physiological source of increased circulating levels of TNF-α in type 2 diabetes remains unclear, but it does not appear to be the muscle itself (18). TNF-α is, however, believed to be a major cytokine involved in the “conversation” between adipose tissue and muscle with increased levels in obesity and type 2 diabetes possibly contributing to insulin resistance in skeletal muscle (19).

With this background in mind, we have used this validated in vitro model of skeletal muscle insulin resistance to explore putative conversation between skeletal muscle and β-cells. A different panel of myokines is secreted by normally sensitive or insulin-resistant myotubes that impact in a beneficial or detrimental way, respectively, on β-cell function, proliferation, and survival.

RESULTS

Impact of TNF-α treatment on human skeletal muscle cells. Human myotubes were cultured for 24 h with 20 ng/mL TNF-α. Cells treated with TNF-α showed decreased IRS-1 protein expression (Fig. 1A), and a diminution of both IRS-1 tyrosine (85% vs. untreated control, P < 0.01) and Akt (Ser473) phosphorylation (54% vs. untreated control, P < 0.01) after insulin stimulation (representative Western blot) (Fig. 1B). This validates the experimental model, confirming that TNF-α induced insulin resistance as expected (17). A concentration of 20 ng/mL TNF-α (as used in the current study) had no impact on human skeletal muscle cell death at any time up to 72 h (Fig. 1C). However, there was a significant decrease of cell death at 24 h using 10 ng/mL TNF-α, but an increase at 40 ng/mL (Fig. 1D), albeit with <2% of the cells being TUNEL-positive even under these latter conditions.

Myokine expression and secretion from human skeletal muscle cells (myotubes) treated with or without TNF-α. Human myotubes were cultured for up to 24 h with (TNF-α–treated insulin-resistant myotubes [TMs]) or without (control myotubes [CMs]) 20 ng/mL TNF-α. After 8 h, mRNA expression of 128 candidate genes for inflammatory cytokines and their receptors were analyzed using Oligo nucleotide array membranes. Insulin-resistant myotubes (+ TNF-α) showed increased expression of 19 cytokine genes (Fig. 2A). This was confirmed and further quantified by quantitative RT-PCR (Fig. 2B). To determine whether such cytokines were released to the medium, conditioned media were collected after 24 h, and candidate cytokines were measured. Using an antibody array allowing for detection of 79 proteins in this large family, we were able to confirm that CCL5, CXCL10, CXCL2, IL-6, IL-8, CCL2, CCL7, CXCL6, CXCL3, and CXCL1 were detectable and increased in the medium from TNF-α–treated human myotubes when compared with untreated myotubes (Fig. 3A and B), with increases ranging from 2.7-fold (ostepostratin) to 87-fold (Gro-α). Absolute concentrations of IL-8, IL-6, and TNF-α in TMs were measured independently (n = 2): IL-8, 3.50 ng/mL; IL-6, 2.5 ng/mL; TNF-α, 18 ng/mL. TNF-α was measured to confirm its stability in the conditioned medium (initial concentration 20 ng/mL).

Impact of conditioned medium from human myotubes with different insulin sensitivity on primary sorted β-cells. We had shown that TNF-α itself inhibits glucose-stimulated insulin secretion (GSIS) but does not impact growth or survival of β-cells (11). We now wished to determine the effect on these β-cell parameters of conditioned medium from TMs or CMs. To this end, β-cells were treated for 48 h with either CMs or TMs, with BrdU added during the last 24 h. Only rat β-cells were used to measure proliferation, because adult human β-cells fail to proliferate to any meaningful extent in vitro (21). Because we

Detection of apoptosis and proliferation. Cell death was measured by TUNEL assay. Proliferation was assessed by BrdU incorporation (22).

RNA interference mediated silencing of MAP4K4. Knockdown of MAP4K4 in rat primary β-cells was achieved by transfection with small interfering RNA as previously described (11). All other standard methods were as described previously (22).

Presentation of data and statistics. Data are mean ± SEM for 3–5 independent experiments (2 replications for each independent experiment for insulin secretion, BrdU incorporation, and TUNEL assay). Statistical significance for differences was evaluated by one- or two-way ANOVA, as appropriate, using Fisher least significant differences test for post hoc determination.
documented the continued presence of TNF-α in TMs (see above), an additional control condition involved CMs “spiked” with 20 ng/mL TNF-α immediately before adding it to the β-cells (CM + TNF-α). There was a dramatic decrease in rat primary β-cell proliferation treated with TMs when compared with CMs or CM + TNF-α (Fig. 4A). This is the first direct demonstration that products secreted from insulin-resistant (but not control) skeletal muscle can impact negatively on β-cells. Interestingly, CMs induced a significant increase in β-cell proliferation when compared with the control condition without CMs, suggesting a beneficial, mitogenic effect of products released by untreated (normally insulin-sensitive) myotubes (Fig. 4A).

Both rat and human primary (sorted) β-cells were used to evaluate the impact of TMs (24 h) on cell death and insulin secretion. Neither CMs nor the addition of TNF-α to such medium (CM + TNF-α) had any effect on β-cell apoptosis. However, TMs induced a sixfold increase in primary β-cell death (Fig. 4B and C). GSIS was significantly decreased in both rat and human primary β-cells treated with either TMs or CM + TNF-α with no change in basal insulin secretion (Fig. 4D and E, respectively). Using both rat and human primary sorted β-cells, CMs alone (but not CM + TNF-α) induced a significant increase in GSIS (Fig. 4D and E), confirming the beneficial effects of CMs from myotubes that were seen for proliferation of rat β-cells.

Taken together, these results document the existence of products secreted from myotubes that impact either positively or negatively on pancreatic β-cell function, proliferation, and survival.

**Dual effects of conditioned medium from skeletal muscle on Akt and extracellular signal–related kinase phosphorylation in β-cells.** We and others have demonstrated that activation of the insulin signaling pathway (via IRS-2) is necessary for β-cell survival, proliferation, and GSIS (22–25). In order to explore the possible impact of conditioned medium from skeletal muscle on this pathway, rat primary β-cells were cultured in the presence of CMs or TMs for 24 h. After this, cells were handled exactly as for the measurement of GSIS. Phosphorylation of both Akt and extracellular signal–related kinase (ERK)
were stimulated by glucose, and TM pretreatment abolished this to the same extent as TNF-α on the background of CM (Fig. 5A and B) suggesting this to be TNF-α–dependent. Conversely, Akt (but not ERK) phosphorylation was increased in CM-treated β-cells under basal (but not glucose-stimulated) conditions (Fig. 5A), which could explain in part increased proliferation of β-cells cultured with such conditioned medium (Fig. 4A)(26).

**Dual effects of conditioned medium from skeletal muscle on phosphorylation of substrates of Akt in β-cells.** We have demonstrated that AS160 is phosphorylated in response to glucose in β-cells and is involved in GSIS as well as β-cell survival (22). As observed for Akt, TM and TNF-α treatment prevented AS160 phosphorylation in response to glucose (Fig. 5C). Because AS160 is just one of the multiple substrates that can be phosphorylated by Akt, we explored the total pattern of such substrates after glucose stimulation. As shown in Fig. 5D, glucose induced the phosphorylation of several Akt substrate proteins, while TM treatment prevented it for nearly all these proteins (Fig. 5D, black crosses). Given the similar pattern obtained using TM versus CM + TNF-α, this effect was again presumed to be driven by the TNF-α that was still present in TM. In the basal condition, phosphorylation of AS160 (Fig. 5C) and other yet-to-be-identified Akt substrate proteins (Fig. 5D, white crosses) were increased when primary rat β-cells were treated 24 h with CMs. Here again, these results can explain the positive impact of CM on survival and GSIS when primary rat β-cells were treated with CMs.

**Dual effects of conditioned medium from myotubes on IRS-1 and -2 mRNA and protein expression in β-cells.** IRS-1 and -2 have been shown to be central in both insulin production and secretion (27,28). Therefore, we explored the impact of conditioned media on IRS-1 and -2 expression. CM treatment induced an increase of protein and mRNA expression for IRS-1 and -2 (Figs. 5E and F).
This was prevented when TNF-α was added to CMs. Surprisingly, TM treatment increased IRS-1 protein expression despite the presence of TNF-α in this medium (Fig. 5E). There was, however, no such change in IRS-1 mRNA levels (Fig. 6A). By contrast, TM treatment decreased both IRS-2 protein and mRNA expression when compared with CMs, whereas TNF-α had only an impact on IRS-2 protein expression (Figs. 5F and 6B).

MAP4K4 mediates the effect of TMs on proliferation, apoptosis, and GSIS in rat primary β-cells. MAP4K4 has been shown to mediate TNF-α action in adipose tissue, skeletal muscle, and β-cells (11,17,29). In rat primary β-cells, the amount of MAP4K4 mRNA was increased by 60 and 40% after either TNF-α or TM treatment for 24 h (Fig. 6C). Unfortunately, there is no available antibody in rats to monitor MAP4K4 protein levels by Western blot, and it also is not possible to measure its activity. We therefore adopted an indirect method to evaluate the importance of MAP4K4 modulation by TMs. Transfection of primary rat β-cells with small interfering RNA decreased MAP4K4 mRNA expression by 60% in primary β-cells treated with TMs (11). Rat primary β-cells lacking MAP4K4 were partially resistant to TM action on proliferation (Fig. 6D) and totally protect against TM-induced apoptosis and its impact on GSIS (Fig. 6E and F). These data suggest that MAP4K4 is an important mediator of TM action on pancreatic β-cells and that TM can impact on MAP4K4 activity independently of TNF-α.

**Impact of IL-1RA, IL-6 receptor blockade, and GLP-1 on effects of TMs on primary rat β-cells.** We show above that TMs decrease β-cell proliferation, survival, and GSIS (Fig. 4). We also show that human myotubes treated with TNF-α for 24 h show an increase of several cytokines including IL-1β (Figs. 2 and 3), which has been shown to be involved in type 2 diabetes (30,31). Therefore, we decided to explore whether pretreatment of β-cells with IL-1RA, the natural soluble IL-1 receptor antagonist, could prevent TM effects on β-cells. IL-1RA treatment failed to prevent TM action on either GSIS (data not shown) or proliferation (Fig. 7A), whereas TM action on cell death was prevented (Fig. 7B). IL-1RA treatment did also induce a decrease of proliferation in the control condition and CM-treated β-cells. This seemingly paradoxical observation can be explained by the fact that β-cells plated as here on extracellular matrix secrete low levels of IL-1β that impact positively on β-cell survival (32), which is in keeping with the established bimodal effects of this cytokine on β-cells (33).
It has been postulated that communication between skeletal muscle and pancreas could be mediated by IL-6 (10). To evaluate this possibility, rat primary β-cells were treated with IL-6 receptor antagonist (Sant-7) or a human anti–IL-6 receptor antibody (AF-227-NA) to block IL-6 action on β-cells. Under the present experimental conditions, neither means of IL-6 receptor blockade was able to block TM effects on rat primary β-cell proliferation, apoptosis, or GSIS (data not shown). Nevertheless, blocking the IL-6 receptor did completely inhibit the increase of β-cell proliferation evoked by CMs (data not shown).

Studies have shown that GLP-1 treatment can protect β-cells from the negative actions of cytokines (34,35) and also enhances primary rat β-cell proliferation (21). Therefore we have explored if GLP-1 treatment could protect β-cells against the detrimental effects evoked by TMs. As expected, primary rat β-cells pretreated with GLP-1 showed an increase in proliferation under basal conditions and also after CM treatment (Fig. 7C). However, pretreatment with GLP-1 induced only a very small—albeit significant—increase in proliferation of cells treated with TMs (Fig. 7C), whereas such treatment protected totally the β-cells from TM action on apoptosis (Fig. 7D). GLP-1 treatment failed to protect against the decrease in GSIS following 24-h culture with TMs (data not shown).

**FIG. 4.** Effect of conditioned medium on primary β-cell proliferation, survival, and GSIS. Conditioned medium was obtained by culturing human myotubes for 24 h with (TM) or without (CM) 20 ng/mL TNF-α. Additional control conditions were as follows: CTRL, culture medium not previously exposed to skeletal muscle cells; CM + TNF, 20 ng/mL TNF-α added to CM immediately before exposure to β-cells. A: Proliferation of rat primary β-cells measured by BrdU incorporation. Cells were grown under standard culture conditions (20% FCS, 11.2 mmol/L glucose) and treated for 48 h with the different conditioned media; BrdU was added for the last 24 h. β-cells were identified by insulin immunofluorescence. n = 7 independent experiments. B and C: Rat and human primary β-cell apoptosis. Cell death was measured by TUNEL. n = 7 (rats) and n = 5 (human) independent experiments. *P < 0.05. D and E: Glucose-stimulated insulin secretion from rat and human primary β-cells measured during 60 min at 2.8 mmol/L glucose (white bars = basal secretion) following by 60 min at 16.7 mmol/L glucose (dark bars = stimulated secretion). Secretion is expressed as a percentage total insulin content. n = 7 (rats) and n = 5 (human) independent experiments. *P < 0.05.
Ethanercept (2 ng/mL) was able to block both TNF-α and TM action on GSIS. This was accompanied by an increase in basal secretion (Fig. 7E). However, trapping of TNF-α remained without effect on TM action on proliferation and apoptosis (Fig. 7F and G), confirming that these effects are because of the impact of factors secreted from TNF-α–treated myotubes and not because of TNF-α present in this conditioned medium. Interestingly, Ethanercept treatment increased apoptosis and decreased proliferation in rat β-cells cultured for 48 h with CMs (Fig. 7E and G). These results lead us to hypothesize that TNF-α at low levels in CMs could act positively by itself or in combination with other myokines on β-cell survival and proliferation. Our combined data with Ethanercept and MAP4K4 silencing reinforce the conclusion that TM action on proliferation and survival is TNF-α–independent and mediated by MAP4K4 activation by factors secreted by insulin-resistant myotubes.

DISCUSSION
To understand the mechanism involved in type 2 diabetes development, peripheral insulin resistance and β-cell function have to be studied in parallel (36,37). Low-grade systemic inflammation is also a feature of obesity and diabetes (38), raising the hypothesis that elevated cytokine levels may contribute to insulin resistance and decreased β-cell functional mass (39). Nonmuscle-derived TNF-α contributes to insulin resistance in human skeletal muscle (16,17) and can also inhibit insulin secretion (11). Nowadays, skeletal muscle is accepted as an endocrine organ (40). This study reports that human myotubes produce and release
different cytokines depending on their state of insulin sensitivity, adding to the list of previously known myokines (41,42). This was achieved using a candidate approach, and an unbiased screening would likely reveal yet more.

We have also focused on the possible endocrine impact of these myokines on sorted primary human and rat β-cells. There was bimodal action with conditioned medium from human CMs that are fully sensitive to insulin-exerted beneficial effects on β-cells with increased proliferation and GSIS, whereas that from insulin-resistant myotubes (TMs) exerted detrimental effects with increased apoptosis, and decreased proliferation and GSIS. Myokines like IL-1β, IL-6, TNF-α, CCL5, MCP-1, IL-8, and CXCL10 are good candidates for these latter, detrimental actions as they have been shown to impact negatively on β-cell function and survival (5,9–13). However, IL-RA, TNF-α trapping, and IL-6 receptor blockade failed to completely rescue β-cells from TM action, exemplifying the complex nature of the cocktail of cytokines in this conditioned medium. Meanwhile, GLP-1 treatment was able to rescue primary β-cells from TM-mediated apoptosis, confirming its antiapoptotic properties (34). The data further indicate that lower concentrations of cytokines secreted by insulin-sensitive myotubes, as previously observed for IL-1β (33) and confirmed here for this cytokine as well as TNF-α and IL-6, could have a positive action on β-cells in keeping with bimodal action depending on concentration and the biological context (33). Nevertheless, other myokines, already identified or not, could be involved in both the positive and negative effects of CMs or TMs.

In order to gain a more detailed understanding of the underlying molecular pathways, we explored the signaling pathways impacted in β-cells by the various conditioned media and possible means to protect them from such action. In β-cells, activation of the insulin-signaling pathway is necessary to mediate glucose action (23) with IRS-2/Akt/AS160 involved in GSIS (22). Moreover, IRS-2 is known to be indispensable for β-cell function (43) while ERK activation has been shown to be involved in GSIS (44). TM
treatment blocks glucose action on ERK, as well as Akt and its substrates including AS160. We also demonstrated that glucose-stimulated phosphorylation of a number of Akt substrates in rat primary β-cells was prevented by TMs. Only a few of these Akt substrates have been identified in muscle and adipose tissue while nothing is known in pancreatic β-cells aside from our previous work on AS160 showing that this Akt substrate was indispensable for GSIS (22). Conversely, CM treatment of primary sorted β-cells increased phosphorylation of Akt and several Akt substrates under basal conditions. This could partly explain the positive impact of CMs on primary β-cells.

Protein and mRNA levels of IRS-2 were decreased by TMs with a compensatory effect seeming to occur between IRS-1 and -2 as both TMs and CMs induced an increase of IRS-1 expression, while only CMs induced an increase of IRS-2. This supports findings in other tissues that IRS-1 and -2 have separate and nonredundant function (45).

It has been shown that insulin resistance can be rescued after MAP4K4 silencing in skeletal muscle and adipose tissue (17,46) while another study reported that silencing of MAP4K4 in macrophages can suppress systemic inflammation and therefore prevent diabetes (29). In β-cells, MAP4K4 silencing protects against TNF-α inhibition of GSIS (11). Here we show that TM treatment increased significantly MAP4K4 gene expression in rat primary β-cells. Its silencing prevented TM action on GSIS, which seems to be TNF-α–dependent, and on β-cell proliferation and apoptosis, which is TNF-α–independent.

Inflammatory mechanisms have been suggested to contribute as causative factors in the development of type 2 diabetes. It has been further postulated that the diabeticogenic environment, including insulin resistance and low-grade systemic and localized islet inflammation, contribute toward β-cell “stunning” (47). Our results lead us to propose a new component of this complex paradigm. We thus
show that induction of insulin resistance in human skeletal muscle by TNF-α leads to secretion of myokines that impact negatively on β-cell proliferation and survival. Of course, this is just one in vitro model of human skeletal muscle insulin resistance, and the panel of myokines secreted by myotubes rendered insulin resistant by other means or taken directly from insulin-resistant patients may be different from that induced by TNF-α: this is worthy of further study. At present, there is little evidence for elevated TNF-α in the skeletal muscle of individuals with type 2 diabetes, whereas its contribution toward skeletal muscle insulin resistance is well established (18). Nevertheless, assuming that it will prove possible to extrapolate from the present artificial situation with myokine concentrations in the conditioned media that are arbitrary and dependent on the in vitro conditions, to the clinical setting, the identification of these myokines and of this new pathway for interorgan communication between skeletal muscle and β-cells offers new insight into the pathological process linking insulin resistance to β-cell failure and opens the possibility for new therapeutic strategies for preservation of functional β-cell mass in type 2 diabetes. Collectively, our results regarding TM action on primary β-cells combined with earlier data in these and other cell types (11,17,29,46) place MAP4K4 as a novel target in the effort to treat or prevent diabetes by addressing both peripheral insulin resistance and the loss of β-cell mass. Our results also indicate a novel mechanism allowing for protection of β-cells by GLP-1. Finally, the beneficial effects of conditioned medium from insulin-sensitive myotubes (CMs) raises the interesting prospect of identifying novel molecules able to improve β-cell function, survival, and proliferation while suggesting that communication from skeletal muscle may contribute toward normal β-cell function and mass in healthy individuals.

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K.B. conducted the experiments, researched data, and wrote the manuscript. P.A.H. analyzed data and wrote the manuscript. T.B. contributed human tissue samples and reviewed the manuscript. P.P., B.K.P., and M.Y.D. contributed to discussion and reviewed the manuscript.

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REFERENCES
1. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 2010;464:1293–1300
2. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52:2789–2798
3. Wentworth JM, Fourlanos S, Harrison LC. Reappraising the stereotypes of diabetes in the modern diabeticogenic environment. Nat Rev Endocrinol 2009;5:483–489
4. Pedersen BK, Steensberg A, Fischer C, et al. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? Proc Nutr Soc 2004;63:203–207
5. Ellingsgaard H, Ehses JA, Hanmur EB, et al. Interleukin-6 regulates pancreatic alpha-cell mass expansion. Proc Natl Acad Sci USA 2008;105:13163–13168
6. Liu Y, Chewchuk S, Lavigne C, et al. Functional significance of skeletal muscle adiponectin production, changes in animal models of obesity and diabetes, and regulation by rosiglitazone treatment. Am J Physiol Endocrinol Metab 2009;297:E657–E664
7. Wijesekara N, Krishnamurthy M, Bhattacharjee A, Suhail A, Sweeney G, Wheeler MB. Adiponectin-induced ERK and Akt phosphorylation protects against pancreatic beta cell apoptosis and increases insulin gene expression and secretion. J Biol Chem 2010;285:33623–33631
8. Maedler K, Schumann DM, Sauter N, et al. Low concentration of interleukin-1beta induces FLICE-inhibitory protein-mediated beta-cell proliferation in human pancreatic islets. Diabetes 2006;55:2713–2722
9. Mandrup-Poulsen T, Pickersgill L, Donath MY. Blockade of interleukin 1 in type 1 diabetes mellitus. Nat Rev Endocrinol 2010;6:158–166
10. 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
11. Bouzaki K, Eibaux P, Halban PA. Silencing mitogen-activated protein 4 kinase 4 (MAP4K4) protects beta-cells from TNF-alpha induced decrease of IRS2 and inhibition of glucose-stimulated insulin secretion. J Biol Chem 2009;284:27882–27888
12. Schultheiss FT, Paroni F, Sauter NS, et al. CXCL10 impairs beta cell function and viability in diabetes through TLR4 signaling. Cell Metab 2009;9:125–139
13. Kolb H, Mandrup-Poulsen T. An immune origin of type 2 diabetes? Diabetologia 2005;48:1038–1050
14. Zierath JR, Krook A, Wallberg-Henriksson H. Insulin action and insulin resistance in human skeletal muscle. Diabetologia 2000;43:821–835
15. Hainaut C, Van Obbergen E, Mothe-Satney I. Role of amino acids in insulin signaling in adipocytes and their potential to decrease insulin resistance of adipose tissue. J Nutr Biochem 2006;17:374–378
16. Plongpaard P, Bouzaki K, Krogh-Madsen R, Middendorf B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. Diabetes 2005;54:233–242
17. Bouzaki K, Zierath JR. MAP4K4 gene silencing in human skeletal muscle prevents tumor necrosis factor-alpha-induced insulin resistance. J Biol Chem 2007;282:7783–7789
18. Benito M. Tissue-specificity on insulin action and resistance; past to recent mechanisms. Acta Physiol (Oxf) 2011;201:297–312
19. Argilés JM, López-Soriano J, Almendro V, Busquets S, López-Soriano FJ. Cross-talk between skeletal muscle and adipose tissue: a link with obesity? Med Res Rev 2005;25:49–65
20. Rouiller DG, Cirulli V, Halban PA. Uvomorulin mediates calcium-dependent aggregation of islet cells, whereas calcium-independent cell adhesion molecules distinguish between islet cell types. Dev Biol 1991;148:312–321
21. Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance via stimulation of integrin-mediated cell adhesion molecules and PI3K/AKT signaling. Am J Physiol Endocrinol Metab 2008;294:E664–E671
22. Assmann A, Hainaut C, Kulkarni RN. Growth factor control of pancreatic islet regeneration and function. Pediatr Diabetes 2009;10:14–32
23. Assmann A, Ueki K, Winnan JN, Kadowaki T, Kulkarni RN. Glucose effects on beta-cell growth and survival require activation of insulin receptors and insulin receptor substrate 2. Mol Cell Biol 2009;29:3219–3228
24. Lin E, Kikugawa Y, Fujii K, Takahashi T, Kojima M, Kikuzaki H, Kadowaki T, Kulkarni RN. Glucose-induced insulin secretion elicits hepatic insulin resistance via activation of the PI3K/Akt pathway. Diabetes 2008;57:1195–1204
25. Lin E, Kikugawa Y, Fujii K, Takahashi T, Kojima M, Kikuzaki H, Kadowaki T, Kulkarni RN. Glucose-induced insulin secretion elicits hepatic insulin resistance via activation of the PI3K/Akt pathway. Diabetes 2008;57:1195–1204
26. Dickson LM, Rhodes CJ. Pancreatic beta-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt? Am J Physiol Endocrinol Metab 2004;287:E192–E198
27. Kulkarni RN, Roper MG, Dahlgren G, et al. Islet secretory defect in insulin receptor substrate 1 null mice is linked with reduced calcium signaling and expression of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA)-2b and -3. Diabetes 2004;53:1517–1525
28. Withers DJ, Gutierrez JS, Towery H, et al. Disruption of IRS-2 causes type 2 diabetes in mice. Nature 1998;391:900–904
29. Aouadi M, Tesz GJ, Nicoloro SM, et al. Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation. Nature 2009;458:1180–1184
30. Elsies JA, Lacraz G, Giroix MH, et al. IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. Proc Natl Acad Sci USA 2009;106:13998–14003
31. Masters SL, Dunne A, Subramanian SL, et al. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. Nat Immunol 2010;11:897–904
32. Ribaux P, Elsies JA, Lin-Marq N, et al. Induction of CXCL1 by extracellular matrix and autocrine enhancement by interleukin-1 in rat pancreatic beta-cells. Endocrinology 2007;148:5582–5590
33. Elsies JA, Perren A, Eppler E, et al. Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 2007;56:2356–2370
34. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. J Biol Chem 2003;278:471–478
35. Corrow M, Yang J, Jaccard E, Poussin C, Widmann C, Thorens B. Glucagon-like peptide-1 protects beta-cells against apoptosis by increasing the activity of an IGF-2/IGF-1 receptor autocrine loop. Diabetes 2009;58:1816–1825
36. Kahn SE, Hall BL, Utschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840–846
37. Ferrannini E. Metabolic syndrome: a solution in search of a problem. J Clin Endocrinol Metab 2007;92:396–398
38. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327–334
39. Donath MY, Schumann DM, Faulenbach M, Ellingsgaard H, Perren A, Elsies JA. Islet inflammation in type 2 diabetes: from metabolic stress to therapy. Diabetes Care 2008;31(Suppl. 2):S161–S164
40. Brandt C, Pedersen BK. The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases (Abstract). J Biomed Biotechnol 2010;2010:520258
41. Izumiya Y, Bina HA, Ouchi N, Akasaka Y, Kharitonenkova A, Walsh K. FGF21 is an Akt-regulated myokine. FEBS Lett 2008;582:3805–3810
42. Ouchi N, Oshima Y, Ohashi K, et al. Follistatin-like 1, a secreted muscle protein, promotes endothelial cell function and revascularization in ischemic tissue through a nitric-oxide synthase-dependent mechanism. J Biol Chem 2008;283:32802–32811
43. Kubota N, Terauchi Y, Tobe K, et al. Insulin receptor substrate 2 plays a crucial role in beta cells and the hypothalamus. J Clin Invest 2004;114:917–927
44. Lawrence M, Shao C, Duan L, McGlynn K, Cobb MH. The protein kinases ERK1/2 and their roles in pancreatic beta cells. Acta Physiol (Oxf) 2008;192:11–17
45. Bouzakri K, Zachrisson A, Al-Khalili L, et al. siRNA-based gene silencing reveals specialized roles of IRS-1/Akt2 and IRS-2/Akt1 in glucose and lipid metabolism in human skeletal muscle. Cell Metab 2006;4:89–96
46. Tesz GJ, Guilherme A, Guntur KV, et al. Tumor necrosis factor alpha (TNFα) stimulates Map4k4 expression through TNFα receptor 1 signaling to c-Jun and activating transcription factor 2. J Biol Chem 2007;282:19302–19312
47. Ferrannini E. The stunned beta cell: a brief history. Cell Metab 2010;11:349–352