Bimodal Effect on Pancreatic β-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) β-cells.

RESEARCH DESIGN AND METHODS—Human skeletal muscle cells were cultured for up to 24 h with tumor necrosis factor (TNF-α) to induce insulin resistance, and mRNA expression for cytokines was analyzed and compared with controls (without TNF-α). Conditioned media were collected and candidate cytokines were measured by antibody array. Human and rat primary β-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 β-cells. Conditioned medium from control myotubes increased proliferation and glucose-stimulated insulin secretion (GSIS) from primary β-cells, whereas conditioned medium from TNF-α-treated insulin-resistant myotubes (TMs) exerted detrimental effects that were either independent (increased apoptosis and decreased proliferation) or dependent on the presence of TNF-α in TM (blunted GSIS). Knockdown of β-cell mitogen-activated protein 4 kinase 4 prevented these effects. Glucagon-like peptide 1 protected β-cells against decreased proliferation and apoptosis evoked by TMs, while interleukin-1 receptor antagonist-deficient only prevented the latter.

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

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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.

RESEARCH DESIGN AND METHODS

Antibodies and reagents. The antibodies and reagents were: human TNF (hTNF)-α (R&D Systems Europe Ltd., Abingdon, U.K.); antiphospho-Akt (Ser473), antiphospho-ERK-1/2-(Thr202/Tyr204) (New England Biolabs, Beverly, MA); antiphospho-ERK-1/2 (Thr202/Tyr204) (New England Biolabs, Beverly, MA); antiphospho-ERK-1/2 (Thr202/Tyr204) (Cell Signaling Technology, Beverly, MA); antiactin (Sigma); anti-IRS-1 (gift from Dr. M.F. White; Children’s Hospital, Harvard University, Boston, MA); Ethaneceptor (Wyeth Pharmaceuticals SA, Zoug, Switzerland); 5-bromo-2-deoxyuridine (BrdU) and transferase-mediated dUTP nick-end labeling (TUNEL) kits (Roche, Mannheim, Germany); IL-1RA (Kineret), Agen (Breda, the Netherlands); and glucagon-like peptide 1 (GLP-1) (Bachem, Bubendorf, Switzerland).

Human skeletal muscle cells. R. ex. abd. was obtained with the informed consent of donors during scheduled abdominal surgery (Geneva University Hospital and Danish Muscle Center approved the study protocols NAC 06-003 and jr. nr. HA-2007-0016). This particular slow-twitch fiber type of skeletal muscle was chosen for ease of surgical access as well as insulin-sensitive glucose uptake using in vitro–derived myotubes. The satellite cells were isolated from the muscle biopsies by trypsin digestion and grown in Ham’s F10 medium with 20% FCS and antibiotics. Confluent myoblasts were allowed to fuse and differentiate into myotubes in Dulbecco’s modified Eagle’s medium, 2% FCS, and antibiotics. Human myotubes were used after induction of differentiation (when most cells are multinucleated) and expressed specific human skeletal muscle markers (17).

Oligo GEArray experiments. Total RNA was extracted with the QiASHredder and RNeasy Mini Kit (QIAGEN, Basel, Switzerland). The UTP-biotinylated cRNA probe was synthesized with the TrueLabeling-AMP 2.0 Kit (SuperArray, Frederick, MD) and purified with the ArrayGrade cRNA Cleanup Kit (SuperArray). The membranes (focused on human inflammatory cytokines and receptors [OHS-011 from SABioscences, Frederick, MD]) were hybridized following the manufacturer’s instructions.

Human chemokine and cytokine antibody arrays. Human chemokine + human cytokineV arrays (Ray Biotech, Norcross, GA) were used following the manufacturer’s instructions, and images were quantified using Multi Gauge software with intensity normalized to internal controls.

Rat primary β-cell purification. Animals were treated according to protocols approved by the State Commissioner on Animal Care. Islets of Langerhans were isolated by collagenase digestion of pancreas from male Wistar rats (150–200 g) followed by Ficol purification (20). β-cells sorted by fluorescence-activated cell sorter were plated on 96-well extracellular matrix (20,21).

Human primary β-cell purification. Human islets were provided by the Islet Cell Resource Centers of Milan (Italy), Geneva (Switzerland), and Lille (France). Human β-cells were sorted by fluorescence-activated cell sorter to >90% purity following labeling with Newport Green (21).

Insulin secretion assay and insulin content. Cells were incubated for 1 h at 37°C with Krebs-Ringer bicarbonate buffer with Heps (KRBH) containing 2.8 mmol/L glucose (basal secretion), followed by 1 h at 37°C with KRBH containing 16.7 mmol/L glucose (stimulated secretion) as previously described (22). Insulin in incubation buffers and cell extracts was measured by radioimmunoassay and secretion expressed as percentage total insulin content.

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

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 (58% vs. untreated control, P < 0.01) and Akt (Ser 473) 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). The impact of TNF-α on myocyte death was also examined. At 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 myocytes (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, CXCL12, IL-6, IL-8, CCL2, CCL7, CXCL6, CXCL8, and CXCL11 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 (osteoprotegrin) 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 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 TMs and CMs spiked with 20 ng/mL TNF-α for 24 h. IRS-1 and Akt protein expression were measured by Western blot (normalized to actin). Mean ± SE, 5 independent experiments. B: 24 h after incubation with TNF-α, human skeletal muscle cells were treated with 100 nmol/L of insulin either for 5 min to induce IRS-1 tyrosine phosphorylation or 10 min to measure Akt phosphorylation. Representative immunoblots are shown for IRS-1 tyrosine (Tyr) phosphorylation (upper panel) and Akt Ser479 phosphorylation (lower panel). C: Effect of different times of TNF-α treatment (20 ng/mL) on human skeletal muscle cell death measured by TUNEL assay. n = 5 independent experiments. D: Effect of different TNF-α concentrations (24 h) on human primary skeletal muscle cell death measured by TUNEL assay. n = 5 independent experiments; *P < 0.05 vs. control. IP, ; IB, . (A high-quality digital representation of this figure is available in the online issue.)
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 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.

**Fig. 2.** Effect of TNF-α on expression of cytokine genes in human skeletal muscle cells. A: Human myotubes were cultured 8 h +/− 20 ng/mL TNF-α, and cytokine mRNA levels were monitored by hybridization to Oligo nucleotide array membranes. White circles show modified mRNA levels between the two conditions on representative membranes out of four different experiments. B: Cytokine mRNA levels were measured by quantitative RT-PCR (qRT-PCR). The data are the mean value of four independent experiments, and mRNA expression levels were normalized to cyclophilin A. HM + TNF, human skeletal muscle cells after 8-h culture with TNF-α; HM, human skeletal muscle cells after 8-h culture without TNF-α. (A high-quality digital representation of this figure is available in the online issue.)
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 and 6A and B). 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

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**FIG. 3. Effect of TNF-α on human skeletal muscle cell cytokine secretion.** A: Human myotubes were cultured 24 h +/- 20 ng/mL TNF-α, and cytokine levels in the conditioned media were monitored by hybridization to human chemokine + cytokine array membranes. White circles show modified protein levels between the two conditions on representative membranes from three different experiments. B: Each membrane was quantified using Multi Gauge software, and the intensity was normalized to internal positive controls for comparison. n = 3 independent experiments. TM, medium from human skeletal muscle cells cultured 24 h with TNF-α; CM, medium from human skeletal muscle cells cultured 24 h without TNF-α. (A high-quality digital representation of this figure is available in the online issue.)
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).
We have published that treatment with TNF-α alone can impair GSIS in primary rat and human β-cells (11) without affecting proliferation of apoptosis. Therefore, we explored directly which actions of TMs on primary rat β-cells were because of the presence of TNF-α in this conditioned medium using an antibody toward TNF-α (Ethanercept). 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. 7F 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 diabetogenic 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 MAPK4 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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