Stimulation of AMP-activated protein kinase (AMPK) in skeletal muscle and liver is seen as an exciting prospect for the treatment of type 2 diabetes. However, we have recently demonstrated that changes in AMPK activity accompany the exposure of pancreatic islet β-cells to elevated glucose concentrations and may be involved in the activation of insulin secretion. Here, we discuss this hypothesis and explore the potential role of changes in AMPK activity in the actions of other secretagogues. Amino acids decreased AMPK activity in MIN6 β-cells with an order of potency for inhibition: arg = leu < gln = leu + glu < glucose, which was closely correlated with the stimulation of insulin release ($r^2 = 0.76$). By contrast, increases in intracellular Ca$^{2+}$ concentration provoked by cell depolarization with KCl activated AMPK in the face of increased free intracellular ATP concentrations. Elevation of intracellular cAMP levels with isobutylmethyxanthine or forskolin had no effect on AMPK activity. We conclude that metabolizable amino acids regulate AMPK in the β-cell via increases in the cytosolic ATP/AMP ratio and via phosphorylation by the upstream kinase LKB1. Intracellular Ca$^{2+}$ ions may activate AMPK by calmodulin kinase 1 kinase–mediated phosphorylation. The latter may act as a novel feedback mechanism to inhibit excessive insulin secretion under some circumstances. Diabetes 53 (Suppl. 3):S67–S74, 2004

Enzymology of AMP-activated protein kinase. AMP-activated protein kinase (AMPK) is a multimeric serine/threonine protein kinase comprising α- (catalytic), β- (scaffold), and γ- (regulatory) subunits (1–3). The enzyme is activated by phosphorylation on threonine-172 of the α-subunit in response to a decrease in cellular energy charge and a fall in ATP/AMP ratios (4).

Regulation of AMPK phosphorylation in mammalian cells is complex (5). Binding of AMP to a regulator γ-subunit appears to render the complex more susceptible to phosphorylation and activation by upstream AMPK kinases (AMPKKs). Very recent data have suggested that the putative mammalian AMPKK may be a homolog of the S. cerevisiae Snf1 (sucrose nonfermenting factor) kinases Elmp1p, Tos3p, or Pak1 (6,7). Subsequently, mammalian LKB1 (derived as a code name for the Peutz-Jeghers syndrome causative gene [8] and also termed STK11, for serine/threonine kinase 11 [9]) has been shown to phosphorylate AMPK in mammalian cells and to mediate the effects of AICAR (5-aminoimidazole-4-carboxamide riboside) (10–12). However, other closer homologs of the yeast Snf1 kinases, including calmodulin kinase 1 kinase (CaMK1K), also exist in mammalian cells. The latter enzyme phosphorylates AMPK in vitro albeit much more poorly than it phosphorylates its usual substrate, CaMK1, and has therefore been considered to play a minor if any role in the regulation of AMPK in mammals (13). However, it is now clear that LKB1 is not a “dedicated” AMPK kinase but also phosphorylates and regulates several members of a wider “AMPK family” (14) whose regulation by nutrients is not yet well understood.

Consequences of AMPK activation for intracellular metabolism. Activation of AMPK is associated with the phosphorylation of enzymes involved in ATP-consuming processes, such as fatty acid (acyetyl-CoA carboxylase) and cholesterol (hydroxymethylglutaryl-CoA dehydrogenase) biosynthesis, and the consequent activation of mitochondrial fatty acid oxidation (15). In this way, regulation of AMPK ensures that cellular ATP is spared during times of nutrient deprivation. As such, the enzyme has aptly been described as the “fuel gauge” of mammalian cells (1). Thus, in most cell types, basal AMPK phosphorylation and activity are low, increasing only during metabolic stress (e.g., during exercise in muscle) (16,17) or after the activation of certain receptors, including those for leptin (18) and adiponectin (ACRP30) (19). However, and as discussed below, AMPK activity is regulated somewhat differently and exerts distinct effects on the metabolism in pancreatic β-cells compared with other tissues.

AMPK in type 2 diabetes. AMPK is increasingly considered a promising target for drug treatment in type 2 diabetes (4). Activation of the enzyme in the liver or skeletal muscle with the cell-permeant AMP analog AICAR...
is associated with diminished gluconeogenesis (20,21) and with enhanced glucose uptake (20,22,23), respectively, and thus potentially with improved glucose homeostasis in type 2 diabetic patients. Correspondingly, treatment of obese diabetic rats with AICAR lowers blood glucose levels (24). Moreover, metformin, used as an antidiabetic agent for >50 years (25), has recently been shown to be an inhibitor of respiratory chain complex 1 (26), which exerts some of its effects through an inhibition of mitochondrial oxidative metabolism and activation of AMPK (20,27). Moreover, the thiazolidinedione class of antidiabetic drugs, usually considered to act principally by binding to the nuclear peroxisome proliferator–activator receptor-γ class of nuclear transcription factors (28), are also able to increase intracellular AMP levels and stimulate AMPK activity (29). At present, the importance of this effect for the antidiabetic effects of these agents is unknown.

By inhibiting the expression of lipogenic genes in the liver (30,31), the activation of AMPK may also contribute to the antisteatotic effects of metformin (32). Indeed, hepatic expression of the lipogenic transcription factor sterol regulatory element binding protein-1 (SREBP-1c) is reduced by metformin (20), decreasing overall liver triglyceride and sterol synthesis. Other targets of AMPK in the liver include the key transcription factor hepatocyte nuclear factor (HNF)-4 (33), which is associated with maturity-onset diabetes of the young-1 (MODY1) (34) and is destabilized by AMPK-mediated phosphorylation (35). The consequent downregulation of apolipoprotein expression (33) may then reduce hepatic lipid export and thereby improve blood lipid profiles.

While decreases in cellular ATP/AMP ratios are likely to explain many of the effects of metformin on AMPK activity, it should be pointed out that evidence has also been provided for a nonmetabolic action of the biguanide on AMPK activity (29,36). However, whether such apparent effects are actually due to changes in free or compartmentalized ATP or AMP concentration, which are masked in measurements of total nucleotide concentrations, remains to be ruled out.

**AMPK and the pancreatic islet β-cell.** In light of the role of pancreatic β-cells as the principal “fuel sensor” for the whole organism, might the functions of AMPK not be well suited as the key intracellular nutrient sensor in these cells? As discussed below, recent findings (rev. in 4) suggest this may well be the case.

Secretion of insulin from pancreatic islets is biphasic in response to an increase in plasma glucose concentration (37). The first phase of secretion (likely to correspond to \(K_{\text{ATP}}\) channel–dependent or “triggering pathway”) (38) involves the rapid release, upon calcium influx, of a “readily releasable pool” of secretory vesicles docked at the plasma membrane (39). The second phase (which may correspond to the \(K_{\text{ATP}}\) channel–independent “amplifying” or “potentiation” pathway) (38) then appears to involve the priming and/or the translocation to release sites of more remotely located secretory granules (40). The second phase is known to be ATP-dependent (41), likely reflecting an energy requirement for the priming and/or docking of secretory vesicles at sites of exocytosis.

In contrast to the situation in liver, muscle, and adipose tissue, AMPK activity is clearly detectable under basal (5.5 mmol/l glucose) conditions in clonal INS-1 (42) and MIN6 (43–46) β-cells as well as in isolated rodent and human islets (44,47), and is chiefly attributable to the activity of the α1 subunit (43). AMPK activity is rapidly (within minutes) decreased by elevations in glucose concentration over the physiological range, concomitant with decreases in the phosphorylation of the α-subunit at Thr-172 (42,44,47,48). Suggesting that changes in AMPK activity contribute to the regulation of insulin secretion, clamping AMPK activity at the elevated levels apparent at low glucose concentrations by the expression of constitutively active AMPKα subunits, the use of AICAR, or use of the drug metformin (47) suppresses glucose-stimulated insulin secretion from MIN6 cells and islets (44–46) as well as from INS1 β-cells (42,49). Conversely, a dominant-negative form of AMPK stimulates insulin release at low glucose concentrations (44–46). The effect on β-cell AMPK activity of the thiazolidinedione class of antidiabetic agents, which has been reported to activate the enzyme in other cell types (see above), has not been reported.

The effects of AMPK activation by AICAR or metformin appear to involve the inhibition of glucose oxidation (44,49) as well as direct effects on secretory vesicle recruitment to the plasma membrane (45) and a diminution of tolbutamide but not KCl-induced Ca\(^{2+}\) influx (44). However, the actions of inactive (dominant-negative) AMPK do not involve the closure of \(K_{\text{ATP}}\) channels or any increase in intracellular Ca\(^{2+}\) concentration (44), indicating that increases in glucose concentration regulate the latter through AMPK-independent mechanisms. Hence, inhibition of AMPK activity by glucose is a necessary, but not usually a sufficient, stimulus for insulin secretion.

**Preservation of β-cell function and mass.** β-Cell mass is profoundly decreased in type 1 diabetes, as a result of immune-mediated destruction (50), and is more modestly decreased in type 2 diabetes (51), by mechanisms that are currently unclear. Activation of AMPK has been reported to enhance β-cell death through apoptosis (48,52) via the activation of the c-Jun NH\(_2\)-terminal kinases JNK1/2. However, these findings contrast with those of other workers (53) who have proposed that by stimulating lipolysis and thus the “detoxification” of fatty acids, active AMPK decreases β-cell death, consistent with an antiapoptotic role in other cell types (12,54–56), and the absence of apoptosis in cells expressing the activated mutant of AMPK catalytic subunits in MIN6 cells (44).

**Role of AMPK in the secretory response to nonglucose secretagogues.** Different secretagogues act at various levels of the insulin secretory machinery (40). Depolarizing agents such as KCl, as well as certain positively charged amino acids including arginine, stimulate Ca\(^{2+}\) influx and the first phase of insulin release but have little effect on the second, sustained phase. By contrast, fuel secretagogues, like glucose or metabolizable amino acids such as leucine and glutamine, enhance both phases through a dual action on cellular metabolism and Ca\(^{2+}\) influx (40). Cyclic AMP-raising agents including GLP-1 (57), the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX), and the adenylate cyclase activator forskolin stimulate both phases of insulin secretion (58) and are believed to act principally via PKA. The latter enzyme appears to phosphorylate voltage-sensitive Ca\(^{2+}\) channels
(59) as well as undefined components of the exocytotic machinery (60). In addition, cAMP may also enhance secretion via a PKA-independent action on the activity of the guanine nucleotide exchange factor Epac/AMP-GEF-II and intracellular Ca\(^{2+}\) mobilization (61). Recent data have suggested that the activation of AMPK may be involved in the regulation by β-adreno receptors of adipose tissue lipolysis (62,63), but the impact of cAMP/PKA on AMPK activity in the β-cell is unknown.

Whereas increases in glucose concentration (42–44, 47,48) inhibit AMPK activity in clonal β-cells and islets (see above), the effects of exposure to amino acids or of changing intracellular free Ca\(^{2+}\) concentrations (64), both important stimuli for insulin release, have not been explored. Given that both can affect intracellular metabolism and thus ATP and AMP levels (61,65), it is conceivable that changes in AMPK activity may play a role in the insulino-tropic effects of each class.

Here, we explore these issues using the model MIN6 β-cell line (66) to monitor changes in AMPK activity and secretion in response to a variety of secretagogues.

**RESEARCH DESIGN AND METHODS**

**Materials.** KN-62 was from Calbiochem. AICAR was from Toronto Research Chemicals (Toronto, ON, Canada). MEM Amino Acids Solution (50X) and MEM Non-Essential Amino Acids Solution (100X) were from GIBCO (Invitrogen; cat. no. 11300051 and 11140060, respectively; www.invitrogen.com) and gave final concentrations of l-amino acids (mmol/l) as follows: gly, 30; asp, 40; glu, 40; pro, and ser (0.1); arg (0.2); cys (0.2); His (0.27); Ile and leu (0.4); lys (0.5); met (0.1); Thr (0.4); Trp (0.05); Tyr (0.2); and val (0.4). All other consumables were from Sigma.

**Antibodies.** Sheep anti-phospho AMPK (T172) antibody was a generous gift of Professor D.G. Hardie (Department of Biochemistry, University of Dundee, U.K.). Rabbit anti-AMPK β1/2 antibody was kindly provided by Dr. D. Carling (MRC Clinical Sciences, London, U.K.). Rabbit anti-phospho Ca\(^{2+}\)/cAMP-response element binding protein (CREB) (S133) was from Cell Signaling (Hong Kong).

**Cell culture.** MIN6 pancreatic β-cells were used between passages nos. 18 and 35 and grown in Dulbecco's modified Eagle's medium (DMEM), containing 25 mmol/l glucose, supplemented with 15% heat-inactivated fetal calf serum (FCS), 4 mmol/l l-glutamine, 100 μmol/l 2-mercaptoethanol, 100 μg/ml streptomycin in a humidified atmosphere at 37°C with 5% CO\(_2\) unless specified otherwise.

**Adenoviruses.** Adenoviruses encoding for enhanced green fluorescent protein (eGFP) only, hereafter named Adapan-GFP (Null), and for cytosolic luciferase (44,67) are described elsewhere. MIN6 cells were infected at a multiplicity of infection (MOI) of 30 pfu/cell for 24–48 h before experiments.

**AMPK activity assay.** AMPK activity assays in MIN6 cells were performed by “SAMS” phospho-transfer assay, as described (47).

**Western (immuno-) blot analysis.** MIN6 cells were cultured and lysed as for AMPK activity assay. Ten to 50 μg of whole cellular extracts were denatured for 5 min at 100°C in 2% SDS and 5% β-mercaptoethanol, resolved by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes before immunoblotting as described (68). Sheep anti-phospho AMPK antibody was used at a dilution of 1:5,000, rabbit anti-AMPK β1/2 was used at 1:5,000 dilution, and rabbit anti-phospho-CREB antibody was used at 1:1,000 dilution.

**Measurements of insulin secretion and luciferase luminescence.** Insulin secretion assays were performed during incubation in Kreb's-HEPES-hbicarbonate buffered medium (KHB) (140 mmol/l NaCl, 3.6 mmol/l KCl, 0.5 mmol/l NaH\(_2\)PO\(_4\), 0.5 mmol/l MgSO\(_4\), 2.0 mmol/l NaHCO\(_3\), 10 mmol/l Hepes [pH 7.4] 0.1% [wt/vol] bovine serum albumin [BSA], and 1.0 mmol/l CaCl\(_2\) equilibrated with O\(_2\)/CO\(_2\) [95:5 vol/vol] and containing the indicated glucose concentrations, as described (47). Cytosolic ATP concentrations were measured as luciferase luminescence in KHB medium without BSA and as described in ref. 67, except that light output was recorded every 1 s and averaged over 20-s intervals.

**Statistics.** Data are shown as the means ± SEM of the number of observations given. Statistical significance was calculated using Student's t test (Microsoft Excel).

**RESULTS**

**Regulation of β-cell AMPK by amino acids: correlation with insulin release.** Amino acids stimulate insulin secretion through a variety of different mechanisms involving an increase in oxidative metabolism and ATP synthesis (65), direct membrane depolarization (arginine, histidine) (69), or a combination of both effects (70).

To determine whether amino acids may affect the activity of AMPK in β-cells, clonal MIN6 cells were incubated with a mixture of all essential and nonessential amino acids, except for glutamine (Fig. 1). In the absence of added amino acids, glucose (3 or 17 mmol/l) caused a marked suppression of AMPK activity, which was reversed by the inclusion of AICAR. These effects were potentiated by amino acids at each glucose concentration in either the absence or presence of AICAR (Fig. 1A). To determine whether the effects of the amino acid mixture might be ascribed, at least in part, to the oxidative metabolism of amino acids and thus increases in cytosolic free ATP concentration ([ATP]<sub>cyt</sub>), the latter was measured dynamically in cell populations transduced with an adenovirus-expressing recombinant firefly luciferase (64). Assayed at

![FIG. 1. Amino acids inhibit AMPK activity in MIN6 cells. A: MIN6 cells were cultured overnight in DMEM containing 3 mmol/l glucose then incubated 60 min in KHB supplemented with glucose, amino acids, and AICAR (as indicated) before cell lysis and AMPK assays, as described in MATERIALS AND METHODS. B: MIN6 cells seeded on coverslips were infected with cytosolic luciferase-expressing adenovirus at an MOI of 30 for 48 h before perfusion with 0, 3, or 17 mmol/l glucose-containing KHB buffer, supplemented with 5 μmol/l luciferin. Amino acids (1×) were added as indicated. Photon counting was performed as described under RESEARCH DESIGN AND METHODS. *P < 0.05, ***P < 0.0005 for the effect of glucose compared with the corresponding 0 mmol/l condition. #P < 0.005, ###P < 0.0005 for the effects of amino acids compared with the same glucose concentration. Data represent means ± SEM of four independent experiments.](image-url)
each glucose concentration tested, the amino acid mixture increased apparent intracellular free ATP concentration, as assessed from the increase in cellular luminescence, with the most substantial increases in the presence of 3 mmol/l glucose (Fig. 1B). Importantly, increasing glucose concentrations still inhibited AMPK activity in the presence of this physiological mixture of amino acids, consistent with the likely ability of the sugar to regulate β-cell AMPK activity in the context of islets in vivo.

We predicted that if changes in AMPK activity are involved in the regulation of insulin secretion in response to amino acids, as well as to glucose (44), then AMPK activity and secretory rate should be inversely correlated (i.e., for a given amino acid or amino acid combination, the highest rates of insulin secretion would be expected when AMPK activity was at its lowest, and vice versa). In agreement with this, AMPK activity was inhibited by l-amino acids with increasing efficiency in the order leu = arg < gln = gln + leu ≤ glucose, while insulin secretion was stimulated in essentially the reverse of this order (Fig. 2A and B). Plotting these two parameters revealed a significant correlation ($r^2 = 0.768$; Fig. 2C).

**Regulation of AMPK activity by cAMP-dependent PKA.** Moule and Denton (62) as well as Yin et al. (63) have provided evidence that AMPK may be a downstream effector of PKA in adipocytes, thus mediating some of the effects of adrenergic agonists on lipolysis in fat tissue. To determine whether increases in PKA activity may influence AMPK activity in β-cells, MIN6 cells were incubated at various glucose concentrations and in the presence of either the phosphodiesterase inhibitor, IBMX, or the adenyl cyclase activator, forskolin.

Whereas either IBMX (Fig. 3B) or forskolin (data not shown) caused substantial increases in the phosphorylation at Ser (133) of CREB, consistent with the expected robust increases in intracellular cAMP concentration and PKA activity, AMPK activity was unaffected under all conditions tested (Fig. 3A). Thus, changes in AMPK activity are unlikely to contribute to the stimulation of insulin secretion by cAMP-raising agents (58).

**Regulation of β-cell AMPK activity by changes in intracellular free Ca$^{2+}$ concentration.** Depolarization of β-cells, leading to increases in cytosolic (71) and consequently mitochondrial (72) free Ca$^{2+}$ concentration, causes a stimulation of oxidative metabolism and consequent increases in cytosolic and mitochondrial free ATP concentration (61,64,67) (Fig. 4C). We reasoned that these increases in free [ATP] (and presumably decreases in free [AMP], given the equilibrium at myokinase) (73) may lead to an inhibition of AMPK activity, which may in turn contribute to the stimulation of secretion under these conditions. We therefore measured AMPK activity in cells incubated at either 0 or 17 mmol/l glucose for various times after the addition of 30 mmol/l KCl (Fig. 4A). Unexpectedly, KCl addition caused clear and highly reproducible increases in AMPK activity at each glucose concentration and at the three time points tested (Fig. 4A).

The SAMS peptide used to quantify AMPK activity may conceivably also report the activity of the Ca$^{2+}$-dependent protein kinase, CaMKI, since the latter enzyme has a similar substrate recognition motif (13). To assess this possibility, we examined changes in AMPK phosphorylation status in intact cells directly by immunoblotting (Fig. 4B). KCl-induced increases in the phosphorylation of AMPK at Thr-172 were clearly evident at both 0 and 17 mmol/l glucose, consistent with the apparent changes in AMPK activity reported by direct phosphotransfer assay (Fig. 4A).

We next assessed the role of 1) intracellular [Ca$^{2+}$] increases and 2) calmodulin function using KN-62, an

![FIG. 2. Inhibition of AMPK activity by individual amino acids correlates with secretory potency. A: MIN6 cells were incubated for 30 min in KHB buffer supplemented with the indicated concentrations of individual amino acids or glucose before cell lysis and measurement of AMPK activity (RESEARCH DESIGN AND METHODS). B: MIN6 cells were incubated as in A for 30 min, except that KHB buffer was supplemented with 0.1% BSA for static insulin secretion assays as described under RESEARCH DESIGN AND METHODS. C: Correlation between AMPK activity and secreted insulin. Data are taken from the experiments shown in A and B. *P < 0.05 and **P < 0.005 for the effects of amino acids or glucose. Data represent means ± SEM of three independent experiments.

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inhibitor of both voltage-gated (l-type) Ca^{2+} channels (74) and calmodulin-dependent kinases (75). While KN-62 caused only a partial (~50%) decrease in KCl-induced Ca^{2+} increases (results not shown), the activation of AMPK was completely inhibited in the presence of this drug (Fig. 4D).

DISCUSSION
Role of AMPK in the regulation of insulin secretion by glucose. Recent data from this laboratory (43–45,47) and others (42,49) have highlighted the likely role of AMPK in the regulation of insulin secretion (rev. in 4). These data contrast with earlier reports of the effects of AICAR (76,77). It should be emphasized that the use of AICAR is not without complications due to uncertainties as to J) the extent to which the compound is accumulated into cells; 2) the proportion converted into the monophosphorylated form (and AMP analog) ZMP, the true activator of AMPK; 3) potential changes in the concentration of endogenous adenine nucleotides (ATP, ADP, and AMP); and 4) the degree of further phosphorylation to the triply phosphorylated ATP analog. The latter phenomenon is likely to complicate results through the closure of ATP-sensitive K^{+} channels, with the consequent activation of insulin secretion, a tendency observed in islets at low but not stimulatory glucose concentrations (42). Thus, the use of recombinant approaches, including adenoviruses (44,47,49) or of mouse transgenic/knockout models, are likely to provide less ambiguous results. It might be mentioned, however, that whereas inactivation of the α2 isoform of AMPK catalytic subunit in mice is without effect on insulin secretion from isolated islets (but leads to glucose intolerance due to elevated catecholamine levels) (78), inactivation of the α1 complex has no effect on either parameter (79). Such findings suggest redundancy in the function of these isoforms in islet β-cells. Double knock-out of both isoforms selectively in β-cells will be required to test this hypothesis.

Given that the dose response for glucose toward both AMPK inhibition and the “amplifying” pathway for insulin secretion are similar (left-shifted compared with that for the triggering pathway), an intriguing possibility is that amplification is mediated, at least in part, by AMPK inhibition. Although direct evidence for this is lacking, it is noteworthy that the recruitment of secretory vesicles to the cell surface, likely to be involved in secretory potentiation, is inhibited by active AMPK (45). The molecular mechanisms through which AMPK acts on vesicle recruitment are unresolved but have been postulated (4) to involve the phosphorylation of microtubule motor proteins including conventional kinesin, given the key role of the latter in insulin vesicle movement (80). By showing that elevated glucose concentrations markedly inhibit AMPK activity even in the presence of a depolarizing concentration of KCl (Fig. 4A), the present observations are consistent with, but do not prove, a role for AMPK in the amplification pathway for glucose-stimulated insulin release.

Regulation of AMPK by amino acids and calcium ions. We show here that β-cell AMPK is regulated by individual amino acids, in good correlation to their insulinotropic effect. We also reveal that substantial increases in intracellular free Ca^{2+} ions activate AMPK in the face of increases in free cytosolic ATP concentration. This result was unexpected given that LKB1-mediated phosphorylation of AMPK catalytic subunits is likely to be inhibited under these conditions. The most straightforward interpretation of these data are that AMPK is phosphorylated by CaMK1K under these conditions, consistent with a complete inhibition of AMPK activation but only partial suppression of KCl-induced Ca^{2+} influx. Activated AMPK may then contribute to a feedback loop to suppress the further stimulation of insulin release.

Conclusions and perspectives: is AMPK in the β-cell a help or a hindrance to the development of antidiabetic therapies? Present thinking in respect to the use of AMPK activators focuses on the actions of these compounds on extrapancreatic tissues. Our own studies, described here and in earlier publications (43–45,47), indicate that the effects of AMPK activation on the β-cell may also need to be considered. Whereas the effects of AMPK on β-cell survival are still a matter of debate, there is a growing consensus that active AMPK reduces insulin secretion. We suspect that, in the context of the whole animal, this effect on pancreatic function may be masked by more marked and beneficial effects on glucose metabolism in the liver and skeletal muscle, lowering the dependency on circulating insulin. Nevertheless, drugs that may be able to selectively regulate different AMPK isoforms—that is, those that target α2 subunits in muscle but are ineffective against the α1 subunits predominant in β-cells (42,43)—may eventually provide advantages with respect to AICAR, metformin, or thiazolidinediones (29), which are not isoform selective. Further studies are therefore
needed to determine the relative levels of expression of each of the AMPK isoforms (α1, α2, β1, β2, γ1, γ2, and γ3) in β-cells and other islet cell types. The finding that leptin stimulates AMPK activity in skeletal muscle (18) but not in β-cells (47) may be of particular importance in this respect because activators of the leptin signaling pathway may provide selective stimulation of extrapancreatic AMPK, including glucose-sensing neurons of the hypothalamus (81,82). Importantly, changes in AMPK in the latter may represent a means of regulating satiety and, thus, the obesity underlying much of the recent increase in the incidence of type 2 diabetes (83).

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