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Control of pancreatic beta cell bioenergetics

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Abstract

The canonical model of glucose-stimulated insulin secretion (GSIS) by pancreatic beta cells predicts a glucose-induced rise in the cytosolic ATP/ADP ratio. Such bioenergetic sensitivity to metabolic fuel is unusual as it implies that ATP flux is governed to a significant extent by ATP supply whilst it is predominantly demand-driven in other cell types. Metabolic control is generally shared between different processes, but potential control of ATP consumption over beta cell bioenergetics has been largely ignored to date. This paper offers a brief overview of experimental evidence that demonstrates ATP flux control by glucose-fuelled oxidative phosphorylation. Based on old and new data it is argued that ATP supply does not hold exclusive control over ATP flux but shares it with ATP demand, and that the distribution of control is flexible. Quantification of the bioenergetic control distribution will be important from basic and clinical perspectives, but precise measurement of the cytosolic ATP/ADP ratio is complicated by adenine nucleotide compartmentalisation. Metabolic control analysis of beta cell bioenergetics will likely clarify the mechanisms by which glucose and fatty acids amplify and potentiate GSIS, respectively. Moreover, such analysis may offer hints as to how ATP flux control shifts from ATP supply to ATP demand during development of type 2 diabetes, and why prolonged sulphonylurea treatment causes beta cell deterioration.

Abbreviations

BAM15	N5,N6-bis(2-fluorophenyl)[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine
Cr	creatine
FCCP	carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
GSIS	glucose-stimulated insulin secretion
K_{ATP} channel	ATP-sensitive potassium channel
PCr	phosphocreatine
T2D	type 2 diabetes

1. Glucose-stimulated insulin secretion

Glucose homeostasis is maintained through biochemical interplay between multiple organs. When the blood glucose concentration increases after consumption of food, pancreatic beta cells secrete insulin, which adjusts the metabolism of various tissues – including skeletal muscle, liver, white adipose and brain – to the risen nutrient availability so that blood glucose is restored to its pre-prandial level. Classical glucose-stimulated insulin secretion (GSIS) by beta cells depends on oxidative fuel catabolism, as a glucose-induced rise in the cytosolic ATP/ADP ratio sets of a series of events that eventually trigger insulin release[1]. An elevated blood glucose concentration stimulates glucose-fuelled oxidative phosphorylation and thus raises the ATP/ADP ratio. This rise leads sequentially to closure of ATP-sensitive potassium (K_{ATP}) channels, plasma membrane depolarisation, activation of voltage-dependent calcium channels, an increased cytosolic free calcium level, and exocytosis (Fig. 1A). Moreover, beta cells rely entirely on oxidative nutrient breakdown to generate the phosphorylation potential needed to drive ATP-demanding processes, because they do not express sufficient lactate dehydrogenase to facilitate anaerobic glycolysis[1]. In addition to general cellular upkeep, the beta cell's energy expenditure includes synthesis and processing of pro-insulin, as well as vesicle trafficking and exocytosis to secrete the produced insulin.

The glucose-responsiveness of the beta cell's cytosolic ATP/ADP ratio has been reviewed recently by Nicholls[2]. The cytosolic ATP/ADP ratio is not generally glucose-sensitive in well-characterised cells such as muscle and liver, and the unusual bioenergetics that are likely responsible for the sensitivity in beta cells are not fully understood. Cellular glucose-sensing ability is often attributed to the kinetic characteristics of the hexokinase-4 isozyme expressed in beta cells, which is also known as glucokinase[3]. Since glucokinase has a relatively low substrate affinity, beta cells do not metabolise glucose below the circulating concentration set point of 4.5 to 5 mM under fasting conditions. In addition, glucokinase has near-complete control over glycolysis[4] and its cooperative glucose kinetics indeed mirror the sigmoidal glucose-dependency of insulin secretion. It is unlikely, however, that canonical GSIS is controlled by glucokinase alone, as hepatocytes express the same isozyme[5] without bioenergetic glucose sensitivity.

To explain how canonical GSIS can be amplified[6] and potentiated[7] by glucose and fatty acids, respectively, additional nutrient-secretion coupling mechanisms have been reported that are not linked to changes in the cytosolic ATP/ADP ratio. Universal acceptance of these non-canonical mechanisms, however, requires conclusive identification of coupling factors[2]. To exclude that GSIS amplification and potentiation are 'mere' bioenergetic phenomena – which would make them special cases of the canonical model – quantification of *cytosolic*

phosphorylation potential is crucial, but measurement of the relevant free adenine nucleotide levels is complicated by their compartmentalisation and tendency to bind to protein[2].

2. Determination of ATP/ADP ratios

Similar to other cell types, beta cells possess distinct cytosolic and mitochondrial adenine nucleotide pools (Fig. 1A). Notably, beta cells also carry adenine nucleotides in their insulin secretory granules with both ATP and ADP present at ~ 4 picogram per microgram granular protein[8]. These adenine nucleotides are co-released with insulin and are thought to regulate insulin secretion via autocrine signalling[9]. Beta cells each hold over 10,000 insulin granules, which constitute 10-20% of total beta cell volume[10], and vesicular adenine nucleotides may account for up to 50% of the beta cell's total adenine nucleotide content[11]. The vesicular ATP/ADP ratio is rather unresponsive to metabolic cues and thus dampens glucose effects on cytosolic phosphorylation potential[11]. By inference, bioenergetic effects of non-canonical metabolic coupling factors may go unnoticed for the same reason. The proportionally high equimolar levels of vesicular ATP and ADP are largely responsible for the relatively low *whole-cell* ATP/ADP ratios that have been reported, with values not much higher than 10-20 (see references cited in[2]) even when beta cells or islets are exposed to stimulatory glucose levels. In accordance, whole-cell ATP/ADP ratios we measure in INS-1E insulinoma cells (a widely used beta cell model[12]) fall within this range (Fig. 1B) and, as expected, ATP/ADP ratios decrease when oxidative phosphorylation is uncoupled with FCCP. The values we see in cells incubated at a non-stimulatory glucose level are higher than those generally reported under such basal conditions (*cf.*[2]), and are a likely additional reason for the apparently low glucose sensitivity we detect (Fig. 1B). Whole-cell ATP/ADP ratios also tend to be relatively low because of mitochondrial adenine nucleotides. Mitochondrial phosphorylation potential is generally much lower than cytosolic potential because of the electrogenic nature of ATP and ADP exchange and because of the pH-driven import of inorganic phosphate[2].

It is difficult to estimate how much the cytosolic ATP/ADP ratio is underestimated by other adenine nucleotide pools, and compartment-specific measurements of adenine nucleotides not bound to protein are needed for precise quantification. Cytosolic ATP/ADP ratios can be determined specifically with fluorescent fusion constructs[13,14], but the dynamic range of such constructs is presently insufficient to detect the high cytosolic ratios that are for example predicted (*cf.* Fig. 1 legend) by the phosphocreatine (PCr) and creatine (Cr) levels reported for skeletal muscle[15]. Moreover, a live-cell imaging approach does not allow quantification of *absolute* cytosolic ATP/ADP ratios unless signals can be calibrated[2]. Pancreatic beta cells contain a PCr/Cr pool[16]. This pool is entirely cytoplasmic and not bound to protein, and may thus be used to monitor the ratio of free cytosolic ATP and ADP

because the reaction catalysed by creatine kinase (Cr + ATP ⇔ PCr + ADP) is at equilibrium[2]. When derived this way in ß-HC9 beta cells[17,18], ATP/ADP ratios are obtained with values that are indeed similar to the high cytosolic phosphorylation potentials seen in skeletal muscle. When cultured with 3 mM Cr, we detected a PCr pool in INS-1E cells that responds to glucose and FCCP as predicted by the canonical GSIS model. Cytosolic ATP/ADP ratios calculated from measured PCr and Cr levels (Fig. 1C) agree with published ß-HC9 values[18]. The precise role of the PCr/Cr pool in beta cells remains to be established conclusively, but a PCr/Cr shuttle has been suggested to facilitate transmission of phosphorylation potential between mitochondria and plasma membrane K_{ATP} channels[16].

3. Control of ATP flux by ATP supply

The ATP/ADP ratio of mammalian cells is generally fairly stable. Most cell types aim to keep a constant phosphorylation potential far removed from thermodynamic equilibrium so that it can drive endergonic processes under a wide range of energetic demands[19]. For instance, skeletal muscle respiratory activity may fluctuate 3 orders of magnitude without discernible effect on the cytosolic ATP/ADP ratio. This relative stability is a direct consequence of the strong control exerted by energy expenditure over myocellular ATP flux. Demand-driven ATP flux ensures that the cytosolic ATP/ADP ratio is kept under tight homeostatic control[20]. In contrast, the apparent lack of such homeostasis in beta cells implies that total ATP flux in these cells must be largely controlled by ATP supply. This implication indeed follows from a GSIS model in which full glucose breakdown via oxidative phosphorylation induces an increase in the cytosolic ATP/ADP ratio (Fig. 1A).

Beta cell respiratory analyses generally offer clear evidence for the strong control of ATP flux by ATP supply: basal oxygen uptake in insulinoma cells as well as in rodent and human islets is increased 1.5 to 3-fold by glucose[21-36]. From oligomycin-sensitive respiration we calculate that glucose raises the ATP supply flux in fuel-deprived INS-1E cells up to almost 6 times (Fig. 2A). The glucose effect on ATP supply is comparably strong because most of the respiratory rise is coupled to phosphorylation[25,36], whilst the proportion of *basal* respiration used to make ATP is exceptionally low[28,33,37-39]. Glucose increases ATP-synthesis-coupled respiration more than it raises respiration linked to mitochondrial proton leak, which means it stimulates both the rate and *coupling efficiency* of oxidative phosphorylation[26,36].

Glucose stimulation of mitochondrial coupling efficiency is decreased by uncoupling protein-2[26], a carrier protein that contributes to the very high proton leak of beta cells[37]. Attenuated bioenergetic glucose sensitivity is mirrored by dampened GSIS[26], which suggests a role for mitochondrial proton leak in the regulation of glucose-sensing. A recent metabolic control

analysis has indeed demonstrated that proton leak exerts significant control over both the magnitude and homeostasis of the mitochondrial membrane potential in insulinoma cells incubated at low glucose levels[39]. Low mitochondrial ATP synthesis efficiency thus appears to ensure that the membrane potential as well as the mitochondrial and cytosolic ATP/ADP ratios are kept relatively low when insulin secretion is not required. Interestingly in this respect, ATPase inhibitory factor 1 has recently also been shown to dampen insulin secretion from insulinoma cells at basal glucose concentrations through inhibition of ATP synthesis[40].

At stimulatory glucose levels, homeostatic control of the mitochondrial membrane potential is almost exclusively exerted by glucose oxidation, a process that is regulated itself by factors that arise downstream from ATP synthesis[39]. These factors have not yet been identified, but positive feedback reinforces bioenergetic responsiveness to glucose and supports the notion that glucose-sensing by beta cells is not accounted for by glucokinase alone (*cf.* section 1). This notion is further supported by the beta cells' responsiveness to pyruvate[33,36,41] that can enter insulinoma cells when added as sodium pyruvate and primary beta cells when given as methyl-pyruvate. We find that INS-1E cells increase their respiratory activity to the same extent in response to 5 mM pyruvate as to 20 mM glucose (Fig. 2B). This bioenergetic response is reflected by pyruvate-stimulated insulin secretion (not shown), and suggests that nutrient-sensing is at least partly controlled by mechanisms downstream from glycolysis. The stimulation of respiration by metabolic fuels is amplified when oxidative phosphorylation is uncoupled (Fig. 2C), which suggests that ATP demand also exerts some control over fuel sensitivity of respiration, and by extension ATP flux, under coupled conditions.

4. Control of ATP flux by ATP demand

Approximately 50% of the total protein synthesis rate in beta cells can be attributed to pro-insulin[42], which implies beta cells need much energy to make insulin. Moreover, trafficking, priming and exocytosis of insulin secretory vesicles are processes that require energy[43,44]. Possible control of ATP demand over cellular bioenergetics has not been quantified in beta cells, although indirect qualitative evidence for such control exist[21,23,24]. Cells in which total ATP flux is demand-driven allow allocation of ATP supply to energy-demanding processes to be approximated from sensitivity of oligomycin-sensitive respiration to experimental inhibition of these processes[45]. Acute cycloheximide effects on oxidative phosphorylation, mediated through increased cytosolic phosphorylation potential, have thus shown that 20-35% of ATP supply is used for protein synthesis in rat thymocytes[45], human embryonic stem cells[46], as well as in rat and human myocytes[47]. It is worth note that the reported percentages may be slight overestimations due to non-specific inhibitor effects[48].

Cycloheximide also appears to lower the respiration of beta cells, a system in which ATP flux is significantly supply-driven as predicted by the canonical GSIS model (*cf.* section 3). Glucose-stimulated oxygen uptake by INS-1E cells (Fig. 3A) and mouse islets (Fig. 3B) is inhibited acutely after addition of 5 µM cycloheximide. This inhibition is reversed when oxidative phosphorylation is uncoupled, which supports the notion that it is mediated via the cytosolic ATP/ADP ratio and excludes the possibility that it arises from cycloheximide effects on fuel oxidation *per se*. From the inhibitory cycloheximide effects on oligomycin-sensitive respiration we estimate that as much as 40% of total ATP supply (*cf.* Fig. 2A) is used to make protein in INS-1E cells and about 30% in mouse islets. Perhaps unsurprisingly, the absolute ATP flux coupled to protein synthesis is stimulated by glucose (Fig. 3C), which is likely owing to lowered AMP-activated protein kinase activity that follows from the glucose-induced increase in cytosolic phosphorylation potential[49].

Acute indirect inhibition of oxidative phosphorylation by cycloheximide demonstrates that energy expenditure linked to protein synthesis exerts significant control over total ATP flux in beta cells. Therefore, ATP flux control is not exclusively derived from glucose-fuelled ATP supply, as might be expected from the bioenergetic glucose sensitivity of beta cells, but also arises from ATP demand. This shared flux control between ATP supply and ATP demand is consistent with differential effects of glucose and sulphonylureas (antidiabetic drugs that act on K_{ATP} channels[50]) on beta cell bioenergetics reported by others[21,23,24]. In accordance with flux control from ATP supply, glucose-induced increases in oxygen uptake are concomitant with increased phosphorylation potential. The increase in oxygen consumption provoked by sulphonylureas (tolbutamide or glybenclamide), on the other hand, coincides with decreased phosphorylation potential[21,23,24], which suggests that the respiratory stimulation is secondary to increased ATP demand. Such increased demand likely emerges from the insulin secretion provoked by sulphonylureas through their inhibition of K_{ATP} channels[50].

5. Why quantitative insight in bioenergetic control is important

The limited information that is available on shared ATP flux control between fuel breakdown and energy expenditure in beta cells is entirely qualitative at present. It will be important from basic and clinical science perspectives to quantify the relative importance of ATP supply and ATP demand for bioenergetic control in pancreatic beta cells. Besides the ATP flux control discussed above, such quantification should also focus on the concentration control exerted by ATP-producing and ATP-consuming processes over the cytosolic ATP/ADP ratio, as it is this ratio that is crucial for nutrient-secretion coupling. It is worth stressing that in the context of this quantitative analysis, the term ‘control’ differs from ‘regulation’ as it reflects a *potential*

to regulate[51]. Distributed control between ATP supply and ATP demand would thus give the beta cell the potential to regulate ATP flux and GSIS by altering either demand or supply.

Modular-kinetic and metabolic control analyses have provided quantitative information on oxidative phosphorylation in beta cells[39] and in isolated beta cell mitochondria[52]. Extending such analyses to cellular energy metabolism surrounding the cytosolic ATP/ADP ratio might offer insight in some unresolved issues in beta cell biology. For instance, amplification and potentiation of GSIS by glucose and fatty acids, respectively, may be owing to an increased ATP supply capacity that would be better able to meet the high energy demand from prolonged insulin secretion. Indeed, sulphonylurea-provoked insulin secretion, which can be amplified by glucose, is reflected by bioenergetic changes that are indicative of increased ATP demand[21,23,24]. This model aligns well with classical GSIS where bioenergetic glucose sensitivity alone mediates nutrient-secretion coupling, and would explain why non-canonical coupling factors remain to be identified conclusively.

Bioenergetic glucose sensitivity implies that total ATP flux is controlled by glucose-fuelled ATP supply (Fig. 4A), and the loss of this sensitivity in type 2 diabetes (T2D)[53,54] indicates that ATP flux control by ATP supply has decreased[38]. The loss of glucose sensitivity in T2D can be managed therapeutically with sulphonylureas that induce insulin release downstream from mitochondria (Fig. 4B). This management shows that glucose-unresponsive beta cells remain bioenergetically competent as ATP is still supplied at the rate necessitated by the high energy demand from insulin synthesis and insulin secretion. It thus appears that control over ATP flux in beta cells shifts from ATP supply to ATP demand during the development of T2D (Fig. 4C)[38]. Metabolic control analysis should clarify whether or not this apparent flux control shift is accompanied by a change in concentration control over the ATP/ADP ratio. Although measured ATP flux and phosphorylation potential will likely be temporal averages in such analysis, and may thus be assumed at steady state as a first approximation, it will be important to account for known oscillatory behaviour of beta cells in due course.

Bioenergetic differences between pancreatic islets from healthy individuals and those with T2D have been shown[38,55-57], but it is unclear which differences account for the apparent shift in ATP flux control. Notably, however, the loss of bioenergetic glucose responsiveness in islets from individuals with T2D can be restored by glucokinase activators, and impaired GSIS is consequently rescued[58]. This effect underscores the importance of glucokinase for GSIS, but also leaves open the possibility that this enzyme is the endpoint of the recently discovered positive feedback loop that is effected by a factor that emerges downstream from mitochondrial ATP synthesis[39]. In this model, bioenergetic glucose sensitivity would be lost

in T2D because of failing feedback. More generally, quantitative insight in the control of beta cell bioenergetics will likely help rationalising therapeutic drug targets.

Finally, it is worth considering that the apparent bioenergetic control shift in dysfunctional beta cells is caused by the persistently high functional demand on beta cells in the insulin-resistant state that leads up to T2D[53]. In this respect, sulphonylurea management of T2D will likely exacerbate the situation as these drugs induce insulin secretion constitutively with a chronic increase in energy demand as result. The inability to meet such demand possibly explains why beta cell function deteriorates during prolonged sulphonylurea treatment[50].

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Declaration of interest

The authors do not have any conflict of interest to declare.

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Author contribution statement

CA conceived the original work included in this review and wrote the manuscript. BA, JB, JEC and AGW designed, executed and analysed the experiments reported in Figs 2A, 3A and 3C (BA), 2B, 2C and 3B (JB), 1B (JEC) and 1B (AGW). All authors commented on drafts of the manuscript and approved its final version.

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Figure legends

Figure 1 – Glucose stimulation of the cytosolic ATP/ADP ratio is the key signal for canonical GSIS. *Panel A*: glucose-fuelled oxidative phosphorylation (OXPHOS) raises the mitochondrial protonmotive force that drives ATP synthesis and thus increases the mitochondrial ATP/ADP ratio. This increase in mitochondrial phosphorylation potential is transferred to the cytosol by the adenine nucleotide translocator and the phosphate carrier. The consequent increase in the cytosolic ATP/ADP ratio leads to closure of K_{ATP} channels, lowering of the plasma membrane potential ($\Delta\psi_p$), an increased cytosolic free Ca^{2+} level, and exocytosis of insulin-containing granules. *Panel B*: Whole-cell ATP levels were measured by a chemiluminescent luciferase-luciferin assay in neutralised perchloric acid extracts[59] obtained from INS-1E cells exposed to 2 mM (G2) or 10 mM (G10) glucose ± 3 µM FCCP. ADP was measured after conversion to ATP using phospho-enolpyruvate and pyruvate kinase in extracts depleted from ATP with sulphurylase[59]. Data are means ± SEM of 5 exposures assayed in triplicate. *Panel C*: cytosolic ATP/ADP ratios were calculated from PCr and Cr levels that were determined by luminometric assay[17] using INS-1E cells incubated at 2 (G2) and 10 (G10) mM glucose ± 3 µM FCCP. Calculations assumed 1 mM free intracellular Mg^{2+}, an intracellular pH of 7.2 and an equilibrium constant for the reaction catalysed by creatine kinase (Cr + ATP $\Leftrightarrow$ PCr + ADP) of 110[17]. Data are means ± SEM of 8 exposures sampled from 2 assays.

Figure 2 – Fuel stimulation of oxidative phosphorylation. Total ATP supply flux (Panel A, J_{ATP}) was calculated in INS-1E cells from the oligomycin-sensitive O_2 uptake assuming a P/O ratio of 2.78 for the complete oxidation of glucose[60]. Data are means ± SEM of 3 separate assays with 5 replicates each. Respiratory traces (Panel B) and data normalised to basal rate (Panel C) showing INS-1E respiratory responses to 2.5 mM glucose, 20 mM glucose and 5 mM sodium pyruvate (grey, white and black symbols, respectively). Cells were incubated in a Seahorse XF24 extracellular flux analyser at 2.5 mM glucose and then treated, sequentially, with additional substrate (S), 3 µM of the mitochondrial uncoupler BAM15 (U) and a mix of 2 µM rotenone and 2 µM antimycin A (R/A), both mitochondrial respiratory inhibitors. Data are means ± SEM of 3 experiments with conditions assayed 2-4 times in each. Differences between means were tested for statistical significance by 2-way ANOVA: responses to 2.5 mM glucose differ from those to 20 mM glucose and 5 mM pyruvate (+/- uncoupler), and all responses in the presence of uncoupler are higher than the equivalent values in the absence of uncoupler (*** $P < 0.001$; **** $P < 0.0001$).

Figure 3 – Oxidative phosphorylation linked to protein synthesis. Respiratory traces showing respiration by INS-1E cells (Panel A) and mouse islets (Panel B) as measured with a Seahorse XF24 extracellular flux analyser. Basal respiration was modulated (at times indicated by dotted lines) with 11 or 28 mM glucose (G11 and G28), 5 or 200 µM cycloheximide (CHX), and with 3 µM FCCP or 3 µM BAM15 in cells and islets, respectively, and with a combination of 2 µM rotenone and 2 µM antimycin A (R/A) in both systems. All activities were normalised to basal respiration. Data are the means ± SEM of 5 wells sampled from 1 assay plate (cells) or 15-20 wells sampled from 3-4 separate assay plates (islets). ATP supply flux (J_{ATP}) used to drive protein synthesis (Panel C) was calculated in INS-1E cells from the responsiveness of oligomycin-sensitive O_2 uptake to cycloheximide assuming a P/O ratio of 2.78 for the complete oxidation of glucose[60]. Data are means ± SEM of 3 separate assays with 5 repeats each.

Figure 4 – Does ATP flux control shift during development of type 2 diabetes? When blood glucose rises, beta cells increase the rate at which they catabolise glucose (A). The resulting rise in ATP/ADP leads to inhibition of K_{ATP} channels, a decrease of the plasma membrane potential ($\Delta\Psi_p$), opening of voltage-gated Ca^{2+} channels (VGCC), influx of Ca^{2+}, and the exocytosis of insulin granules. GSIS is impaired in type 2 diabetes, but insulin secretion can still be stimulated with sulphonylureas (SUs), which inhibit K_{ATP} channels (B). Loss of glucose sensitivity and SU-responsiveness suggest the control over ATP flux shifts from ATP supply to demand during development of type 2 diabetes (C).