Review Article

Nutritional Regulation of Insulin Secretion: Implications for Diabetes

***Philip Newsholme[1] and Mauricio Krause[2,3]**

[1]School of Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, WA 6845, Australia; [2] Biomedical Research Group, Department of Science, ITT Dublin, Ireland, [3]School of Public Health, Physiotherapy & Population Science, UCD Dublin 4, Ireland.

*For correspondence: Professor Philip Newsholme, philip.newsholme@curtin.edu.au

Abstract

Pancreatic β-cells are exquisitely organised to continually monitor and respond to dietary nutrients, under the modulation of additional neurohormonal signals, in order to secrete insulin to best meet the needs of the organism. β-cell nutrient sensing requires complex mechanisms of metabolic activation, resulting in production of stimulus-secretion coupling signals that promote insulin biosynthesis and release. The primary stimulus for insulin secretion is an elevation in blood glucose concentration and β-cells are particularly responsive to this important nutrient secretagogue via the tight regulation of glycolytic and mitochondrial pathways at steps such as glucokinase, pyruvate dehydrogenase, pyruvate carboxylase, glutamate dehydrogenase and mitochondrial redox-shuttles. With respect to development of type-2 diabetes (T2DM), it is important to consider individual effects of different classes of nutrient or other physiological or pharmacological agents on metabolism and insulin secretion and to also acknowledge and examine the interplay between glucose metabolism and that of the two other primary nutrient classes, amino acids (such as arginine and glutamine) and fatty acids. It is the mixed nutrient sensing and outputs of glucose, amino and fatty acid metabolism that generate the metabolic coupling factors (MCFs) essential for signalling for insulin exocytosis. Primary MCFs in the β-cell include ATP, NADPH, glutamate, long chain acyl coenzyme A and diacylglycerol. It is the failure to generate MCFs in a coordinated manner and at sufficient levels that underlies the failure of β-cell secretion during the pathogenesis of T2DM.

Introduction

Glucose-stimulated insulin secretion (GSIS) is central to normal control of metabolic fuel homeostasis, and its impairment is a hallmark of β-cell failure in type 2 diabetes (T2DM).[1] β-cells are often referred to as 'fuel sensors' as they continually monitor and respond to dietary nutrients, under the modulation of additional neurohormonal signals, in order to secrete insulin to best meet the needs of the organism.[2] β-cell responses to nutrients require metabolic activation, resulting in the production of stimulus-secretion coupling signals that promote insulin biosynthesis, movement of insulin containing vesicles to the cell surface and the release of the cargo of insulin. The primary stimulus for insulin secretion is glucose and islet β-cells are particularly responsive to this important nutrient secretagogue.[2]

Glucose and other nutrients such as amino acids and fatty acids exert some of their effects on insulin secretion via their metabolism in β-cells to generate stimulus/secretion coupling factors, including a rise in the ATP/ADP ratio, which serves to suppress ATP-sensitive potassium (K_{ATP}) channels and activate voltage-gated Ca^{2+} channels, leading to stimulation of insulin granule exocytosis.[1] In addition to the primary stimulus of glucose, specific amino acids may acutely and chronically regulate insulin secretion from pancreatic β-cells *in vivo* and *in vitro*.[3] Mitochondrial metabolism is crucial for the coupling of glucose, alanine, glutamine and glutamate recognition with exocytosis of insulin granules. The positively charged amino acid L-arginine is now recognised as not only a powerful secretagogue, but also an essential synergic compound for nutrient-dependent insulin secretion.[4] In addition to the known acute effects of some amino acids on β-cells, chronic exposure to specific amino acids may influence gene expression in the β-cell, which has an impact on insulin secretion and cellular integrity. Therefore amino acids may play a direct or indirect (via generation of putative messengers of mitochondrial origin) role in insulin secretion.[3]

The third group of nutrients that are known to influence β-cell function are the fatty acids.[5] In particular, non-esterified fatty acids (NEFA) are known to induce both stimulatory and detrimental effects on pancreatic β-cells. Acute exposure of the pancreatic β-cell to high glucose concentrations and/or saturated NEFA results in a substantial increase in insulin release, whereas chronic exposure results in desensitisation and suppression of secretion, followed by the induction of apoptosis. Therefore, changes in the islet levels of NEFA are likely to be important for the regulation of β-cell function and viability under physiological conditions.

Understanding the molecular mechanisms by which glucose, amino acids and fatty acids regulate insulin secretion and cell integrity may identify novel targets for future diabetes therapies. In this review, we aim to present a summary of the latest research regarding the effects of nutrients on β-cell function and their therapeutic use as nutritional support for the treatment of conditions related to insulin resistance and diabetes.

Glucose Dependent Metabolic Stimulus-Secretion Coupling in β-cells

Insulin secretion from the pancreatic islet β-cell is regulated by a number of factors, but the predominant stimulatory signal is the rise in blood glucose that occurs with the ingestion of carbohydrate containing meals. Glucose not only directly stimulates insulin secretion from β-cells via its metabolism but also modulates the action of several other effectors, including free fatty acids, amino acids and incretin hormones (group of hormones secreted in response to nutrients from a meal, e.g. glucagon-like peptide-1 (GLP-1)).[6]

As is widely reported, the traditional model of GSIS involves the transport of glucose into the β-cell via GLUT-2 or GLUT-1 transporters followed by glucose phosphorylation to produce glucose 6-phosphate catalysed by glucokinase, the production of ATP from both glycolytic and mitochondrial sources and the generation of key stimulus secretion-coupling factors essential for insulin secretion, such as NADPH, acyl coenzyme A (acyl-CoA) and some amino acids. It is the mixed nutrient sensing and stimulus-secretion outputs of glucose, amino and fatty acid metabolism that generate the metabolic coupling factors (MCFs) critical for insulin exocytosis. Briefly, the triggering signal for insulin exocytosis is ATP produced in glycolysis and mitochondrial glucose oxidation. Efficient shuttling of reducing equivalents from the cytosol to the mitochondrial electron transport chain is required as lactate dehydrogenase levels are low in the β-cell (Figure). The resultant increase in the ATP/ADP ratio inhibits K_{ATP} channels, resulting in an inhibition of K^+ efflux, plasma membrane depolarisation, activation of voltage-gated Ca^{2+} channels, and influx of extracellular Ca^{2+}, which serves to activate granule exocytosis.[7]

This markedly unusual arrangement of β-cell metabolism ensures that generation of metabolic stimulus secretion coupling factors is rapid and efficient so that the β-cell response to a rise in blood glucose is proportional to the concentration and is swift. As described above, the glucose is transported into the pancreatic β-cell by the non-insulin-dependent glucose transporter GLUT2 in rodents and by both GLUT1 and GLUT2 in humans.[5] Once inside the cells, glucose is phosphorylated by the low-affinity (Km of 6-11 mmol/L) hexokinase IV (glucokinase).[3] The glycolytic flux in pancreatic islet β-cells is therefore regulated by a combination of the rate of glucose uptake and the glucokinase activity, although it is unlikely that glucose uptake is rate-limiting under most conditions.[8] The unique metabolic design of the β-cell is characterised by (i) utilisation of glucose in the physiologically relevant range (2-20 mmol/L); (ii) low lactate dehydrogenase and plasma membrane monocarboxylate pyruvate/lactate transporter activity and correspondingly high activity in the mitochondrial malate/aspartate shuttle activity; and (iii) high activity of both pyruvate dehydrogenase and pyruvate carboxylase, ensuring that both anaplerotic and oxidative metabolism of glucose/pyruvate can co-exist.[1,3,5,9,10] Acetyl-CoA formed from pyruvate can condense with oxaloacetate forming citrate for metabolism in the tricarboxylic acid cycle, leading to NADH and $FADH_2$ production.[2] All these specific metabolic adaptations are geared to enhance mitochondrial tricarboxylic acid cycle activity, oxidative phosphorylation and efficient ATP production. An enhancement of the ATP/ADP ratio results in closure of ATP-sensitive K^+ channels and depolarisation of the plasma membrane as described above, eventually resulting in fusion of insulin-containing granules with the plasma membrane.[11,12]

However, the K_{ATP} channel dependent component of GSIS does not fully describe the β-cell response to glucose, and signals (MCFs) in addition to changes in the ATP/ADP ratio are also implicated as important regulators of insulin secretion.[1] Primary MCFs in the β-cell include ATP (triggering MCF), NADPH, glutamate, long chain acyl-CoA and diacylglycerol (all amplifying MCFs).[2] Mitochondrial metabolism is also crucial for the glucose-induced movement of the insulin-containing granules. Mitochondria generate ATP but the subsequent elevation in cytosolic Ca^{2+} cytosol is necessary for full development of sustained insulin secretion. Hence mitochondria generate ATP and other coupling factors serving as fuel sensors for the control of the exocytotic process. Numerous studies have sought to identify the factors that mediate the amplifying pathway over the Ca^{2+} signal in nutrient-stimulated insulin secretion. Predominantly, these

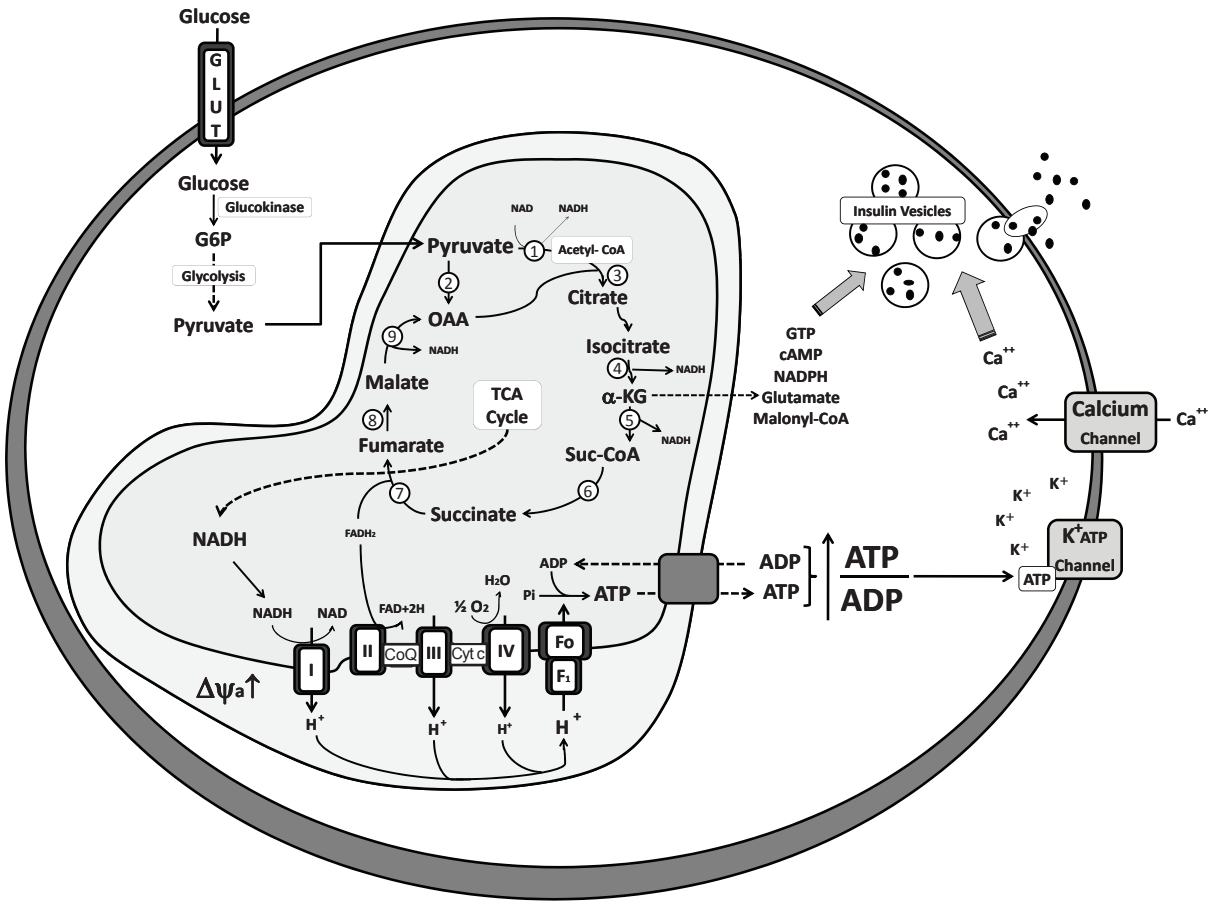

Figure. General view of the mitochondrial metabolism in pancreatic β-cells. Products of carbohydrate, protein and fat metabolism can be converted to CO_2 and water by the mitochondria, using key enzymes of the TCA cycle and the electron transport chain; NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome bc1 (Complex III), and cytochrome *c* oxidase (Complex IV). During these reactions, protons (H^+) are pumped from the matrix to the space between the inner and outer membranes, establishing a proton gradient. Protons diffusing back along this gradient drive the synthesis of ATP by the F_0F_1ATP synthase complex. Mitochondria generate cellular energy through TCA cycle activity and the associated electron transport chain of the inner membrane. The reducing equivalents (NADPH and FADH2) produced from the TCA cycle are reoxidised via a process that involves transfer of electrons through the electron transport chain and associated translocation of protons across the mitochondrial inner membrane, creating the transmembrane electrochemical gradient which is used to provide the electrochemical potential to make ATP through the ATP synthase complex. In the case of β-cells, the increased ATP/ADP ratio leads to the closure of the K^+_{ATP} channels leading to membrane depolarisation followed by calcium influx that, together with other co-factors (such as glutamate, NADPH, malonyl-CoA, cAMP, GTP) induces the translocation and exocytosis of the insulin vesicles. ①Pyruvate dehydrogenase, ②Pyruvate carboxylase, ③Citrate synthase, ④Isocitrate dehydrogenase, ⑤α-ketoglutarate dehydrogenase, ⑥Succinate thiokinase, ⑦Succinate dehydrogenase, ⑧Fumarase, ⑨ Malate dehydrogenase.

factors are nucleotides (GTP, ATP, cAMP and NADPH), although metabolites have also been proposed, such as long-chain acyl-CoA derivatives and the key amino acid glutamate.[1,13]

Fatty Acid Dependent Metabolic Stimulus-Secretion Coupling in β-cells

Long-chain fatty acids can be transported into the cell by free diffusion with no requirement for active transport.[14] For most mammalian cells, fatty acid metabolism is mainly controlled by substrate supply.[5] In the fasted state, fatty acids are converted into long-chain acyl-CoA by acyl-CoA synthetase (ACS) and enter the mitochondria via carnitine palmitoyl transferase 1 (CPT-1), where they are oxidised via the β-oxidation pathway for energy production,[15] maintaining the basal levels of insulin secretion. After a carbohydrate-containing meal, fatty acid oxidation is inhibited, since the regulatory molecule malonyl-CoA is synthesised by acetyl-

CoA carboxylase (ACC) from an acetate group derived from citrate which is elevated following synthesis from glucose and/or amino acids.[16] Malonyl-CoA inhibits CPT-1, thus blocking transport of long chain acyl-CoA into the mitochondria.[17] Accumulation of long chain acyl-CoA in the cytosol leads to an increase of intracellular Ca^{2+} levels and to changes in acylation state of proteins involved both in regulation of ion channel activity and exocytosis.[18] In addition, long-chain acyl-CoA can also enhance the fusion of insulin-secretory vesicles with the plasma membrane and insulin release.[19]

The crosstalk between fatty acids and glucose resulting in controlled levels of ATP production can be regulated through the activation of the AMP-activated kinase (AMPK), since this regulatory enzyme is known to inhibit the activation of ACC.[20] AMPK may additionally regulate β-cell function more chronically by changing the levels of expression of key transcription factors controlling lipogenic and glycolytic enzymes, such as sterol-regulatory-element-binding protein 1c (SREBP1c) and hepatocyte nuclear factor 4α (HNF-4α). Esterification of long-chain acyl-CoA to triacylglycerol (TAG) also occurs in β-cells in the presence of glycerol 3-phosphate provided by glucose metabolism.[5] Endogenous lipolysis is also a regulatory process for insulin secretion since pancreatic β-cells express hormone sensitive lipase (HSL) which can generate NEFAs and other lipid signalling molecules, a mechanism that may explain, at least in part, the action of incretins such as GLP-1 on the potentiation of GSIS.[22]

On the other hand, it is known that chronic exposure of β-cells to high levels of saturated fatty acids can impair glucose oxidation, resulting in a fall in the ATP/AMP ratio and activation of AMPK, phosphorylation and inhibition of ACC, reduction in fatty acid synthesis and promotion of fatty acid oxidation, thus impairing GSIS. The chronic inhibitory effect of NEFAs on GSIS may be due to a metabolic effect. Reduced glucose oxidation may result from decreased conversion of pyruvate into acetyl-CoA, a consequence of a decline in islet pyruvate dehydrogenase activity due to the inhibitory action of increased NADH production via NEFA β-oxidation,[23] an increase in pyruvate dehydrogenase kinase activity or via changes in the expression of key metabolic genes or transcription factors.[24]

Effects of fatty acids on glucose-induced insulin secretion are directly correlated with chain length and the degree of unsaturation, where long-chain fatty acids (such as palmitate or linoleate) *acutely* increase but *chronically* reduce insulin release in response to glucose stimulation.[25] A recent study by the authors demonstrated that chronic incubation (24 hours) of β-cells with a polyunsaturated fatty acid (arachidonic acid) increased insulin secretion while, on the other hand, exposure of a clonal pancreatic β-cell line (BRIN-BD11) for 24 hours to a saturated fatty acid (palmitic acid) resulted in inhibition of insulin secretion.[26]

A recent advance in the understanding of the mechanisms by which NEFAs modulate insulin secretion *in vivo* was the discovery of high levels of expression of the membrane-bound G-protein-coupled receptor (GPR). The latter is a putative NEFA receptor in human and animal islet β-cell preparations[27] and the levels of its mRNA were shown to be positively correlated with the insulinogenic index.[27] Details on the signalling mechanisms that enable GPR40 to influence insulin secretion are still not fully resolved, but the mechanism appears to involve changes in intracellular Ca^{2+} mobilisation.[28] In addition, recent evidences have shown that GPR41 and GPR119 are also important in islet physiology.[29,30]

Amino Acid Dependent Metabolic Stimulus-Secretion Coupling in β-cells

In addition to glucose and fatty acids, some amino acids are known to acutely and chronically regulate insulin secretion from pancreatic β-cells *in vivo* and *in vitro*.[3] Amino acids such as glutamine, alanine, arginine and others are known to cause increments in GSIS, indicating that β-cell amino acid and glucose metabolism share common pathways. Specifically, mitochondrial metabolism is crucial for the coupling of amino acid and glucose recognition to exocytosis of insulin granules.

The nucleotide ATP, mainly generated by mitochondrial metabolism, is the main coupling factor in insulin secretion as discussed above but amino acids can generate further MCFs via cytosolic or mitochondrial metabolism.

This scenario further emphasises the importance of the key enzymes, e.g. glutamate dehydrogenase, the aspartate and alanine aminotransferases, and the malate-aspartate shuttle in the control of insulin secretion as well as amino acid transporters. In addition, after chronic exposure, amino acids may influence gene expression in the β-cell, which subsequently impacts on insulin secretion. Therefore amino acids may play a direct or indirect role in insulin secretion.[3] Also, after chronic exposure, specific amino acids may influence cellular integrity.[4,31-36] Individual amino acids do not evoke insulin-secretory responses when added at physiological concentrations, but combinations of amino acids or high concentrations of individual amino acids are much more effective. Understanding the molecular mechanisms by which amino acids regulate insulin secretion may identify novel targets for future diabetes therapies. The effects of some amino acids are listed following.

Arginine

This amino acid is known for stimulating insulin release through electrogenic transport into the β-cell via the mCAT2A amino acid transporter, resulting in membrane depolarisation, a rise in intracellular Ca^{2+} through opening of voltage-gated Ca^{2+} channels, and then insulin secretion.[37] Arginine may also be converted to L-glutamate and thus influence insulin secretion by the generation of further MCFs.[35] It was recently shown that L-arginine exerts many positive influences on β-cell metabolism: i) stimulation of β-cell insulin secretion; ii) provision of anti-oxidant and protective responses (glutathione synthesis); iii) increasing glucose consumption; and iv) inducing basal glutamate synthesis.[4] It was suggested that, in some situations, arginine could exert a negative effect on β-cell insulin release. The potentially detrimental effect of arginine metabolism hinges on arginine-derived nitric oxide (NO) through the action of inducible nitric oxide synthase (iNOS). High levels of NO are known to interfere with β-cell mitochondrial function and the generation of key stimulus-secretion coupling factors, which could lead to a reduction in cellular insulin output. However, high concentrations of NO alone are not harmful for β-cells unless accompanied by superoxide production.[38] In addition, incubation of β-cells in the absence of L-arginine but in the presence of pro-inflammatory cytokines resulted in β-cell damage and death, but on marginally increasing L-arginine concentrations, β-cell viability was maintained.[4]

L-glutamine

The average extracellular concentration of L-glutamine is 0.7 mmol/L.[39] L-glutamine is consumed at high rates by both primary islets and BRIN-BD11 β-cells.[4] L-glutamine is rapidly taken up and metabolised by islets, however, alone it does not stimulate insulin secretion or enhance glucose-induced insulin secretion. Activation of glutamate dehydrogenase (GDH) by addition of leucine enhances insulin secretion by increasing the entry of glutamine carbon into the tricarboxylic acid cycle.[40] The production of γ-aminobutyric acid (GABA) from glutamine[41] has been proposed as an explanation for the paradox that glutamine alone does not stimulate insulin release.[42] Under this scheme, glutamine is preferentially metabolised to GABA and L-aspartate, with the release of $^{14}CO_2$ from L-[U-^{14}C] glutamine in the process. There is no oxidation of glutamine in the process and thus stimulus-secretion coupling via ATP would be minimal.

Using ^{13}C-labelled glutamine and nuclear magnetic resonance (NMR) spectroscopy, we showed that the major products of L-[1,2^{13}C] glutamine metabolism are L-[1,2-^{13}C] glutamate and L-aspartate labelled at positions C-1 and C-4 in BRIN-BD11 β-cells.[43] L-aspartate is formed after entry of L-glutamate into the tricarboxylic acid cycle. Additionally, the L-glutamate produced from glutamine entered the γ-glutamyl cycle and resulted in an increased production of glutathione.[43] A recent paper reported that L-glutamate was released from the BRIN-BD11 β-cell into the extracellular medium.[32] Chronic glutamate release may cause glutamate receptor activation and desensitisation of the cell to further insulinotropic signals, representing a novel autocrine mechanism for regulation of β-cell function.[32] Indeed, sub-lethal concentrations of pro-inflammatory cytokines significantly increased glucose consumption and glutamate export from a clonal β-cell line, suggesting a novel mechanism to explain the phenomenon of cytokine-dependent inhibition of insulin secretion.[44] As glutamate is known to inhibit glucagon secretion from the pancreatic α-cell, glutamate release from the β-cell may additionally represent a novel paracrine mechanism for pancreatic islet hormone release.

Intracellular L-glutamate

Maechler and Wollheim[45] proposed that L-glutamate participates in nutrient-induced stimulus–secretion coupling, as an additive factor in the amplifying pathway of glucose-stimulated insulin secretion. Confusion over the importance of glutamate in glucose-stimulated insulin secretion has arisen over the years as a result of opposing findings: total cellular glutamate levels have been reported to increase in response to to a glucose stimulus in human, mouse and rat islets, as well as in clonal β-cells by some groups,[35,46,47] whereas others reported no such change.[48,49] The finding that mitochondrial activation in permeabilised β-cells directly stimulates insulin exocytosis[50] pioneered the identification of glutamate as a putative intracellular messenger.[45,51] It has been suggested that glutamate is transported into secretory granules, thereby promoting Ca^{2+}-dependent insulin secretion.[51,52] Other support for the glutamate hypothesis arises from a study in which β-cells overexpressing L-glutamate decarboxylase showed a reduced glutamate content and a reduction in glucose-stimulated insulin secretion.[47] More recently glutamate transporters in secretory vesicles have been demonstrated to play a role in the insulin secretory process.[53]

L-alanine

Addition of 10 mmol/L alanine at basal glucose concentrations increased insulin secretion 3- and 1.6-fold for BRIN-BD11 β-cells and islets respectively.[33] L-alanine was also reported to be oxidised by islets from the *ob*/*ob* mouse, the latter being an animal model for diabetes.[54] In RINm5F cells, the insulinotropic action of L-alanine has been reported to be a result of co-transport with Na^+, which resulted in membrane depolarisation leading to an increase in intracellular Ca^{2+}.[55,56] Additionally, by use of the respiratory poison oligomycin, the metabolism and oxidation of alanine were shown to be important for its ability to stimulate insulin secretion.[46]

Prolonged exposure to alanine has previously been reported to reduce subsequent alanine-induced insulin secretion.[33]

Homocysteine

Homocysteine can inhibit insulin section.[57] Homocysteine is a sulfhydryl-containing amino acid formed during the metabolism of methionine and which can be taken up by cells mainly via cysteine transporters.[58] Elevated plasma homocysteine levels have been reported in hyperinsulinaemic obese subjects and in subjects with T2DM with pre-existing coronary vascular disease.[59] In contrast to all the amino acids discussed above, homocysteine has a negative impact on insulin secretion in pancreatic β-cells. The inhibition of insulin secretion by homocysteine was reported to occur rapidly, reversibly and in a dose-dependent manner, impairing the insulin secretory response to low and high glucose concentrations and also to other stimulatory components without alterations in cell viability.[60] In particular, homocysteine caused a significant and dose-dependent inhibition of insulin secretion with initial effects at 50 μmol/L. Although the precise mechanism of homocysteine action is unclear, it could act by interactions with key molecules, by modulating enzyme activities or by protein modification[58] and also by causing oxidative stress damage.[61] NMR spectroscopy studies revealed that homocysteine caused a significant reduction in the labelling of glucose-derived TCA cycle-dependent end products, which may subsequently affect the triggering and potentiation of insulin secretion. The effects of homocysteine were not limited to glucose but also impaired amino acid-stimulated insulin secretion. Acute incubation with homocysteine resulted in concentration-dependent inhibition of alanine, arginine, and ketoisocaproic acid induced insulin secretion. A novel mechanism by which homocysteine blunts insulin secretion is by its effect on NO production. Homocysteine is a known precursor of asymmetric (N^G, N^G) dimethylarginine (ADMA), which is an endogenous methylated amino acid that inhibits the constitutive endothelial and neuronal isoforms of nitric oxide synthase (NOS) but a less potent inhibitor of the iNOS isoform.[62] Homocysteine is also an inhibitor of the enzyme dimethylarginine dimethylhydrolase (DDAH), a key regulatory enzyme which metabolises ADMA. Thus homocysteine is capable of inducing a further increment in ADMA and therefore decreasing the availability of $NO\cdot$.[63] Since a constant low production of $NO\cdot$ is essential for insulin secretion and β-cell function, homocysteine may cause further damage. Further studies into the mechanism of homocysteine-mediated reduction in insulin secretion should shed some light on the possible role of hyperhomocysteinaemia in the development of T2DM.

Branched Chain Amino Acids

It is known from numerous recently published articles that there is a strong positive correlation between plasma levels of branched chain amino acids (BCAA) and the level of insulin resistance.[64] The major tissues that will contribute to changes in plasma BCAA concentrations in the development of Type-2 diabetes are skeletal muscle, liver and kidney, all of which are associated with insulin resistance. Expression of genes associated with BCAA catabolism are also known to be associated with insulin resistance.[64]

Obesity, Low-grade Inflammation and Pancreatic β-cell Dysfunction

Impaired insulin secretion might be induced by insufficient β-cell mass, by functional defects within the β-cells themselves, or by both of these conditions.[65] Reductions in β-cell mass and abnormalities of β-cell function can both be demonstrated in patients with T2DM and individuals at increased risk for diabetes.[66] A genetic element clearly underlies β-cell dysfunction and insufficient β-cell mass; however, a number of modifiable factors are also linked to β-cell deterioration, most notably chronic hyperglycaemia and elevated free fatty acid (FFA) levels.[65] Evidence has also been found for a link between increased pro-inflammatory cytokines and the impairment of insulin-signalling pathways in the β-cells, as well as the potential roles of islet amyloid deposition and fibrotic islet destruction.[67]

The incidence of T2DM has increased dramatically over the last decades, and this seems to be driven by growing rates of obesity.[68] Obesity is a multifactorial condition and the causes include genetic and environmental factors. Glucose homeostasis is critically dependent on a finely regulated balance between insulin sensitivity and output in the pancreas. Insulin resistance demands a corresponding rise in insulin output in order to maintain normal glycaemia.[68] However, this compensation is lost in individuals predisposed to T2DM, resulting in overt hyperglycaemia. Furthermore, insulin resistance related to excess adiposity is linked to several abnormalities which impact on β-cell function and viability.[68] These include glucotoxicity, lipotoxicity, increased oxidative stress and inflammation. In addition, insulin signalling in the β-cell is essential to its own functionality and viability, and obesity-related abnormalities in insulin signalling are known to induce failure of insulin secretion and hyperglycaemia.

Obesity is linked to a chronic pro-inflammatory state, since the adipose tissue expansion results in the release of several cytokines such as tumour necrosis factor alpha (TNF-α), which leads to the activation of serine threonine kinases, c-jun amino terminal kinase (JNK) and IκB, kinase kinase

(IKK) in target cells.[69] It is known that both JNK and IKK phosphorylate insulin receptor substrate 1 (IRS-1) on Ser-307, leading to the inactivation of the insulin receptor.[69] In addition, lipid oversupply and hyperglycaemia can lead to increased deposition of lipid species such as diacylglycerol and ceramide, which can also activate JNK and IKK in liver and/or skeletal muscle, leading to insulin resistance[70] and causing sustained hyperglycaemia and hyperlipaemia. Hyperglycaemia is also known to be involved in inflammation and vascular complications associated with diabetes, arising from reactive oxygen species generation and action.[71,72] Chronic hyperglycaemia induces the production of reactive oxygen species (ROS), through the glycation reaction,[73] in a great many tissues in addition to other production routes, as described above. ROS increases levels of protein oxidation, DNA oxidation and lipid peroxidation. Consequently, oxidative stress originating from hyperglycaemia would be a major cause of impaired islet function at the level of insulin synthesis and secretion.

In addition to ceramide and TNF-α signalling, excessive ROS are also known activators of JNK and IKK.[74] Excessive levels of ROS not only directly damage cells by oxidising DNA, protein and lipids, but indirectly damage cells by activating a variety of stress-sensitive intracellular signalling pathways such as NF-kB, p38 MAPK, JNK/SAPK, hexosamine and others. Activation of these pathways results in the increased expression of numerous gene products that may cause cellular damage and play a major role in the aetiology of late complications in type 2 diabetics. In addition, recent data *in vitro* and *in vivo* suggest that activation of the same or similar stress pathways results in insulin resistance and impaired insulin secretion.[75] Accordingly, it has been proposed that links exist between the hyperglycaemia- and FFA-induced increases in ROS and oxidative stress, activation of stress-sensitive pathways and the eventual development of not only diabetes late complications, but also insulin resistance and β-cell dysfunction.[76]

Obesity also causes a vicious cycle where adipose tissue expansion increases the levels of FFAs and pro-inflammatory cytokines, which together with hyperglycaemia increase the synthesis and accumulation of intramyocellular triglycerides (IMCT). Sedentary behaviour and aging are related with a decreased mobilisation of IMCTs resulting in an increased synthesis of toxic fatty-acid-delivered metabolites (FADM). These metabolites cause a elevation in the production of ROS and reactive nitrogen species, resulting in oxidative stress, mitochondrial dysfunction and the activation of stress signals such as NF-κB followed by the increased production and release of pro-inflammatory cytokines (TNF-α and others). TNF-α is one of the major molecules involved with insulin resistance in skeletal muscle cells and, in addition, this cytokine can also induce activation of stress signals in pancreatic β-cells, leading to mitochondrial dysfunction that culminates in cell dysfunction and death.[77]

Interestingly, growing evidence indicates that ROS are critical for normal β-cell glucose responsiveness.[78] Thus under some circumstances, ROS can act as a 'second-messenger signal' in response to hormone/receptor activation that serves as part of the 'relay' to trigger the ultimate biological response.[78] Short-term ROS production may play a role for physiological regulation of glucose-induced insulin secretion while long-term exposure to high glucose induces oxidative stress in β-cells.[79] It is intriguing that pancreatic β-cells are considered to be particularly vulnerable to oxidative damage, as they express relatively low levels of peroxide-metabolising enzymes such as catalase and glutathione peroxidase,[80] which would contribute to lipotoxicity, glucotoxicity or a combination (termed glucolipotoxicity) in β-cells chronically exposed to nutrients, favouring apoptosis.[5,80,81] Thus, specific manipulation in antioxidant defences may result in different outcomes[82] and, since anti-oxidant systems are also dependent on the nutritional state,[83] this opens the door for nutritional supplementation therapies including amino acids and fatty acids. Tables 1 and 2 summarise some of the potential beneficial systemic effects that individual fatty acid (Table 1) and amino acid (Table 2) supplementation exerts in the metabolic syndrome, diabetes and obesity. Surprisingly, for most amino acids there are few studies of the supplementation effects in humans.

Conclusions

In this article we have reviewed some of the known effects of the nutritional compounds on insulin secretion and β-cell metabolism. Understanding the molecular mechanisms by which glucose, amino acids and fatty acids regulate insulin secretion and cell integrity may identify novel targets for future diabetes therapies. Although there is growing evidences suggesting the beneficial effects of nutrients such as amino acids and fatty acids for the treatment of diabetes, most of the research has been performed only in cell and animal models. With respect to the treatment of T2DM, more research is needed to investigate and identify the potential effects of individual nutrient (specific amino acid and fatty acid) supplementation in human clinical trials. In addition, we believe that nutrient supplementation could be more effective in the early steps of β-cell dysfunction and, for this reason, the time of the nutritional intervention could be critical for the treatment of the disease.

Table 1. Beneficial effects of fatty acid supplementation in humans with type-2 diabetes.

Fatty Acid	Range of Supplementation	Role for Type-2 Diabetes Treatment	Reference
Lipoic Acid	600 mg/day	Reduces levels of asymmetric (N^G, N^G) dimethylarginine in diabetic end-stage renal disease patients on haemodialysis. Reduces NF-κB activation and inflammation. Reduces oxidative stress.	[84-86]
Linolenic acid	3.2-8 g/day	Improves insulin sensitivity. Increases GLUT4 expression in skeletal muscle. Reduces IL-6 and TNFα expression. Increase adiponectin levels. Decreases adipose tissue mass and increase lean mass.	[87-89]
Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA)	2-4 g/day	Reduces lipoperoxidation and oxidative stress. Reduces triglyceride level. Improves microvascular function.	[90-92]

Table 2. Beneficial effects of amino acid supplementation in humans with type-2 diabetes.

Amino Acid	Range of Supplementation	Role for Type-2 Diabetes Treatment	Reference
Taurine	1.5-3 g/day	Decreases triglyceride level and atherogenic index. Reduces body weight. No effect on insulin secretion or sensitivity.	[93], [94]
Glycine	1.33 mmol/kg/day	Increases glutathione. Plasma oxidative stress and lipid peroxides decreases.	[95]
Arginine	6.4g-8.3 g/day	Reduces adiposity. Improves endothelial function. Increases insulin and adiponectin. Increases insulin sensitivity index. Decreases postprandial plasma glucose. Decreases IL-6 production. Increases nitric oxide availability. Decreases asymmetric (N^G, N^G) dimethylarginine. Reduces oxidative stress.	[96-102]
Cysteine	0.81 mmol/kg/day of cysteine (given as n-acetylcysteine)	Increases glutathione. Plasma oxidative stress and lipid peroxides decreases. Improves endothelial function and reduces oxidative stress (when together with arginine).	[95], [98], [103]
Glutamine	15-30 g acute effect (single dose administration)	Increases circulating GLP-1, glucagon and insulin. Reduces postprandial glycaemia and augments GLP-1.	[104, [105]
Branched chain amino acids (BCAA; leucine, valine and isoleucine)	5-7.5 g/day	Higher dietary branched chain amino acid intake is associated with lower prevalence of overweight/obesity. No changes in body composition, muscle mass, glycaemic control and/or lipidaemia (in patients consuming adequate dietary protein).	[106-109]

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