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Amino acids and diabetes: implications for endocrine, metabolic and immune function

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1. ABSTRACT

Aberrant alterations in glucose and lipid concentrations and their pathways of metabolism are a hallmark of diabetes. However, much less is known about alterations in concentrations of amino acids and their pathways of metabolism in diabetes. In this review we have attempted to highlight, integrate and discuss common alterations in amino acid metabolism in a wide variety of cells and tissues and relate these changes to alterations in endocrine, physiologic and immune function in diabetes.

2. STRUCTURE AND HORMONES OF THE PANCREATIC ISLETS

The regulation of energy metabolism is a fundamental homeostatic mechanism. The main hormones that control glycaemic homeostasis are insulin and glucagon. Both these hormones are synthesized and secreted in the endocrine tissue of the pancreas – the islets of Langerhans. Basically, four cell types constitute the islets, in which beta- and alpha-cells are the major contributors to secretion of the antagonistic hormones that

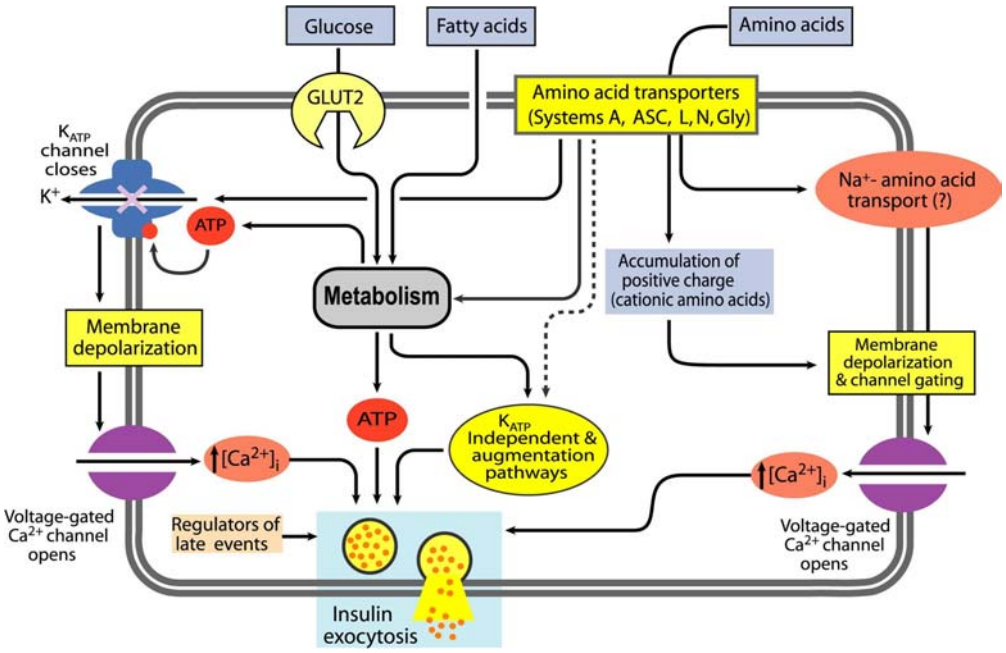

Figure 1. Common mechanisms of nutrient and amino acid stimulated insulin secretion. Glucose metabolism is essential for stimulation of insulin secretion. The mechanisms by which amino acids enhance insulin secretion are understood to primarily rely on (i) direct depolarization of the plasma membrane (e.g. cationic amino acid, L-arginine); (ii) metabolism (e.g. alanine, glutamine, leucine); and (iii) co-transport with Na^+ and cell membrane depolarization (e.g. alanine). Notably, rapid partial oxidation may also initially increase both the cellular content of ATP (impacting on K_{ATP} channel closure prompting membrane depolarization) and other stimulus secretion coupling factors. In the absence of glucose, fatty acids may be metabolised to generate ATP and maintain basal levels of insulin secretion. Adapted with permission from (35).

regulate blood glucose levels. Beta-cells are the most abundant cells in islets and are responsible for the synthesis and secretion of insulin in response to increased extracellular glucose concentrations. Insulin is the only hormone with hypoglycaemic action. Alpha cells secrete glucagon, a hormone whose actions in the liver culminate with release of glucose into the blood and an increase in plasma glucose concentration. The other cell types found in islets are delta-cells, which secrete somatostatin (which has an inhibitory paracrine effect on insulin and glucagon secretion) and PP-cells, which secrete pancreatic polypeptide, a hormone whose physiological significance is still unresolved.

3. GLUCOSE STIMULATED INSULIN SECRETION FROM THE BETA CELL

The pancreatic beta cell will transport and metabolise glucose in proportion to the extracellular concentration. The combination of glucose transport and phosphorylation at the glucokinase step determines a major part of metabolic flux through glycolysis in the beta cell. Pyruvate may be further metabolised by pyruvate dehydrogenase and pyruvate carboxylase, resulting in enhanced tricarboxylic acid cycle activity and subsequent ATP generation (1). This results in closure of the ATP-sensitive K^+ channels, decreasing the hyperpolarising outward K^+ flux, depolarizing the plasma membrane and opening voltage-gated Ca^{2+} channels to increase the influx of extracellular Ca^{2+}, which is a primary driver of insulin exocytosis. Glucose metabolism also results in formation of other metabolic stimulus–secretion coupling factors such as NADPH, some amino acids and lipid derived metabolites (2–8). Importantly, amino acids may potentiate the generation of metabolic stimulus–secretion coupling factors via a number of distinct mechanisms that are ultimately related to changes in intracellular Ca^{2+} concentration (1) (Figure 1). The glucose-dependent oscillations in intracellular Ca^{2+} concentration can stimulate mitochondrial generation of reactive oxygen species (ROS), whereas Ca^{2+}, via protein kinase C activation, may enhance NADPH oxidase (NOX)-dependent generation of ROS (9–11). The subsequent formation of sustained levels of H_2O_2, unless rapidly removed, can suppress beta cell metabolic activity resulting in inhibition of insulin secretion (1). In addition, specific amino acids including glutamine, cysteine and glycine may contribute to antioxidant defense (see below).

4. MECHANISMS UNDERLYING BETA CELL ACTIONS OF AMINO ACIDS

Under appropriate conditions, amino acids enhance glucose-stimulated insulin secretion (GSIS) from primary islet beta cells and beta cell lines (12 - 19). *In vivo*, L-glutamine and L-alanine are quantitatively the most abundant amino acids in blood and extracellular fluids, closely followed by the branched chain amino acids (20). However, individual amino acids do not evoke insulin-secretory responses *in vitro* when added at physiologic

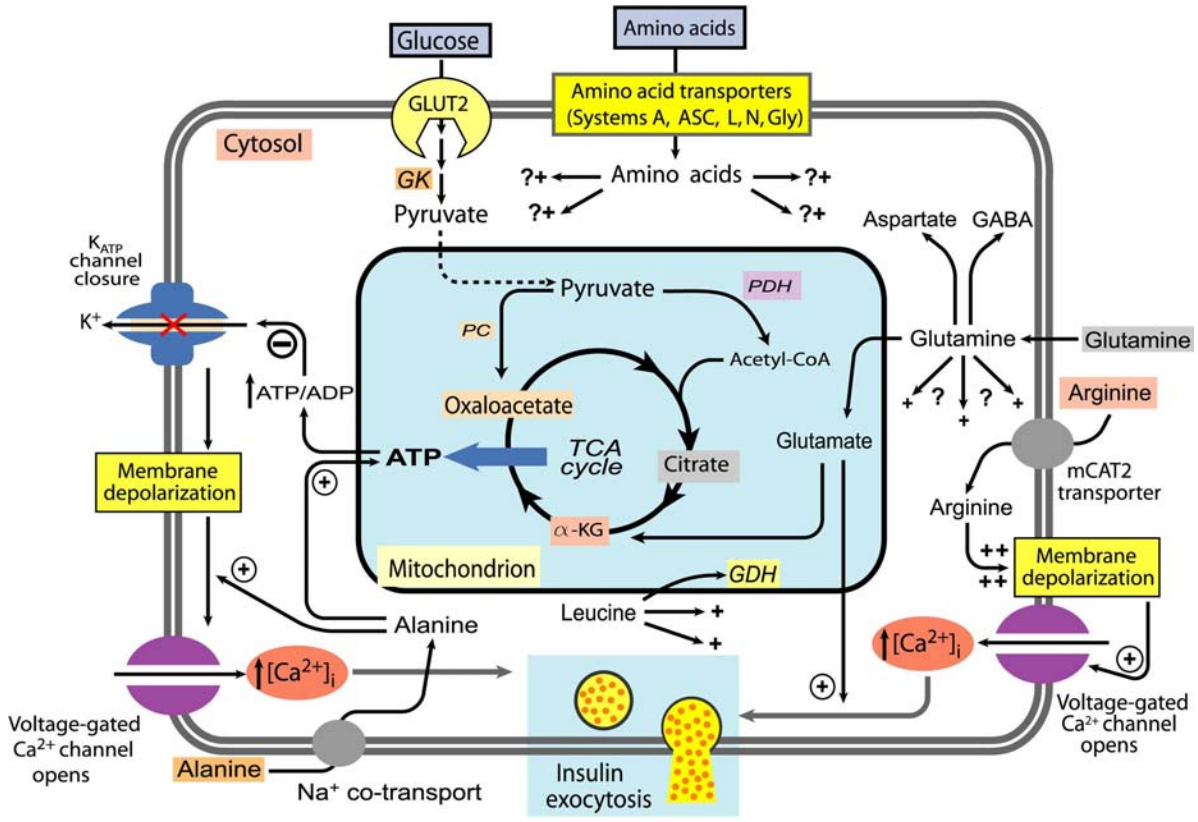

Figure 2. Glucose, alanine, glutamine, leucine and arginine are the major nutrient drivers of insulin secretion. Metabolism of glucose, alanine and glutamine result in enhanced TCA cycle activity and generation of metabolic secretion coupling factors including ATP, Ca^{2+} and glutamate. Leucine may enhance glutamine oxidation via activation of glutamate dehydrogenase (GDH). Arginine may depolarise the plasma membrane by net import of positive charge thus causing opening of voltage gated Ca^{2+} channels. The key sites of metabolic control in the beta cell are indicated; glucokinase (GK), pyruvate dehydrogenase (PDH), pyruvate carboxylase (PC), glutamate dehydrogenase (GDH). Adapted with permission from (35).

concentrations, rather, combinations of physiologic concentrations of amino acids or high concentrations of individual amino acids are much more effective. *In vivo*, amino acids derived from dietary proteins and those released from intestinal epithelial cells, in combination with glucose, stimulate insulin secretion, thereby leading to protein synthesis and amino acid transport in target tissues such as skeletal muscle (1).

While amino acids can potentially affect a number of aspects of beta cell function, a relatively small number of amino acids promote or synergistically enhance insulin release from pancreatic beta cells (21, 22). As illustrated in Figure 2, the mechanisms by which amino acids enhance insulin secretion are understood to primarily rely on: (i) direct depolarization of the plasma membrane (e.g., cationic amino acid, L-arginine); (ii) metabolism (e.g., L-glutamine, L-leucine); and (iii) co-transport with Na+ and cell membrane depolarization (e.g., L-alanine). Notably, partial oxidation, e.g., L-alanine (7) may also initially increase the cellular content of ATP impacting on K^{+}_{ATP} channel closure prompting membrane depolarization, Ca^{2+} influx and insulin exocytosis. Additional mitochondrial signals that affect insulin secretion may also be generated (Figure 2) (23-25).

In beta cells, specific amino acids have been shown to dose-dependently stimulate the mTOR-signalling pathway in the presence of glucose. The mTOR pathway enhances cell growth and survival (26). However, at present, the mechanism by which amino acids activate the mTOR complex in beta cells has not been fully elucidated, but it is interesting to note that there is synergy between beta-cell activation-derived cAMP and Ca^{2+} with amino acid stimulation of mTOR (26-28). In this sense, mTOR signaling could potentially be a novel mechanism for beta cell protection in the context of diabetes. In a recently published study, the plasma levels of both glutamine and arginine were significantly reduced (by approximately 20%) in patients with type-2 diabetes compared to controls (29). The reduction in plasma glutamine and arginine may have important consequences to beta-cell survival, perhaps in part mediated by a reduction in mTOR activity.

4.1. Alanine

Effects of L-alanine have been studied in BRIN-BD11 cells and primary rat islet cells, which consume high amounts of this amino acid (9). Moreover, L-alanine is known to potentiate GSIS by enhancing glucose utilization and metabolism (7), and numerous studies have highlighted L-alanine as a potent initiator of insulin release. The

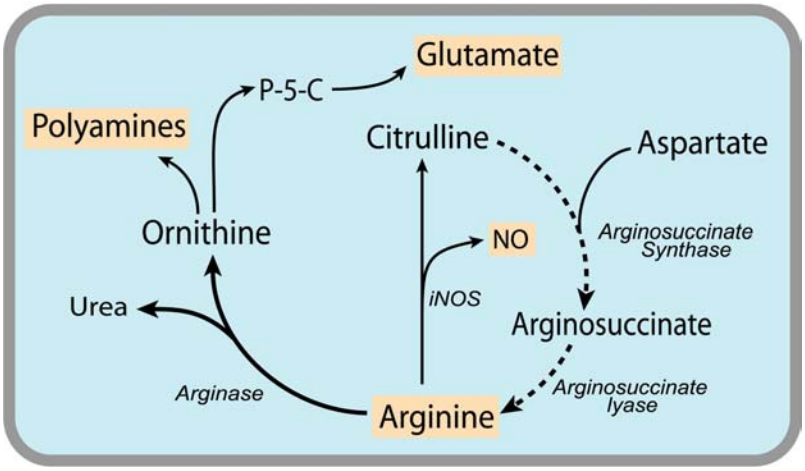

Figure 3. Arginine synthesis and degradation in the beta cell.Arginine may be synthesized from citrulline by the enzymes arginosuccinate synthetase and arginosuccinase. Arginine may be utilized as a substrate by arginase, so providing ornithine for polyamine or glutamate synthesis or arginine may be converted to NO and citrulline by the action of indicible nitric oxide synthase (iNOS) (1). The metabolites highlighted are key substrates and products.

authors have utilized BRIN-BD11 cells to study the actions of L-alanine on beta cells demonstrating an influence on GSIS by electrogenic Na^+ transport, and exploited ^{13}C nuclear magnetic resonance technologies to trace L-alanine metabolism, demonstrating generation of glutamate, aspartate and lactate. Additionally, studies using the respiratory poison oligomycin indicated the importance of metabolism and oxidation of alanine for its ability to stimulate insulin secretion (7).

4.2. Arginine

This amino acid stimulates insulin release through electrogenic transport into the beta cell via the mCAT2A amino acid transporter, thereby increasing membrane depolarization, resulting in a rise in intracellular Ca^{2+} through opening of voltage-gated Ca^{2+} channels and promotion of insulin secretion (30). However, in some situations, arginine principally through its metabolism is understood to exert a negative effect on beta 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 beta cell mitochondrial function and generation of key stimulus–secretion coupling factors, which could lead to a reduction in cellular insulin output (31).

Arginine metabolism is critical to the function of several diverse cell types such as pancreatic beta cells, vascular endothelial cells and immune cells. The fact that arginine may be cleaved by arginase to produce urea and ornithine is normally considered to be important only in the liver. However, if ornithine is not utilized to form citrulline it may be converted to glutamate or into polyamine synthesis such as putriscine (via ornithine decarboxylase) and thus enter pathways associated with redox regulation and cell proliferation (Figure 3)

4.3. Cysteine

This is a sulfur-containing amino acid that importantly acts as a donor of thiol components. Cysteine is also a substrate for glutathione synthesis and could be considered a rate-limiting precursor for glutathione, an important cellular reducing agent. Acutely, the administration to rat pancreatic islets of L-cysteine and its permeable analogue, N-acetyl-L-Cysteine (NAC), has previously been reported to enhance insulin secretion in the presence of high glucose levels (32). The same effect was also observed by the incubation of mouse islets in the presence of the cysteine donor (L-2-Oxothiazolidine-4-carboxylic Acid), which increased insulin release at basal and high glucose concentrations. Such positive effect was paralleled by the increase in intracellular calcium influx in isolated islet cells (33). Despite these positive reports of the acute effects of cysteine on insulin secretion, higher cysteine concentrations were reported to impair GSIS. This negative effect was attributed to excessive hydrogen sulfide (H_2S) generation, a consequence of cysteine metabolism (34).

Oxidative damage is believed to participate in the process of pancreatic β-cell failure during diabetes progression (35), which may be associated with the low levels of some antioxidant enzymes (36). Cysteine treatment mediated protection of insulin-producing cells from damage induced by hydrogen peroxide (37, 38) and by a lipid peroxidation product, 4-hydroxy-2-nonenal (4-HNE) (38). The cysteine protection correlated with an increase in the content of glutathione in insulin-producing cells (37, 38, 39) and was accompanied by the suppression of translocation of the transcription factor pancreatic and duodenal homobox 1 (PDX-1) to the nucleus (required for insulin transcription) induced by 4-HNE (38). Interestingly, beta cell dysfunction associated with exposure to high glucose, was attenuated by H_2S production from cysteine which protected cells

from glucotoxicity, preserving the secretory response of mouse β-cells (39).

4.4. Glutamine

Among the amino acids, glutamine is considered one of the most important, playing an essential role in promotion and maintenance of functionality of various organs and cells, including pancreatic beta cells (40). Both rat islets and BRIN-BD11 beta-cells consume glutamine at high rates (9), but notably while glutamine can potentiate glucose-stimulated insulin secretion (GSIS) and interact with other nutrient secretagogues, it does not initiate an insulin-secretory response (22). In rat islets, glutamine is converted to γ-amino butyric acid (GABA) and aspartate (Figure 2), and in the presence of leucine oxidative metabolism is increased (due to allosteric activation of glutamate dehydrogenase) leading to enhanced rates of TCA cycle activity, generation of ATP and other stimulus-secretion coupling factors. More recently, a potential glutamine synthetase inhibitor – methionine sulfoximide – was demonstrated to completely abolish GSIS in normal mouse islets (41), a phenomenon reversed by addition of glutamine or a non-metabolizable analogue. However, it is important to note that this inhibitor may block a number of glutamate-utilizing enzymes and so the outcome cannot be attributed to a specific action on glutamine synthetase.

Indeed dietary supplementation with glutamine can offer protection against cell stress and damage. Isolated rat islets cultured with glutamine for 24 hours increased their HSP70 and Bcl-2 expression and GSH content, which correlated with the attenuation of IL-1β-inducing injury (which is partially dependent on NO production, (42)).

4.5. Glutamate

The ability of glutamate to stimulate insulin secretion from beta cells has been hotly debated. Intracellular generation of L-glutamate has been proposed to participate in nutrient-induced stimulus–secretion coupling as an additive factor in the amplifying pathway of GSIS (43). During glucose stimulation, total cellular glutamate levels have been demonstrated to increase in human, mouse and rat islets, as well as clonal beta cells (7, 9, 43, 44), whereas other studies have reported no change (45, 46). The observation that mitochondrial activation in permeabilized beta cells directly stimulates insulin exocytosis (32) pioneered the identification of glutamate as a putative intracellular messenger (43, 47). However, in recent years, the role of L-glutamate in direct actions on insulin secretion has been challenged (46, 48). For example, stimulatory (16.7 mM) glucose did not increase intracellular L-glutamate concentrations in rat islets in one study (46), and while L-glutamine (10 mM) increased the L-glutamate concentration ten-fold, this was not accompanied by a stimulation of insulin release. In a separate study, incubation with glucose resulted in a significant increase in L-glutamate concentration in depolarized mouse and rat islets, but L-glutamine while increasing L-glutamate content did not alter insulin secretion (48). Additionally, in this latter study, BCH-induced activation of GDH lowered L-glutamate levels, but increased insulin secretion. However, it is probable that experimental conditions in which L-glutamine is used as L-glutamate precursor may lead to saturating concentrations of L-glutamate without necessarily activating the K^+_{ATP}-dependent pathway and associated increase in insulin secretion (44). It is likely that during enhanced glucose metabolism, the concentration of the key TCA cycle intermediate alpha-ketoglutarate (2-oxoglutarate) is elevated and a proportion of this metabolite is subsequently transaminated to glutamate (49). It is the opinion of the authors that the glutamate so formed may indirectly stimulate insulin secretion through additive actions on the malate–aspartate shuttle. This follows from the fact that glutamate is a substrate for the mitochondrial membrane aspartate/glutamate carrier 1, and thus may increase the capacity of the shuttle (49). Also glutamate is one of the three amino acids required for glutathione synthesis and could contribute to the formation of glutathione and subsequent positive effects on cellular redox state and mitochondrial function (for further detail see (49)). Glutamate release from beta cells has recently been reported (50), adding complexity to this story and offering the intriguing possibility of other beta cell actions, perhaps mediated through glutamate receptors which could influence insulin release. However, considering that the expression of vesicular glutamate transporter type 1 and 2 (VGLUT1 and VGLUT2) was not observed in rat beta-cells (51), and VGLUT1 was also not expressed in human islets (52), then glutamate release by beta cells could be mediated by membrane glutamate transporters. This and other aspects of beta cell glutamate signaling and actions are currently under investigation by the authors.

Indeed, it has been reported that glutamate can act on beta cells as an extracellular signaling molecule. This amino acid can act at the cell surface by ionotropic (iGluR) and metabotropic (mGluR) receptors. The expression and possible functional roles of iGluR in islet cells was reported by Inagaki *et al.* 1995 (53). Those authors found mRNA expression of AMPA/kainate and NMDA iGluRs that correlated with activation of beta-cells in electrophysiological and intracellular calcium recordings. However, only AMPA/kainate activation was positively correlated with increase in insulin secretion. In contrast, in another study only NMDA activation was able to increase insulin secretion from rat islets (54). On the other hand in human, monkey and mouse islets, glutamate, AMPA and kainate failed to elicit insulin secretory responses at low glucose concentrations. Similar results were also observed *in vivo* in mice (52). This thus raises the possibility that glutamate *via* iGluRs does not trigger insulin secretion, but rather could enhance glucose-stimulated insulin secretion via the amplifying pathway.

Additionally, another class of glutamate receptors, mGluRs, was shown to be expressed in human islets (mGluR3 and 5), rat islets (mGluR3, 5, 4 and 8) as well as in insulin-secreting cell lines (mGluR2, 3, 5 and 8) (55) .The mGluR3 and 5 were widely distributed in these tissues, being constituents of the mGluR group II receptors which are coupled to adenylate cyclase activation and group I receptors which are coupled to phospolipase C activation, respectively. These receptors are coupled to

stimulatory transduction mechanisms, suggesting that glutamate could indeed enhance glucose-stimulated insulin release through modulation of intracellular signaling pathways, as suggested from the observation that agonists for classes I, II and III mGluR increased insulin secretion at low glucose concentration, from beta cell lines (55).

The specific source of glutamate production in pancreatic islets is also an unresolved issue. Although glutamate production by beta cells has been previously reported (see above), some authors argue that glutamate production also occurs in alpha cells, as there was intense immunoreactivity for glutaminase reported in alpha cells (53) in addition to the observation of vesicular glutamate transporters 1 and 2 (VGLUT1, 2) in alpha cells, contrary to the absence of VGLUTs in beta cells (56). Thus the identification of VGLUTs in alpha cells suggests that glutamate may be stored and co-secreted with glucagon in these cells.

Glutamate production and secretion by alpha cells could allow a paracrine action from alpha to beta cells via mGluRs, considering that glutamate has been shown to be proportionally co-secreted with glucagon (51, 52). Indeed the stimulatory effect of alpha-cell secretory products on insulin secretion, usually attributed to glucagon, may be also partially due to alpha cell glutamate secretion and activation of mGluRs in beta-cells.

Another important function of glutamate in beta-cells is as a precursor of gamma-amino-butyric acid (GABA). GABA was believed to accumulate inside synaptic-like microvesicles (57). However, more recent evidence showed that GABA is also co-localised with dense-core granules and co-released with insulin (58). As discussed below, GABA is a potent beta-cell derived inhibitor of glucagon secretion via activation of the $GABA_A$ receptor chloride channel. Interestingly, Brice *et al.* 2002 (55) demonstrated the expression of metabotropic $GABA_BR1$ a/b and $GABA_BR2$ receptors in human islets and MIN6 cells and expression was correlated with suppression of insulin secretion induced by high glucose in MIN6 cells. Metabotropic $GABA_B$ receptors are coupled to G_i proteins, what could lead to inhibition of insulin secretion via cAMP reduction and activation of K_{ATP} channels, with consequent inhibition of voltage-dependent calcium channels (55). However, electrophysiologic experiments showed that $GABA_B$ activation did not have a pronounced effect on calcium currents, but rather directly inhibited the exocytotic machinery of rat beta-cells via activation of the serine/threonine phosphatase calcineurin (59). Thus, GABA in addition to its role as a paracrine modulator of alpha-cells can be an autocrine modulator of beta-cell function.

4.6. Homocysteine

Homocysteine is a sulfhydryl-containing amino acid formed during the metabolism of methionine that can be taken up by cells mainly via cysteine transporters (60). 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 (60). Moreover, it could also cause oxidative stress damage. Insulin resistance has been associated with elevated plasma homocysteine in healthy non-obese subjects (61) and hyperhomocysteinemia has also been described in obese hyperinsulinemic subjects (62) and in diabetic patients (63-65). Positive correlation between fasting insulin and homocysteine plasma levels was observed. It is possible that the observed increase in homocysteine was associated with insulin resistance (62). *In vitro* the presence of homocysteine in the incubation medium was reported to impair insulin secretion from mouse islets (66) and from insulin secreting cells (66-68). This inhibition 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 (67, 68). Although homocysteine increases hydrogen peroxide levels and could cause oxidative stress damage, probably due to its auto-oxidation, its inhibitory effect on insulin secretion was not related to hydrogen peroxide production (69). Moreover, overnight exposure of insulin secreting cells to homocysteine also impaired the insulin secretory responsiveness to moderate and high glucose concentration. Interestingly, after an overnight recovery from homocysteine exposure the detrimental effects of homocysteine on the actions of insulinotropic stimuli were completely reversed. This clearly shows that this phenomenon is not due to cellular dysfunction, decreased viability or cell death (70). Thus, hyperhomocysteinemia during insulin resistance may contribute to impairment of pancreatic β-cell function.

4.7. Leucine

Prolonged exposure of rat islets to leucine increases ATP, cytosolic Ca^{2+}, and potentiates glucose-stimulated insulin secretion. In addition, chronic exposure to leucine leads to an increase in both ATP synthase and glucokinase, which can sensitize pancreatic beta cells to glucose-induced insulin secretion (71). Leucine-induced insulin secretion involves allosteric activation of glutamate dehydrogenase (GDH) leading to an increase in glutamine → glutamate → alpha-ketoglutarate flux, elevated mitochondrial metabolism and an increase in ATP production leading to a membrane depolarization (Fig 2). Interestingly, in rats fed a low protein diet, GDH protein expression was reduced. However its expression could be rescued by leucine supplementation, suggesting that this amino acid also modulates GDH at the protein level (72). Additionally transamination of leucine to α-ketoisocaproate (KIC) and entry into TCA cycle via acetyl-CoA can contribute to ATP generation by increasing the oxidation rate of the amino acid and thus stimulation of insulin secretion. Moreover, it has been reported that α-keto acids (including KIC) can directly block K_{ATP} channel activity and exert additional K_{ATP} channel-independent effects thereby inducing insulin secretion (73, 74). Notably, a recent study reported patients with mutations in the regulatory (GTP binding) site of GDH had increased beta cell responsiveness to leucine, presenting with hypoglycaemia after a protein rich meal (73, 75). In addition, mice harbouring a beta cell-specific GDH deletion exhibit a marked decrease (37%) in glucose-induced insulin

secretion, supporting an essential role of GDH in insulin release (76).

4.8. Taurine

Taurine is an example of a beta amino acid, where the NH_2 group is not attached to the carbon adjacent to the carboxyl carbon of the carboxylic acid group but to the next in sequence carbon atom (the beta carbon atom). One of the most important functions of taurine is osmoregulation of the cell and thus control of cell volume. Remarkably in intact pancreatic islets taurine is associated with pancreatic alpha and delta cells but not beta cells (77). However, beta cell lines may accumulate taurine in conditions of cell culture but the physiological relevance is not clear. Dietary taurine supplementation for 30 days in mice increased GSIS and PDX 1 localization to the nucleus of islet cells (and thus positively regulated insulin gene expression). The studies also reported an increase in glucose tolerance and insulin sensitivity in peripheral tissues (78). In addition, taurine supplementation increased leucine-induced insulin secretion by a mechanism that is not dependent on allosteric GDH activation (79). Perhaps taurine and possibly leucine and glutamine can be considered for future use in type 2 diabetes therapy, as part of a dietary multi-supplement strategy.

5. MECHANISMS UNDERLYING ALPHA CELL ACTIONS OF AMINO ACIDS

A robust inhibitory effect of glucose on glucagon secretion is part of the primary endocrine system regulation of glycaemia, though it is still a matter of debate whether the intrinsic sensing of glucose by the alpha cells (80, 81) or paracrine signals from beta (82 - 85) and delta cells (86) are the main contributors to this glucose effect. As ATP production in alpha-cells is poorly enhanced by glucose (87), metabolism-independent mechanisms may also regulate glucagon secretion in alpha-cells. Amino acids are potent stimulators of glucagon secretion, as they are also stimuli for insulin secretion. Though insulin and glucagon may have antagonic effects on blood glucose levels, this common effect of amino acids on the secretion of both hormones may have an adaptive value. Amino acid-induced glucagon release could actually constitute an anticipatory (feedforward) response to prevent hypoglycaemia potentially caused by amino acid-stimulated insulin secretion after protein-rich meals (88).

Arginine is considered to be the most potent amino acid for glucagon release, while alanine and glycine are less potent and valine, leucine and isoleucine are not effective (88). Interestingly, infusion of arginine acutely enhances glucagon secretion in both control and diabetic individuals, but diabetic patients show an increased secretory response during chronic stimulation (89). Arginine greatly enhances electrical activity in alpha cells (90). This effect of arginine is attributed to the fact that, being a positively charged aminoacid, its uptake by the alpha-cell causes membrane depolarization and thus elicits glucagon secretion via opening of voltage-dependent calcium channels and calcium-induced granule exocytosis. However, sustained alpha-cell membrane depolarisation has been shown to induce calcium channel inactivation (91). Indeed, although arginine increases the action potential firing frequency in isolated mouse alpha-cells, the amplitude of the action potential is reduced (92), suggesting reduction of some of the currents involved. Ca^{2+} mobilization in each action potential was however, able to sustain enhanced glucagon secretion. Thus, the rate of uptake of arginine by the alpha cells appeared insufficient to result in chronic depolarization of the membrane that could decrease glucagon release due to Ca^{2+} channel inactivation. One should also bear in mind that arginine can be a source of NO radicals that in turn activate guanylate cyclase. However, the effect of NO and cGMP on alpha-cell function is controversial. NO donors and the membrane-permeable cGMP analogue 8-Br-cGMP were shown to inhibit Ca^{2+} influx via L-type channels from rat alpha-cells (93). On the other hand, experiments performed in the mouse clonal cell line alphaTC6 showed that both NO and cGMP stimulate glucagon secretion leading to the suggestion that part of the effect of arginine on glucagon secretion could be attributable to its role as NO precursor, as the stimulatory effect of arginine was significantly reduced in the presence of the NO-synthase inhibitor L-NAME (94). Clearly, further investigation must be pursued in order to understand nitric oxide action in the alpha cell.

A neurotransmitter and amino acid – GABA (γ-aminobutyric acid) – is a potent inhibitor of glucagon secretion. The main extraneuronal site of GABA synthesis in the body is the pancreatic beta-cell, where it is produced from glutamate by glutamate decarboxylase, mainly the isoform GAD65 (57). Most of the beta cell associated GABA is packed in small vesicles that are distinct from the large dense core insulin granules (57). However, it was recently shown that 15% of the insulin granules are associated with GABA immunoreactivity and that GABA release coincides with exocytosis of insulin granules in rat beta-cells (58). Also, both GABA content and release are increased when rat beta-cells are incubated with increasing concentrations of glutamine (95). Alpha-cells are the only cell type in the islet that express ionotropic $GABA_A$ receptor channels (83). However, there is no evidence of metabotropic $GABA_BR1$ expression or function in alpha-cells (60). Upon GABA binding, $GABA_A$ receptors open, increasing chloride permeability, which has an inhibitory effect on electrical activity and Ca^{2+} entry. It has been shown that GABA released from beta-cells can activate $GABA_A$ receptors from neighbouring alpha-cells (83). In this sense, GABA can be recognized as a potent signal from beta-cells to inhibit glucagon release. Interestingly, the circulating levels of GABA are reduced in diabetic patients (29). Moreover, chronically elevated glucose levels reduce GABA release from rat beta-cells (95). Though this may not directly correlate with intra-islet GABA levels, it is tempting to speculate that, reduced GABA release in diabetic patients would favour increased glucagon secretion in the presence of hyperglyaemia, which has previously been reported (96). Though glutamate may be an indirect source of alpha-cell inhibition due to its role as a precursor of GABA in beta-cells, glutamate released from the alpha-cells apparently enhances glucagon secretion in humans (52). Intracellular glutamate is probably produced from

glutamine, which is transported across the plasma membrane by the SAT2 transporter, differently from beta-cells that express the glutamine transporter SN1 (97). Cytosolic glutamate is transported into secretory granules in alpha-cells via vesicular glutamate transporter 2 and co-secreted with glucagon (51). In perfused rat pancreas, glutamate transiently enhanced glucagon secretion via ionotropic AMPA/kainate receptors in rat alpha-cells (98). However, this evidence was later challenged (99), as glutamate was demonstrated to act on metabotropic glutamate type 4 (mGluR4) receptors in rat alpha-cells. Glucagon secretion would therefore be decreased via Gi-mediated inhibition of cAMP synthesis by adenylate cyclase. At low glucose, human islets are stimulated to secrete glucagon via ionotropic AMPA/kainate glutamate receptors and glutamate was co-secreted with glucagon by alpha-cells (52). Thus glutamate release from alpha-cells may constitute a positive feedback loop to stimulate glucagon secretion via AMPA/kainate receptor-induced membrane depolarization and consequent calcium influx (52, 100). In the presence of beta-cell inhibitory signals, such as GABA, this positive feedback would be attenuated. However, in conditions when beta-cell signals are chronically reduced then desensitization of ionotropic glutamate receptors in alpha-cells by a steady binding of glutamate could occur thus explaining poor glucagon secretion in response to hypoglycaemia, that can be observed in diabetics (52, 100). In this sense, as previously discussed above, the observation of impaired GABA release from beta-cells at sustained high glucose levels (95) and possibly in type 2 diabetes (29) could result in the hyperglucagonemia observed in conditions of hyperglycemia and reduced glucagon secretion in response to hypoglycemia that are associated with diabetes.

6. AMINO ACIDS AND MUSCLE FUNCTION IN DIABETES

Skeletal muscle hypotrophy is common in patients with long term diabetes mellitus and the association between insulin deficiency and/or peripheral insulin resistance with muscle protein wasting has been reported (101, 102). Indeed, evidence for reduced muscle protein synthesis but increased muscle protein breakdown and amino acid oxidation in the diabetic state has been published (103). The plasma levels of branched-chain amino acid (BCAA) and urea production are increased markedly with increased efflux of amino acids from muscle to splanchnic tissues (104, 105). However, the plasma concentration of some amino acids is decreased in both the insulin resistant and diabetic condition. There was a significant reduction in the circulating concentrations of gamma-aminobutyric acid (GABA), arginine, glutamine, and phosphoethanolamine and increase in concentration of valine in patients with type 2 diabetes compared with controls (29). In patients with impaired glucose tolerance, there was a significant decrease in the plasma concentrations of GABA and increased concentration of tyrosine (29).

Skeletal muscle from both type 1 and type 2 diabetes is associated with deficiency of some amino acids but an increased content of other amino acids. The diaphragm muscle from streptozotocin-diabetic rats (an experimental model of type 1 diabetes) oxidizes two to threefold more BCAA than controls when it is incubated in the presence of pyruvate (106). In these animals, there was a decrease in the skeletal muscle content of histidine and increased content of proline, valine, isoleucine, and leucine (107). Diaphragm muscle of streptozotocin-diabetic rats contained significantly more content of taurine, glutamate, and BCAA and less content of glutamine, serine, asparagine, lysine, arginine, histidine, threonine, citrulline, and carnosine (beta-alanyl-L-histidine) when compared to controls (108). Glutamine and alanine are released from skeletal muscle in significantly increased quantities in streptozotocin-diabetic rats (107). The Zucker diabetic fatty (ZDF) rats are characterized by expression of defective leptin receptors and are used as experimental model for the study of type 2 diabetes (109). Amino acid metabolism in the ZDF rat during the pre-diabetic insulin-resistant stage (5 weeks) and the frank type 2 diabetic stage (11 weeks) was investigated by Wijekoon and co-workers (2004). In these animals, there is a decrease of content of threonine, serine, asparagine, glutamine, glycine, citrulline, lysine, histidine, anserine, carnosine and arginine and an increase of content of taurine, aspartic acid, proline, alanine, isoleucine and leucine in skeletal muscle after 5 weeks, corresponding to the hyperinsulinemic phase (110). At 11 weeks, there was decreased content of threonine, serine, asparagine, glutamine, glycine, tyrosine, lysine and arginine and an increased content of alanine, valine and leucine (110). From the fifth to the eleventh week, there was a decrease in plasma insulin level and increase of glycemia (by 3.6-fold) associated with an increase in content of valine and decrease in content of tyrosine in skeletal muscle (110). The analyzed sample consisted predominately of slow-twitch muscle fibers (obtained from soleus muscle).

The reduction in the concentration of various amino acids in patients with diabetes may argue in favor of selective amino acid supplementation, but there is little direct evidence of beneficial effects of amino acid supplementation on skeletal muscle function in the diabetic or glucose-intolerant state. However, other effects of amino acid supplementation, such as increased insulin secretion, improved insulin sensitivity, endothelium-dependent vasodilation, metabolic efficiency and redox control may indirectly improve skeletal muscle function (111, 112), further described below.

Glutamine is the most abundant amino acid in the organism and in skeletal muscle tissue. In the diabetic state the content of glutamine in both plasma and skeletal muscle is reduced (110, 29). Glutamine supplementation improved insulin signalling in skeletal muscle (113). L-alanyl-L-glutamine dipeptide was effective in increasing the concentrations of glutamine and glutamate in skeletal muscle tissue (114). Muscle protein synthesis increased when glutamine was included in the diet as a free amino acid (115). The alanyl-glutamine dipeptide prevented skeletal muscle wasting in rats treated with glucocorticoid (116). Reactive oxygen species (ROS) may be important to

signaling pathways that lead to muscle dysfunction in diabetes. The overexpression of catalase abolished the transactivation of both transcription factors nuclear factor kappaB (NF-kappaB) and forkhead box O (Foxo), thereby attenuating the loss of muscle mass in an experimental model of skeletal muscle hypotrophy (117). Supplementation with the L-alanyl-L-glutamine dipeptide increased the content of glutathione (GSH) improving redox control in the rat soleus muscle (114). Dietary supplementation with L-arginine decreased NF-kappaB activity in skeletal muscle (118). The supplementation with arginine and ornithine also increased glutamine content in skeletal muscle and may be protective for muscle mass and the preservation of muscle function in diabetes (119).

As described above, skeletal muscle in diabetes has a low content of arginine (110). L-arginine supplementation had a stimulatory effect on muscle mass gain, increasing mTOR signaling pathway activity and enhancing skeletal muscle protein synthesis and whole-body growth (120, 121). In obese rats, L-arginine supplementation improved insulin sensitivity and increased muscle mass independently of changes in serum concentrations of insulin (122). L-arginine will stimulate insulin secretion *in vivo* in humans (89). Arginine supplementation increased NO production and attenuated endothelial dysfunction in diabetic state (119, 120) (123, 124). The increased NO availability induced by the long-term supplementation with L-arginine also has an important role in the increase of peripheral insulin sensitivity in type 2 diabetes (125, 126). NO is an important regulator of blood flow and vascular conductance in skeletal muscle during exercise (127). NO also contributes to the augmented vasodilatation observed during hypoxic exercise (128), stimulating an increase in glucose uptake in skeletal muscle fibers during contraction (129). NO improves mechanical and metabolic muscle power through a cGMP-mediated effect. However, the effect of arginine supplementation on protein balance in skeletal muscle occurs by a mechanism which is independent of NO production (130). Dietary supplementation with essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) plus arginine for 16 weeks improved muscle strength in glucose intolerant individuals (131).

Arginase, which produces urea and ornithine from arginine, may have significant importance unconnected with the urea cycle. Supplementation with L-ornithine, a precursor of the free radical scavenger spermine, could ameliorate mitochondrial respiratory chain activity and oxidative stress in skeletal muscle (132). The regulation of ornithine decarboxylase in many tissues is severly impaired in diabetes and starvation. Polyamine formation *in vivo* is an integral component of the growth-promoting effect of insulin or of other insulin-dependent factors in skeletal muscle (133, 134). Polyamines are essential for cell proliferation and their role in stimulation of RNA, DNA, and protein synthesis is clearly established. However, in diabetes the activity of ornithine decarboxylase is reduced (133).

Leucine is a potent stimulator of protein synthesis in skeletal muscle but this process may be impaired in diabetes. Leucine both promotes protein synthesis and inhibits protein degradation (135, 136). Leucine supplementation improves muscle protein synthesis independently of other amino acids (137). Leucine stimulates protein synthesis in skeletal muscle by enhancing eukaryotic initiation factor 4F (eIF4F) activation independently of insulin (138). Defective leucine stimulation of protein synthesis is observed in aged skeletal muscle (139). Curiously, there is an increase in content of leucine in skeletal muscle in experimental models of type 1 and type 2 diabetes but protein synthesis is decreased. This may be due to insulin deficiency and/or muscle insulin resistance. Insulin appears be important for promotion of leucine dependent modulation of synthesis of muscle proteins. Leucine stimulates the activation of mTOR synergistically with insulin and both signaling pathways converge at the level of activation of phosphoinositol 3-kinase (PI3K) (140). The addition of insulin to the medium in a perfused hemicorpus preparation caused a rapid reversal of a diabetes induced block in initiation in mixed fast-twitch muscles but had no effect in the slow-twitch muscle in rats (141). According to Flaim *et al.*, (1980) long-term diabetes resulted in the development of an additional impairment in protein synthesis that also affected the soleus and is not restored by insulin. Fatty acids (FAs) are also increased in diabetes and may play a role in muscle wasting. FAs decrease the stimulatory effect of leucine in protein synthesis (142). FAs can also induce oxidative stress in skeletal muscle. Recently, Marzani and co-workers demonstrated that the supplementation with antioxidant mixture (rutin, vitamin E, vitamin A, Zn^{2+}, and selenium) restored defective leucine stimulation of protein synthesis in skeletal muscle from aged rats (139).

7. AMINO ACIDS AND LIVER FUNCTION IN DIABETES

The large amount of information concerning hepatic metabolism which has been published over the past 50 years has led to the view that the liver plays an essential role in the metabolism of amino acids. Moreover, it has been clearly established that the pivotal role of the liver occurs not only in physiologic conditions (49) but also in pathological disorders, most notably hypoglycemia (143), cancer (144)), infection (145) and diabetes (146). In this section we will review the main aspects of the liver metabolism of amino acids with emphasis to the diabetic condition.

Traditionally, diabetes is considered as a disorder primarily of carbohydrate metabolism and thus glycemia has become the main marker for monitoring and directing treatment. This focus on blood glucose levels ignores the participation of amino acids in the pathophysiology of diabetes. Considering that insulin is the key anabolic regulator for peripheral protein metabolism, then it stands to reason that blood amino acid availability will be altered in diabetic patients (see above). It is well established that uncontrolled type 1 diabetes in humans and experimental animals, the blood availability of amino acids to the liver is increased and is associated with muscle protein wasting (147). These amino acids are converted at an accelerated rate to glucose by the liver contributing to hyperglycemia.

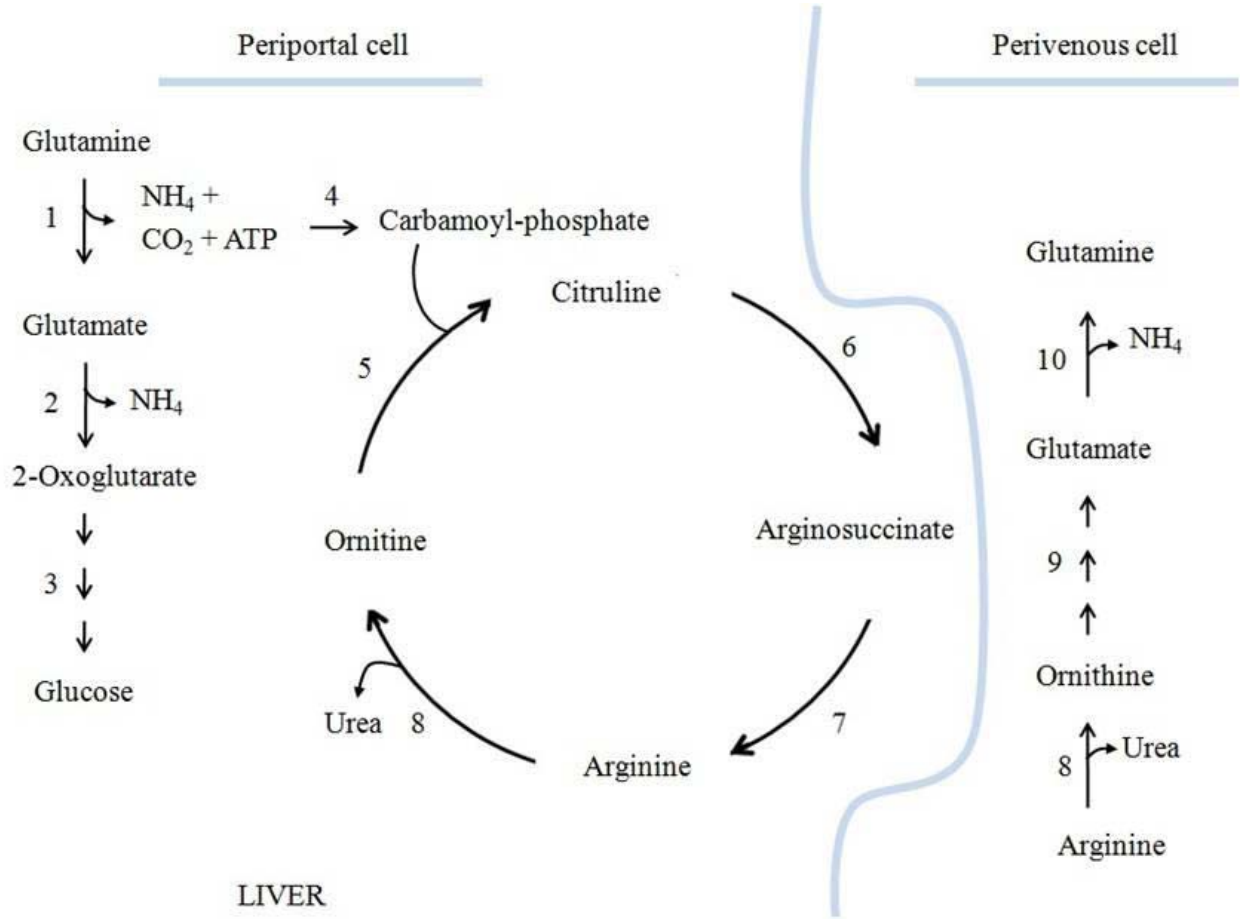

Figure 4. Overview of glutamine metabolism in in the periportal and perivenous liver cells. 1, Phosphate-dependent glutaminase; 2, glutamate dehydrogenase; 3, enzymes of the gluconeogenesis pathway; 4, carbamoyl-phosphate synthetase; 5, ornithine transcarbamoylase; 6, argininosuccinate synthetase; 7, argininosuccinate lyase; 8, arginase; 9, enzymes of glutamate synthesis; 10, glutamine synthetase. Adpapted from (150).

Glutamine is the main source of glutamate for GABA synthesis in the neurons (148) and represents an important substrate for the synthesis of arginine in the liver (149). Furthermore, since the contribution of glutamine to the synthesis of arginine was higher in with respect to enteral compared with intravenous administration, then the importance of the participation of the liver in the de novo synthesis of arginine was established (149).

Compartimentalization of liver glutamine metabolism ensures that glutamine taken up by the periportal liver cells can be cleaved by glutaminase to yield glutamate and ammonia (Figure 4). Glutamate that has been produced in the periportal cells may be further metabolized to produce other amino acids by transamination or may enter the TCA cycle as an anaplerotic substrate or may enter the pathway of gluconeogenesis via formation of phosphoenolpyruvate from oxaloacetate (150). Moreover, gluconeogenesis from glutamine is higher in the diabetic state (151). On the other hand glutamine formation and release from the liver, occurs mainly in the perivenous region. The hepatocytes in this area are rich in glutamine synthetase (150). The substrates for glutamine synthesis are glutamate and ammonia. Glutamate may be produced via glucose conversion to alpha-ketoglutarate and subsequent conversion to glutamate via glutamate dehydrogenase or transamination reactions. In addition, it has been suggested that arginine catabolism may provide glutamate for the glutamine synthetase reaction (152).

It must be emphasized that the potential beneficial effect of glutamine supplementation in type 2 diabetes goes beyond that of a simple source of glutamine, glutamate, GABA and arginine as previously assumed, i.e., glutamine can act as signaling molecule to alter expression (gene) of several enzymes. The ´insulin like effects´ of glutamine on gene expression includes activation of lipogenesis, glycogen synthesis and inhibition of ketone body production and gluconeogenesis (153, 154). Clearly the ´insulin like effects´ of glutamine are not evident in the absence of insulin. However, in type 2 diabetes, these actions of glutamine favor the normalization of blood glucose levels. In support of this suggestion, it was demonstrated recently that glutamine supplementation reduces insulin resistance in the liver (113).

8. AMINO ACIDS AND ENDOTHELIAL CELL FUNCTION IN DIABETES

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among people with diabetes accounting for up to 65% of patient deaths in the United States (155). The risk of CVD is two- to fourfold greater among adults with diabetes than among adults who do not have diabetes (156).

Vascular tone is regulated by a number of vasorelaxant and contractile factors synthesized and released from endothelial cells – for example vasodilators (nitric oxide - NO, prostaciclin, badykinin, adrenomedullin) and vasoconstrictors (endothelin-1, angiotensin-II, prostaglandins, hydrogen peroxide, reactive oxygen species) (157, 158). The L-arginine-NO pathway is thought to be the most important vasodilator source. In addition to its function as a regulator of vasodilation, NO released from endothelial cells reduces vascular permeability, is a potent inhibitor of platelet aggregation and adhesion to the vascular wall, controls the expression of proteins involved in atherosclerotic plaque formation and decreases the expression of the chemoattractant protein moncyte (MCP-1) and of surface adhesion molecules such as CD11/Cd18, P-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule (ICAM-1) and inhibits proliferation of vascular smooth muscle cells (159). NO is synthesized by a family of oxidoreductases, called NO synthases (NOS). In the endothelium eNOS is activated by a number of agonists acting on G protein-coupled receptors and by physical stimuli such as shear stress and changes in oxygen levels that lead to the activation of stretch-opetated nonselective cation channels. NO is synthesized by the oxidation of guanidine nitrogen of L-arginine and this process requires NADPH as a source of electrons plus a number of cofactors: Flavin adenine dinucleotide (FAD), heme, flavin mononucleotide (FMN), calmodulin, and tetrahydrobiopetrin (BH4) (160).

NO is an important tonic inhibitory factor for controlling mitochondrial respiration, and thus a decrease in eNOS activity (or NO bioavailabitlity) will result in an increase in $\bullet O_2^-$ production by mitochondria. In particular, overproduction of $\bullet O_2^-$ may be detrimental because of its rapid interaction with NO, which leads to the loss of NO bioavailability and a reduction in the antiatherogenic effects of NO (161, 162). Inadequate intake of protein from the diet reduces systemic NO synthesis by eNOS, resulting in cardiovascular abnormalities and compromised immune functions (163).

In humans the status of the endothelium is an important surrogate marker of atherosclerosis progression (164). It is not surprising that endothelial dysfunction is a hallmark of vascular disease states (123, 125) and is associated with NO scavenging due to increased superoxide ($\bullet O_2^-$) production. A majority of patients with diabetes die from vascular disease which is linked to dysfunction of the endothelium (166, 167). In addition, vascular dysfunction can continue to progress after a hyperglycemic insult despite the subsequent maintenance of normal blood glucose leves (168, 169).

Concentrations of amino acids in plasma are maintained relatively constant in the post-absorptive state of healthy adults. However, circulating levels of most amino acids undergo marked changes during the neonatal period, under catabolic conditions and in disease (170, 171, 172). Perhaps this is not surprising as amino acids directly participate in cell signaling (173, 174), cell specific metabolism of nutrients (175), oxidative stress (176, 177), and efficiency of utilization of dietary protein (178).

Thus, an optimal balance among amino acids in the diet and circulation is crucial for whole body homeostasis. There is growing recognition that besides their role as building blocks of proteins and polypeptides, some amino acids regulate key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity. These amino acids include arginine, cysteine, glutamine, leucine, proline, and tryptophan. Dietary supplementation with one or a mixture of these amino acids may be beneficial for ameliorating health problems at various stages of the life cycle e.g., fetal growth restriction, neonatal morbidity and mortality, weaning-associated intestinal dysfunction and wasting syndrome, obesity, diabetes, cardiovascular disease, the metabolic syndrome, and infertility (179).

Leucine is an inhibitor of protein degradation in incubated skeletal muscle (180, 181) and the perfused liver (182). As discussed above, leucine stimulates muscle protein synthesis both *in vitro* and *in vivo* (181, 183) and glutamine and leucine increase insulin release from pancreatic beta-cells (184). In contrast to leucine which inhibits NO production by the endothelium, high concentrations of arginine in plasma enhance NO availability and improve vascular insulin sensitivity (185).

Arginine can be synthesized from glutamine, glutamate, and proline in humans (186, 187). However, increasing arginine provision beyond its intake from the regular diet can beneficially regulate many organ functions, particularly under conditions associated with a sub-optimal concentration of arginine in the circulation. There is evidence that the plasma arginine concentration is reduced in diabetic patients (188) probably due to reduced endogenous synthesis and enhanced catabolism by extraintestinal tissues (189). When arginine and BH4 are deficient, (e.g. in diabetes) the enzymatic reduction of molecular oxygen by eNOS produces relatively large amounts of superoxide in endothelial cells (190). The superoxide reacts with NO to yield peroxynitrite, further reducing NO bioavailability. Recent work has shown that dietary supplementation with arginine or watermelon (rich in citrulline) reduced plasma levels of glucose, homocysteine, and asymmetric dimethylarginine (ADMA), which are considered as risk factors for the metabolic syndrome (191), while improving endothelium-dependent relaxation (an indicator of cardiovascular function) in both type 1 and type 2 models of diabetes mellitus (192, 193).

Studies *in vitro* have shown that increasing extracellular L-arginine contractions dose-dependently increases NO production by cultured endothelial cells in the presence of physiological glutamine concentration (194). Increasing plasma L-arginine concentrations also increases *in vivo* NO synthesis by eNOS in mammals (195), indicating that endothelial NO synthesis can be regulated by changes in extracellular or intracellular arginine concentrations. Previous clinical studies have found that L-arginine induced beneficial effects over endothelial function and insulin sensitivity both in healthy individuals and in type 2 diabetes mellitus patients (196, 197). Moreover, other studies have found that, by increasing the bioavailability of NO through an oral administration of L-arginine, it is possible to decrease ADMA levels and improve endothelial dysfunction in patients affected by hypertension (198, 199), explaining, at least in part, the beneficial effects of L-arginine on endothelial function. L-arginine supplementation also decreased important markers of inflammation such as IL-6 and MCP-1 levels. Lower MCP-1, a regulator of monocyte migration into the sub-endothelial layer of the intima where they differentiate into macrophages (200), may beneficially alter cardiovascular risk.

The other AA that deserves special attention with respect to endothelial function is L-glutamine. Increased concentrations of glutamine and glucose inhibit nitric oxide synthesis in endothelial cells and impair endothelial-dependent relaxation (201, 202, 203, 204). Elevated levels of glucose activate the glucosamine synthetic pathway, as well as the production of superoxide and other ROS in endothelial cells (162, 205). Thus, given an important role for ROS in the metabolic syndrome (206) and endothelial dysfunction associated with diabetes (207), activation of the glucosamine-synthetic pathway by hyperglycemia may have important implications for vascular insulin resistance in obesity and diabetes. Increased glucosamine synthesis may play an important role in mediating impaired endothelium-dependent relaxation, oxidative injury, and vascular insulin resistance in obese or diabetic patients (205). *In vitro* L-glutamine at lower concentrations, can regulate rat aortic glutamate content and can modulate NO formation and the contractile response of the thoracic aortic wall (208).

Thus some amino acids, especially glutamine and arginine may be useful to treat or indeed avoid pathologies associated with the vasculature. Much of the published data remains preliminary and more needs to be done to elucidate the exact role of each amino acid with respect to endothelial function.

9. AMINO ACIDS AND IMMUNE FUNCTION IN DIABETES

It is clear from the scientific literature that the role of amino acids in immune function has been explored utilizing a number of specific cell types as described below. We also highlight the implications for diabetes.

9.1. Neutrophils

Neutrophils act as first-line-of-defense cells in the plasma and perform phagocytosis in isolation or in cooperation with antigen-specific defenses. Neutrophils contain a characteristic lobulated chromatin-dense nucleus, which has given rise to the term polymorphonuclear leukocyte, and are 9–12 μm in diameter. At any time, 90% of the neutrophil population is located as newly differentiated cells within the bone marrow. The remaining neutrophils are distributed between the circulation and the vascular endothelium, where they are attached to marginated pools or located within specific tissues. Along with the onset of phagocytosis of bacteria or tissue fragments by neutrophils, a number of different cellular processes, including motility, respiratory burst, and secretion of cytoplasmic (proteolytic) enzymes and immunomodulatory compounds, are initiated. The combination of these processes assists in the killing and digestion of the engulfed bacteria and, if prolonged, the development of a local inflammation. Increase of respiratory burst involves a sudden stimulus-induced increase in non-mitochondrial oxidative metabolism, which results in the production of the superoxide anion and associated reactive oxygen species (209). The processes of endocytosis, secretion of active compounds, and generation of reactive oxygen species had been assumed to be almost entirely dependent on glucose metabolism in neutrophils (210).

It has been demonstrated by at least one of the authors of this review that glutamine is actively used by neutrophils in addition to glucose and that the rate of glutamine consumption was higher than glucose (770 nmol/h/mg protein *vs* 440 mmol/h/mg protein, respectively (211)). As is the case with macrophages and lymphocytes, neutrophils express a kidney-type phosphate-dependent glutaminase activity as a key enzyme of glutamine metabolism (211). These results were confirmed in human neutrophils (212). Glutamine is an important precursor of peptides and proteins, amino sugars, purines and pyrimidines and thus of nucleic acid and nucleotides (213, 214, 115). Glutamine metabolism provides ideal conditions for the synthesis of key redox regulatory molecules, such as glutathione (GSH) (215, 216). Nuclear, mitochondrial, and plasma membrane events associated with apoptosis in rat and human neutrophils cultured in the presence or absence of glutamine have been investigated (217, 218). Glutamine protected both rat and human neutrophils from agents capable of triggering and executing apoptosis. This protective effect of glutamine against neutrophils apoptosis was accompanied by an increase in Bcl-2 expression (217). Acute exercise leads to marked changes in expression of pro- and anti-apoptotic genes of neutrophils in mature rats (90 days old). The effect of exercise on gene expression was not observed in neutrophils obtained from immature rats (60 days old). This suggests that the changes in the pro and anti-apoptotic genes expression induced by exercise are dependent on sexual maturation (219). The same was observed for the effect of glutamine administration. Glutamine treatment ($1 g.kg^{-1}$ body weight) decreased bax and bcl-xs expression in neutrophils from mature rats but had not effect on cells of immature rats.

In neutrophils from burned and post-operative patients, glutamine augmented *in vitro* bacterial killing

activity (220) and it also optimized production of reactive oxygen species (221). The NADPH-oxidase enzyme has a pivotal role in this process for the production of a number of reactive oxidants, including oxidized halogens, free radicals, and singlet oxygen (222). These enzyme catalyses the production of the superoxide anion ($\bullet O_2^-$) by the one-electron reduction of oxygen, using NADPH as the electron donor and comprises five components. In the resting cell, three of these five components, $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$, exist in the cytosol as a complex. The other two components, $p22^{phox}$ and $gp91^{phox}$, are located in the membrane, where they are present as a heterodimeric flavohaemoprotein known as cytochrome b_{558} (223). When the resting cell is exposed to PKC activators such as PMA the resulting phosphorylation of the cytosolic NADPH oxidase components (especially $p47^{phox}$) results in translocation to the plasma or phagosome membrane so allowing association with other NADPH oxidase components. This chain of events triggers the production of $\bullet O_2^-$ (224). The effect of glutamine on $\bullet O_2^-$ production by neutrophils maintained previously for 3 hours in medium deprived of this amino acid was tested (225). Under these conditions, glutamine at 1 and 2 mM increased $\bullet O_2^-$ production in the presence of PMA by 100% and 74%, respectively. PMA increased the expression of $gp91^{phox}$, $p22^{phox}$ and $p47^{phox}$ mRNAs. In the absence of PMA, glutamine (0.5 and 2 mM) increased the expression of the three proteins. Glutamine at 2 mM also increased the expression of these three proteins in the presence of PMA. Therefore, glutamine can regulate of the expression of the components of NADPH oxidase (225). Additional studies have also found that glutamine is important for the production of reactive oxygen species in both neutrophils and monocytes from post-operative patients (220). In fact, stressful conditions such as prolonged exercise (226, 219) and injuries (227), glutamine utilization and oxidation by neutrophils are reduced. Adrenaline (228, 229) as well as glucocorticoids modulate several aspects of neutrophil function such as adhesion, migration, phagocytosis and oxidative burst. These functional effects of dexamethasone may result from changes in reduction of glutamine utilization by neutrophils (230).

Glutamine administered to mice during hypoxia attenuates hyperoxia-induced acute lung injury and improves survival of these animals (231). In an experimental model of abdominal sepsis, a single dose of intravenous glutamine (0.75g/kg) may modulate the inflammatory process by increasing IL-10 in peritoneal lavage fluid at 18 h and broncoalveolar fluid at 48 h, but decreasing CINC-1 and IL-6 in bronchoalveolar and peritoneal lavage fluid at 18 hour (232).

The mechanisms by which glutamine decreases pro-inflammatory cytokines and improves health in patients has been investigated. This amino acid can enhance stress-inducible heat shock protein (HSP 70), and suppress NF-κB signal transduction activity, decreasing production of cytokines and neutrophil infiltration (233, 234). Houdijk *et al.* (235) indicated that citrulline formed from glutamine can serve as a substrate for arginine synthesis in the kidney. Indeed this pathway has also been reported to be active in monocytes and Macrophages (236). Thus glutamine supplementation may be the most appropriate mechanism to increase availability of utilizable arginine (237).

Patients with diabetes mellitus have increased susceptibility to and severity of infections. Several studies have demonstrated impairment of neutrophil function, a disorder that contributes to the high incidence of infections in diabetes. Metabolism in neutrophils obtained from the peritoneal cavity of streptozotocin-induced diabetic rats was investigated (238). Phagocytosis and phorbol myristate acetate (PMA)-stimulated H_2O_2 production were decreased in neutrophils from diabetic rats. The activities of glucose 6-phosphate dehydrogenase and glutaminase were decreased, whereas that of PFK was increased in the diabetic state. The activities of the remaining enzymes were not changed. Diabetes decreased levels of oxidation as determined by CO_2 production from (1-^{14}C)glucose and (U-^{14}C)glutamine. However, (6-^{14}C)glucose and (U-^{14}C)palmitic acid oxidation levels were increased. These observations indicate that changes in pathways of metabolism may play an important role in the impaired neutrophil function observed in diabetes. The treatment with insulin abolished the changes induced by the diabetic state but with no marked change in glycemia. Therefore, insulin may have a direct effect on neutrophil metabolism and function (238).

9.2. Lymphocytes

Lymphocytes are an example of mature immunological cells that recirculate via blood and lymph through lymphoid tissues in a relatively quiescent state until stimulated to proliferate during, for example, a bacterial or viral infection. T lymphocyte stimulation by mitogens, such as lectins and anti-CD3 antibodies, promotes IL-2 production and cell proliferation leading to the propagation of immune response and to up-regulate specific receptors on the T-cell surface, which will further enhance rates of proliferation (239). The modifications associated with autoimmunity are generally specific to T cell responses. Key studies in the early/mid 1980s demonstrated that immune cells such as lymphocytes could utilize glutamine at high rates in addition to glucose (240, 241, 242, 243). Glucose and glutamine metabolism play a pivotal role in lymphocyte proliferation (244, 245). The pathway of glutamine metabolism in lymphocytes is postulated to be similar to that reported for neutrophils and macrophages, where glutamine is only partially oxidized. In leukocytes, glutaminolysis occurs mainly in the mitochondria through the left hand side of the Krebs cycle: glutamine → glutamate → alpha-ketoglutarate → succinyl-CoA → succinate → fumarate → malate → oxaloacetate →pyruvate (244, 246, 247).

Glutamine was consumed by the three leukocyte types in the order: neutrophils>lymphocytes> monocytes. The average amount of glutamine utilized by the cells, expressed as mmol per hour per mg protein was 0.11 for neutrophils 0.047 for monocytes and 0.055 for lymphocytes. The production of glutamate was not remarkably different among the three cells types. The amount of glutamate produced by neutrophils was the

lowest as compared to the remaining cell types after 24 hours in culture. The mean values of glutamate produced by the cells, expressed as mmol per hour per mg protein, were 0.027 for neutrophils, and 0.033 for monocytes and lymphocytes. These findings led us to postulate that these cells may oxidize glutamine at different rates (248). Glutamine plays an important role in regulating cell proliferation. This effect of glutamine has been observed in a large variety of cell types including lymphocytes (249, 250, 251). A significant increase in the plasma lymphocyte number after 10 days of treatment with glutamine was reported (252). The concentration of extracellular glutamine appears to regulate T-lymphocyte proliferation, the rate of interleukin (IL)-2 production and IL-2 receptor expression (253, 254, 255). Glutamine is required for the expression of key lymphocyte cell surface markers related to activation such as CD25, CD45RO and CD71 (216). These results indicate an overall stimulating effect of glutamine on the immune system.

As described above, glutamine can be metabolized in key tissues and organs such as liver to increase plasma arginine levels. Arginine availability is essential for normal T-cell proliferation and function. T lymphocytes depend on arginine for multiple key biological processes, including proliferation, the expression of the TCR complex and the ζ-chain peptide, and the development of memory in B cells (256, 257, 258). However, Bjurström *et al.* (259) demonstrated that GABA may affect T lymphocytes entering the brain. These cells express functional GABA channels that could be activated by low GABA concentrations. GABA inhibits T cell proliferative responses to anti-CD3. This inhibitory effect was associated with greatly reduced IL-2 production by antigen-primed T cells (260). Several alternative mechanisms for the reduction of T cell proliferation by GABA are also possible, including a loss in cell viability.

10. CONCLUDING REMARKS

The effects of various amino acids on cell and tissue function are wide-ranging and influential. Amino acids may impact on receptor and enzyme activity, metabolism, anti-oxidant status and gene expression thus impacting on cell function. Impairement in amino acid supply and metabolism may contribute to disease pathogenesis as evidenced by endothelial cell dysfunction, vascular disease and diabetes. Amino acid supplementation, parenteral or enteral, may therefore be useful as strategy to treat or reduce risk of metabolic disease.

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Abbreviations: AA, amino acid; GABA, gamma amino butyric acid; GSIS, glucose stimulated insulin secretion; mTOR, mammalian target of rapamycin; NADPH oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; NFkB, Nuclear Factor of activated B cells; NO, Nitric oxide; PKC, Protein kinase C; PMA, phorbol myristate acetate; ROS, Reactive oxygen species

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