**Name of journal: World Journal of Diabetes**

**ESPS Manuscript No: 9129**

**Columns: Review**

Molecular mechanisms of protein induced hyperinsulinaemic hypoglycaemia

Chandran S *et al*. Protein induced hypoglycaemia

Suresh Chandran, Fabian Yap, Khalid Hussain

**Suresh Chandran,**Department of Neonatology, KK Women’s and Children’s Hospital, Singapore 229899, Singapore

**Fabian Yap,** Department of Paediatric Endocrinology, KK Women’s and Children’s Hospital, Singapore 229899, Singapore

**Suresh Chandran, Fabian Yap,** Duke-NUS Graduate Medical School, Singapore 169857, Singapore

**Suresh Chandran,** Yong Loo Lin School of Medicine, National University of Singapore, Singapore117598, Singapore

**Suresh Chandran, Fabian Yap,** Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 308232, Singapore

**Khalid Hussain,**Departments of Paediatric Endocrinology, Great Ormond Street Hospital for Children NHS Foundation Trust, London WC1N 3JH, United Kingdom

**Khalid Hussain,** Developmental Endocrinology Research Group, Institute of Child Health, University College London, London WC1N 1EH, United Kingdom

**Author contributions:** Suresh C and Yap F wrote the review under the supervision of Hussain K; Hussain K contributed to abstract, introduction written and final editing of the manuscript.

**Correspondence to: Dr. Khalid Hussain, Reader/Honorary Consultant Paediatric Endocrinologist,** Developmental Endocrinology Research Group, Molecular Genetics Unit, Institute of Child Health, University College London, 30 Guildford Street, London WC1N 1EH, United Kingdom. khalid.hussain@ucl.ac.uk

**Telephone:** +44-20-79052128 **Fax:** +44-20-74046191

**Received:** January 21, 2014 **Revised:** April 29, 2014

**Accepted:** May 28, 2014

**Published online:**

**Abstract**

The interplay between glucose metabolism and that of the two other primary nutrient classes, amino acids and fatty acids is critical for regulated insulin secretion. Mitochondrial metabolism of glucose, amino acid and fatty acids generates metabolic coupling factors (such as ATP, NADPH, glutamate, long chain acyl-CoA and diacylglycerol) which trigger insulin secretion. The observation of protein induced hypoglycaemia in patients with mutations in *GLUD1*gene*,* encoding the enzyme glutamate dehydrogenase (GDH) and *HADH* gene, encoding for the enzyme short-chain 3-hydroxyacyl- CoA dehydrogenase has provided new mechanistic insights into the regulation of insulin secretion by amino acid and fatty acid metabolism. Metabolic signals arising from amino acid and fatty acid metabolism converge on the enzyme GDH which integrates both signals from both pathways and controls insulin secretion. Hence GDH seems to play a pivotal role in regulating both amino acid and fatty acid metabolism.

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**Key words:** Hyperinsulinaemic hypoglycaemia; KATP channel; Glutamate dehydrogenase; Hyperinsulinism/Hyperammonaemia syndrome; Short-chain-3-hydroxyacyl-CoA dehydrogenase; Glutamine

**Core tip:** The interplay between glucose, amino acid and fatty acid metabolism is critical for regulated insulin secretion. Mitochondrial metabolism of glucose, amino acid and fatty acids generates metabolic coupling factors (such as ATP, NADPH, glutamate, long chain acyl-CoA and diacylglycerol) which trigger insulin secretion. The observation of protein induced hypoglycaemia in patients with mutations in*GLUD1* (encoding for the enzyme glutamate dehydrogenase GDH) and *HADH* genes, has provided novel mechanistic insights into the regulation of insulin secretion by amino acid and fatty acid metabolism. Metabolic signals arising from amino acid and fatty acid metabolism converge on the enzyme GDH which integrates both signals from both pathways and controls insulin secretion. Hence GDH seems to play a pivotal role in regulating both amino acid and fatty acid metabolism.

Chandran S, Yap F, Hussain K. Molecular mechanisms of protein induced hyperinsulinaemic hypoglycaemia. *World J Diabetes* 2014; In press

**INTRODUCTION**

Glucose, amino acids and fatty acids are the substrates available for metabolic homeostasis and play important roles in insulin secretion. Pancreatic β-cells synthesise and secrete insulin in response to signals generated from glucose, amino acid and fatty acid metabolism but glucose is the prime stimulus for insulin secretion. Regulated insulin release requires tight coupling in the β-cell between glucose metabolism and insulin secretory response. As β-cells are continually exposed to a complex milieu of nutrients and other circulating factors (like incretins), it is important to understand the interplay between glucose metabolism and that of the two other primary nutrient classes, the amino acids and fatty acids. Specific amino acids are now known to acutely and chronically regulate insulin secretion from pancreatic β-cells in vivo and in vitro and lipid metabolism in the β-cell is critical for the regulation of insulin secretion[1].

The metabolism of glucose, amino acids and fatty acids results in the generation of metabolic coupling factors involved in regulating insulin exocytosis. These metabolic coupling factors generated from the metabolism of glucose, amino acids and fatty acids in the β-cell include ATP, NADPH, glutamate, long chain acyl-CoA and diacylglycerol[2]. Each of these coupling factors plays a key role in regulating insulin secretion. The exocytotic process is closely controlled by signals generated from nutrient metabolism as well as by neurotransmitters and circulating hormones.

Under normal physiological conditions the metabolism of glucose, amino acids and fatty acids is intricately controlled and will result in the regulated secretion of insulin. The secretion of insulin is precisely regulated to keep fasting blood glucose concentrations between 3.5-5.9 mmol/L. In some pathological states the signals generated from glucose, amino acid and fatty acid metabolism cause insulin hyper-secretion or dysregulation of insulin secretion. In these states insulin secretion becomes inappropriate for the level of blood glucose causing hyperinsulinaemic hypoglycaemia (HH).

HH is a major cause of persistent hypoglycaemia in the childhood period[3]. In the newborn and infancy periods HH can be either congenital or secondary to certain risk factors (such as intrauterine growth retardation). Congenital forms of HH are due to defects in key genes (*ABCC8, KCNJ11, GLUD1, GCK, HADH, HNF4/HNF1A, SLC23A*, and *UCP2*) involved in regulating insulin secretion[4]. Loss of function mutations in the genes *ABCC8* and *KCNJ11* (which encode for the SUR1 and KIR6.2, components of the β-cell potassium (KATP) channel subunits respectively) lead to the most severe forms of HH, which is usually medically unresponsive[5]. Clinically HH presents with fasting hypoglycaemia but in some patients the HH is typically triggered by the ingestion of protein (amino acids). Protein induced HH is observed in patients with gain of function mutations of *GLUD1*[6], loss-of-function mutations of *ABCC8/KCJN11* [7] and loss of function mutations in the *HADH*[8].

This state of the art review article will firstly discuss the molecular mechanisms of glucose, amino acid and fatty acid regulated insulin secretion and then focus on the current understanding of the molecular mechanisms involved in protein induced HH.

**GLUCOSE MEDIATED INSULIN SECRETION BY THE PANCREATIC Β-CELL**

Glucose mediated secretion of insulin is initiated by the uptake of glucose by the β-cells *via* the glucose transporter (GLUT2). Glucose is then phosphorylated to glucose-6-phosphate by islet-specific glucokinase (GK). Further metabolism of glucose increases the cellular ATP: ADP ratio, which closes ATP-dependent KATP channels in the β-cell membrane, causing membrane depolarization and influx of calcium. Intracellular free calcium then promotes margination of secretory granules, which fuse with the cell membrane before releasing their contents into the extracellular space by exocytosis (Figure 1)[9]. The functional integrity of both SUR1 and KIR 6.2 proteins is necessary for KATP channel function and the genes encoding for these two proteins are localized very closely to each other on the short arm of chromosome 11(11p14-15.1).

Although KATP channels have an essential role in linking the metabolism of glucose to the secretion of insulin, there is now evidence that there may well be other mechanisms of insulin secretion, the so-called KATP channel independent pathways of insulin secretion[10]. This pathway leads to augmented insulin release in the presence of raised cytosolic calcium (Ca2+) concentrations. Increases in the intracellular Ca2+ concentration in the pancreatic β-cell cause modest increases in insulin secretion, which can be dramatically increased by modulators of protein kinases and phosphatases. This suggests that steps distal to the elevation of cytosolic Ca2+ are of greater quantitative importance in controlling insulin secretion. It has also been shown that glucose can cause pronounced insulin secretion in Ca2+ depleted islets in the presence of activators of protein kinases A and C[11].

Given the key role of pancreatic β-cell KATP channels in regulating insulin secretion it is no surprise that genetic defects in the genes regulating the function of these channels lead to severe forms of HH. Recessive inactivating mutations in KATP channel subunits are the most common cause of HH[5, 12]. So far, over 150 mutations have been identified in the *ABCC8* and 25 in *KCNJ11*[13]. These include missense, frame shift, nonsense, insertions/deletions, splice site and regulatory mutations, either present in homozygous or compound heterozygous state. In the Ashkenazi Jewish population, two common (F1388del and c.3992-9G4A) mutations account for 90% of all cases of congenital HH[4].

The molecular basis of recessive inactivating *ABCC8* and *KCNJ11* mutations involves multiple defects in KATP channel biogenesis and turnover, in channel trafficking from the endoplasmic reticulum and Golgi apparatus to the plasma membrane and alterations of channels in response to both nucleotide regulation and open state frequency.

**AMINO ACID MEDIATED INSULIN SECRETION**

The observations that plasma levels of insulin increase consistently and significantly when healthy subjects ingest protein meals[14] or when intravenous mixtures of amino acids are administered[15], provide fundamental scientific evidence of the relationship between protein metabolism, amino acids and insulin secretion.

Protein metabolism begins when dietary proteins are broken down to amino acids by intestinal enzymes[16]. Large differences in capacity of individual amino acids to stimulate insulin release are noted in both animal and human studies[15, 17]. For example, when 30 g each of 10 amino acids in a mixture was administered individually, arginine proved the most effective and histidine the least in stimulating insulin release[15]. Although leucine itself can stimulate insulin secretion, the phenomenon of protein meal or amino acid stimulated insulin secretion does not solely or largely depend on the presence of leucine[14, 15].

Amino acids, alone[18] or in combination[19], act synergistically with glucose to potentiate the release of insulin. Synergism was also observed between amino acid pairs, where the synergistic effect was significantly greater with arginine-leucine than with arginine-phenylalanine and their combined effects greater than when amino acids were administered alone[20]. Indeed, the oral ingestion of amino acid mixtures in combination with carbohydrates produce stronger insulinotropic effects compared with carbohydrate-only preparations[21], a phenomenon mediated by the incretin hormones GIP and GLP-1[22]. Amino acids shown to have the highest insulinotropic effect include leucine, valine, lysine, and isoleucine[23]. Metabolism of amino acids can occur either by transamination or by oxidative deamination.

Transamination is an early step in the degradation of most amino acids and involves a chemical reaction between two molecules, an amino acid (with an amine NH2 group) and a keto acid (with a keto = O group), catalysed by a family of enzymes known as aminotransferases. Different aminotransferases are each specific for an amino acid or a group of chemically similar ones such as branch chain amino acids (BCAA). The keto acid that accepts the amino group is always alpha-ketoglutarate (α-KG), a metabolically important biological compound and key intermediate in the citric acid cycle. For example, alanine transaminase catalyses the transfer of an amino group from alanine to α-KG giving rise to pyruvate and glutamate.

On the other hand, oxidative deamination involves conversion of an amino acid into the corresponding keto acid by removing the amine group as ammonia, which goes into the urea cycle. As glutamate is the end product of many transamination reactions, oxidative deamination occurs primarily on glutamate, generating α-KG[16, 24]. The main enzyme involved in oxidative deamination is GDH.

Glutamine and alanine are the most abundant amino acids in the blood and extracellular fluids. Whereas glutamine and alanine require the presence of glucose for insulin secretion, leucine is able to stimulate insulin secretion independently through the allosteric activation of GDH[25-28], generating α-KG. The further metabolism of α-KG is then involved in insulin production in two ways. First, by entering the TCA cycle, the ATP: ADP ratio is raised causing closure of the KATP channel and depolarisation of the β-cell. The voltage dependant calcium channel opens leading to an increase in cellular calcium concentration, triggering the release of insulin from storage granules (Figure 1)[29]. Second, α-KG inhibits isocitrate dehydrogenase resulting in increased cytosolic citrate needed for the synthesis of short and long chain acyl-CoA, which are coupling factors closely involved in insulin secretion[30].

**LEUCINE**

Leucine is one of the most potent insulin secretagogues among the BCAA that facilitates glucose-induced insulin release from pancreatic β-cells[31]. It does so *via* several mechanisms. First, in pancreatic β-cells, leucine and its non-metabolizable analogue 2-aminobicyclo [2.2.1] heptane-2-carboxylic acid (BCH), stimulate the secretion of insulin by acting indirectly as a positive allosteric activator of GDH to enhance glutaminolysis. Activated GDH facilitates the oxidation of glutamate to α-KG, which raises the ATP: ADP ratio resulting in closure of KATP channel, cellular depolarization, influx of calcium and exocytosis of insulin from the storage granules **(**Figure 1)[32]. Second, the transaminated product of leucine, α-ketoisocaproate (KIC) can cause insulin secretion through direct inhibition of the KATP channel[33]. Glucose completely blocks the effects of leucine but not of KIC on stimulation of insulin secretion by β-cells[34]. Third, leucine plays an important role in the regulation of the mammalian target of Rapamycin (mTOR) pathway, which was recently recognized as a critical regulator of metabolic response to nutrients and growth factors[35]. Recent data strongly suggest that leucine down-regulates the surface expression of α2 adrenergic receptors (α2ARs) in pancreatic islets through activation of mTOR, leading to insulin secretion[36].

**GLUTAMINE**

As the most abundant amino acid found in the blood, glutamine has both nutritive and non-nutritive effects[37]. Glutamine is physiologically important for maintaining cellular function in tissues of the intestine, kidney, brain and liver[38]. It is an important precursor substrate for the synthesis of peptides, proteins and nucleotides[39], in particular ATP which is central in the β-cell signalling pathway. In SUR1 knockout (KO) β-cells models, isolated pancreatic islets respond briskly to a physiological mixture of 20 amino acids even though these islets cannot be stimulated by glucose or by leucine. Glutamine played an important role in mediating amino acid stimulation of insulin release as 60% of the insulin response was attributable to glutamine even though it comprised 16% of the amino acid load[7].

Although glutamine itself functions as a key precursor for nucleic acids and nucleotides, in many physiological circumstances it acts to provide glutamate, which promotes a wider array of metabolic functions compared to glutamine. By oxidative deamination of glutamate, GDH liberates free ammonia and the α-KG is then oxidized in the TCA cycle, raising ATP levels that close KATP channels and depolarize the cell membrane to release insulin. Ammonia is added to glutamate by glutamine synthetase to form glutamine, the major inert-organ carrier for ammonia.

Glutamine can be cleaved by glutaminase to yield glutamate and NH3. The mitochondrial carbamoyl phosphate synthetase (CPS 1) then can catalyze the conversion of ammonia to carbamoyl phosphate. The CPS 1 enzyme is allosterically activated by N-Acetyl glutamate (NAG) produced from glutamate by NAG synthase and may thus be indirectly regulated by glutamate concentration. Carbamoyl phosphate thus formed combines with ornithine in the urea cycle. Thus glutamate also aids in ammonia detoxification and promotion of urea synthesis in the liver (Figure 2)[40, 41]. However, the exact mechanism of glutamine linked hyperinsulinaemia remains less well understood. Glutamine can also potentiate insulin secretion by stimulating enteroendocrine L-cells to synthesise and secrete the incretin GLP-1. This effect is attributable to a triggering pathway that elevates intracellular Ca2+ and an amplifying pathway mediated by elevated cAMP[42].

**ALANINE**

The mechanism of action of alanine as an insulin secretagogue is still unclear. Upon entry into the β-cell cytosol, alanine is deaminated and takes part in the TCA cycle through pyruvate and acetyl-CoA. This results in increase of the cellular content of ATP, closure of the KATP channel, depolarization of the plasma membrane, activation of voltage-gated calcium channel, increase in calcium influx and insulin exocytosis (Figure 3)[43]. Insulinotropic property of alanine has been reported by Dunne *et al*[44] and McClenaghan *et al*[45] to be the result of co-transport with Na+, leading to β-cell membrane depolarization and increase in cellular calcium. Current evidence suggests that the mode of action of alanine as an insulin secretagogue involves a combination of increased ATP generation, co-transport with Na+ and signal transduction[26, 46].

**ARGININE**

The mechanism of insulin release by arginine involves the mCAT2A amino acid transporter which electrogenically transports arginine into the β-cell, leading to increased intracellular calcium[47]. Accumulation of intracellular arginine leads to membrane depolarization, a further rise in intracellular calcium through opening of voltage-gated calcium channels, and insulin secretion[48]. Arginine can also influence insulin secretion by its conversion to glutamate, which allows the generation of metabolic coupling factors[49], however the detailed metabolism of arginine in the β-cell remains to be investigated.

**FATTY ACID Β-OXIDATION PATHWAY**

During the fasting state fatty acids (FA) are the most important substrates for ketogenesis to provide the brain with an “alternative fuel” source. Triglycerides are broken down to FA and glycerol in the process of lipolysis. β-oxidation of FA occurs in the peroxisomes and mitochondria. Short and medium chain FA can diffuse directly into the mitochondria and are then activated by acyl-CoA synthetase to acyl-CoA in the mitochondrial matrix, whereas long and very long chains FA are activated by acyl-CoA synthetase on the mitochondrial outer membrane. The “carnitine shuttle” allows acyl-CoA to penetrate the outer and inner mitochondrial membranes, catalysed by carnitine palmitoyltransferase-I and II (CPT- I and II) respectively, facilitated by the inner membrane exchange transporter, carnitine-acylcarnitine translocase[50].

In the mitochondrial matrix, acetyl-coA is generated by β-oxidation of acyl-CoA *via* a 4-step process involving dehydrogenation, hydration, oxidation and thiolysis (Figure 4)[50]. Acetyl- CoA finally enters the Krebs cycle. The short-chain 3-hydroxyacyl- CoA dehydrogenase (SCHAD), an intramitochondrial homodimer enzyme is essential for catalysing the penultimate reaction of 3-hydroxyacyl CoA to 3-ketoacyl-CoA. Possible molecular mechanisms involved in the pathogenesis of HH due to deficiency of SCHAD have been reported recently[8, 51].

**PROTEIN INDUCED INSULIN SECRETION- HISTORICAL PERSPECTIVE**

The history of protein induced hypoglycaemia dates back to 1956 when Cochrane described three children with severe hypoglycaemia while on low carbohydrate and high protein diet. Even though amino acid-stimulated insulin secretion by pancreatic β-cells was known for long[52], the molecular mechanisms involved in the dysregulated islet cell function leading to HH due to genetic mutations remain poorly understood. In 1970 researchers reported that amino acids could induce insulin secretion only in the presence of glucose except in case of leucine where the insulin–stimulatory effect is abolished in presence of glucose[53]. Animal studies have suggested that amino acid oxidation and signalling effects are two vital steps in which the amino acid amplifies insulin release from the stored vesicles following β-cell depolarisation and influx of calcium. By early 80’s leucine’s property to induce insulin secretion by allosterically stimulating GDH was identified[29].

**MOLECULAR MECHANISMS OF AMINO ACID INDUCED HYPERINSULINAEMIC HYPOGLYCAEMIA**

Amino acids are known to enhance insulin secretion from primary islet β-cell lines under appropriate conditions. Leucine can stimulate insulin release on its own by allosterically activating GDH. In the β-cell mitochondria, GDH can stimulate insulin secretion by oxidative deamination of glutamate by raising α-KG, NADH/NAD and NADPH/NADP ratios. Protein sensitive hyperinsulinaemic hypoglycaemia occurs in three forms; gain-of-function mutations of *GLUD1*[6], loss of function mutations of *ABCC8/KCNJ11*[7] and loss of function mutations in *HADH*[8].

**HYPERINSULINISM/HYPERAMMONAEMIA SYNDROME**

Hyperinsulinism/Hyperammonaemia syndrome (HI/ HA) syndrome is the second most common cause of congenital hyperinsulinism(CHI), characterized by both fasting and protein sensitive hypoglycaemia together with persistently elevated plasma ammonia levels [6]. HI/HA is likely the disorder described by Cochrane *et al*[52] in 1955, with leucine sensitive hypoglycaemia in a child and her father. Zammarchi *et al*[54] first reported a case of hyperammonaemia with leucine sensitive hypoglycaemia. Activating mutations in the GLUD1 gene were reported to be the cause of HI/HA syndrome by Stanley *et al*[6, 32] in 1998. Children usually present with recurrent symptomatic hypoglycaemic episodes (leucine sensitive) and persistent hyperammonaemia.

***Molecular basis of HI/HA***

The enzyme, GDH has a complex allosteric regulatory mechanism and is highly expressed in the pancreas, liver, kidney and brain. GDH catalyses the reversible oxidative deamination of glutamate to α-KG and ammonia, using NAD or NADP as co-factors. GDH is allosterically inhibited by GTP and activated by ADP and leucine[55].

In patients with HI/HA syndrome there is impairment of allosteric inhibition of GDH by GTP leading to gain-of GDH function. This causes increased leucine induced glutamate oxidation to α-KG, which explains the leucine sensitivity following a protein meal and postprandial hypoglycaemia. These patients on fasting develop hypoglycaemia following release of alanine and glutamine from skeletal muscle, which can stimulate insulin release mediated through GDH[56]. The mechanism of hyperammonaemia in HI/HA syndrome is still unclear. In liver, increased GDH activity may lead to hyperammonaemia through 2 possible mechanisms: elevated activity of GDH causing increased levels of ammonia from glutamate and excessive depletion of glutamate pool, reducing the availability of NAG *via* NAG synthase reaction. NAG is an allosteric activator of CPS 1 and deficiency of this can impair urea synthesis[32, 57]. An alternative hypothesis for hyperammonaemia in HI/HA syndrome is that the excessive ammonia is due to abnormal muscle catabolism[58]. More recently the source of the hyperammonaemia in the HI/HA syndrome is thought to be the kidney[59].

***Sirtuins and insulin secretion***

Sirtuins are a family of NAD+ dependant enzymes having a critical role in metabolic adaptation to stress. Sirtuin4 (SIRT4), an intramitochondrial enzyme highly expressed in pancreatic β-cells, also regulates GDH. SIRT4 repress the activity of GDH by ADP-ribosylation in pancreatic β-cell mitochondria, down regulating insulin secretion mediated through amino acids. In normal glucose states, SIRT4 blunts amino acid-induced insulin secretion by repressing the activity of GDH[60, 61]. In contrary GDH is released from the SIRT4-mediated inhibition *via* an undefined mechanism during fasting, thereby enhancing amino acid-induced insulin secretion[61]. In SIRT4 knockout mice, GDH activity is enhanced in β-cells, leading to the enhancement of glucose and amino acid-stimulated insulin secretion[61]. So loss of function mutation of SIRT4 can present with a phenotype similar to gain of function mutation of GLUD1. However no humans have yet been described with protein induced hyperinsulinism due to SIRT4 mutations[62].

***Clinical presentation of HI/HA***

The infants with HI/HA syndrome are usually born at term and not macrosomic. The major clinical feature is recurrent episodes of symptomatic HH after first few months of life. These may occur with fasting or can be provoked by protein feeding. Hypoglycaemia in HI/HA syndrome is not as severe as seen in HH due to KATP channel mutations. Hyperammonaemia, a characteristic biochemical marker of HI/HA syndrome, is typically mild to moderate (up to 3-5 times the upper limit of normal) and is not associated with lethargy, irritability, or coma. The plasma amino acid profile remains normal in HI/HA syndrome in contrast to abnormal profile observed in the other causes of hyperammonaemia[62, 63].

Protein diet or blood glucose levels do not affect the plasma ammonia levels in patients with HI/HA syndrome[54, 64]. Kapoor *et al*[62] reported some patients who have mutations in GLUD1 with HH but with normal serum ammonia levels and the authors proposed that this could be due to mosaicism for the mutation in the liver, where the mutation is absent or seen in < 50% in hepatocytes. Hyperammonaemia is resistant to detoxification compounds (sodium benzoate and N-carbamylglutamate) or protein-restricted diet[65].

Kapoor *et al*[62] have published the clinical characteristics of patients with HI/HA due to GLUD mutations. Of the twenty patients most of them were appropriate for the gestational age and presented at a mean age of 23.4 wk. Nineteen of them had hyperammonaemia. Thirteen of the 17-screened probands had 7 different heterozygous mutations and three novel mutations were identified (N410D, D451V, P436L). More than 90% cases responded to diazoxide. Seizure was the most common (94%) symptom, 43% of them developed generalized epilepsy with a higher preponderance in cases with mutations in exons 6 and 7 of GLUD1 gene[62,66].

Earlier in 2004, Stanley *et al*[32,57] has reported that over activity of GDH in the brain decreases the levels of glutamate and glutamine, protecting the central nervous system from the neurotoxicity of its accumulation.

GDH transgenic mice harbouring the human GDH-HI *H454Y* mutation develop a hypoglycaemia phenotype[67] and insulin secretion studies in these mice are associated with increased oxidative deamination of glutamate *via* GDH, this confirming the key role of GDH in amino acid stimulated insulin secretion.

Using a β-cell-specific GDH KO mouse model (βGlud1 (-/-)) islets isolated from these mice showed diminished of insulin release when stimulated by glutamine combined with 2-aminobicyclo [2.2.1] heptane-2-carboxylic acid or l-leucine[68]. Further studies in these mice showed that permissive levels of glutamate were required for the full development of glucose-stimulated insulin secretion and that GDH plays an indispensable role in this process.

***Management of HI/HA***

Treatment of HI/HA is aimed at correction of fasting and protein induced hypoglycaemia. Diazoxide remains the main stay of treatment and affected patients are well controlled with a dose of 5-15 mg/kg per day[69]. Being a KATP channel agonist, diazoxide prevents β-cell membrane depolarization and inhibits insulin secretion by keeping KATP channels open. Diazoxide is usually combined with hydrochlorothiazide in neonates to counteract its fluid retention side effects. Hypertrichosis seen in infants on diazoxide usually resolves on discontinuation[70].Recent reports of large symptomatic pericardial effusion in infants on diazoxide, warrants meticulous cardiovascular monitoring while on treatment[71].

***Green tea flavonoids and HI/HA***

Naturally occurring compounds from green tea, discovered by the Chinese Emperor Shen-Nung in 2737 B.C. has been used as a remedy to treat a number of ailments, including diabetes mellitus[72]. Green tea is a significant source of a type of flavonoid called catechin, which includes epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate (ECG) and epicatechin, of which EGCG and ECG have a strong inhibitory effect on GDH function[72,73].

Animal studies have shown that ECG binds to the same site as the allosteric regulator ADP and hijacks the ADP activation site. In pancreatic islet cells of transgenic mice expressing a human HI/HA form of GDH, a hyper-response to glutamine caused by dysregulated GDH is blocked by the addition of EGCG[73]. Above all EGCG has the property to inhibit GTP-insensitive GDH mutations, opening the window of therapeutic potential to treat GDH hyperinsulinism. EGCG also has been shown to block glutamine stimulated calcium influx and insulin secretion in GDH transgenic mice islets[74].

Several novel GDH inhibitors are identified and are under trial[75]. Current evidence support the pathological basis of hyperammonaemia to be due to gain in GDH activity and excessive oxidation of glutamate, reducing the level needed for the synthesis of NAG and thereby slowing the clearance of ammonia (Figure 2). In this context N-carbamylglutamate (Carglumic acid), a carbamoyl phosphate synthetase activator has a potential role in the treatment of hyperammonaemia in HI/HA syndrome[69,76,77]. De novo mutations in GLUD1 have been reported in 70% of GDH-HI cases with the remainder inherited in an autosomal dominant pattern[69].

**PROTEIN INDUCED HYPOGLYCAEMIA DUE TO DEFECTS IN KATP CHANNEL GENES**

Mutations in the *ABCC8/KCNJ11* genes are the most common cause of CHI[5]. The observation that patients with KATP channel null mutations can develop HH following high protein meal in the absence of leucine sensitivity[78], demonstrates that amino acids can induce HH *via* GDH and KATP channel independent pathways. Patient with GLUD1 mutations show leucine sensitive hypoglycaemia whereas those with *ABCC8/KCNJ11* mutations are not leucine sensitive. Thus, protein-induced HH is not necessarily synonymous with leucine-sensitive HH. The GDH and KATP channel independent mechanism of protein induced HH can be explained through the direct induction of insulin release by glutamine, formed by the ATP-dependent condensation of glutamate with ammonia, catalysed by glutamine synthetase (GS) (Figure 5).

***Role of glutamine in insulin secretion in patients with KATP channel defects***

Glutamine plays a pivotal role in glucose and amino acid stimulated insulin secretion as a signalling molecule, which is followed by β-cell depolarization and influx of calcium and insulin release. Prerequisites for glutamine to function in β-cell include elevated ATP levels and increased cytosolic calcium[7]. Role of glutamine in stimulation of insulin release has been shown in patients with mutations of SUR1[78]. Animal studies have shown that β-cells of SUR1 -/- mice are markedly sensitive to glutamine stimulation[7, 67]. Li *et al*[7, 67] has shown that β- cells lacking SUR 1 protein were hyper-responsive to glutamine and amino acid mixture but were refractory to glucose stimulation. This amino acid response was reduced by 60% when glutamine was omitted from the amino acid mixture[7]. Two possible mechanisms are considered but still remain unsettled: Metabolism of amino acids is enhanced while glucose is impaired in SUR1 lacking β-cell which could be the result of persistent elevation of cytosolic calcium and secondly glutamine may be triggering insulin release by a hypothetical novel mechanisms like activation of protein kinase pathways[11, 78, 79].

**PROTEIN INDUCED HYPERINSULINAEMIC HYPOGLYCAEMIA DUE TO LOSS OF FUNCTION MUTATION IN HADH GENE**

Mutations causing genetic defects have been described in many of the enzymes involved in mitochondrial fatty acid oxidation. Recently, mutations in the penultimate enzyme in the fatty acid oxidation chain have been described that result in quite different symptoms from those normally seen. Patients with the mutations in *HADH* present with protein (leucine)-induced HH, suggesting a link between fatty acid oxidation, amino acid metabolism and insulin secretion[80].

Short-chain-3-hydroxyacyl-CoA dehydrogenase catalyses the penultimate reaction of the β-oxidation cycle for medium and short chain 3-hydroxy fatty-acyl-CoA’s. SCHAD deficiency impairs short chain fatty acid oxidation. First insights into the molecular mechanism involved in SCHAD deficiency came with the observation of Clayton *et al*[8] (2001) that fatty acid beta oxidation defect is associated with HH, supporting the concept of lipid signalling pathway in the control of insulin secretion[81].

***Clinical aspects of patients with HADH mutations***

Affected children with SCHAD deficiency on fasting as well as following a protein meal, either present with mild late onset hypoglycaemia or severe neonatal hypoglycaemia with raised levels of fatty acid metabolites including plasma hydroxybutyrylcarnitine and urinary 3-hydroxyglutaric acid[82,83]. Most often they present with hypoglycaemic seizures. Kapoor *et al*[84] in 2009 reported for the first time that human mutations of *HADH* gene cause severe dietary protein sensitivity leading to HH and they may have normal acylcarnitine and urinary organic acid profiles. These cases had novel *HADH* gene mutations. The enzymes GDH and SCHAD have a direct protein-protein interaction, which is lost in patients with HADH mutations causing leucine induced HH. Leucine sensitivity is evident in patients with *HADH* gene mutations (Figure 3). There is no associated loss of inhibitory effect of GTP on GDH, as seen with *GLUD1* mutations[85].

***The interaction between SCHAD and GLUD1***

SCHAD has a vital role in insulin secretion, suggested by the high degree of expression of *HADH* gene in β-cells of pancreas[86]. Hardy *et al*[87], using RNA interference, identified *HADH* gene as one of the 4 essential genes required for normal insulin secretion. FOXA2, a transcription factor encoded by the gene *FOXA2*, is essential for β-cell differentiation and its function has been shown to regulate *HADH* expression[88]. Additionally, severe hyperinsulinism after leucine tolerance testing was reported in all patients having *HADH* gene mutations [85]. Further reports on the loss of protein-protein interaction in human cases between SCHAD and GDH were published[51, 83, 89]. Heslegrave *et al*[85] made a similar observation of the loss of interaction between SCHAD and GDH in lymphoblasts.

Sund *et al*[90] showed severe HH in FOXA2 β-cell KO mice. Islets from these mice were shown to have reduced expression of both SCHAD and Kir6.2 and had severe HH. Li *et al*[51] showed that HADH KO mice developed a hyperinsulinaemic response following leucine loading and an exacerbation of the same on addition of glutamine and alanine. When glutamine and leucine were removed from the amino acid mixture, KO mice islets failed to induce HH, suggesting the role of GDH activation for abnormal insulin secretion.

Recent studies on HADH KO mice showed an increased sensitivity to amino acid stimulated insulin secretion indicating activation of the glutaminolysis pathway *via* GDH to increase ATP production and thereby insulin. Binding of SCHAD to GDH was also shown in immunoprecipitation experiments. These research works indicate that hyperinsulinism in SCHAD-deficient states is caused by loss of “moonlighting function” (a protein having additional functions in other pathways) of SCHAD protein, which otherwise provides a direct inhibitory regulation of GDH in β-cells[51, 91]. So in pancreatic β-cells, mutations resulting in the absence of SCHAD protein leads to abnormal activation of GDH, causing hyperinsulinism.

The activation of GDH in *HADH* gene mutant patients or mouse KO models is limited to pancreatic β-cells and hence deficiency of SCHAD enzyme does not lead to hyperammonemia unlike in HI/HA syndrome[51,92]. Further evidence for protein-protein interaction between enzymes came from Zhang *et al*[93]. They showed the co-precipitation of GDH with SCHAD when anti- SCHAD antibody was used as bait in wild type mouse liver mitochondria, confirming the previous observation that GDH activation in SCHAD deficiency is due to loss of protein-protein interaction.

Diazoxide remains the treatment of choice in HH due to *HADH* gene mutations. This also confirms the intactness of KATP channel in patients with SCHAD deficiency[8, 82, 84 , 92].

**CONCLUSION**

The interplay between glucose metabolism and that of the two other primary nutrient classes, amino acids and fatty acids is critical for regulated insulin secretion. Protein induced HH is observed in patients with mutations in *GLUD1, HADH* and *ABCC8/KCNJ11*. GDH and SCHAD play important roles in integrating amino acid and fatty acid signals for insulin secretion.

**REFERENCES**

1 **Newsholme P**, Brennan L, Rubi B, Maechler P. New insights into amino acid metabolism, beta-cell function and diabetes. *Clin Sci (Lond)* 2005; **108**: 185-194 [PMID: 15544573]

2 **Maechler P**, Carobbio S, Rubi B. In beta-cells, mitochondria integrate and generate metabolic signals controlling insulin secretion. *Int J Biochem Cell Biol* 2006; **38**: 696-709 [PMID: 16443386 DOI: 10.1016/j.biocel.2005.12.006]

3 **Aynsley-Green A**, Hussain K, Hall J, Saudubray JM, Nihoul-Fékété C, De Lonlay-Debeney P, Brunelle F, Otonkoski T, Thornton P, Lindley KJ. Practical management of hyperinsulinism in infancy. *Arch Dis Child Fetal Neonatal Ed* 2000; **82**: F98-F107 [PMID: 10685981 DOI: 10.1136/fn.82.2.F98]

4 **James C**, Kapoor RR, Ismail D, Hussain K. The genetic basis of congenital hyperinsulinism. *J Med Genet* 2009; **46**: 289-299 [PMID: 19254908 DOI: 10.1136/jmg.2008.064337]

5 **Thomas PM**, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 1995; **268**: 426-429 [PMID: 7716548 DOI: 10.1126/science.7716548]

6 **Stanley CA**, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E, Poncz M. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med* 1998; **338**: 1352-1357 [PMID: 9571255 DOI: 10.1056/NEJM199805073381904]

7 **Li C**, Buettger C, Kwagh J, Matter A, Daikhin Y, Nissim IB, Collins HW, Yudkoff M, Stanley CA, Matschinsky FM. A signaling role of glutamine in insulin secretion. *J Biol Chem* 2004; **279**: 13393-13401 [PMID: 14736887 DOI: 0.1074/jbc.M311502200]

8 **Clayton PT**, Eaton S, Aynsley-Green A, Edginton M, Hussain K, Krywawych S, Datta V, Malingre HE, Berger R, van den Berg IE. Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. *J Clin Invest* 2001; **108**: 457-465 [PMID: 11489939 DOI: 10.1172/JCI11294]

9 **Inagaki N**, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J. Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 1995; **270**: 1166-1170 [PMID: 7502040]

10 **Henquin JC**. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 2000; **49**: 1751-1760 [PMID: 11078440 DOI: 10.2337/diabetes]

11 **Ammälä C**, Eliasson L, Bokvist K, Berggren PO, Honkanen RE, Sjöholm A, Rorsman P. Activation of protein kinases and inhibition of protein phosphatases play a central role in the regulation of exocytosis in mouse pancreatic beta cells. *Proc Natl Acad Sci U S A* 1994; **91**: 4343-4347 [PMID: 8183910]

12 **Thomas P**, Ye Y, Lightner E. Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 1996; **5**: 1809-1812 [PMID: 8923010 DOI: 10.1093/hmg/5.11.1809]

13 **Flanagan SE**, Clauin S, Bellanné-Chantelot C, de Lonlay P, Harries LW, Gloyn AL, Ellard S. Update of mutations in the genes encoding the pancreatic beta-cell K(ATP) channel subunits Kir6.2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. *Hum Mutat* 2009; **30**: 170-180 [PMID: 18767144 DOI: 10.1002/humu.20838]

14 **Floyd JC**, Fajans SS, Conn JW, Knopf RF, Rull J. Insulin secretion in response to protein ingestion. *J Clin Invest* 1966; **45**: 1479-1486 [PMID: 5919349 DOI: 10.1172/]

15 **Floyd JC**, Fajans SS, Conn JW, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. *J Clin Invest* 1966; **45**: 1487-1502 [PMID: 5919350 DOI: 10.1172/JCI105456]

16 **Bateman KS**, Chernaia MM, Tarasova NI, James MN. Crystal structure of human pepsinogen A. *Adv Exp Med Biol* 1998; **436**: 259-263 [PMID: 9561228]

17 **Rocha DM**, Faloona GR, Unger RH. Glucagon-stimulating activity of 20 amino acids in dogs. *J Clin Invest* 1972; **51**: 2346-2351 [PMID: 4639019]

18 **Floyd JC**, Fajans SS, Pek S, Thiffault CA, Knopf RF, Conn JW. Synergistic effect of essential amino acids and glucose upon insulin secretion in man. *Diabetes* 1970; **19**: 109-115 [PMID: 4905636]

19 **van Loon LJ**, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH, Keizer HA. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. *Diabetes Care* 2003; **26**: 625-630 [PMID: 12610012 DOI: 10.2337/diacare.26.3.625]

20 **Floyd JC**, Fajans SS, Pek S, Thiffault CA, Knopf RF, Conn JW. Synergistic effect of certain amino acid pairs upon insulin secretion in man. *Diabetes* 1970; **19**: 102-108 [PMID: 5414363]

21 **van Loon LJ**, Saris WH, Verhagen H, Wagenmakers AJ. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am J Clin Nutr* 2000; **72**: 96-105 [PMID: 10871567]

22 **Asmar M**, Holst JJ. Glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide: new advances. *Curr Opin Endocrinol Diabetes Obes* 2010; **17**: 57-62 [PMID: 19881341 DOI: 10.1097/MED.0b013e3283339051]

23 **Nilsson M**, Stenberg M, Frid AH, Holst JJ, Björck IM. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. *Am J Clin Nutr* 2004; **80**: 1246-1253 [PMID: 15531672]

24 **Smith MB**, March J. Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th ed. Wiley, 2001

25 **Malaisse WJ**, Sener A, Carpinelli AR, Anjaneyulu K, Lebrun P, Herchuelz A, Christophe J. The stimulus-secretion coupling of glucose-induced insulin release. XLVI. Physiological role of L-glutamine as a fuel for pancreatic islets. *Mol Cell Endocrinol* 1980; **20**: 171-189 [PMID: 7002657 DOI: 10.1016/0303-7207(80)90080-5]

26 **Sener A**, Malaisse WJ. The stimulus-secretion coupling of amino acid-induced insulin release. Insulinotropic action of L-alanine. *Biochim Biophys Acta* 2002; **1573**: 100-104 [PMID: 12383948 DOI: 10.1016/S0304-4165(02)00337-9]

27 **Malaisse WJ**, Malaisse-Lagae F, Sener A. The stimulus-secretion coupling of amino acid-induced insulin release metabolic interaction of L-asparagine and L-leucine in pancreatic islets. *Biochim Biophys Acta* 1984; **797**: 194-202 [PMID: 6365174]

28 **Sener A**, Malaisse WJ. L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. *Nature* 1980; **288**: 187-189 [PMID: 7001252]

29 **Arya VB**, Senniappan S, Guemes M, Hussain K. Neonatal hypoglycemia. *Indian J Pediatr* 2014; **81**: 58-65 [PMID: 23904063 DOI: 10.4103/2230-8210.107822]

30 **Deeney JT**, Gromada J, Høy M, Olsen HL, Rhodes CJ, Prentki M, Berggren PO, Corkey BE. Acute stimulation with long chain acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI beta-cells). *J Biol Chem* 2000; **275**: 9363-9368 [PMID: 10734079 DOI: 10.1074/jbc.275.13.9363]

31 **Hutton JC**, Sener A, Malaisse WJ. Interaction of branched chain amino acids and keto acids upon pancreatic islet metabolism and insulin secretion. *J Biol Chem* 1980; **255**: 7340-7346 [PMID: 6993486]

32 **Stanley CA**. Hyperinsulinism/hyperammonemia syndrome: insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. *Mol Genet Metab* 2004; **81 Suppl 1**: S45-S51 [PMID: 15050973 DOI: 10.1016/j.ymgme.2003.10.013]

33 **Bränström R**, Efendić S, Berggren PO, Larsson O. Direct inhibition of the pancreatic beta-cell ATP-regulated potassium channel by alpha-ketoisocaproate. *J Biol Chem* 1998; **273**: 14113-14118 [PMID: 9603910]

34 **Gao Z**, Young RA, Li G, Najafi H, Buettger C, Sukumvanich SS, Wong RK, Wolf BA, Matschinsky FM. Distinguishing features of leucine and alpha-ketoisocaproate sensing in pancreatic beta-cells. *Endocrinology* 2003; **144**: 1949-1957 [PMID: 12697702 DOI: 10.1210/en.2002-0072]

35 **Avruch J**, Long X, Ortiz-Vega S, Rapley J, Papageorgiou A, Dai N. Amino acid regulation of TOR complex 1. *Am J Physiol Endocrinol Metab* 2009; **296**: E592-E602 [PMID: 18765678 DOI: 10.1152/ajpendo.90645.2008]

36 **Yang J**, Dolinger M, Ritaccio G, Mazurkiewicz J, Conti D, Zhu X, Huang Y. Leucine stimulates insulin secretion via down-regulation of surface expression of adrenergic α2A receptor through the mTOR (mammalian target of rapamycin) pathway: implication in new-onset diabetes in renal transplantation. *J Biol Chem* 2012; **287**: 24795-24806 [PMID: 22645144 DOI: 10.1074/jbc.M112.344259]

37 **Williamson DH**, Brosnan JT. Concentrations of metabolites in animal tissues. In: Bergmeyer HU (Editor), Methods in Enzymatic Analysis. Academic Press, New York and London,1974

38 **Newsholme P**, Lima MM, Procopio J, Pithon-Curi TC, Doi SQ, Bazotte RB, Curi R. Glutamine and glutamate as vital metabolites. *Braz J Med Biol Res* 2003; **36**: 153-163 [PMID: 12563517 DOI: 10.1590/S0100-879X2003000200002]

39 **Young VR**, Ajami AM. Glutamine: the emperor or his clothes? *J Nutr* 2001; **131**: 2449S-259S; discussion 2449S-259S; [PMID: 11533293]

40 **Cho SW**, Ahn JY, Lee J, Choi SY. Identification of a peptide of the guanosine triphosphate binding site within brain glutamate dehydrogenase isoproteins using 8-azidoguanosine triphosphate. *Biochemistry* 1996; **35**: 13907-13913 [PMID: 8909287 DOI: 10.1021/bi9618575]

41 **Wrzeszczynski KO**, Colman RF. Activation of bovine liver glutamate dehydrogenase by covalent reaction of adenosine 5'-O-[S-(4-bromo-2,3-dioxobutyl)thiophosphate] with arginine-459 at an ADP regulatory site. *Biochemistry* 1994; **33**: 11544-11553 [PMID: 7918368]

42 **Tolhurst G**, Zheng Y, Parker HE, Habib AM, Reimann F, Gribble FM. Glutamine triggers and potentiates glucagon-like peptide-1 secretion by raising cytosolic Ca2+ and cAMP. *Endocrinology* 2011; **152**: 405-413 [PMID: 21209017 DOI: 10.1210/en.2010-0956]

43 **Brennan L**, Shine A, Hewage C, Malthouse JP, Brindle KM, McClenaghan N, Flatt PR, Newsholme P. A nuclear magnetic resonance-based demonstration of substantial oxidative L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic beta-cell line: metabolism of L-alanine is important to the regulation of insulin secretion. *Diabetes* 2002; **51**: 1714-1721 [PMID: 12031957 DOI: 10.2337/diabetes.51.6.1714]

44 **Dunne MJ**, Yule DI, Gallacher DV, Petersen OH. Effects of alanine on insulin-secreting cells: patch-clamp and single cell intracellular Ca2+ measurements. *Biochim Biophys Acta* 1990; **1055**: 157-164 [PMID: 2242384 DOI: 10.1016/01674889(90)]

45 **McClenaghan NH**, Barnett CR, Flatt PR. Na+ cotransport by metabolizable and nonmetabolizable amino acids stimulates a glucose-regulated insulin-secretory response. *Biochem Biophys Res Commun* 1998; **249**: 299-303 [PMID: 9712690 DOI: 10.1006/]

46 **Dura E**, Jijakli H, Zhang HX, Oguzhan B, Sener A, Malaisse WJ. Insulinotropic action of amino acids at their physiological concentrations: I. Experiments in incubated islets. *Int J Mol Med* 2002; **9**: 527-531 [PMID: 11956661 DOI: 10.3892/ijmm.9.5.527]

47 **Sener A**, Best LC, Yates AP, Kadiata MM, Olivares E, Louchami K, Jijakli H, Ladrière L, Malaisse WJ. Stimulus-secretion coupling of arginine-induced insulin release: comparison between the cationic amino acid and its methyl ester. *Endocrine* 2000; **13**: 329-340 [PMID: 11216645 DOI: 10.1385/ENDO: 13: 3: 329]

48 **Malaisse WJ**, Plasman PO, Blachier F, Herchuelz A, Sener A. Stimulus-secretion coupling of arginine-induced insulin release: significance of changes in extracellular and intracellular pH. *Cell Biochem Funct* 1991; **9**: 1-7 [PMID: 1712259]

49 **Broca C**, Brennan L, Petit P, Newsholme P, Maechler P. Mitochondria-derived glutamate at the interplay between branched-chain amino acid and glucose-induced insulin secretion. *FEBS Lett* 2003; **545**: 167-172 [PMID: 12804769 DOI: 10.1016/S0014-]

50 **Wood PA**. Defects in mitochondrial beta-oxidation of fatty acids. *Curr Opin Lipidol* 1999; **10**: 107-112 [PMID: 10327278]

51 **Li C**, Chen P, Palladino A, Narayan S, Russell LK, Sayed S, Xiong G, Chen J, Stokes D, Butt YM, Jones PM, Collins HW, Cohen NA, Cohen AS, Nissim I, Smith TJ, Strauss AW, Matschinsky FM, Bennett MJ, Stanley CA. Mechanism of hyperinsulinism in short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency involves activation of glutamate dehydrogenase. *J Biol Chem* 2010; **285**: 31806-31818 [PMID: 20670938 DOI: 10.1074/]

52 **COCHRANE WA**, PAYNE WW, SIMPKISS MJ, WOOLF LI. Familial hypoglycemia precipitated by amino acids. *J Clin Invest* 1956; **35**: 411-422 [PMID: 13306783 DOI: 10.1172/JCI103292]

53 **Pace CS**, Stillings SN, Hover BA, Matschinsky FM. Electrical and secretory manifestations of glucose and amino acid interactions in rat pancreatic islets. *Diabetes* 1975; **24**: 489-496 [PMID: 1092581]

54 **Zammarchi E**, Filippi L, Novembre E, Donati MA. Biochemical evaluation of a patient with a familial form of leucine-sensitive hypoglycemia and concomitant hyperammonemia. *Metabolism* 1996; **45**: 957-960 [PMID: 8769351]

55 **Fahien LA**, MacDonald MJ, Kmiotek EH, Mertz RJ, Fahien CM. Regulation of insulin release by factors that also modify glutamate dehydrogenase. *J Biol Chem* 1988; **263**: 13610-13614 [PMID: 3047128]

56 **Chang TW**, Goldberg AL. The metabolic fates of amino acids and the formation of glutamine in skeletal muscle. *J Biol Chem* 1978; **253**: 3685-3693 [PMID: 649596]

57 **Stanley CA**, Fang J, Kutyna K, Hsu BY, Ming JE, Glaser B, Poncz M. Molecular basis and characterization of the hyperinsulinism/hyperammonemia syndrome: predominance of mutations in exons 11 and 12 of the glutamate dehydrogenase gene. HI/HA Contributing Investigators. *Diabetes* 2000; **49**: 667-673 [PMID: 10871207 DOI: 10.2337/diabetes.49.4.667]

58 **Kamoun P**, Rabier D, Saudubray JM. Muscular origin of hyperammonaemia. *Eur J Pediatr* 1998; **157**: 868 [PMID: 9809835]

59 **Treberg JR**, Clow KA, Greene KA, Brosnan ME, Brosnan JT. Systemic activation of glutamate dehydrogenase increases renal ammoniagenesis: implications for the hyperinsulinism/hyperammonemia syndrome. *Am J Physiol Endocrinol Metab* 2010; **298**: E1219-E1225 [PMID: 20332361 DOI: 10.1152/ajpendo.00028]

60 **Haigis MC**, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW, Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* 2006; **126**: 941-954 [PMID: 16959573 DOI: 10.1016/j.cell.2006.06.057]

61 **Ahuja N**, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, Maechler P, Verdin E. Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. *J Biol Chem* 2007; **282**: 33583-33592 [PMID: 17715127 DOI: 10.1074/jbc.M705488200]

62 **Kapoor RR**, Flanagan SE, Fulton P, Chakrapani A, Chadefaux B, Ben-Omran T, Banerjee I, Shield JP, Ellard S, Hussain K. Hyperinsulinism-hyperammonaemia syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. *Eur J Endocrinol* 2009; **161**: 731-735 [PMID: 19690084 DOI: 10.1530/EJE-09-0615]

63 **Kapoor RR**, James C, Hussain K. Advances in the diagnosis and management of hyperinsulinemic hypoglycemia. *Nat Clin Pract Endocrinol Metab* 2009; **5**: 101-112 [PMID: 19165222 DOI: 10.1038/ncpendmet1046]

64 **Miki Y**, Taki T, Ohura T, Kato H, Yanagisawa M, Hayashi Y. Novel missense mutations in the glutamate dehydrogenase gene in the congenital hyperinsulinism-hyperammonemia syndrome. *J Pediatr* 2000; **136**: 69-72 [PMID: 10636977 DOI: 10.1016/S0022-]

65 **De Lonlay P**, Benelli C, Fouque F, Ganguly A, Aral B, Dionisi-Vici C, Touati G, Heinrichs C, Rabier D, Kamoun P, Robert JJ, Stanley C, Saudubray JM. Hyperinsulinism and hyperammonemia syndrome: report of twelve unrelated patients. *Pediatr Res* 2001; **50**: 353-357 [PMID: 11518822]

66 **Bahi-Buisson N**, El Sabbagh S, Soufflet C, Escande F, Boddaert N, Valayannopoulos V, Bellané-Chantelot C, Lascelles K, Dulac O, Plouin P, de Lonlay P. Myoclonic absence epilepsy with photosensitivity and a gain of function mutation in glutamate dehydrogenase. *Seizure* 2008; **17**: 658-664 [PMID: 18321734 DOI: 10.1016/j.seizure.2008.01.005]

67 **Li C**, Matter A, Kelly A, Petty TJ, Najafi H, MacMullen C, Daikhin Y, Nissim I, Lazarow A, Kwagh J, Collins HW, Hsu BY, Nissim I, Yudkoff M, Matschinsky FM, Stanley CA. Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. *J Biol Chem* 2006; **281**: 15064-15072 [PMID: 16574664 DOI: 10.1074/jbc.M600994200]

68 **Vetterli L**, Carobbio S, Pournourmohammadi S, Martin-Del-Rio R, Skytt DM, Waagepetersen HS, Tamarit-Rodriguez J, Maechler P. Delineation of glutamate pathways and secretory responses in pancreatic islets with β-cell-specific abrogation of the glutamate dehydrogenase. *Mol Biol Cell* 2012; **23**: 3851-3862 [PMID: 22875990 DOI: 10.1091/mbc.E11-08-0676]

69 **Palladino AA**, Stanley CA. The hyperinsulinism/hyperammonemia syndrome. *Rev Endocr Metab Disord* 2010; **11**: 171-178 [PMID: 20936362]

70 **Arnoux JB**, de Lonlay P, Ribeiro MJ, Hussain K, Blankenstein O, Mohnike K, Valayannopoulos V, Robert JJ, Rahier J, Sempoux C, Bellanné C, Verkarre V, Aigrain Y, Jaubert F, Brunelle F, Nihoul-Fékété C. Congenital hyperinsulinism. *Early Hum Dev* 2010; **86**: 287-294 [PMID: 20550977 DOI: 10.1016/]

71 **Avatapalle B**, Banerjee I, Malaiya N, Padidela R. Echocardiography monitoring for diazoxide induced pericardial effusion. *BMJ Case Rep* 2012; **2012**: [PMID: 22761225 DOI: 10.1136/bcr.03.2012.6110]

72 **Yang CS**, Wang ZY. Tea and cancer. *J Natl Cancer Inst* 1993; **85**: 1038-1049 [PMID: 8515490 DOI: 10.1093/jnci/85.13.1038]

73 **Li C**, Allen A, Kwagh J, Doliba NM, Qin W, Najafi H, Collins HW, Matschinsky FM, Stanley CA, Smith TJ. Green tea polyphenols modulate insulin secretion by inhibiting glutamate dehydrogenase. *J Biol Chem* 2006; **281**: 10214-10221 [PMID: 16476731 DOI: 10.1074/jbc.M512792200]

74 **Li C**, Li M, Chen P, Narayan S, Matschinsky FM, Bennett MJ, Stanley CA, Smith TJ. Green tea polyphenols control dysregulated glutamate dehydrogenase in transgenic mice by hijacking the ADP activation site. *J Biol Chem* 2011; **286**: 34164-34174 [PMID: 21813650 DOI: 10.1074/jbc.M111.268599]

75 **Li M**, Smith CJ, Walker MT, Smith TJ. Novel inhibitors complexed with glutamate dehydrogenase: allosteric regulation by control of protein dynamics. *J Biol Chem* 2009; **284**: 22988-23000 [PMID: 19531491 DOI: 10.1074/jbc.M109.020222]

76 **Huijmans JG**, Duran M, de Klerk JB, Rovers MJ, Scholte HR. Functional hyperactivity of hepatic glutamate dehydrogenase as a cause of the hyperinsulinism/hyperammonemia syndrome: effect of treatment. *Pediatrics* 2000; **106**: 596-600 [PMID: 10969108 DOI: 10.1542/peds.106.3.596]

77 **Daniotti M**, la Marca G, Fiorini P, Filippi L. New developments in the treatment of hyperammonemia: emerging use of carglumic acid. *Int J Gen Med* 2011; **4**: 21-28 [PMID: 21403788 DOI: 10.2147/IJGM.S10490]

78 **Fourtner SH**, Stanley CA, Kelly A. Protein-sensitive hypoglycemia without leucine sensitivity in hyperinsulinism caused by K(ATP) channel mutations. *J Pediatr* 2006; **149**: 47-52 [PMID: 16860127 DOI: 10.1016/j.jpeds.2006.02.033]

79 **Li C**, Nissim I, Chen P, Buettger C, Najafi H, Daikhin Y, Nissim I, Collins HW, Yudkoff M, Stanley CA, Matschinsky FM. Elimination of KATP channels in mouse islets results in elevated [U-13C]glucose metabolism, glutaminolysis, and pyruvate cycling but a decreased gamma-aminobutyric acid shunt. *J Biol Chem* 2008; **283**: 17238-17249 [PMID: 18445600 DOI: 10.1074/jbc.M709235200]

80 **Heslegrave AJ**, Hussain K. Novel insights into fatty acid oxidation, amino acid metabolism, and insulin secretion from studying patients with loss of function mutations in 3-hydroxyacyl-CoA dehydrogenase. *J Clin Endocrinol Metab* 2013; **98**: 496-501 [PMID: 23253615 DOI: 10.1210/jc.2012-3134]

81 **Prentki M**. New insights into pancreatic beta-cell metabolic signaling in insulin secretion. *Eur J Endocrinol* 1996; **134**: 272-286 [PMID: 8616523]

82 **Hussain K**, Clayton PT, Krywawych S, Chatziandreou I, Mills P, Ginbey DW, Geboers AJ, Berger R, van den Berg IE, Eaton S. Hyperinsulinism of infancy associated with a novel splice site mutation in the SCHAD gene. *J Pediatr* 2005; **146**: 706-708 [PMID: 15870679 DOI: 10.1016/j.jpeds.2005.01.032]

83 **Molven A**, Matre GE, Duran M, Wanders RJ, Rishaug U, Njølstad PR, Jellum E, Søvik O. Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. *Diabetes* 2004; **53**: 221-227 [PMID: 14693719 DOI: 10.2337/diabetes.53.1.221]

84 **Kapoor RR**, James C, Flanagan SE, Ellard S, Eaton S, Hussain K. 3-Hydroxyacyl-coenzyme A dehydrogenase deficiency and hyperinsulinemic hypoglycemia: characterization of a novel mutation and severe dietary protein sensitivity. *J Clin Endocrinol Metab* 2009; **94**: 2221-2225 [PMID: 19417036]

85 **Heslegrave AJ**, Kapoor RR, Eaton S, Chadefaux B, Akcay T, Simsek E, Flanagan SE, Ellard S, Hussain K. Leucine-sensitive hyperinsulinaemic hypoglycaemia in patients with loss of function mutations in 3-Hydroxyacyl-CoA Dehydrogenase. *Orphanet J Rare Dis* 2012; **7**: 25 [PMID: 22583614 DOI: 10.1186/1750-1172-7-25]

86 **Agren A**, Borg K, Brolin SE, Carlman J, Lundqvist G. Hydroxyacyl CoA dehydrogenase, an enzyme important in fat metabolism in different cell types in the islets of Langerhans. *Diabete Metab* 1977; **3**: 169-172 [PMID: 334589]

87 **Hardy OT**, Hohmeier HE, Becker TC, Manduchi E, Doliba NM, Gupta RK, White P, Stoeckert CJ, Matschinsky FM, Newgard CB, Kaestner KH. Functional genomics of the beta-cell: short-chain 3-hydroxyacyl-coenzyme A dehydrogenase regulates insulin secretion independent of K+ currents. *Mol Endocrinol* 2007; **21**: 765-773 [PMID: 17185391 DOI: 10.1210/me.2006-0411]

88 **Lantz KA**, Vatamaniuk MZ, Brestelli JE, Friedman JR, Matschinsky FM, Kaestner KH. Foxa2 regulates multiple pathways of insulin secretion. *J Clin Invest* 2004; **114**: 512-520 [PMID: 15314688 DOI: 10.1172/JCI21149]

89 **Filling C**, Keller B, Hirschberg D, Marschall HU, Jörnvall H, Bennett MJ, Oppermann U. Role of short-chain hydroxyacyl CoA dehydrogenases in SCHAD deficiency. *Biochem Biophys Res Commun* 2008; **368**: 6-11 [PMID: 18036338 DOI: 10.1016/j.bbrc.207.10.188]

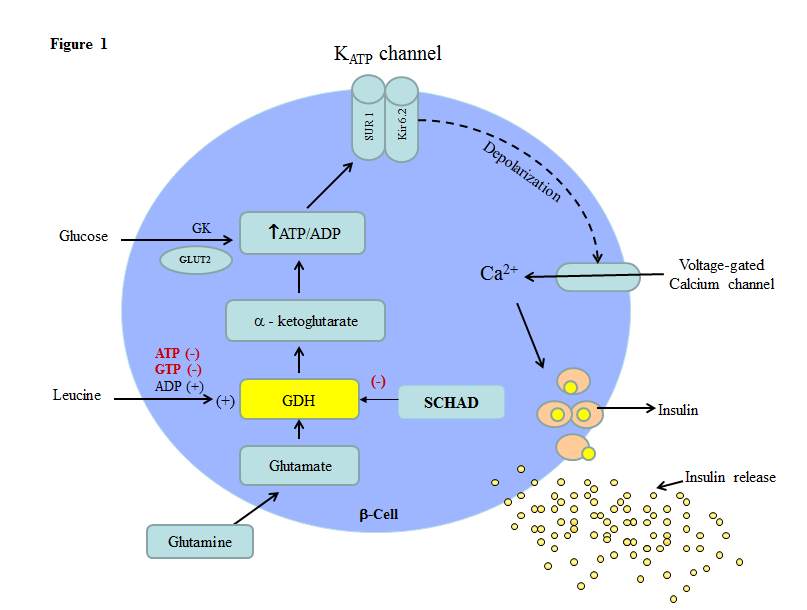
90 **Sund NJ**, Vatamaniuk MZ, Casey M, Ang SL, Magnuson MA, Stoffers DA, Matschinsky FM, Kaestner KH. Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. *Genes Dev* 2001; **15**: 1706-1715 [PMID: 11445544 DOI: 10.1101/gad.901601]

91 **Jeffery CJ**. Moonlighting proteins--an update. *Mol Biosyst* 2009; **5**: 345-350 [PMID: 19396370 DOI: 10.1039/b900658n]

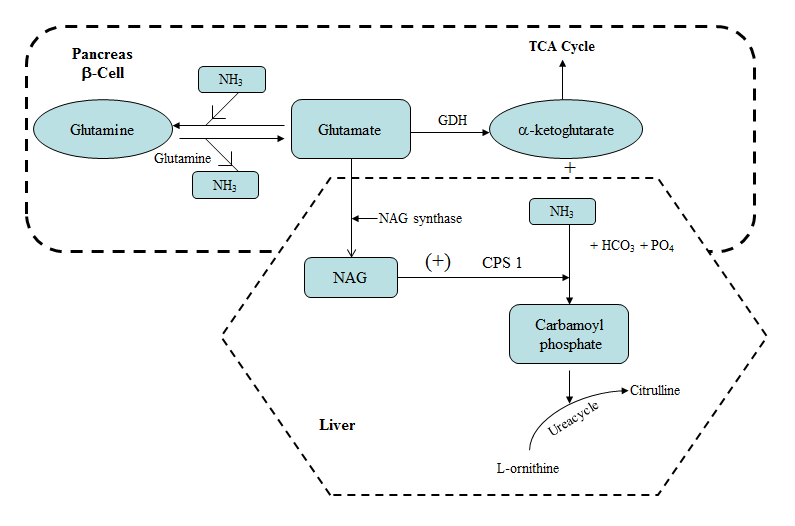
92 **Stanley CA**. Two genetic forms of hyperinsulinemic hypoglycemia caused by dysregulation of glutamate dehydrogenase. *Neurochem Int* 2011; **59**: 465-472 [PMID: 21130127 DOI: 10.1016/j.neuint.2010.11.017]

93 **Zhang T**, Li C. Mechanisms of amino acid-stimulated insulin secretion in congenital hyperinsulinism. *Acta Biochim Biophys Sin (Shanghai)* 2013; **45**: 36-43 [PMID: 23212075 DOI: 10.1093/abbs/gms107]

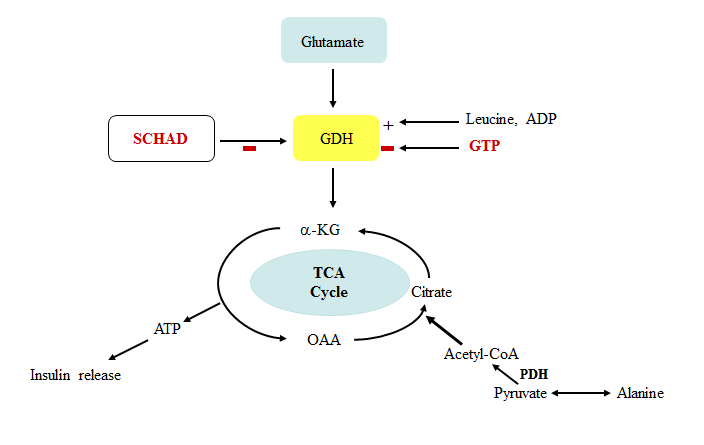
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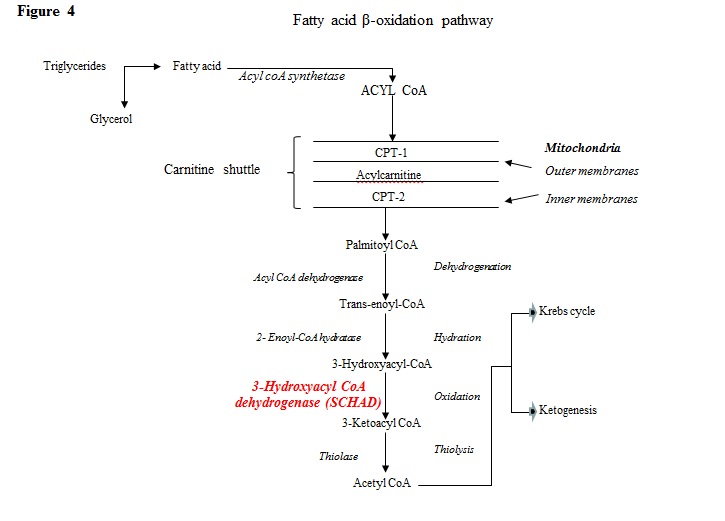


**Figure1 Glucose and protein mediated insulin secretion in the beta cell of pancreas.** GDH: Glutamate dehydrogenase; SCHAD: Short-chain 3-hydroxyacyl- CoA dehydrogenase; GK: Glucokinase.

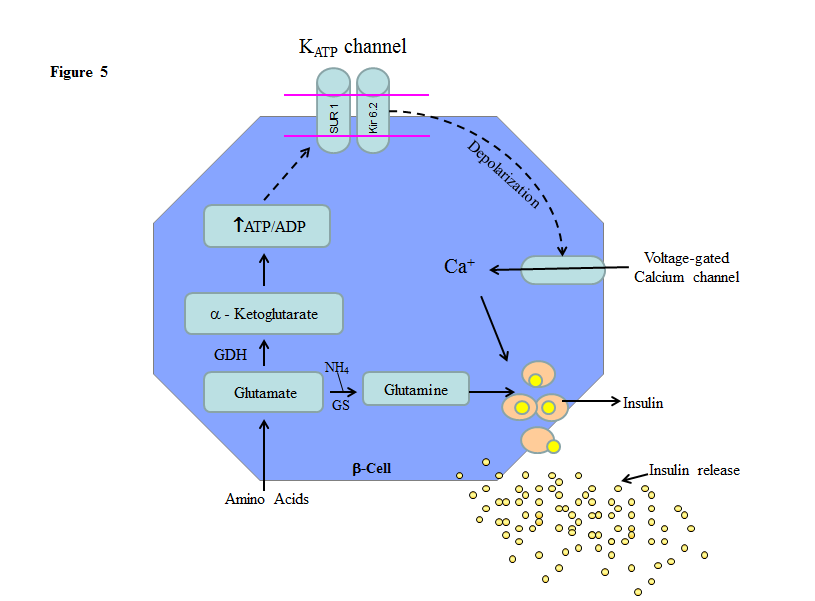


**Figure 2 Glutamate metabolism**. Oxidation of glutamate by glutamate dehydrogenase (GDH) liberates free ammonia (NH3) and alpha ketoglutarate (α-KG), which enters TCA cycle and generates ATP. In the liver glutamate also generates N-acetylglutamate (NAG), which in turn allosterically activates carbomyl phosphate synthetase (CPS) to regulate ammonia detoxification into urea. Glutamine provides a substrate for ammonia buffering, by adding ammonia to glutamate to form glutamine. TCA: Tricarboxylic acid cycle.

**Figure 3 Glutamate and alanine as insulin secretagogues.** Protein induced hyperinsulinaemic hypoglycaemia due to loss of function mutation in HADH gene (SCHAD). Alanine is deaminated to pyruvate and pyruvate dehydrogenase (PDH) converts it to acetyl CoA, which can enter TCA cycle to generate ATP for closing KATP channel.



**Figure 4 β-oxidation of fatty acids. Acyl-CoA is converted to acetyl-CoA through dehydrogenation, hydration, oxidation and thiolysis.** Acetyl-CoA can enter the Krebs cycle or can lead to ketogenesis. CPT-I: Carnitine palmitoyltransferase-I; CPT-2: Carnitine palmitoyltransferase -2; MCAD: Medium-chain Acyl-CoA dehydroxgenase; LCHAD:Long - chain3-hydroxyacyl-CoA dehydrogenase.



**Figure 5 Protein Induced Hypoglycaemia due to defects in KATP channel genes.** GS: glutamine synthetase.