Nutrient regulation of insulin secretion and action

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Abstract

Pancreatic β-cell function is of critical importance in the regulation of fuel homoeostasis, and metabolic dysregulation is a hallmark of diabetes mellitus (DM). The β-cell is an intricately designed cell type that couples metabolism of dietary sources of carbohydrates, amino acids and lipids to insulin secretory mechanisms, such that insulin release occurs at appropriate times to ensure efficient nutrient uptake and storage by target tissues. However, chronic exposure to high nutrient concentrations results in altered metabolism that impacts negatively on insulin exocytosis, insulin action and may ultimately lead to development of DM. Reduced action of insulin in target tissues is associated with impairment of insulin signalling and contributes to insulin resistance (IR), a condition often associated with obesity and a major risk factor for DM. The altered metabolism of nutrients by insulin-sensitive target tissues (muscle, adipose tissue and liver) can result in high circulating levels of glucose and various lipids, which further impact on pancreatic β-cell function, IR and progression of the metabolic syndrome. Here, we have considered the role played by the major nutrient groups, carbohydrates, amino acids and lipids, in mediating β-cell insulin secretion, while also exploring the interplay between amino acids and insulin action in muscle. We also focus on the effects of altered lipid metabolism in adipose tissue and liver resulting from activation of inflammatory processes commonly observed in DM pathophysiology. The aim of this review is to describe commonalities and differences in metabolism related to insulin secretion and action, pertinent to the development of DM.

Key Words
- diabetes
- insulin
- insulin resistance
- nutrients
- metabolism
- inflammation

Journal of Endocrinology (2014) **221**, R105–R120

Journal of Endocrinology

Introduction

Homoeostatic regulation of fuel metabolism in the body is a tightly controlled process and dysregulation can lead to pathological conditions such as diabetes mellitus (DM), cardiovascular disease, stroke, renal disease and other manifestations of the metabolic syndrome. Glucose, the body's primary metabolic fuel source, is ingested usually in polymeric form following the consumption of a mixed meal, and the subsequent postprandial elevation in blood glucose level is stringently modulated by the release of the pancreatic hormones insulin and glucagon. These hormones target metabolically active tissues such as muscle, adipose tissue and liver in order to maintain blood glucose concentration within narrow limits (~4.0–6.0 mmol/l). However, dysregulation of metabolic processes may result in chronic hyperglycaemic, dyslipidaemic or glucolipotoxic conditions that may negatively impact a wide variety of tissues and organs including pancreatic islets, skeletal muscle, adipose tissue and the liver and are frequently observed in DM.

According to the International Diabetes Federation (IDF), in 2011, 336 million of the world's population

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(~6.4%) had either type 1 (T1DM) or type 2 DM (T2DM), yet the prevalence is continuing to rise at rapid rates and is projected to exceed 550 million by 2030 (Whiting *et al.* 2011). Furthermore, T2DM is by far the most common form of the disease, representing about 90–95% of DM cases. Pancreatic islets are specialised and highly vascularised structures that monitor the nutrient contents of the blood stream and consist of mainly five cell types; α-cells, β-cells, δ-cells, ghrelin cells (γ-cells) and pancreatic peptide (PP)-secreting cells (Wierup *et al.* 2014). Islets continually sample blood from the branches of the splenic and pancreaticoduodenal arteries (Gray's Anatomy 1995) and react to elevated dietary nutrients by directly secreting insulin from β-cells into the blood stream or glucagon from α-cells (in response to nutrient-deprived states such as fasting and starvation). Interestingly, β-cells are the most studied cell of the islet and account for ~50% of the islet cell mass in humans, while α-cells comprise about 35–40%, with the remainder made up of somatostatin-secreting δ-cells, γ-cells and PP-cells that play a regulatory function (Cabrera *et al.* 2006, Newsholme *et al.* 2011, Rorsman & Braun 2013, Wierup *et al.* 2014). As β-cells are responsible for the biosynthesis and distribution of insulin into the blood stream, they have an important clinical impact. Diminished glucose-stimulated insulin secretion (GSIS) and β-cell failure correlate with DM development (Jensen *et al.* 2008, Newsholme & Krause 2012), and consequently it is important to determine the metabolic stimulus–secretion coupling factors that influence β-cell insulin production and release, with a view to develop novel therapeutic strategies.

However, the progression of DM does not solely rest with β-cell dysfunction, as IR is a major contributor and a condition normally associated with obesity. Importantly, downstream of β-cell insulin production/release, receptor binding and insulin signalling in target tissues and organs are essential for metabolic responses. Failure to respond adequately to insulin, following ligation of its receptor in target tissues, results in excess blood glucose. This is a key determinant in the development of T2DM and ultimately overwhelms the capacity of pancreatic β-cells to produce sufficient insulin, contributing to excessive blood glucose. The present review explores the metabolic factors that regulate insulin biosynthesis and release from pancreatic β-cells and also describes the metabolic consequence of attenuated insulin action in target tissues. This review unites the metabolic facets of insulin secretion and action that are pertinent to the development of DM.

Metabolic regulation of β-cell insulin secretion

Biochemical mechanisms of insulin secretion

Insulin exocytosis is a highly controlled process, and many factors actively promote insulin release (refer to Fu *et al.* (2013) for more detail). As carbohydrates are normally the primary source of fuel in food and glucose is the primary insulin secretagogue (see Flatt & Lenzen (1994) and Fu *et al.* (2013)), traditional models of insulin exocytosis are based on an increase in the β-cell intracellular ATP:ADP ratio, following elevated glucose metabolism. Enhanced flux through the glycolytic pathway and tricarboxylic acid cycle (TCA) results in elevated mitochondrial ATP generation, following substrate-level phosphorylation and electron transport in the mitochondria utilising the electron donors NADH and $FADH_2$. The enhanced ATP:ADP ratio induces plasma membrane depolarisation by closure of β-cell K^+_{ATP}-sensitive channels, and subsequently the opening of voltage-gated calcium channels (Fig. 1; Jensen *et al.* 2008, Newsholme & Krause 2012). The resultant influx of Ca^{2+} leads to insulin export through fusion of a readily releasable pool of insulin-containing vesicles with the plasma membrane (Komatsu *et al.* 2013). This triggering mechanism of K_{ATP}-dependent GSIS is responsible for the first phase of the insulin secretory response, over 5–10 min, but the second, more sustained phase of insulin release over a period of 30–60 min is absolutely dependent on metabolic stimulus–secretion coupling and was first described in rat and mouse β-cells in 1992 (Fig. 2). Experimentally, when K^+_{ATP}-sensitive channels were prevented from closing by addition of diazoxide (Gembal *et al.* 1992, Sato *et al.* 1992) in the presence of glucose, insulin release was still possible. This was also demonstrated in mice with genetically disrupted or deleted K^+ channels and pointed to an additional secretory mechanism that regulated sustained insulin release (Miki *et al.* 1998, Remedi *et al.* 2006). Termed K_{ATP}-independent GSIS, this mechanism is initiated by TCA intermediates and associated products (anaplerosis), phospholipase C/protein kinase C (PKC) signalling, alterations in intracellular levels of lipids and/or elevation in cAMP levels, together enhancing cytosolic Ca^{2+} flux and exocytosis (Sugden & Holness 2011, Komatsu *et al.* 2013). Furthermore, various reports have suggested that multiple coupling factors may amplify K_{ATP}-independent GSIS, such as NADPH, NADH, glutamate and malonyl-CoA (Komatsu *et al.* 2001). As the precise biochemical mechanisms of K_{ATP}-dependent and -independent GSIS are not the sole focus of this article, readers are advised to refer to recent review

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DOI: 10.1530/JOE-13-0616

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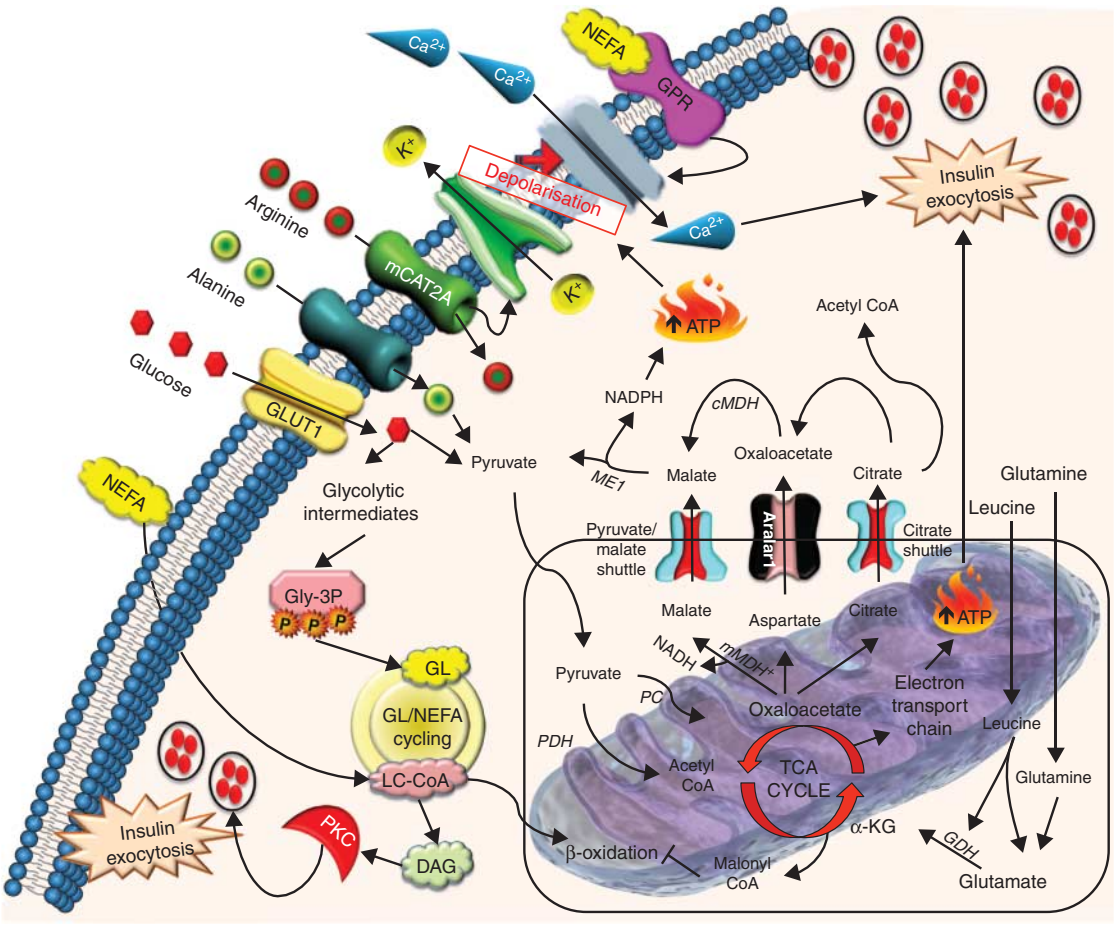

Figure 1
Mechanisms of nutrient stimulus–secretion coupling in the pancreatic β-cell. Nutrients promote insulin exocytosis by a variety of mechanisms, including plasma membrane depolarisation by enhancement of the ATP:ADP ratio via glycolytic and TCA metabolism and generation of reducing equivalents and production of lipid signalling molecules that promote vesicle trafficking and calcium influx. GPR, G-protein-coupled receptor; NEFA, non-esterified fatty acids; GL, glycerolipid; Gly-3, glycerol-3-phosphate; LC-CoA, long-chain CoA; DAG, diacylglycerol; PKC, protein kinase C; TCA, tricarboxylic acid; α-KG, α-ketoglutarate; *PDH*, pyruvate dehydrogenase; *PC*, pyruvate carboxylase; *GDH*, glutamate dehydrogenase; *ME1*, malic enzyme 1; *cMDH*, cytosolic malate dehydrogenase; *mMDH*, mitochondrial malate dehydrogenase.

articles (Fu *et al.* 2013, Komatsu *et al.* 2013) for more detail. However, it is clear that β-cell nutrient metabolism is central and critical to the insulin secretory mechanism described above, and consequently elevated glucose and lipid levels, as observed in T2DM patients, can chronically impact insulin secretion. Thus, the impact of specific nutrient groups on pancreatic β-cell insulin secretion will be discussed.

Carbohydrate metabolism and insulin secretion

The 'fuel-sensing' β-cell is exquisitely designed to release insulin when stimulated by dietary nutrients, particularly glucose. Several adaptions allow continuous monitoring of the plasma glucose load, and these are coupled to rapid oxidative and anaplerotic metabolism, which transduces the elevated nutrient signal and maximises ATP generation for insulin exocytosis. These features include the ability to 'sense' glucose in the physiological range with high K_m glucose transporters and the 'glucokinase' enzyme (2–20 mmol/l), reduced expression of lactate dehydrogenase (LDH), high expression of redox shuttles to regenerate reducing equivalents, and increased pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) activity, that ensure efficient oxidative metabolism in the presence of high glucose (Newsholme & Krause 2012).

The influx of glucose is regulated by specific insulin-independent GLUT membrane transporter proteins (GLUT1 (SLC2A1) in human and GLUT2 in rodent β-cells) (De Vos *et al.* 1995, McCulloch *et al.* 2011, Rorsman

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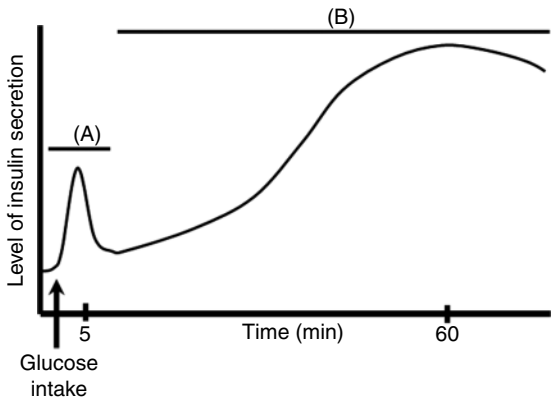

Figure 2
The pancreatic β-cell biphasic insulin secretion response. After administration of stimulatory levels of glucose, β-cell insulin secretion is immediately initiated and follows biphasic kinetics. The initial phase of insulin secretion (A) is rapid and is dependent on generation of ATP and the subsequent intracellular influx of calcium ions. The second, prolonged secretion of insulin (B) is dependent on mitochondrial metabolism, generation of coupling factors and calcium influx.

& Braun 2013). The K_m of these GLUT1 and GLUT2 transporters for glucose is high (6 and 11 mM respectively), indicating that they are only active at high extracellular glucose, as observed in postprandial conditions. Following glucose uptake, glycolytic degradation to pyruvate generates ATP, which is an important stimulus–secretion coupling factor as outlined above. Glucokinase (GCK) is a sophisticated hexokinase enzyme that also acts as a glucose sensor and has a high K_m for glucose (6 mM). However, unlike other hexokinases, it is not inhibited by its product glucose-6-phosphate and maintains high glycolytic flux in the presence of elevated glucose, coupling carbohydrate sensing to insulin secretion in the β-cell (Bedoya *et al.* 1986, Alvarez *et al.* 2002, Newsholme & Krause 2012). Alterations in the activity of important glycolytic enzymes such as GCK and phosphofructokinase can modulate GSIS, and this may lead to impaired glucose metabolism and insulin secretion (Nielsen *et al.* 1998, Westermark & Lansner 2003, Gloyn *et al.* 2005). Furthermore, chronic hyperglycaemic conditions, as observed in T2DM, can negatively regulate the expression of several important glucose metabolising β-cell genes including *SLC2A2*, *GCK*, Ca^{2+} channels and insulin transcription factors pancreatic and duodenal homoeobox 1 (*Pdx1*), neurogenic differentiation 1 (*NeuroD1*) and v-maf musculoaponeurotic fibrosarcoma oncogene homologue A (*MafA*) (Cnop *et al.* 2005, Newsholme *et al.* 2010). In the diabetic state, reduced expression of these gene products leads to reduced glucose utilisation by the β-cells and consequently insulin-sensitive tissues. The decrease in glucose disposal by these tissues maintains elevated plasma glucose, which propagates glucotoxic conditions.

Interestingly, glycolytic intermediates may be diverted from glycolytic processes via the glycerol-3-phosphate (Gly-3-P) shuttles and impact on insulin secretion (Fig. 1). Gly-3-P formation from fructose 1,6-bisphosphate can enhance glycerolipid/non-esterified fatty acid (GL/NEFA) cycling, which promotes insulin secretion via generation of lipid signalling molecules such as long-chain acyl-CoA (LC-CoA) and diacylglycerol (DAG) (Fig. 1; Nolan & Prentki 2008, Newsholme *et al.* 2010). Furthermore, Gly-3-P can be converted to dihydroxyacetone phosphate on entry into the mitochondria by mitochondrial Gly-3-P dehydrogenase (mGPDH), which generates $FADH_2$ and thereby contributes to ATP production (Jitrapakdee *et al.* 2010).

As pancreatic β-cells express low levels of LDH, they mostly recycle NAD^+ by expressing high levels of mitochondrial redox shuttles (NADH/NADPH) such as pyruvate/malate and pyruvate/citrate (Maassen *et al.* 2006). Central to the operation of pyruvate shuttles is the production of oxaloacetate from pyruvate by PC (Fig. 1). Oxaloacetate is converted to malate by mitochondrial malate dehydrogenase and enters the cytosol from the mitochondria. Malic enzyme 1 (ME1) regenerates pyruvate from malate (created from oxaloacetate), while simultaneously creating NADPH (Fig. 1). Pyruvate can then re-enter the mitochondria to continue the process, generating more NADH and increasing ATP levels (Jitrapakdee *et al.* 2010). Alternatively, oxaloacetate can be transferred to the cytosol by first condensing with acetyl CoA (provided by PDH) to form citrate, which is then translocated via the citrate carrier (Fig. 1). Citrate is converted back to oxaloacetate and acetyl CoA, and ME1 generates NADPH as described above, while acetyl CoA promotes NEFA accumulation through malonyl-CoA formation (dependent on acetyl CoA carboxylase (ACC)) and subsequent insulin secretion (Jitrapakdee *et al.* 2010). Understandably, studies have shown that siRNA knockdown of *Pc* (*Pcx*) in murine models reduces β-cell proliferation and GSIS (Hasan *et al.* 2008, Xu *et al.* 2008), while enhanced expression increases GSIS (Xu *et al.* 2008), thus illustrating the importance of key β-cell enzymes for insulin secretion via participation in NADH redox shuttles.

Another vital redox shuttle that regulates reducing equivalent regeneration and consequently ATP production in β-cells is the malate/aspartate shuttle, along with the mitochondrial malate/2-oxoglutarate and

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DOI: 10.1530/JOE-13-0616

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aspartate/glutamate carriers. Cytosolic malate dehydrogenase (cMDH) converts oxaloacetate to malate and NAD^+, and malate enters the mitochondrion via the malate/2-oxoglutarate. Here, it is oxidised back to oxaloacetate by mitochondrial MDH (mMDH), while NAD^+ is reduced to NADH (Fig. 1). Mitochondrial oxaloacetate can then be transaminated to aspartate in the presence of glutamate and return to the cytosol through the aspartate/glutamate carrier, Aralar1 (Fig. 1; Newsholme *et al.* 2007*a*). This carrier plays an important role in mediating GSIS, and deletion in INS-1 β-cells was shown to elicit a complete loss of malate/aspartate shuttle activity and a 25% decrease in insulin secretion (Marmol *et al.* 2009). By contrast, enhanced expression of Aralar1 improves GSIS and amino acid-stimulated insulin secretion in BRIN-BD11 cells (Bender *et al.* 2009).

Finally, current reports have suggested that β-cells express carbohydrate receptors that may directly signal to factors that regulate insulin secretion. Nakagawa *et al.* (2009) have shown that the murine β-cell line MIN6 expressed the functional sweet taste receptors T1R2 (TAS1R2) and T1R3 (TAS1R3), which are normally expressed in enteroendocrine cells and taste buds of the tongue. The MIN6 β-cell line was able to respond to a variety of receptor carbohydrate agonists, such as sucralose, by enhancing insulin secretion. Furthermore, other artificial sweeteners could modulate GSIS in this cell line, and evidence was provided to show that changes in insulin secretion were modulated through alterations in β-cell Ca^{2+} and/or cAMP handling (Nakagawa *et al.* 2013). Although not clearly understood, these data implicate a direct role for carbohydrate-mediated G-protein-coupled receptor (GPR) activity and signalling in the regulation of insulin secretion from the β-cell.

Taken together, carbohydrates impact on insulin secretion through a variety of mechanisms, and central to these is the generation of ATP and enhancement of mitochondrial metabolism including generation of NADH and $FADH_2$. However, metabolism of other nutrient classes such as amino acids and lipids can also impact insulin secretion and this is discussed in the following sections.

Amino acid metabolism and insulin secretion

Anabolic and catabolic metabolism of amino acids is critical for a variety of cellular functions including protein and nucleotide synthesis. However, amino acids as a nutrient source are also key modulators of pancreatic β-cell insulin secretion. They can elicit either positive and/or negative effects on insulin release *in vitro* and *in vivo*, and this is mostly dependent on the amino acid type, duration of exposure and concentration (Newsholme & Krause 2012). Interestingly, amino acids administered alone at physiological concentrations do not modulate GSIS, but supplied in specific combinations at physiological concentrations or individually at elevated concentrations can elevate GSIS (Newsholme *et al.* 2007*a*, Newsholme & Krause 2012). Amino acids regulate both the triggering and amplification pathways of insulin secretion by i) acting as a substrate for the TCA cycle and/or redox shuttles with subsequent generation of ATP, ii) direct depolarisation of plasma membrane by transport of positively charged amino acids into the cell via specific amino acid membrane transporters and iii) co-transport of Na^+ ions along with the amino acid on entry into the cell, resulting in plasma membrane depolarisation (Nolan & Prentki 2008, Newsholme *et al.* 2010).

Glutamine is the most abundant amino acid in blood and extracellular fluids (Nolan & Prentki 2008, Newsholme *et al.* 2010). Cell culture medium is regularly supplemented with glutamine to maintain cell proliferation and function *in vitro*. It is consumed at a rapid rate by many cell types including β-cells (Dixon *et al.* 2003). However, glutamine does not increase insulin exocytosis when administered alone (Dixon *et al.* 2003, Newsholme *et al.* 2010, Newsholme & Krause 2012). Moreover, pharmacological impairment of glutamine metabolism attenuates GSIS (Li *et al.* 2004, Newsholme *et al.* 2010). Thus, the high demand and uptake of glutamine by β-cells indicate that it is essential for other cellular processes, perhaps protein, pyrimidine and purine synthesis (Newsholme *et al.* 2007*a*, Newsholme & Krause 2012). Glutamine metabolism leads to aspartate and glutamate production (Brennan *et al.* 2003), and when administered in combination with leucine, insulin exocytosis is increased through activation of glutamate dehydrogenase (GDH) and entry of glutamine carbon into the TCA cycle (Fig. 1; Henquin 2000, Newsholme *et al.* 2007*a*), enhancing the formation of reducing equivalents and activation of mitochondrial carrier proteins (Fig. 1; Sener & Malaisse 1980, Nolan & Prentki 2008).

Production of glutamate from glutamine may also contribute to β-cell antioxidant defence with entry into the γ-glutamyl cycle, thereby enhancing glutathione synthesis (Brennan *et al.* 2003, Newsholme & Krause 2012). Therefore, glutamine derivatives possibly protect β-cells from oxidative insult. Interestingly, glutamate may also play a significant role in mediating insulin secretion directly, but the exact mechanisms are not entirely clear due to inconsistent observations published in the

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DOI: 10.1530/JOE-13-0616

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literature. Increased glutamate levels have been detected following exposure to glucose in islets and β-cell lines (Brennan *et al*. 2002, Broca *et al*. 2003), but others did not detect any significant changes (Danielsson *et al*. 1970, MacDonald & Fahien 2000). Indeed, glutamate can accumulate within insulin vesicles and, potentially, be transported into the surrounding matrix during insulin exocytosis (Hoy *et al*. 2002, Newsholme & Krause 2012). Glutamate released in this way may influence β-cell glutamate receptor activation (Corless *et al*. 2006). In addition, it may regulate glucagon secretion from adjacent glutamate-sensitive pancreatic α-cells and is possibly an additional paracrine regulatory mechanism for maintenance of blood carbohydrate levels (Corless *et al*. 2006).

Alanine and arginine have also been noted to stimulate insulin secretion significantly. We have consistently shown that alanine is consumed by β-cell lines and islet cells and increases insulin secretion (Dixon *et al*. 2003, Newsholme *et al*. 2010, Newsholme & Krause 2012, Salvucci *et al*. 2013), findings that are supported by observations from other β-cell lines including murine and human (Dunne *et al*. 1990, McCluskey *et al*. 2011, Kasabri *et al*. 2012). More recently, we have created an integrated mathematical model which predicted that increased intracellular ATP and Ca^{2+} levels were critical for glucose plus amino acid-stimulated insulin secretion in BRIN-BD11 cells (Salvucci *et al*. 2013). Furthermore, additional analyses demonstrated that alanine-mediated Na^+ co-transport acted synergistically with membrane depolarisation and led to K^+_{ATP}-independent Ca^{2+} influx (McClenaghan *et al*. 1998, Newsholme & Krause 2012, Salvucci *et al*. 2013). However, the mechanism of action of alanine-induced insulin secretion is multifactorial and includes conversion to pyruvate (Salvucci *et al*. 2013), glutamate, aspartate and lactate (Fig. 1; Newsholme *et al*. 2010).

Arginine-induced insulin secretion is dependent on changes in plasma membrane potential, leading to opening of Ca^{2+} ion channels, Ca^{2+} influx and ultimately insulin exocytosis (McClenaghan *et al*. 1998, Sener *et al*. 2000, Newsholme & Krause 2012). Arginine, a positively charged amino acid, enters the β-cell via the electrogenic transporter mCAT2A causing direct depolarisation of the membrane (Fig. 1; Newsholme & Krause 2012). Interestingly, physiological concentrations of arginine have a cytoprotective role and attenuate cytokine-mediated apoptosis in β-cells, while partially boosting insulin secretion (Krause *et al*. 2011). This was facilitated by the conversion of arginine to glutamate, with enhancement of antioxidant levels (Krause *et al*. 2011). However, negative effects of high concentrations of arginine have been reported and these stem from the effect of enhancing velocity of inducible nitric oxide synthase (iNOS) through substrate stimulation, which may be harmful to the β-cell if cellular antioxidant defences are overwhelmed (Newsholme & Krause 2012).

Finally, branched-chain amino acids (BCAAs), consisting of leucine, isoleucine and valine, are also reported to play an influential role in mediating insulin exocytosis (Newsholme & Krause 2012, Gaudel *et al*. 2013), while enhanced plasma levels correlated with increased IR in the presence of elevated lipids (Newgard 2012, Lu *et al*. 2013). Interestingly, consumption of dairy products, which are a rich source of BCAAs, has been associated with improvements in both weight loss and T2DM management (Tremblay & Gilbert 2009, Jakubowicz & Froy 2013); consumption of whey protein hydrolysates have been suggested to improve fasting insulin levels, insulin release and glycaemic control in *in vivo* animal models and in obese and T2DM human subjects (Gaudel *et al*. 2013, Jakubowicz & Froy 2013). However, the precise mechanism of these positive effects are not fully understood, but are believed to involve increased protein synthesis and possibly thermogenesis via activation of mammalian target of rapamycin (mTOR) signalling, increased anaplerosis and, in the case of leucine, enhanced allosteric activation of GDH that leads to increased TCA activity in the β-cell (Fig. 1; Yang *et al*. 2006, Newsholme *et al*. 2010, Jakubowicz & Froy 2013). Conversely, recent reports utilising metabolomic profiling have suggested that BCAA catabolism is associated with decreased insulin sensitivity in obese patients (Newgard *et al*. 2009). Comparable *in vivo* data showed that while animals on a high-fat diet with BCAAs (HF/BCAAs) did not consume as much food as animals receiving high fat alone (HF), they were as insulin resistant as this HF group, but retained a similar body weight to that of standard chow animals. In addition, chronic activation of mTOR by BCAAs, along with increased phosphorylation of insulin receptor substrate 1 (IRS1) in skeletal muscle, potentially promoted IR (Newgard *et al*. 2009). The accumulation of acylcarnitines from BCAA catabolism and their interplay with these signalling mechanisms appear to be of critical importance. However, while progress has been made to identify the precise effects of BCAAs on insulin release and action, more work is required to fully understand the mechanisms underlying the links between BCAAs and the development of metabolic disease. In conclusion, published evidence suggests that several key amino acids play a crucial role in mediating insulin secretion in a range of β-cell models and that these effects

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are mediated through a variety of biochemical and physiological mechanisms. Potentially, the positive effects of amino acids may be harnessed to combat DM progression and aid DM management.

Lipid metabolism and insulin secretion

Lipids and NEFAs are crucial for β-cell function and insulin release, but elevated extracellular levels, usually associated with dyslipidaemia, are also associated with IR, β-cell failure and T2DM (Nolan *et al.* 2006*a*). In fasting or starvation, lipids are metabolised by β-oxidation in the mitochondria to produce ATP in many cells and tissues including liver and muscle (Newsholme *et al.* 2010). Entry into the mitochondria, where β-oxidation occurs, is regulated by a series of enzymatic and shuttle mechanisms (Hamilton & Kamp 1999). Cytosolic NEFAs are first converted to LC-CoA by acyl-CoA synthase (ACS) and then translocated to the mitochondrial matrix by the action of carnitine palmitoyltransferases 1 and 2 (CPT1 and CPT2) (Berne 1975, Newsholme & Krause 2012). In the matrix, LC-CoA molecules are oxidised to produce CO_2, NADH and $FADH_2$, and thus ATP. In the pancreatic β-cell, in the presence of sufficient nutrients, NEFAs can influence insulin secretion by three distinct metabolic signalling mechanisms. This 'trident model' was first described by Nolan *et al.* (2006*a*) and includes TCA/malonyl-CoA metabolic signalling, GL/NEFA cycling and direct activation of GPRs (Fig. 1).

CPT activity and TCA/malonyl-CoA metabolism are closely connected to the regulation of β-oxidation. In the presence of NEFAs and excess carbohydrate, CPT activity is directly inhibited by the formation of malonyl-CoA from TCA intermediates by ACC (Fig. 1; Carpentier *et al.* 2000, Nolan *et al.* 2006*a*). Subsequently, accumulation of cytosolic lipids can enhance insulin secretion by i) altering the activity of regulatory ion channel proteins, ii) increasing Ca^{2+} influx, iii) generating insulinotropic lipids including LC-CoA and DAG and iv) enhancing insulin vesicle interaction with the plasma membrane (Deeney *et al.* 2000, Haber *et al.* 2006, Newsholme *et al.* 2010, Newsholme & Krause 2012). Crucially, AMP-activated protein kinase (AMPK) is central to the regulation of NEFA metabolism and reduces the levels of malonyl-CoA by inhibiting ACC and enhancing malonyl-CoA decarboxylase (MCD) activity (Ruderman & Prentki 2004, Nolan *et al.* 2006*a*, Newsholme & Krause 2012). AMPK is sensitive to the cell energy status and is stimulated by a high AMP:ATP ratio, and thereby increasing β-oxidation (Nolan *et al.* 2006*a*). Interestingly, overexpression of MCD (MLYCD) in the presence of NEFAs significantly reduced GSIS in INS832/13 β-cells and islets, which demonstrated the importance of AMPK, malonyl-CoA and lipid metabolism to insulin secretion (Mulder *et al.* 2001, Roduit *et al.* 2004, Nolan *et al.* 2006*a*).

Cycling of GL/NEFA in β-cells also impacts upon insulin exocytosis and is a convergence point of glucose and NEFA metabolism. Formation of Gly-3-P from inbound glucose (25%) and generation of NEFAs, GLs and LC-CoA by lipolysis amplify the insulin secretory response in elevated glucose (Fig. 1). NEFA esterification and lipolysis can be initiated simultaneously in β-cells by glucose challenge in the presence of NEFA, while β-oxidation is inhibited (Nolan *et al.* 2006*a*,*b*). This mechanism provides insulinotropic lipid signalling molecules that aid vesicle manufacture and exocytosis. Specifically, DAG and LC-CoA enhance the exocytotic function of key vesicle priming and docking proteins such as MUNC13, synaptosomal-associated protein 25 (SNAP25) and synaptotagmin, while also modulating signal transduction by PKC activity (Fig. 1; Nolan *et al.* 2006*a*, Newsholme *et al.* 2007*b*, Nolan & Prentki 2008, Newsholme *et al.* 2010, Rorsman & Braun 2013). The main advantages of GL/NEFA cycling are that conversion of glucose to Gly-3-P protects β-cells and deviates inbound glucose carbon away from excessive oxidative metabolism, and offers an alternative mechanism to generate supplementary secretion coupling factors that are independent of TCA cycle and oxidative processes (Nolan & Prentki 2008).

Lipids also augment insulin secretion through activation of GPRs (Shapiro *et al.* 2005), and recent reports have demonstrated that they are highly expressed in β-cells (Shapiro *et al.* 2005, Tomita *et al.* 2006, Newsholme & Krause 2012). Isoforms including GPR40 (FFAR1), GPR41 (FFAR3), GPR119 and GPR120 (FFAR4) are important in β-cell physiology (Newsholme & Krause 2012), and reduced levels or knockdown in rat β-cells, islets and Gpr40-deficient mice was shown to lower NEFA-induced amplification of GSIS (Itoh *et al.* 2003, Latour *et al.* 2007). It is believed that NEFA activation of GPRs leads to GSIS amplification through alterations in Ca^{2+}-handling mechanisms, including efflux from the endoplasmic reticulum (ER) (Nolan & Prentki 2008), and appears to be dependent on glucose-mediated activation of L-type calcium channels (Fig. 1; Shapiro *et al.* 2005, Nolan & Prentki 2008). Interestingly, current evidence has examined the use of GPR agonists as potential therapeutic treatments for hyperglycaemia in T2DM patients, and TAK-875 was shown to reduce HbA1c and hypoglycaemic

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DOI: 10.1530/JOE-13-0616

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effects in DM patients (Burant *et al.* 2012). Indeed, dietary supplementation with ω3 fatty acids may also promote insulin sensitisation and anti-inflammatory effects, as shown in obese mice models, and this appears to be facilitated by interaction with GPR120 (Oh *et al.* 2010).

The insulinotropic effects of NEFAs are dependent on lipid type, level of saturation, length of the carbon chain, and whether treatment is under acute or chronic conditions (Newsholme & Krause 2012). Palmitic acid and stearic acid, both saturated NEFAs, can chronically decrease GSIS *in vitro* (Hosokawa *et al.* 1997, Keane *et al.* 2011), while unsaturated oleic acid or arachidonic acid enhances insulin secretion (Vassiliou *et al.* 2009, Keane *et al.* 2011). However, prolonged exposure of β-cells to high circulatory lipid levels, such as in T2DM, impairs glucose oxidation and consequently results in an increased AMP:ATP ratio and AMPK activation (Newsholme & Krause 2012). As fatty acid oxidation is promoted, fatty acid synthesis is inhibited along with NEFA-mediated amplification of insulin secretion (Newsholme & Krause 2012). In this scenario, AMPK functions to promote oxidation and avoids the toxic effect of the incoming lipids, mirroring the effect of physical activity, but at the cost of decreased insulin secretion (Towler & Hardie 2007). This is particularly important when high glucose and high lipid levels are present together, potentially contributing to glucolipotoxicity.

In addition, inhibition of AMPK activity leads to cytosolic accumulation of lipids and these may also promote lipotoxicity by inducing ER stress and ceramide formation (Lang *et al.* 2011). Studies have shown that chronic palmitic acid exposure has a deleterious effect on β-cell ER morphology, depletes ER Ca^{2+} levels and causes increased NEFA esterification, which impair processing/transport functions of the ER and ultimately cause ER stress (Cnop 2008, Cunha *et al.* 2008). Moreover, ceramide has been shown to be toxic to β-cells and islets (Lupi *et al.* 2002, Lang *et al.* 2011). The precise pro-apoptotic mechanism is not fully understood, but ceramide is thought to contribute to detrimental cell signalling (Lang *et al.* 2011).

The chronic intracellular accumulation of lipids, particularly in the presence of high glucose, can elicit damaging effects in β-cells through excessive reactive oxygen species (ROS) generation by increased TCA metabolite turnover, enhanced electron transport chain activity and elevated ER stress (conditions resulting in glucolipotoxicity (for further details, see review Newsholme *et al.* (2012*b*))). Excessive ROS can activate key inflammation pathways including nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) and c-Jun NH_2-terminal kinase (JNK) signalling (Morgan & Liu 2011). It is widely accepted that inflammation is a major mediator of islet dysfunction not only in T1DM, but also in T2DM (Turley *et al.* 2003, Donath & Shoelson 2011, Bending *et al.* 2012). The inflammatory pathophysiology of pancreatic islets in T1DM and T2DM is characterised by the presence of immune cell infiltration, apoptotic islet cells, high expression of cytokines or adipokines (e.g. interleukin 1β (IL1β, IL1B)), tumour necrosis factor α (TNFα (TNF), leptin) and amyloid deposits (islet amyloid polypeptide, IAPP) (Donath *et al.* 2008). In fact, some researchers have reported that hyperglycaemic conditions can promote IL1β production from pancreatic islets (Maedler *et al.* 2002, Böni-Schnetzler *et al.* 2008), but this remains controversial, as others have failed to observe a similar response (Welsh *et al.* 2005). Interestingly, IAPP has been detected in 90% of T2DM islets postmortem (Clark *et al.* 1988, Back *et al.* 2012), and we have shown that IAPP oligomers can activate and induce IL1β production from dendritic cells and macrophages *in vitro* (Masters *et al.* 2010). Other researchers have demonstrated that ceramide can promote IL1β production from macrophages in high-fat diets (Vandanmagsar *et al.* 2011) and that NEFAs can activate the NLRP3 inflammasome in haematopoietic cells leading to IR (Wen *et al.* 2011). These studies elegantly illustrate the connection between nutrient metabolism and inflammation. Our own recent work has revealed that activation of the inflammasome is dependent on both glucose and fatty acid metabolism in macrophages (Masters *et al.* 2010). The inflammasome is a protein complex that is responsible for generation of IL1β and IL18 from their immature structure to the active, mature state. Masters *et al.* also observed that both glucose and minimally modified LDL (mmLDL), which is elevated in T2DM (Yano *et al.* 2004), were required for full IAPP-mediated 'priming' of NLRP3 inflammasomes in bone marrow-derived macrophages. The precise mechanism behind these observations remains unknown. However, interplay with *Tlr4* appears to be vital, as 'priming' was not possible in C3H/HeJ mice with non-functional *Tlr4* (Masters *et al.* 2010).

The role of the inflammasome in complex diseases has been reviewed recently (Masters 2013), and these data clearly implicate that nutrient metabolism plays an important function in mediating islet inflammation and possibly pancreatic β-cell death. Nutrient effects in β-cells are complex and elevated glucose and NEFA levels are primary risk factors in relation to inflammation, obesity and T2DM (Cunha *et al.* 2008). However, the impact of excess nutrients, high insulin and elevated IR in

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insulin-sensitive tissues is also critical in the progression of DM. Consequently, the influence of insulin action in target tissues and organs is discussed next.

Nutrient metabolism and insulin action in target tissues

Skeletal muscle, insulin and nutrients

Skeletal muscles play a major role in glucose metabolism, being responsible for ~75% of whole-body insulin-stimulated glucose uptake (Shulman *et al*. 1990, Corcoran *et al*. 2007). Maintenance of skeletal muscle represents an important factor related to quality and longevity of life.

A variety of muscle cell functions can be altered by chemical and mechanical stimuli, establishing a cause and effect in relation to glucose homoeostasis and insulin signalling (Newsholme *et al*. 2012*a*). In both T1DM and T2DM, skeletal muscle cells show an imbalance between protein synthesis and degradation, resulting in increased myofibrillar protein breakdown and muscle wasting (Russell *et al*. 2009), with a concomitant increase in glycated end products and vascular complications (Newsholme *et al*. 2011, Krause *et al*. 2012).

Once released by pancreatic β-cells into the circulation, insulin initiates its anabolic effects through binding of the transmembrane insulin receptor (IR) in target tissues. The IR is a heterotetrameric tyrosine kinase receptor composed of two chain subunits (α- and β-chains) and is a member of the growth factor receptor family (Fig. 3). This interaction promotes the autophosphorylation of the receptor and activation of intracellular proteins known as insulin receptor substrates (IRSs). There are more than 13 different IRSs; however, isoforms 1 and 2 deserve more attention as they are widely distributed among different cell types and mainly activated in muscle tissue (Corcoran *et al*. 2007). IRS1, and to a lesser extent IRS2, promotes the phosphorylation of phosphoinositide 3-kinase (PI3K) and subsequent intracellular events, resulting in 3-phosphoinositide-dependent protein kinase 1 (PDK1) activation. This pathway is related to the glucose transport machinery via migration and activation of protein kinase B (PKB/Akt) and atypical PKC (aPKC) (White 2003). While there are three isoforms of *Akt*, *Akt2* is recognised as the most abundant isoform in insulin-sensitive tissues, promoting the translocation of GLUT4 to the cell membrane and, consequently increasing glucose uptake from the blood (Taniguchi *et al*. 2006). The essential role of *Akt2* in GLUT4 translocation was highlighted in *Akt2* knockout mice, which exhibited increased IR (Cho *et al*. 2001).

Skeletal muscle protein synthesis is highly responsive to *Akt* signalling, with several effector pathways acting downstream, such as mTOR (Nader 2007), and the negative regulator of glycogen synthesis, glycogen synthase kinase 3 (GSK3), resulting in enhanced glycogen synthesis (van der Velden *et al*. 2007). Experimentally, it was shown that cultured myotubes exposed to growth factors demonstrated hypertrophy, and this was stimulated by pathways downstream of Akt/mTOR and resulted in attenuation of GSK3 activity (Rommel *et al*. 2001). Many initiation factor complexes (e.g. eIF2 and eIF4F), which are assembled from multiple subunits, are sensitive to activation by the mTOR cascade (Sartorelli & Fulco 2004) or indirectly stimulated by an increase in cell volume that is promoted by glucose transport and storage (Usher-Smith *et al*. 2009). These pathways can also be activated by other extracellular nutrients, such as amino acids, especially leucine. More recently, glutamine availability was identified as a limiting step for the mTOR complex 1 (mTORC1) activation pathway, a major regulator of cell size and tissue mass in both normal and diseased states (Nicklin *et al*. 2009). Moreover, mTORC1 activation has been related to increased transport of amino acids in skeletal muscle, and thus protein synthesis (Fig. 3).

In skeletal muscle, amino acid transport can occur through systems such L-type (Na^+-independent), A-type (Na^+-dependent), proton-coupled amino acid transporter (PAT-type) and cationic amino acid transporter (CAT-type) (Palacin *et al*. 1998, Drummond *et al*. 2010, Nicastro *et al*. 2012). L- and A-type systems are sensitive to mTORC1 activation, which in turn may be activated by insulin and other growth factors (Roos *et al*. 2009, Drummond *et al*. 2010). In mammalian cells, mTORC1 is essential for the phosphorylation and activation of S6K1, which directly impacts cell growth via eukaryotic initiation factor translation complexes (Ma *et al*. 2008). AMPK will attenuate energy-requiring processes, such as protein synthesis, by interfering with anabolic signalling mediated by the PI3K-Akt-mTOR-S6K1 cascade, resulting in inhibition of protein synthesis (Ohanna *et al*. 2005, Nader 2007). Hence, glucose and amino acid availability may impact insulin-mediated effects on muscle protein synthesis (Fig. 3).

The plasma levels of BCAAs and urea production are increased markedly with increased efflux of amino acids from muscle to splanchnic tissues (Nair *et al*. 1995, Newsholme *et al*. 2011). However, the plasma and muscular concentrations of some amino acids such as γ-aminobutyric acid (GABA), arginine and glutamine (Menge *et al*. 2010) are decreased in both insulin-resistant

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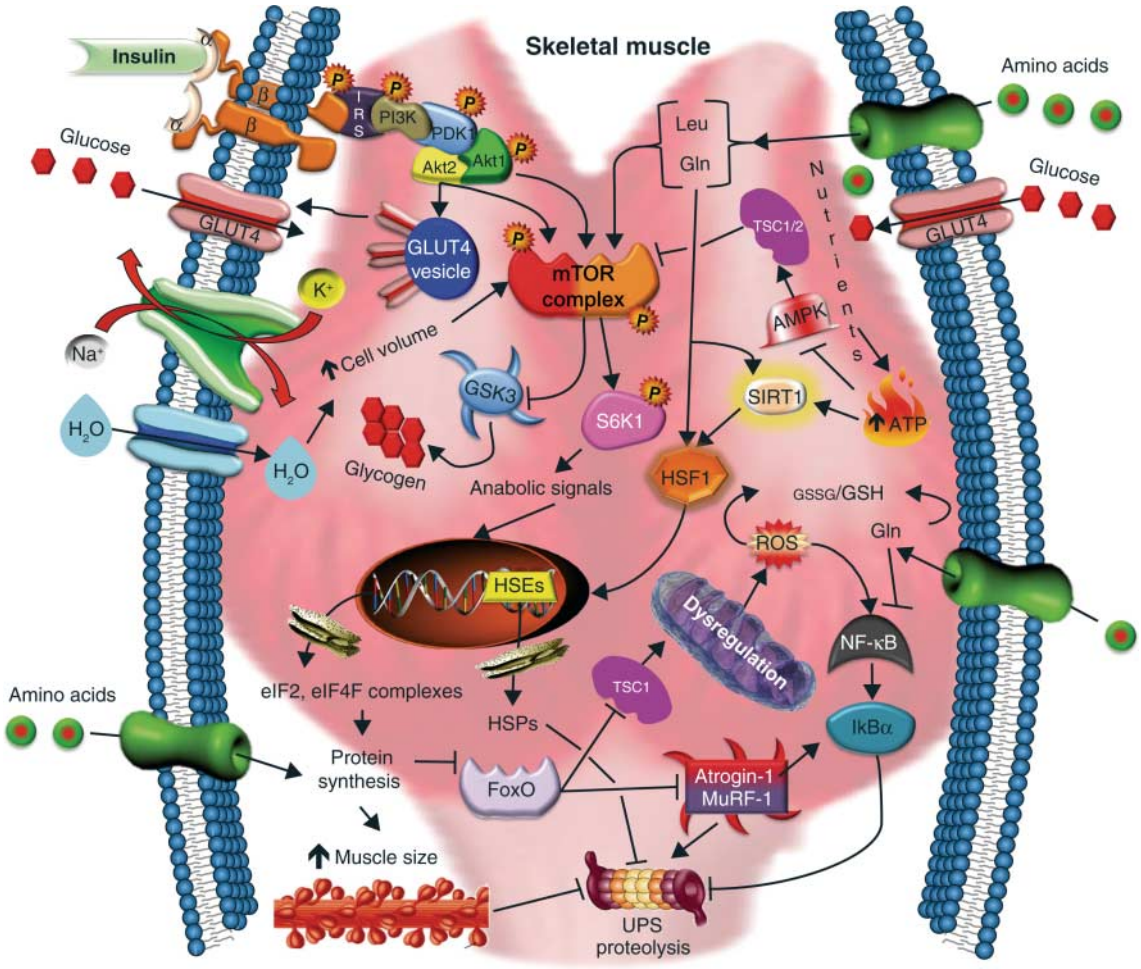

Figure 3

Canonical pathway mediated by insulin in skeletal muscle protein synthesis and degradation. Insulin binds to the insulin receptor, which initiates subsequent intracellular phosphorylation events through the PI3K-Akt-mTOR-S6K1 pathway, inhibiting the negative regulator of glycogen synthesis, GSK3, and stimulating glucose transport inside the cell. The gene expression of initiation factors begins to promote protein synthesis and inhibit protein degradation by FoxO–Atrogin1–MuRF1–UPS. Moreover, protein synthesis is dependent on amino acid availability and transport, especially leucine and glutamine. Glutamine may serve as a substrate for the *de novo* synthesis of GSH and possibly in the heat shock protein response via nutrient sensors such as SIRT1. IRS, insulin receptor substrate; PI3K, phosphoinositide 3-kinase; PDK1, phosphoinositide-dependent kinase 1; AKT1, protein kinase B or serine/threonine protein kinases 1; AKT2, protein kinase B or serine/threonine protein kinases 2; GLUT4, glucose transporter 4; mTOR, mammalian target of rapamycin; GSK3, glycogen synthase kinase 3; S6K1, ribosomal S6 kinase 1; Leu, L-leucine; Gln, L-glutamine; HSF1, heat shock factor 1; HSEs, heat shock elements; HSPs, heat shock proteins; eIF2, eukaryotic initiation factor 2; eIF4F, eukaryotic initiation factor 4F; FoxO, Forkhead box O; UPS, ubiquitin–proteasome system; TSC1/2, tuberous sclerosis 1 and 2; MURF1 (TRIM63), muscle RING-finger protein 1; ROS, reactive oxygen species; GSSG, GSH, glutathione; AMPK, AMP-activated protein kinase; SIRT1, sirtuin 1; NFκB, nuclear factor κ-light-chain-enhancer of activated B cells; IkBα, nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, alpha.

and diabetic conditions, independent of the stage of the disease (Wijekoon *et al*. 2004, Menge *et al*. 2010, Newsholme *et al*. 2011).

The tripeptide glutathione (GSH, L-γ-glutamyl-L-cysteinylglycine) is the most important non-enzymatic soluble intracellular antioxidant and has many protective and metabolic functions in cellular metabolism, including attenuation of oxidative stress and inflammation (Cruzat & Tirapegui 2009, Cruzat *et al*. 2010). However, *de novo* synthesis of GSH is dependent on glutamine, the immediate precursor of glutamate (Cruzat *et al*. 2013). Recently, Krause *et al*. (2012) revealed that T2DM patients exhibited lower concentrations of nitrite and nitrate in skeletal muscle, which may contribute to elevated IR and partially explain muscle wasting in these circumstances (Newsholme *et al*. 2011). Moreover, the altered redox state of the cell and high inflammatory profile observed in diabetes may lead to activation of JNK, which is a key

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player in the progression of impaired insulin signalling in tissues such as skeletal muscle (Sabio *et al*. 2010, Zhang *et al*. 2011). Furthermore, glutamine is a potent modulator of the heat shock protein (HSP (HSP90B2P)) response, through the activation of the glucosamine pathway (Hamiel *et al*. 2009) and phosphorylation of eIF2. Indeed, glutamine may increase HSP70, BCL2 expression and GSH content, which may reduce TNFα- and IL1β-induced injury and inflammation, an effect partially dependent on nitric oxide (NO) production (Newsholme *et al*. 2011). Although the mechanism is not completely understood, recent work has demonstrated that other HSPs, such as HSP27 (HSPB1) and HSP90 (HSP90AA1), operate as chaperones promoting protection (Cruzat *et al*. 2013, 2014). HSPs provide a protein management system including quality control of damaged proteins, such as those impacted by oxidation and degradation, via the ubiquitin–proteasome system (UPS) (Pratt *et al*. 2010).

Adipose tissue and the liver

Low-grade chronic inflammation is clearly linked to obesity-related metabolic diseases. The obesity/type 2 diabetes and inflammation connection was firmly established when, in 1993, a report was published demonstrating that the expression of TNFα in adipose tissue in mice was increased during the development of obesity, but on blocking TNFα, IR was attenuated (Hotamisligil *et al*. 1993). It was subsequently determined that TNFα suppressed insulin signalling by inhibiting insulin receptor tyrosine kinase activity (Hotamisligil *et al*. 1996), thus attenuating insulin-driven changes in cell function and metabolism. Normally, insulin will drive fatty acid incorporation into triacylglycerol, utilising the substrate of Gly-3-P, which is derived from glucose. This ensures a coordinated uptake of fatty acids and glucose into adipose tissue, followed by esterification into triacylglycerol. Clearly, any interference in this process will lead to elevated circulating levels of glucose and fatty acids. Adipose tissue will release NEFAs and glycerol, under the action of various lipases, in periods of fasting, starvation and exercise.

In addition to secreted metabolites, adipose tissue can produce and release endocrine factors (adipokines). Leptin was identified in 1994 as a secretory bioactive molecule, which impacted food intake and energy expenditure through neuroendocrine circuits in the hypothalamus (Zhang *et al*. 1994). Adipokines are now known to alter the insulin sensitivity of the major insulin-sensitive organs, including liver and skeletal muscle, via the circulation. An impressively large number of adipokines have been identified (Tateya *et al*. 2013). TNFα and IL6 may be considered as adipokines under appropriate circumstances. TNFα activates pro-inflammatory signalling but attenuates insulin receptor signalling (Tateya *et al*. 2013), thus being the major factor linking adipose tissue inflammation with IR. By contrast, some other adipokines can promote insulin sensitivity and better control glucose homoeostasis. Adiponectin is an example of an adipokine that positively regulates insulin sensitivity. Adiponectin-deficient mice are severely insulin resistant (Tateya *et al*. 2013). Chronic adipose tissue inflammation can result in impairment of adipokine secretion, leading to systemic IR.

Macrophage accumulation in adipose tissue Macrophages are known to accumulate in adipose tissue in obesity and release pro-inflammatory cytokines such as TNFα, which then impact on adipose tissue metabolism, including glucose and lipid metabolism (Tateya *et al*. 2013). TNFα and IL6 inhibit lipoprotein lipase, thus elevating triacylglycerol concentration in the blood and TNFα additionally stimulates hormone-sensitive lipase in adipose tissue, resulting in NEFA release into the blood. TNFα also reduces insulin-stimulated glucose uptake via effects on GLUT4 translocation, as a result of impaired insulin signalling. All of these effects will tend to reduce lipid accumulation within adipose tissue, but increase blood lipid levels. Tateya *et al*. (2013) showed that macrophages defined as F4/80$^+$ CD11b$^+$ are resident in lean adipose tissue, representing 5% of the stromal vascular fraction, but may be increased in obesity by up to 30%. Furthermore, they demonstrated that chronic weight loss reduced the macrophage content in adipose tissue, but fasting or acute weight loss elicited their accumulation.

Inflammatory activation of myeloid cells in the liver Macrophages are terminally differentiated cells of the mononuclear phagocyte system including dendritic cells, circulating blood monocytes and committed myeloid progenitor cells in the bone marrow. Macrophage activation is defined by a model that postulates two separate polarisation states, M1 (pro-inflammatory) and M2 (anti-inflammatory). In M1, classically activated macrophages will be formed on stimulation by inflammatory mediators such as lipopolysaccharide (LPS), TNFα and interferon γ (IFNγ), and in turn release TNFα, IL1 and IL6. In M2, alternatively activated macrophages have vastly reduced inflammatory characteristics but release high levels of anti-inflammatory cytokines, for example, IL10. As the attenuation of

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macrophage M1 activation and the maintenance of M2 activity are believed to be important for appropriate levels of glucose and lipid formation and release from the liver, stimuli which control the formation of one over the other form of macrophage are important for liver metabolism. TNFα, for example, increased hepatic lipogenesis due to a combination of inhibition of intracellular lipases and provision of intracellular fatty acids for triacylglycerol synthesis. Hepatic gluconeogenesis is also increased by TNFα, as well as reduced hepatocyte glycogen content, by a mechanism dependent on NAPDH oxidase 3 activation and ROS generation, leading to elevated blood glucose levels (Gao *et al*. 2010).

Consumption of a high-fat diet activated Kupffer's cells (the resident macrophages of the liver) in mice, resulting in an increased M1-polarised population; an event associated with the pathogenesis of obesity-induced IR and fatty liver disease (Gao *et al*. 2010). Interestingly, chemical deletion of Kupffer's cells improved insulin sensitivity during high-fat feeding. The range of immune cells in the liver is complex and heterogeneous, but it is clear that the Kupffer's cells contribute to both IR and hepatic steatosis.

In summary, diets rich in fat facilitate activation of macrophages, which then exert a negative influence on metabolic processes in both adipose tissue and liver during the onset of DM. Further, DM is associated with the production of monocytes from the bone marrow (Hu *et al*. 2013), thus contributing to inflammation and continued repression of insulin secretion and insulin signalling.

Concluding remarks

In conclusion, carbohydrate, lipid and amino acid metabolism plays an important role in regulating pancreatic β-cell insulin secretion. Furthermore, the nutrient handling capabilities of other insulin-sensitive tissues, such as skeletal muscle, adipocytes and liver, dictate the whole-body nutrient homoeostasis. However, nutrient overconsumption leads to an increased risk of β-cell dysfunction and impaired insulin action that is routinely observed in DM. The startling epidemic rise in obesity and diabesity, due to diminished physical activity and excessive consumption of carbohydrate- and lipid-laden diets, highlights the need for new therapies and strategies to combat increased DM. Consequently, further understanding of the complexities of β-cell function, insulin action and metabolism may generate novel targets and treatments for the metabolic syndrome.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements
We thank the School of Biomedical Sciences and Faculty of Health Sciences, Curtin University, Perth, Western Australia, for provision of excellent research facilities and support.

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Received in final form 5 March 2014
Accepted 13 March 2014
Accepted Preprint published online 25 March 2014

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0616

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