

Signal transduction mechanisms in nutrient-induced insulin secretion

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Summary The knowledge of the mechanism whereby glucose and other fuel stimuli promote the release of insulin by the pancreatic beta cell remains fragmentary. The closure of metabolically sensitive K^+ channels and a rise in cytosolic free Ca^{2+} are key features of beta-cell metabolic signal transduction. However, these two signalling events do not account for the dose dependence of glucose-induced insulin secretion. In fact, recent evidence indicates that there are K_{ATP} channel and Ca^{2+} independent pathway(s) of beta-cell activation which remain to be defined. In this review, we have limited our attention to the recent developments in our understanding of the mode of action of nutrient secretagogues. A particular emphasis is placed in summarising the evidence in support of two new concepts: 1) oscillations in the glyco-

lytic pathway and beta-cell metabolism contribute to the oscillatory nature of beta-cell ionic events and insulin secretion; 2) malonyl-CoA and long chain acyl-CoA esters may act as metabolic coupling factors in beta-cell signalling. Finally, we propose that the altered expression of genes encoding enzymes in the pathway of malonyl-CoA formation and fatty acid oxidation contributes to the beta-cell insensitivity to glucose in some patients with non-insulin-dependent diabetes mellitus. [Diabetologia (1997) 40: S32–S41]

Keywords Insulin secretion, malonyl-CoA, long chain acyl-CoA, fatty acid, acetyl-CoA carboxylase, carnitine palmitoyl-transferase I, glycolytic oscillations, anaplerosis, gene expression, non-insulin-dependent diabetes mellitus.

The secretion of insulin by the pancreatic beta cell is modulated by various nutrients, neurotransmitters and peptide hormones [1]. Glucose is the only nutrient secretagogue capable of promoting alone in vitro the release of insulin at concentrations within its physiological range. Nonetheless, many additional nutrients including fatty acids, amino acids and keto acids influence secretion as well. Thus, the islet of

Langerhans can be viewed as a 'fuel sensor' which simultaneously integrates the signals of many nutrients and modulators to secrete insulin according to the needs of the organism. The unique feature of the beta cell is that it possesses a transduction system for calorogenic nutrient signals which is entirely different from that of neuromodulators or peptide hormones. Indeed, fuel stimuli must be metabolised in the beta cell to cause secretion [2–5]. By contrast, neuromodulators, such as the potent incretin GLP-I [6], influence the secretory process following their interaction with specific cell-surface receptors.

Despite considerable effort, the exact biochemical nature of the signals which couple glucose metabolism to insulin secretion have remained poorly defined. The difficulty of 'cracking the code' of nutrient signalling may stem from the fact that it is perhaps one of the most complex transduction systems which exists because metabolism, whose regulation is so

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Abbreviations: ACC, Acetyl-CoA carboxylase; CPT I, carnitine palmitoyl-transferase I; NEFA, non-esterified fatty acid; LC-CoA, long chain acyl-CoA ester; L-PK, liver-type pyruvate kinase; PC, pyruvate carboxylase; PFK-1, 6-phosphofructo-1-kinase; PFK-2, 6-phosphofructo-2-kinase; NIDDM, non-insulin-dependent diabetes mellitus.

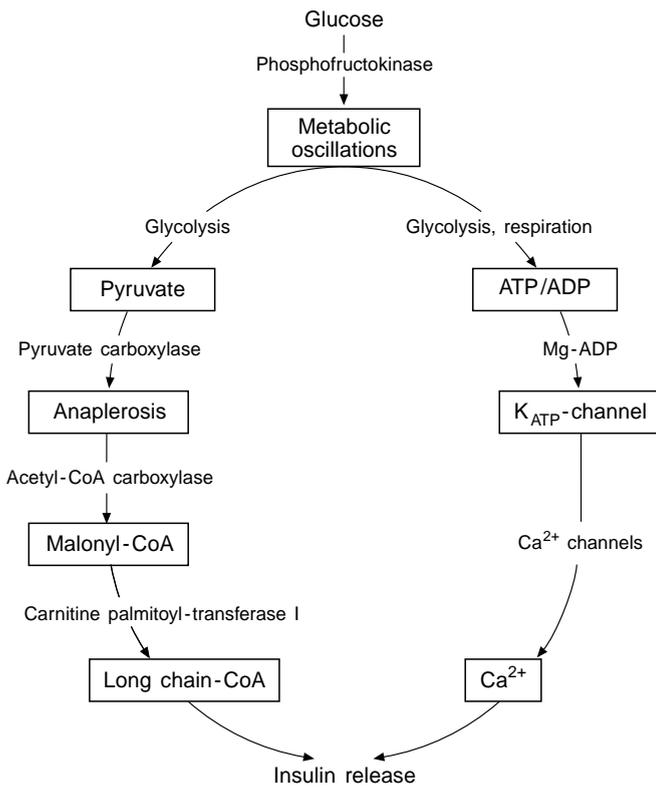


Fig. 1. Model illustrating the role of glycolytic oscillations and the two arms in beta-cell signalling. Oscillations in the metabolism of glucose generate oscillatory O_2 consumption, cytosolic ATP/ADP ratio, K_{ATP}^+ channel opening probability, membrane potential fluctuations and free Ca^{2+} in the cytoplasm. On the other hand, glucose-derived pyruvate is carboxylated to oxaloacetate by pyruvate carboxylase. This anaplerotic reaction favours the formation of malonyl-CoA by acetyl-CoA carboxylase. Malonyl-CoA in turn inhibits carnitine palmitoyl-transferase I with a resulting elevation of long chain acyl-CoA esters in the cytoplasm. Elevated free Ca^{2+} and fatty acyl-CoA synergize to cause full induction of insulin release

complex, is intimately implicated in the process. In addition, the heterogeneity of islet tissue and the scarcity of the material amenable for biochemical studies has added considerable difficulty.

Until recently, it was thought that oxidative events and accelerated ATP generation are the central events causally implicated in insulin secretion. The widely accepted view summarised in the right part of Figure 1 is that metabolically sensitive K^+ -channels close in response to physiological variations of ATP and/or ADP with resulting opening of voltage-gated Ca^{2+} channels. As a consequence of Ca^{2+} influx, cytosolic Ca^{2+} rises which triggers the exocytotic release of insulin [7]. However, the recent evidence indicates that the picture may be more complex and that what may be named the ' K_{ATP}^+/Ca^{2+} pathway' does not fully account for the action of nutrient stimuli. Indeed, K^+ -induced insulin secretion, which elevates Ca^{2+} maximally, causes transient secretion of insulin whereas glucose-induced secretion is sustained [1].

Furthermore, several recent reports showed that glucose stimulates a normal amount of insulin release under conditions where K_{ATP} channel closure is bypassed (use of diazoxide) provided that Ca^{2+} is elevated (use of high K^+) [8, 9]. These observations underscore the existence of K_{ATP} channel and Ca^{2+} -independent pathways in the insulinotropic action of glucose [8].

Role of metabolic oscillations in beta-cell signalling

Oscillations in insulin secretion and intracellular free Ca^{2+} . Insulin secretion in vivo in man and animals and from the perfused pancreas is pulsatile [10, 11]. This pulsatility is lost in patients with non-insulin-dependent diabetes mellitus (NIDDM) and in their near relatives [12]. This suggests that abnormal oscillatory insulin secretion may be an early phenomenon in the development of NIDDM [12]. We [13, 14] and others [15, 16] have demonstrated oscillations in insulin secretion from groups of perfused islets. In addition, transplanted pancreata exhibit oscillations in insulin secretion before the establishment of neural connections [17]. These studies indicate that the oscillatory behaviour is not dependent on a neural network, but rather is an inherent property of the islet. It is therefore attractive to believe that the oscillatory nature of the beta cell is characteristic of the stimulus secretion coupling process, and that at least in the case of glucose-stimulated insulin secretion, it derives from the oscillatory metabolism of glucose [13, 18], not increased glycolytic flux per se (Fig. 1).

Spontaneous glycolytic oscillations have been observed widely (see below); they involve oscillations in ADP and the ATP/ADP ratio, which in the pancreatic beta cell could cause opening and closing of K_{ATP} channels, leading to oscillations in membrane potential, in the influx of Ca^{2+} through voltage-gated channels and insulin release. In agreement with this hypothesis oscillations in intracellular free Ca^{2+} have been observed in single rat [13] and mouse islets [19, 20] and in single pancreatic beta cells [21, 22]. In addition, cytosolic free Ca^{2+} and insulin secretion oscillations are tightly correlated as demonstrated in single islets [19, 23].

Glycolytic oscillations. Oscillations in the glycolytic pathway have been studied in various cell types including skeletal muscle [24, 25], yeast cells [26, 27], heart [28] and ascites tumour cells [29]. The mechanism of the oscillations in skeletal muscle extracts involves a very unusual form of enzyme regulation in which the product of the phosphofruktokinase reaction (PFK-1) activates the enzyme [30, 31]. Indeed, this key glycolytic enzyme is activated by fructose-1, 6-bisphosphate (F16BP) in the micromolar range in an AMP-dependent manner [31]. An extract of

beta-cell cytosol exhibiting glycolytic oscillations has not been isolated yet due to the difficulty in obtaining enough biological material. As an alternative approach we have used permeabilized insulinoma cells supplemented with a glycolysing cell free muscle extract. Using this system linked oscillations of free Ca^{2+} and the ATP/ADP ratio were observed [32]. Interestingly, when glucokinase replaced hexokinase as the glucose phosphorylating enzyme, Ca^{2+} oscillations were induced with increasing glucose from 2 to 8 mmol/l [32].

Oscillations in NADH fluorescence have been associated with every oscillating glycolytic system so far studied and have in fact been used for monitoring the yeast and heart extract systems. Oscillations in NAD(P)H fluorescence occur in single beta cells on stimulation with glucose [21, 22]. Importantly, the initial rise in NAD(P)H precedes that of Ca^{2+} , consistent with Ca^{2+} changes being secondary to oscillations in metabolism [22]. Also consistent with the idea that glycolytic oscillations occur in the beta cell are reports indicating that oscillations in O_2 consumption [13] and lactate production [18] occur in glucose-stimulated islets with the same periodicity as for Ca^{2+} and insulin release. Rapid NAD(P)H and Ca^{2+} oscillations of similar frequency have been identified in a sub population of islet cells in culture [22]. The authors hypothesised that subpopulations of beta cells might electrically pace oscillatory Ca^{2+} influx in islet micro domains. This may explain why NAD(P)H oscillations are not observed in single islets [33] in contrast to the electrically coupled Ca^{2+} transients. Finally, we have observed oscillations in the ATP/ADP ratio and glucose 6-phosphate in a suspension of beta cells synchronised by clonidine treatment [34]. Thus, there is much experimental support for the idea that oscillations in glycolysis and the ATP/ADP ratio contribute at least in part to the oscillations in Ca^{2+} , beta cell electrical activity and insulin release.

Advantages of oscillations. Berridge and Rapp [35] have emphasised the advantages of oscillatory behaviour in signalling, especially the higher signal-to-noise ratio. Simply stated, a relatively infrequent but high pulse is more easily discernible above background than is a constant but small elevation. Frequency-dependent rather than amplitude-dependent signals have been proposed to operate in Ca^{2+} signalling in other biological systems [35, 36]. In the case of signalling via intracellular free Ca^{2+} , there may also be a practical limitation that favours transient elevations, since a prolonged Ca^{2+} rise might overload the capacity of Ca^{2+} storage organelles. Furthermore, sustained elevations in cytosolic Ca^{2+} may be detrimental to mitochondrial metabolism and ATP generation.

There are additional advantages of regulation by metabolic oscillations in the beta cell. There is economy of energy expenditure since excess ATP is

produced only transiently when required to participate in triggering the secretory event. A greater sensitivity to variations in external glucose is another possibility since oscillations in the generation of coupling molecules and hormone secretion are rapidly turned on or off upon variations in the concentration of the sugar. Oscillatory behaviour may also have certain advantages specifically related to hormone action. It has been suggested that oscillations in hormone levels may reduce down-regulation of receptors, and thereby enhance hormone action. There is evidence that pulsatile administration of some hormones, including insulin, enhances their efficiency [11].

It must be underlined that regulation by the amplitude of the signal, in particular the absolute peak and trough values reached, not just the oscillatory behaviour may also be of importance. In this respect it is attractive to hypothesize that the peaks of the ATP/ADP ratio reached during metabolic oscillations provide the linkage to the Ca^{2+} oscillations in the islet by virtue of their effect on the K_{ATP} channels.

Phosphofructokinase. 6-phosphofructo-1-kinase (PFK-1) is a key enzyme implicated in the generation of glycolytic oscillations. There are three mammalian isoforms of PFK-1 with differing tissue distribution [37–39]. Adult muscle has only the M-type subunit, and liver mainly the L-type subunit. Brain has C-type as well as M and L-type subunits [38]. They are transcribed from separate genes and have slightly different apparent molecular weights by SDS-PAGE [40]. PFK-1 isozymes have the same set of activators and inhibitors; however, there are some large differences in affinity for certain regulators. In particular, skeletal muscle PFK-1 is strongly activated by micromolar levels of F16BP [41, 42], whereas there is little if any activation of liver PFK-1 by F16BP in the presence of near physiological concentrations of ATP, AMP and F6P [43]. Thus, the autocatalytic activation of PFK-1 by micromolar levels of F16BP, which is the basis of the glycolytic oscillations in the muscle extract system, would not be expected to occur with the L and C isozymes. The situation is actually even more complicated where multiple isozymes are expressed in a given tissue. Indeed, PFK-1 is a tetramer and the subunits most likely associate in all combinations, not just the M_4 , L_4 and C_4 complexes. The kinetics of the hybrids are probably intermediate between those of the homotetramers, but have not been investigated.

The few studies of PFK-1 activity in islet extracts have shown inhibition by ATP and citrate and activation by AMP and fructose-2,6-bisphosphate (F26BP) [44–46]. This is a characteristic feature of all the isozymes to various degrees. Recent PFK-1 protein analyses and kinetic studies with pancreatic islets and clonal beta cells (INS-1) have revealed the presence of both the M and C isoforms, with a smaller

amount of the L isozyme [47]. Kinetic studies of PFK-1 activity in INS-1 cell extracts showed strong activation by micromolar levels of F16BP at near physiological concentrations of ATP, AMP and fructose 6-phosphate, indicative of the M-type isoform. Activation by submicromolar concentrations of F26BP and potent inhibition by citrate were also observed. The F16BP stimulated activity was about half that caused by F26BP [47]. These experiments demonstrate the presence in beta cells of the M-type isoform of PFK-1 which has the requisite regulatory properties for generating glycolytic oscillations.

There are also tissue-specific isozymes of 6-phosphofructo-2-kinase (PFK-2), the enzyme which synthesises F26BP, a strong activator of PFK-1 and glycolysis [30, 43]. Glucose stimulation has been reported to increase F26BP substantially in normal islets [45], but this was called into question by other authors [48]. Hence, the role of PFK-2 in beta cell glycolysis remains to be defined.

Mitochondrial metabolism and the generation of coupling factors

Oxidative events and anaplerosis. Various mitochondrial uncouplers and respiration inhibitors impair the secretion of insulin promoted by glucose and by nutrients that are directly metabolised in the mitochondrion [2]. This has lent support to the 'fuel hypothesis' proposing that mitochondrial oxidative events and ATP generation are key factors in beta cell activation [2]. Although this view is attractive, recent evidence suggests that accelerated production of acetyl-CoA, which is required for enhanced O_2 consumption and ATP generation, does not solely account for full induction of insulin secretion. As indicated above, there is ample pharmacological evidence demonstrating the existence of K_{ATP} channel independent secretion. Furthermore, substrates which are directly metabolised to acetyl-CoA, i.e. fatty acids and ketone bodies, require the presence of glucose to elicit secretion [1]. Finally, leucine, which is metabolised to acetyl-CoA, synergizes with glutamine which directly enters the citric acid cycle via alpha-ketoglutarate [49]. These considerations led us to propose that anaplerosis, which allows the direct replenishment of the Krebs' cycle with intermediates, is also a key event in beta cell signalling (Fig. 1) [50, 51]. Consistent with this view, the islet tissue contains large amounts of pyruvate carboxylase (PC) which catalyses the formation of the intermediate oxaloacetate. Thus, 0.4% of islet protein is PC [52]. It is generally believed that PC is abundant only in gluconeogenic and lipogenic tissues. However, the islets of Langerhans do not synthesise glucose de novo [53] and have a very low lipogenic capacity [54]. It is attractive to hypothesize that a

reason for the abundance of PC is that accelerated flux through the enzyme will permit a rise in citrate, the carbon precursor of the putative coupling factor malonyl-CoA. Consistent with this idea, glucose causes a rapid elevation of both citrate [55] and malonyl-CoA in clonal β cells [50, 56]. Another possibility is that the abundance of PC allows the existence of a pyruvate/malate shuttle for cytosolic NADPH production [52].

Adenine nucleotides and reducing equivalents. Nutrients which are initially metabolised via diverse pathways before entering the Krebs' cycle elicit similar changes in various parameters of beta cell activation [57]. It is therefore attractive to believe that the beta cell coupling factors are high energy molecules which are common to the metabolism of all fuel stimuli, rather than particular metabolites specific to a given pathway, such as a glycolytic intermediate. The identification of these factors remains a formidable task and is central to the understanding of how glucose causes the release of insulin. As illustrated in Figure 2 the currency molecules or energy parameters which are common to all fuel stimuli include adenine nucleotides, the redox state and acyl-CoA compounds.

Adenine nucleotides are prime candidate coupling factors because they modulate the activity of the K_{ATP} channel. However, it remains uncertain whether they are the true physiological regulators of the K_{ATP} channel because the activity of this channel is also altered by numerous compounds including various non-esterified fatty acids (NEFA), phospholipids, fatty acyl-CoA esters, and pyridine nucleotides [58, 59] whose cellular contents are expected to vary following beta cell activation by fuels. Furthermore, recent studies have shown that above 5 mmol/l glucose does not cause [60] or causes a very modest [61] elevation in islet ATP. It is possible that local cytoplasmic elevations in ATP play a role or that important variations in cytosolic ATP and the ATP/ADP ratio are not detected by the presently available technology because of the oscillatory nature of beta cell metabolism. In fact it is more attractive to consider that variations in ADP rather than ATP regulate the K_{ATP} channel. Indeed, very small differences in the beta cell ATP content must be associated with large differences in ADP because the concentration of ATP is around 3000 $\mu\text{mol/l}$ whereas free ADP is present at a concentration of 30 $\mu\text{mol/l}$ in islet tissue [60]. ATP and ADP have antagonistic actions on the K_{ATP} channel [62]. Furthermore there is evidence for the existence of two sites of regulation of the K_{ATP} channel by adenine nucleotides [63]. The first site binds ATP and the second binds Mg ADP acting as an antagonist since the channel opening probability decreases when ADP decreases [63] (Fig. 2). Thus, ADP rather than ATP now emerges as a putative

formally proven, is summarised below (see also Figs. 1 and 3).

1) Glucose causes a large increase in beta (HIT) cell and islet malonyl-CoA which precedes secretion and is sustained for more than 30 min [50]. Only those nutrients and combinations of nutrients which elevate malonyl-CoA are secretagogues [56]. 2) Similarly, glucose rapidly elevates citrate, the carbon precursor of malonyl-CoA and only nutrients or combined nutrients which elevate citrate are efficient secretagogues [55]. 3) Some authors [50, 72], but not all [73], reported that glucose causes a rise in the C-kinase activator diacylglycerol. This is consistent with the view that esterification processes are promoted by glucose due to the availability of glucose-derived glycerol 3-phosphate and of fatty acyl-CoA due to CPT I inhibition (Fig. 3). 4) Exogenous long chain fatty acids acutely cause a rise in the beta-cell content of LC-CoA and potentiate glucose-induced insulin secretion [56]. 5) The CPT I inhibitors 2-bromopalmitate and etomoxir induce secretion at low glucose levels [56, 74]. 6) 2-bromopalmitate restores sensitivity to islets from fasted animals [75, 76], fetal islets and islets from very young rats [77]. 7) Inhibition of the pathway of malonyl-CoA formation with hydroxy-citrate, which impairs citrate lyase activity, curtails glucose-induced secretion [74]. 8) Basal secretion in cells preincubated for 48 h at various glucose concentrations correlates with the beta (INS) cell content of acetyl-CoA carboxylase (ACC), the enzyme which forms malonyl-CoA [78]. 9) Fatty acid synthase, the enzyme which uses malonyl-CoA for palmitate synthesis, is present at an extremely low level in islet tissue [55]. This should favour the rapid accumulation of malonyl-CoA and indicates that the principal function of malonyl-CoA in the beta cell is not to serve as substrate for lipid biogenesis but is rather to inhibit CPT I and fatty acid oxidation.

It must be underlined that there is not a close correlation between the beta-cell content of malonyl-CoA and secretion stimulated by various fuel stimuli but rather between LC-CoA and insulin release [56]. This observation and the fact that CPT I inhibitors which bypass the formation of malonyl-CoA induce secretion [56, 74] gives support to the idea that LC-CoA rather than malonyl-CoA acts as a 'coupling factor effector'. This however does not dismiss the importance of malonyl-CoA which acts as a 'coupling factor regulator'. Thus, by inhibiting CPT I it allows the accumulation of LC-CoA in the cytoplasm (Fig. 3). It remains to be shown that LC-CoA increases specifically in the cytoplasmic compartment upon glucose stimulation. This is not an easy task due to the lipid and protein binding properties of the LC-CoA molecule and that the vast majority of LC-CoA is bound to high affinity fatty acyl CoA binding proteins [79].

There are several ways by which malonyl-CoA may influence beta-cell metabolism and insulin secretion. First, it may play an important role in the metabolism of glucose by redirecting islet metabolism from fatty acid oxidation at low glucose levels to glucose utilisation at elevated concentrations of the sugar. Second, and as discussed above, by inhibiting CPT I it allows the cytosolic accumulation of LC-CoA acting themselves directly as effectors or indirectly via the formation of complex lipids such as lyso-phosphatidic acid, phosphatidic acid and diacylglycerol. With respect to a direct action of LC-CoA, three possibilities must be mentioned. LC-CoA may alter the activity of C-kinase enzymes [80] or change the palmitoylation state of signal transducing proteins such as trimeric G proteins implicated in exocytosis [81]. LC-CoA may also induce the fusion of secretory granules with the plasma membrane. This suggestion is derived by analogy to the observation that LC-CoA accelerate the traffic of vesicles between Golgi elements, in particular by promoting the fusion of *cis*-Golgi-derived vesicles to the *trans*-Golgi compartment [82]. Third, by changing the concentration of LC-CoA, malonyl-CoA may have pronounced effects on the expression level of genes encoding metabolic enzymes involved in the fuel sensing process (see below) and other beta-cell processes. Thus, there is little doubt that malonyl-CoA will prove to be of central importance in the regulation of several islet functions. The challenge now is the formal identification of these processes using a combination of experimental approaches, i.e. biochemical and biophysical methods, pharmacological agents and the tools of molecular biology.

Glucose and fatty acid regulation of genes encoding metabolic enzymes

In addition to their actions on insulin release and biosynthesis, nutrients have pronounced effects on the expression level of genes encoding metabolic enzymes. This new aspect of beta-cell research has implications for our understanding of the pathogenesis of obesity and NIDDM. Using pancreatic islets or INS cells it has been shown that glucose induces the transporter GLUT2 [83], the PFK-1 isozymes (C, M and L) [84], glyceraldehyde 3-phosphate dehydrogenase [84] and the L-PK [84, 85] genes in the glycolytic pathway. Glucose also increases the level of expression of the anaplerotic enzyme PC [86] and ACC [78] in the pathway of malonyl-CoA formation. Interestingly, glucose decreases the expression of the branched-chain α -keto-acid dehydrogenase transcript in cultured islets [87]. These findings suggest that glucose, when abundant, favours the expression of enzymes implicated in its metabolism and represses

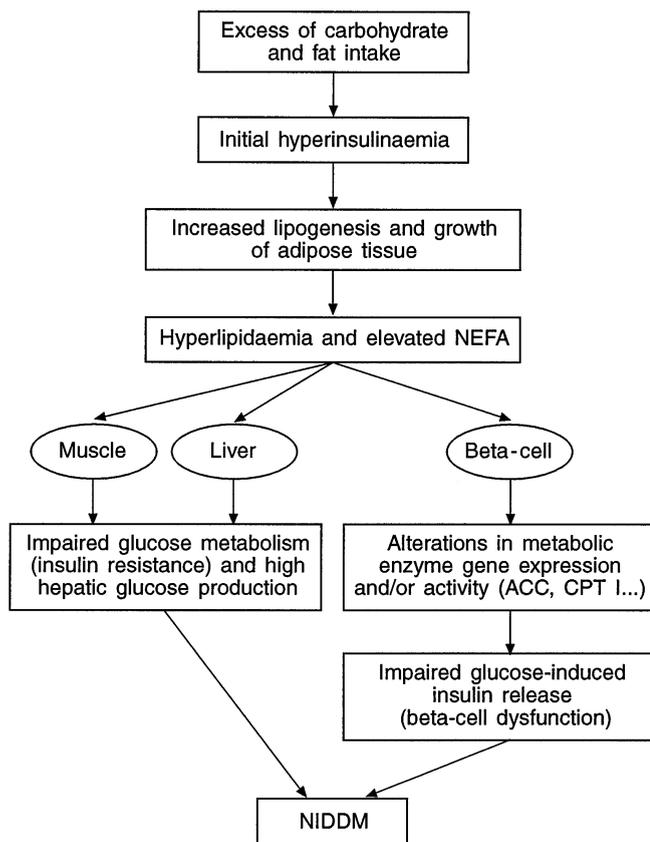


Fig. 4. A nutritional model for the pathogenesis of NIDDM. Excessive intake of both carbohydrate and fat affects the target tissues of insulin and the beta cell by changing the expression level of genes encoding metabolic enzymes, in particular acetyl-CoA carboxylase and carnitine palmitoyl-transferase I

others that specifically metabolise other fuel stimuli such as leucine.

In contrast to all expectations, the mechanism by which glucose regulates genes coding for metabolic enzymes is entirely different from that by which it causes secretion. Glucose induction of ACC mRNA is highly specific since, except for mannose, other fuels do not induce the gene [78]. Furthermore, it does not require metabolism of the hexose beyond the glucokinase step and is not mediated by the Ca^{2+} , cAMP and C-kinase transduction systems [78]. The action of glucose on this class of genes is apparently transcriptional. A carbohydrate response element has been identified in the promoter of the rat liver S14 and L-PK [88] genes. It most likely mediates induction in the beta cell as well [85]. Thus, glucose causes a co-ordinated induction of gene encoding metabolic enzymes in the glycolytic pathway as well as in the pathway of malonyl-CoA formation from pyruvate in the β (INS) cell [84].

Long chain fatty acids (NEFA) also change the expression of key metabolic enzymes. Using INS cells we have observed that various NEFA counteract the action of glucose on the ACC gene [89] and markedly

induce the liver type CPT I gene at the transcriptional level [90]. These late actions of fatty acids correlated with elevated fatty acid oxidation and altered insulin secretion [89, 90]. Hence, glucose and fatty acids have pronounced long-term effects on the expression levels of several genes in the glycolytic and anaplerotic/malonyl-CoA pathways. Much remains to be learned about the mechanism by which they exert these effects and the biological implications of this phenomenon.

Nutritional and clinical implications

Obesity and NIDDM are heterogeneous diseases from a genetic point of view. Mutations in the glucokinase gene [91] and a number of other genes have been observed in some forms of NIDDM. However, all the mutations which have been found so far account for a very small number of all the cases of NIDDM [92]. Thus, many additional genes involved in obesity and NIDDM remain to be identified. If the anaplerotic/malonyl-CoA pathway is implicated in the regulation of insulin secretion or beta-cell proliferation, it becomes very attractive to hypothesize that mutations in genes coding for enzymes in this pathway may cause diabetes. In this respect, ACC is a prime candidate because it is a highly regulated enzyme which catalyses malonyl-CoA formation and the rate-limiting step in fatty acid biogenesis. Another excellent candidate is an isoform of CPT I because it catalyses the rate-limiting step of fatty acid oxidation. Thus, fuel partitioning, which is the balance of fatty acid oxidation, synthesis, esterification and glucose utilisation, is primarily controlled by metabolic fluxes through ACC and CPT I [69]. Alterations in the activity of these two enzymes should affect glucose and lipid metabolism in the beta cell and in the target tissues of insulin as well [71].

The environmental and nutritional factors implicated in the pathogenesis of NIDDM are also poorly defined. On the basis of what we have discussed above, we would like to propose that exaggerated intake of both lipids and carbohydrates may, in genetically susceptible individuals, cause obesity and NIDDM via the altered expression level of metabolic enzymes, in particular ACC and CPT I (Fig. 4). Elevated NEFA are known to cause muscle insulin resistance and favour exaggerated production of glucose by the liver [93]. With respect to the beta cell, exaggerated fat intake in rat [94] and long-term exposure of islets to elevated NEFA in vivo [95] or in vitro [90, 96, 97], impair glucose-induced insulin secretion. Interestingly, various NEFA induce CPT I mRNA and repress the ACC gene in β (INS) cells (see above). In this aetiology of the disease, brief episodes of elevated glucose and fatty acids would initially favour hyperinsulinaemia which may precipitate

insulin resistance by promoting hyperlipidaemia [93]. It must be mentioned for the sake of clarity that short-term exposure of beta cells to NEFA potentiates glucose-induced insulin secretion [56] whereas long-term exposure of islets to NEFA impairs glucose-induced insulin release [97]. Clearly, at this stage, our proposal remains a hypothesis; but it is experimentally testable and is entirely compatible with the data in the presently available literature.

Note added in proof: NADH fluorescence oscillations indicative of glycolytic oscillations have been demonstrated in islet extracts (Civelek VN, Deeney JT, Fusonie GE, Corkey BE, Tornheim K (1997) Oscillations in oxygen consumption by permeabilized clonal pancreatic beta cells (HIT) incubated in an oscillatory glycolyzing muscle extract. Roles of free Ca^{2+} , substrates and the ATP/ADP ratio. *Diabetes* 46: 51–56

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