A case for glucosamine

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A long-standing notion in the pathophysiology of diabetes mellitus has been that prolonged hyperglycemia can – among many effects – per se induce and worsen insulin resistance (so called gluco-toxicity) mainly in skeletal muscle (1–4). In patients with non-insulin-dependent diabetes mellitus (NIDDM), induction of euglycemia with weight reduction (5), sulfonylureas (6, 7), or insulin therapy (8, 9) results in significant amelioration of insulin sensitivity as well as increased glucose transport rates measured in adipocytes (10, 11) and skeletal muscle (11). Similarly, patients with insulin-dependent diabetes mellitus (IDDM) in poor glycemic control exhibit insulin resistance which can be reversed by intensified insulin therapy designed to achieve near normal glycemia (12, 13). Rats made diabetic by subtotal pancreatectomy or streptozotocin exhibit marked impairment in insulin’s action to stimulate glucose uptake in muscle and fat which can be reversed by induction of euglycemia by exogenous insulin or by promotion of glycosuria with phlorizin (14–16). Among the explanations for this phenomenon are that intermediary products of glucose metabolism accumulate and inhibit by various mechanisms (allosteric inhibition, (de-)phosphorylation) some rate-limiting enzymes of intermediary metabolism. Thus, during glucose-induced insulin resistance, glucose would enter insulin-responsive cells (mainly skeletal muscle and adipose tissue) through the facilitated glucose transporter, GLUT4, be phosphorylated and intracellular glucose-6-phosphate (G6P) would accumulate due to inhibition of further downstream processing enzymes.

A similar situation is envisioned when insulin resistance is induced by free fatty acids (FFA) as described in the Randle cycle (17 and references therein). However, in experimental situations of both prolonged hyperglycemia and hyperlipidemia an accumulation of G6P is only found transiently which then recedes to lower than baseline levels, leading to the conclusion that glucose transport and/or phosphorylation into insulin-responsive cells is disturbed (17). Thus, less glucose appears to be transported into the cells. However, in insulin resistant states cellular GLUT4 content is not necessarily reduced, but some evidence exists that upon insulin stimulation GLUT4 is not translocated from intracellular compartments to the site of action at the surface of the cells (18, 19). The metabolic pathways which lead to glucose- and FFA-induced reduction/inhibition of cellular glucose uptake now appear to be unravelled and point towards a role for glucosamine. Furthermore, glucosamine, a product of the hexosamine biosynthesis pathway, appears to be a molecular energy-sensing device which is involved in the signaling of the energy status of peripheral tissues and the brain via leptin.

In 1991 Marshall et al. (20), using primary cultures of adipocytes proposed that the down-regulation of the glucose transport system observed after prolonged incubation with high insulin and glucose required the metabolism of hexose-phosphates in a quantitatively minor pathway of intracellular glucose utilization, i.e. the glucosamine (GlcN) pathway (Fig. 1). Based on their previous findings that desensitization of the insulin-responsive glucose transport system required three components, glucose, insulin, and glutamine (and was independent of changes in insulin receptor binding (21, 22)), they postulated and confirmed that the routing of incoming glucose through the hexosamine biosynthesis pathway plays a key role in the development of insulin resistance in primary cultured adipocytes. Inhibition of the rate-limiting enzyme in the hexosamine pathway, glutamine:fructose-6-phosphate amidotransferase (GFAT) inhibited desensitization towards insulin action of cultured adipocytes (20). Further, glucosamine, an agent which enters the hexosamine pathway downstream of GFAT could induce insulin resistance without the presence of either high glucose or glutamine (20).

Additional in vitro experimental support for the glucosamine hypothesis comes from findings indicating that GlcN induces insulin resistance in isolated rat muscle (23) and may modulate insulin’s and/or glucose’s effects on pyruvate kinase (24), glycogen synthase (23, 25) and transforming growth factor-α (26). Incubation of rat adipocytes in glucosamine for 4 h decreases the intrinsic activity of GLUT4; and longer incubation (16 h) leads to a decrease in the amount of GLUT4 in the plasma membrane (27). Moreover, in vivo experiments have revealed that GlcN infusions induce insulin resistance in normoglycemic but not hyperglycemic rats (28) and are accompanied by attenuating insulin-induced GLUT4 translocation to the cell surface in skeletal muscle (29). Transgenic mice overexpressing GFAT selectively in skeletal muscle develop insulin resistance (30). GlcN infusion has also been reported to influence insulin sensitivity not only in skeletal muscle and adipose tissue but also in heart muscle and liver (31).

Fat-induced insulin resistance – another animal model with features resembling NIDDM – is also accompanied by increased accumulation of metabolites of the hexosamine pathway (32). An important observation in this study was that the persistence of fat-induced insulin resistance once established did not

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depend upon the continuous presence of elevated circulating FFA. Furthermore, the FFA-induced impairment in the actions of insulin on glucose uptake and glycogen synthesis was associated with an early increase in the skeletal muscle concentrations of hexose-phosphates and with the accumulation of end products of the GlcN pathway (32). Thus, glucosamine appears to provide a common biochemical substrate in glucose and FFA-induced insulin resistance.

An experimental hint towards how metabolites influence glucose transport in skeletal muscle is given by the fact that insulin-stimulated glucose uptake in skeletal muscle correlates significantly with the cellular amount of UDP-acetyl-glucosamine (UDP-GlcNAc) and that glycosylation of GLUT4 in skeletal muscle is increased 100-fold after GlcN infusion compared with controls (33). Thus, the glycosylation status of GLUT4 may be involved in its ability to translocate to the cell surface. Another proposed mechanism for the effects of glucosamine is activation of protein kinase C (PKC). PKC is well known to mediate hyperglycemia-induced impaired glucose transport. Both high glucose and glucosamine induce PKC in cultured rat adipocytes, and an inhibitor of PKC effectively reverses the impaired glucose uptake induced by either glucose or glucosamine (34). Troglitazone, a pharmacological agent which renders peripheral tissues sensitive to insulin action has recently been reported to prevent hyperglycemia-induced but not glucosamine-induced insulin resistance (35). This observation may point towards the site(s) of action of troglitazone (proximal of entry of glucosamine into the hexosamine pathway) or may point out that glucose-induced insulin resistance is mediated by more pathways than the hexosamine pathway.

In addition to the effects of the hexosamine pathway on insulin action, storage of glucose as glycogen also appears to be influenced by glucosamine. Down-regulation of glycogen synthase can be induced by high glucose levels in Rat-1 fibroblasts (25), and overexpression of the rate-limiting enzyme in the hexosamine biosynthesis pathway glutamine:fructose-6-phosphate amidotransferase makes the cells more sensitive to the effects of glucose. Both basal and insulin-stimulated glycogen synthase activities are down-regulated by high glucose. These effects can be efficiently mimicked by glucosamine in place of glucose (36).

The observations outlined so far suggest a physiological role for the hexosamine pathway in that it may be a cellular ‘fuel sensor’ linking the rates of glucose and free fatty acid flux to metabolic actions of insulin; also it may be a regulator of molecular events involved in cellular uptake, processing, and storage of glucose. Several questions arise at this point. Is there a link between this putative cellular sensor of carbon-energy flux and signaling of the body’s nutritional status to the brain? If there is a link to the presently known signal leptin, is the hexosamine pathway the biochemical link between (i) incoming energy and expression of the leptin encoding ob gene and (ii) insulin resistance and leptin expression, which can be associated without the presence of adiposity and fat distribution patterns (37, 38)?

**Figure 1** After transport and phosphorylation of glucose to glucose-6-phosphate (G6P), the latter is primarily utilized in two major pathways i.e. glycogen synthesis and glycolysis. However, 1–3% of the incoming glucose which is converted to fructose-6-phosphate (F6P) enters the hexosamine biosynthetic pathway through the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT) whose major end products, UDP-N-acetyl-glucosamine (UDP-GlcNAc) and UDP-N-acetyl-galactosamine, serve as substrates in the synthesis of glycoproteins. Alternatively, infused GlcN enters cells directly via the glucose transport system and is phosphorylated to GlcN-6-P. Further metabolites are formed by subsequent acetylation and uridylation of GlcN-6-P. Increased FFA availability generates increased acetyl co-enzyme A which inhibits pyruvate dehydrogenase and thus ultimately the rate of glycolysis. This results in increased accumulation of F6P and hence increased substrate for the GFAT enzyme.
Here an important new study (39) fills the gap and substantially increases our understanding of the biochemical mechanisms by which energy homeostasis at the cellular level and satiety signals to the brain are linked. Wang et al. tested the hypothesis that the hexosamine pathway is linked to ob gene expression and leptin production by conduction studies with infusions of GlcN, infusions of uridine, prolonged hyperglycemia and prolonged hyperlipidemia (see Fig. 1) in combination with euglycemic, hyperinsulinemic clamps in conscious rats (39). All procedures, except the control situation resulted in peripheral insulin resistance. Plasma leptin levels were increased 2- to 3-fold in all experiments which led to increased GlcN flux. Expression of the ob gene mRNA was increased in adipose tissue (mixed white-, brown-, and subcutaneous white adipose tissue) and – somewhat surprisingly and considerably less abundant as compared with adipose tissue – in skeletal muscle. Immunohistochemical analysis of tissue confirmed the expression of leptin in skeletal muscle after 3 h GlcN infusion. The positive staining disappeared 10 h after the GlcN infusion had been terminated. Expression of leptin was also found in Zucker fatty rat (defect in leptin signaling, insulin resistance, diabetes) skeletal muscle tissue. These findings also lend some weight to the notion that leptin may act locally in the regulation of energy expenditure and lipid oxidation (40, 41).

Extending their observations in vivo, Wang et al. also describe induction of leptin gene expression in vitro in cultured 3T3 preadipocytes and L6 myocytes, establishing cellular models for extended studies on the regulation of gene expression by GlcN, carbon flux and nutrients (39). Gene regulation has previously been shown to be regulated by GlcN (42–44). A putative mechanism on gene regulation by UDP-GlcNAc has already been described. UDP-GlcNAc, which is required for N- and O-glycosylation of proteins regulates transcriptional activity of the transcription factor Sp1 by protecting it from degradation by O-glycosylation (45). Thus, a novel biochemical link between cellular energy availability, regulation of fuel combustion and storage, satiety signaling as well as regulation of gene expression has been established.

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