Contribution of defects in glucose production and uptake to carbohydrate intolerance in insulin-resistant subjects

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INTRODUCTION

Insulin resistance can be defined as a reduced reponse to insulin. The resulting defect in insulin-mediated uptake and utilization of glucose, i.e. glucose intolerance, is extensively studied in man, animal models of diabetes, and cell culture. Insulin resistance is usually characterized by a reduction in insulin-stimulated storage of glucose as glycogen in skeletal muscle and liver. In muscle cells, the primary mechanism responsible appears to be a blocking of the glucose transport/phosphorylation step.

In healthy individuals, glucose homeostasis is maintained by an insulin-regulated balance between glucose input to and removal from the blood stream. In the post-absorptive state, glucose is derived from hepatic glucose production, in the post-prandial state supplemented by glucose from the gastrointestinal tract. After a meal, glucose and insulin rises resulting in suppression of endogenous glucose production (EGP) and stimulation of glucose utilization, so that glucose eventually drops to basal level. The prevailing plasma glucose concentration thus reflects the balance between glucose appearance into the systemic circulation and the rate of glucose uptake into hepatic and extra-hepatic tissues. The regulation of these processes is complicated and involves a complex interaction between a variety of substrates, hormones and receptors.

In healthy individuals, the post-absorptive glucose concentration is usually 4-6 mmol/l with food ingestion producing an abrupt rise to peak levels that rarely exceed 8 mmol/l [1-4]. Glucose intolerance, i.e. abnormally high glucose concentrations in response to carbohydrate ingestion, may result from decreased insulin secretion [5-8], impaired insulin action [9-12] and alterations in both glucose effectiveness (defined as the ability of glucose to enhance its own disposal and to suppress its own release) [13-16] and glucagon secretion [1, 17-19]. Few studies have explored the relative contribution of and the mechanisms by which these defects cause glucose intolerance in insulin-resistant subjects. In particular, recent reseach indicates that glucose effectiveness has a pivotal role, but it remains unknown whether it is impaired in states of deficient insulin action and if so how this affects glucose tolerance [16, 20].

The present paper reviews the mechanisms regulating glucose production and uptake in healthy individuals and discusses how these are altered in insulin-resistant and glucose intolerant subjects in the presence of variable glucose and insulin concentrations.

REGULATION OF CARBOHYDRATE METABOLISM IN HEALTHY INDIVIDUALS

THE POST-ABSORPTIVE STATE

The post-absorptive state refers to the time period six to twelve hours after a meal, when the body is neither in the post-prandial nor in the fasting state. In the post-absorptive state, about 70% of the absorbed glucose is disposed by insulin-independent tissues like nervous tissue (~50%) and splanchnic organs (20%), and about 15% by insulin-dependent tissues, mainly skeletal muscle. Adipose tissue (5%) and tissues such as red blood cells and renal medulla (10%) accounts for minor amounts of glucose uptake [2].

EGP in the post-absorptive state and during fasting

Glucose homeostasis is achieved by the release of glucose from the liver at rates equal to the glucose disposal rate of peripheral tissues. Hepatic glucose uptake and glycogen storage increase after carbohydrate ingestion, whereas glycogen breakdown and gluconeogenesis minimize changes in EGP during a prolonged fast [21-23]. The liver is the major source of glucose production. It may produce glucose as a result of either glycogenolysis or gluconeogenesis. Studies using magnetic resonance spectroscopy (MR) have determined that hepatic glycogenolysis accounts for 45±6% of EGP in healthy individuals following an overnight fast [24]. Consequently, 55% of the glucose released into the systemic circulation is a result of gluconeogenesis. Confirming these results, Chandramouli et al. reported that gluconeogenesis accounts for 54±2% of glucose formation as assessed by the ^2H_2O technique [25], and Gay et al. reported that gluconeogenesis accounts for 51±5% of glucose formation when a stable isotope approach is used in combination with indirect calorimetry [26]. In contrast, using mass isotopomer distribution analy-

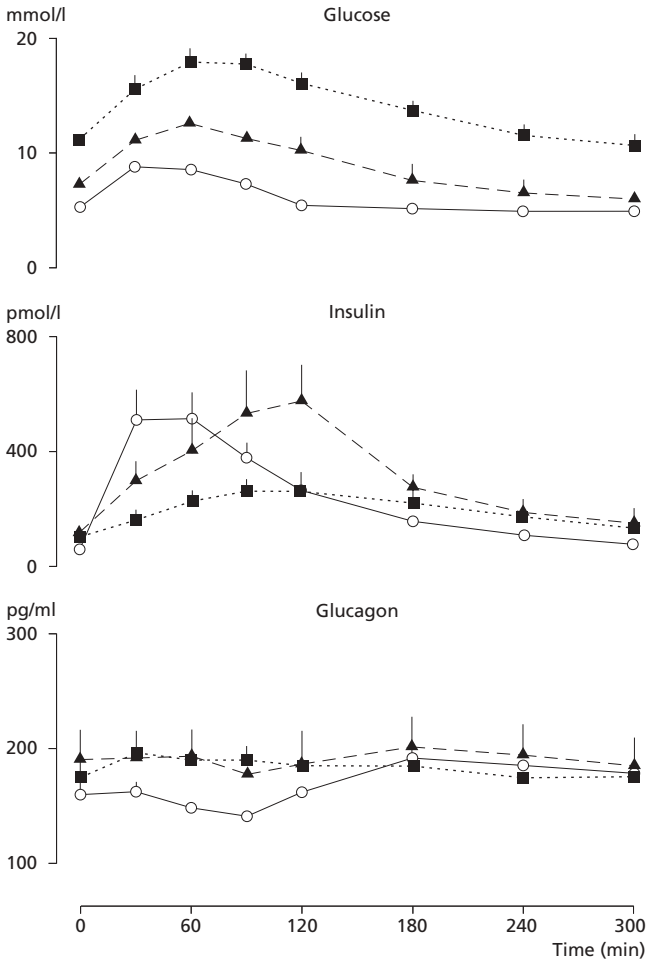

Figure 1. Glucose, insulin and glucagon concentrations prior to and following ingestion of a mixed meal in non-diabetic (○), glucose-intolerant (▲) and type 2 diabetic subjects (■).

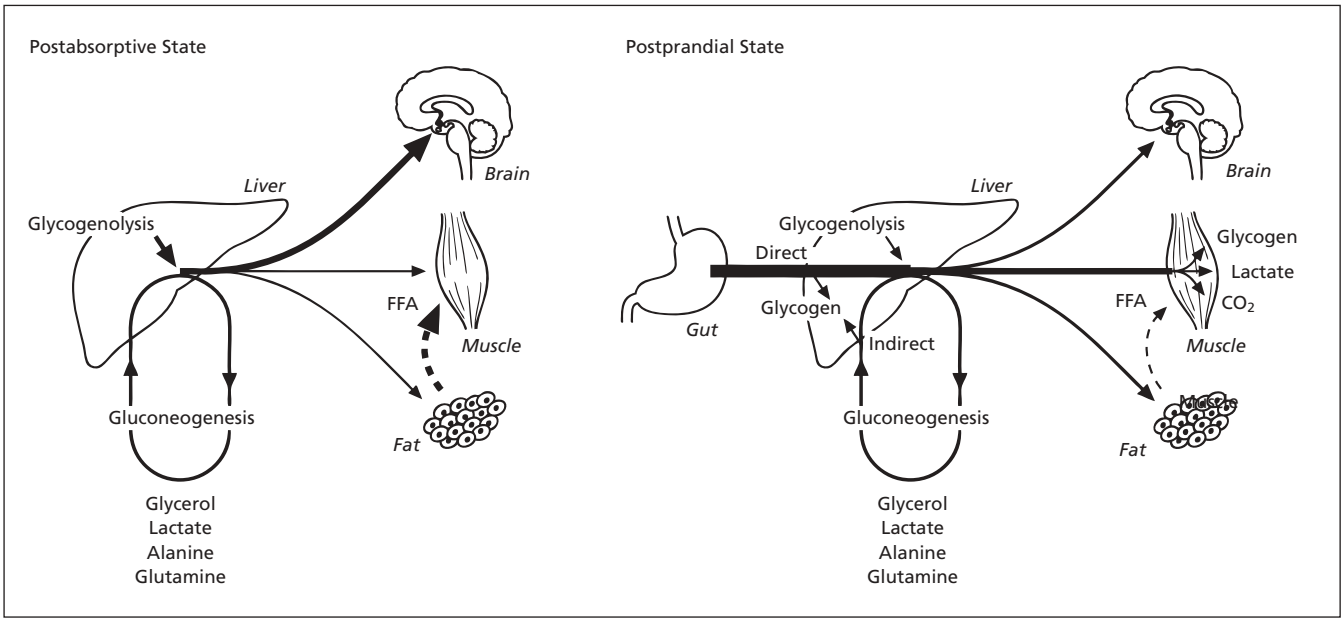

Figure 2. Regulation of glucose metabolism in the post-absorptive and post-prandial states. Following carbohydrate ingestion glucose uptake diverts from insulin-independent (i.e. nervous tissues) to insulin-dependent tissues (i.e. liver, muscle and adipocytes). In the post-absorptive state, EGP is derived almost exclusively from the liver. In the post-prandial state, HGP is supplemented by glucose absorbed from the gastrointestinal tract. The synthesis of hepatic glycogen may occur through either the indirect (gluconeogenic) pathway or the direct pathway. In the post-prandial state, FFA uptake by muscle tissue is decreased.

sis (MIDA), Hellerstein et al. reported a slightly lower contribution of 36% [27].

A prolonged fast may cause gluconeogenesis to contribute excessively to EGP. Landau et al. found that gluconeogenesis accounted for 67% of glucose production after 22 hours, and for 93% after 42 hours [28]. Chen et al. reported that it rose from 50.3% of EGP 16 hours after the last meal to 61.7% after 24 hours [29]. When expressed in absolute values, however, gluconeogensis in these experiments did not seem to increase (5.3±0.5 at 16 hours vs. 5.3±0.6 μmol/kg/min at 24 hours). The percentage rise in gluconeogenesis seems to occur because EGP declines due to a decrease in glycogenolysis. Similar results have been reported by Rothman et al. using ^{13}C MR spectroscopy to measure the decrease in hepatic glycogen concentration and magnetic resonance imaging (MRI) to measure liver volume [30]. Gluconeogenesis rates were calculated by subtracting glycogenolysis rate from EGP rate. The mean gluconeogenesis rate in this study was 7.9±1.0 μmol/kg/min or 46±5% of EGP between 0 and 22 hours of fasting, 7.1±0.5 μmol/kg/min or 82±5% of EGP between 22 and 46 hours and 8.3±0.5 μmol/kg/min or 96±1% between 46 and 64 hours of fasting. Similarly, using using [U-^{13}C] glucose, Katz and Tayek reported gluconeogenesis levels of 5.3 μmol/kg/min (0.96 mg/kg/min) equivalent to 41% of EGP after a 12-hour fast, 7.1 μmol/kg/min (1.29 mg/kg/min) accounting for 71% of EGP after a 20-hour fast and 9.0 μmol/kg/min (1.64 mg/kg/min) reaching 92% of EGP after a 40-hour fast [31]. These data demonstrate that during prolonged fasting, glycogenolysis and EGP declines, whereas gluconeogenesis remains essentially unchanged (but increases relative to EGP), and that gluconeogenesis accounts for approximately 40 to 50% of EGP in the post-absorptive state rising to over 90% following a 40-h fast.

Effects of FFA on glucose production

Free fatty acids (FFA) have been demonstrated to stimulate gluconeogenesis both in vivo and in perfused livers [29, 32]. The proposed mechanisms underlying their stimulatory effects include enhanced gene expression of phosphoenolpyruvate carboxykinase [33] and the increased generation of fructose-1,6-biphosphatase [34]. Chen et al. studied the effects of acute changes in endogenously derived FFA's in healthy individuals whose plasma levels were lowered with nicotinic acid [29]. The result was a decrease in gluconeogenesis and a simultaneous increase in glycogenolysis. EGP was accordingly virtually unaltered (EGP declined to the same extent as observed in the control group). Discontinuation of nicotinic acid after 4 hours caused a rebound in plasma FFA levels that was associated with a rise in gluconeogenesis and a concomitant decline in glycogenolysis.

Several investigators have analysed the effects of intravenously infused FFA. Boden et al. reported an elevated EGP [35], as apposed to Roden et al. who found augmented gluconeogenesis and a trend suggesting a rise in EGP [36]. It appears that decreasing plasma FFA levels acutely decrease gluconeogenesis and increase glycogenolysis, whereas increasing plasma FFA levels have the opposite effect. These reciprocal changes produce only minor or no changes in EGP; a phenomenon that has been named "hepatic autoregulation" [37].

Renal contribution to whole-body glucose production

The magnitude and importance of renal glucose release is controversial and has been heavily debated. Cells in the renal medulla display an appreciable glucose-phosphorylating and glycolytic enzyme activity and, like nervous tissue, they are obligate glucose users [38]. In contrast, cells in the renal cortex harbour gluconeogenic enzymes, including glucose-6-phosphatase, and thus have the enzymatic capacity to make and release glucose through gluconeogenesis. Moreover, studies in rats have shown that kidney cortex is capable of producing glucose from pyruvate [39]. These cells, however, display little phosphorylating activity and cannot synthesize appreciable amounts of glycogen [40, 41].

The controversy has centred on the quantitative contribution of renal gluconeogenesis. Net balance experiments have indicated little or no difference between arterial and renal vein glucose concentrations in post-absorptive normal individuals [42, 43]. However studies combining the isotope dilution technique and measurements of glucose and tracer specific activity gradients across the kidneys have challenged this hypothesis [44-50]. These studies indicate that in the post-absorptive state, renal glucose release may account for 15-30% of the isotopically determined rate of EGP, while glucose utilization accounts for 10-20% of whole-body glucose uptake [48, 50-55]. These turnover rates suggest that the kidneys may play a hitherto unrecognised role in the regulation of glucose homeostasis. In support of this are experiments by Cersosimo et al. who demonstrated that renal glucose release was doubled and its contribution to glu-

cose appearance increased during hypoglycaemia [55]. Furthermore, Meyer et al. demonstrated that renal glucose release was increased threefold, while HGR was only increased 1.4 fold during hypoglycaemia compared with hyperinsulinaemic-euglycaemic control experiments [56]. In addition, the kidneys may be a potential site for lactate [49] and glycerol [57] disposal. Moreover, the stimulation of renal glucose release resulting from enhanced gluconeogenesis at epinephrine concentrations equivalent to those observed during hypoglycaemia may have important implications for human counter-regulation [58]. In contrast, data provided by Ekberg et al. shoved no significant gradient of labelled glucose across the kidneys and post-absorptive renal glucose production accounted at most for 5% of total glucose production [59].

An attempt to resolve the controversy pertaining to the renal contribution to glucose homeostasis Møller et al. determined renal glucose production by using both stable and radioactive isotopes [51]. These experiments indicate that renal tracer dilution may vary from 0.5 to 2.8% depending on the method used. They also demonstrated a percentage contribution of post-absorptive renal glucose production to EGP in the range of 4-18%, which implies that the estimate is susceptible to considerable methodological variability. This variability may be explained by a large renal blood flow, which will tend to dilute a subtle difference in glucose concentration and tracer specific activity across the kidneys to an extent that approaches the detection limit. Moreover, it has been suggested that the contribution of renal glucose production may have been overestimated [60]. Based on these observations, it appears appropriate to conclude that renal gluconeogenesis is not just a theoretical construct. However, it seens unlikely that the contribution to total glucose production exceeds 10% in the post-absorptive state.

Glucose uptake following carbohydrate ingestion

Following an overnight fast, EGP equals approximately 2 mg/kg/min. Since glucose concentration is at steady state utilization also equals 2 mg/kg/min. Because insulin concentration is at a basal level, most of the glucose uptake (~80%) occurs in insulin-insensitive tissues like nervous and splanchnic tissues. Only a small contribution to the overall glucose uptake is provided by muscle. Muscle cells along with liver, heart and kidneys derive most of their energy through oxidation of FFAs. The relationship between carbohydrate and fat oxidation is thought to be reciprocal, since FFA oxidation may inhibit glucose uptake through the so-called Randle Cycle [61].

Contribution of EGP to hyperglycaemia

Excess glucose production may contribute to post-absorptive hyperglycaemia. Consequently, the question of the extent to which glucose production is abnormal in insulin resistance has been extensively studied, in particula in patients with type 2 diabetes mellitus. Several studies have shown that such patients have elevated post-absorptive rates of both EGP and gluconeogenesis [22, 62-64]. However, this defect in glucose production seems to be depending on the glycaemic control, since glucose production and gluconeogenesis have both been reported to be increased in individuals with fasting glucose concentrations above 10 mmol/l, whereas EGP appears to be unaltered in diabetic subjects with mild to modest fasting hyperglycaemia [65, 66]. Most of these studies have employed the isotope dilution method, but measuring glucose production in the fasting state requires steady state conditions, which may be difficult to obtain, in particular in subjects with post-absorptive hyperglycaemia.

This methodological concern has been addressed by Hother-Nielsen et al. in a series of experiments, where they investigated the effects of a constant versus an adjusted priming dose of the tracer on glucose turnover assessment [67-70]. Based on these experiments it was suggested that the optimal tracer technique should rely on an adjustment of the priming dose to the prevailing glucose concentration and that glucose turnover should be calculated using the non-steady state equations of Steele et al [67]. Applying these improved tracer methods basal EGP rates were calculated to be 12% increased in type 2 diabetic subjects, which appears to fit with recent estimates of gluconeogenesis reported to be increased by about 10% using the deuterated water technique [63, 71].

A strong positive correlation has been reported between EGP and fasting blood glucose concentration [72]. Reevaluation of the tracer data using the optimised tracer dilution technique have confirmed the positive correlation, but it appears to be weaker than previously anticipated [73]. This obervation has prompted the hypothesized that the primary role of the liver is to produce sufficient glucose to keep plasma glucose at a level, which by mass action can maintain glucose uptake (specifically in skeletal muscle) within the normal range. Accordingly, fasting hyperglycaemia in type 2 diabetes appears to result from insulin resistance, whereas increased glucose production only to a minor degree contributes to hyperglycaemia in these individuals [72].

THE POST-PRANDIAL STATE

The post-prandial state describes the metabolic condition present during the immediate hours following meal ingestion. In this state, the amount of glucose entering systemic circulation reflects the difference between the amounts of glucose absorbed from the gut and taken up by the liver [2]. Passing through the splanchnic bed, some of the glucose is taken up, phosphorylated and converted into glycogen via the indirect pathway where it passes through lactate in the Cori cycle or is exchanged with the alanine and glycerol pools [21]. Via the direct glucose-6-phosphate pathway or the indirect lactate, alanine and glycerol pathway it may also be stored as glycogen [21]. The amount of glucose entering systemic the circulation is, therefore, equivalent to the difference between the amounts of glucose absorbed from the gut and taken up by the liver. During the post-prandial period, glycogenolysis and gluconeogenesis are suppressed, which serves to minimize the amount of glucose released into the blood stream.

Glucose kinetics following carbohydrate ingestion

In healthy individuals, the post-prandial glucose concentration rarely exceeds 8 mmol/l. Homeostasis is essentially maintained through the dynamic interaction between glucose appearance and disappearance. A rise in blood glucose levels induces a delay before glucose utilization is increased and glucose production is suppressed, even when there is a concomitant increase in insulin concentration [74-76]. This contributes to the postprandial increase in glucose concentration seen in healthy subjects following meal ingestion. However, glucose utilization eventually exceeds production and the blood concentration drops to fasting levels. The regulation of these processes is determined predominantly by the prevailing plasma glucose, insulin and glucagon concentrations. In insulin-resistant subjects, these regulatory mechanisms are disturbed, which results in glucose intolerance.

Effects of variable insulin and glucagon concentrations on glucose metabolism

Glucose derived from food appears in the systemic circulation approximately within 5 to 10 minutes after eating. Splanchnic tissues (i.e. the liver and the gastrointestinal tissues) extract approximately 10-25% of the ingested glucose, whereas the rest is deposited in predominantly insulin-sensitive tissues like muscle and adipose tissue. In healthy individuals insulin concentrations promptly increase and glucagon concentrations promptly decrease after carbohydrate ingestion. These hormonal changes act in concert to minimize the postprandial rise in glucose concentration. It is anticipated that the interaction between the increase in plasma glucose and insulin and the simultaneous decrease in plasma glucagon lead to the suppression of approximately 60% of endogenous glucose release 30-60 minutes following meal ingestion. The consequent decline in glucagon concentration, which in healthy individuals decreases by 20-

30% immediately following carbohydrate ingestion [1, 77], aims to facilitate insulin-induced glycogen synthesis and the suppression of glycogenolysis. Moreover, it has been demonstrated that abolishing the transient fall in glucagon secretion, which typically lasts for two hours after meal ingestion, may induce glucose intolerance [18]. As entry of the ingested glucose decreases, glucose and insulin will fall and glucagon increase causing EGP to rise. This complicated relationship will ensure that systemic glucose delivery approximates glucose uptake, thereby preventing post-prandial hypoglycaemia.

Hepatic glucose metabolism in the post-absorptive state

In the post-prandial state, both hepatic and extra-hepatic tissues contribute to the distribution of the oral carbohydrate load. As insulin secretion increases and glucagon concentration declines, the liver takes up glucose to replenish the glycogen used during earlier fasting. This occurs predominantly through the direct pathway of glycogen synthesis i.e. glucose is phosphorylated to glucose 6-phosphate and then converted into glycogen. However, experiments suggest that a substantial fraction (up to 60%) of newly synthesized glycogen is derived indirectly from gluconeogenic precursors such as glycerol or lactate [78-80].

The liver is not only a target of insulin action; it is also a principal regulating factor determining systemic insulin concentrations. Thus, it is anticipated that approximately 50% of the insulin presented to the liver is extracted in healthy individuals (20-80%) [81]. The degree to which insulin is extracted varies [82] and may be regulated by both glucose [81] and FFA [83]. Moreover, hepatic insulin depletion is impaired in states like liver cirrhosis [84-86] and obesity, particularly abdominal obesity [87, 88]; states that are characterized by hyperinsulinaemia and insulin resistance. Hyperinsulinaemia, when chronic, may itself cause insulin resistance [89-91], thus suggesting a mechanism whereby the bypassing of the liver could cause both hyperinsulinaemia and insulin resistance.

Glucose uptake in the post-prandial state

In extra-hepatic tissues, the post-prandial rise in glucose and insulin will produce an increase in glucose uptake mediated by insulin receptors and GLUT 4 translocation to the cell surface membrane. This occurs predominantly in insulin-responsive tissues such as muscle and adipose tissues. Moreover, lipid oxidation decreases and glucose oxidation increases. It is assumed that approximately 50% of the glucose taken up is oxidized, 35% is stored and 15% released as lactate and alanine, which are then available for hepatic glycogen synthesis through the Cori cycle [92]. As post-prandial hepatic glucose release is suppressed by about 60% and does not return to baseline levels in healthy individuals until approximately 3 hours following meal ingestion, it is apparent that the liver and peripheral tissues are both important tissues for maintaining post-prandial glucose homeostasis.

Effects of glucose effectiveness on glucose tolerance following carbohydrate ingestion

While the rise in insulin concentration undoubtedly is a major regulator of the hepatic and extra-hepatic responses to glucose, recent studies suggest that glucose itself plays a significant role in regulating both its own production and disposal. Animal [93, 94] and human studies [13, 95] have demonstrated that in the presence of permissive insulin concentrations, hyperglycaemia has a potent ability both to stimulate glucose utilization and to suppress glucose release by inhibiting glycogenolysis and gluconeogenesis. This insulin independent stimulation of glucose metabolism is referred to as glucose effectiveness [16, 20]. In states of deficient insulin action e.g. in individuals with type 2 diabetes, glucose effectiveness is assumed to play a substantial role in determining glucose tolerance both during fasting and post-prandial conditions. In fact, it has been suggested that up to 50% of glucose disposal in normal individuals during an oral glucose tolerance test (OGTT) can be ascribed to glucose effectiveness and not to the dynamic insulin response [16]. This may have therapeutic benefits in as much as both insulin action and glucose effectiveness have been demonstrated to be impaired in several conditions associated with insulin resistance [13, 20, 95, 96].

INSULIN ACTION

Insulin increases the uptake, storage and oxidation of glucose. Skeletal muscle is the principle site of insulin-mediated glucose disposal (75%) and the major site of peripheral insulin resistance [97, 98]. Moreover, the suppressive effect of insulin on EGP is well established [95, 99]. Glucose uptake is achieved through activation of a

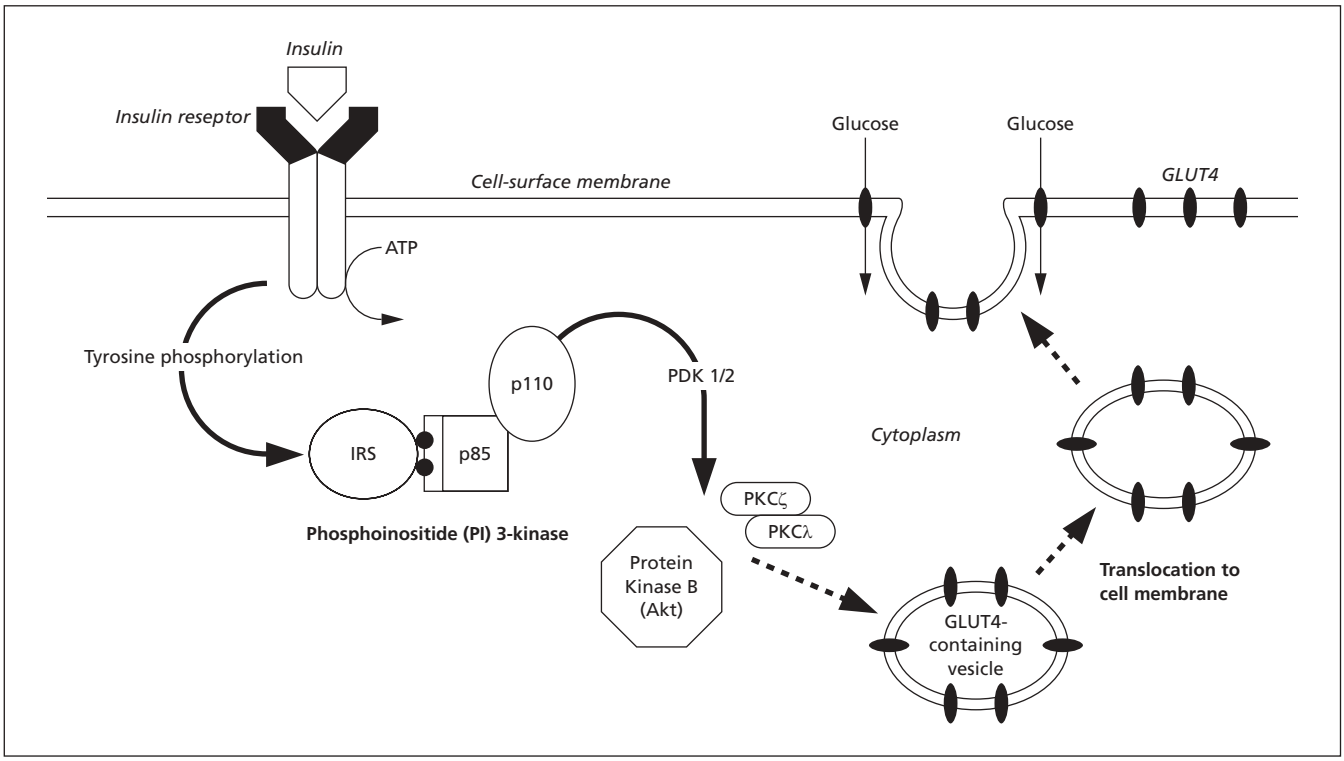

Figure 3. Major pathways of insulin-induced glucose uptake.

signal transduction cascade that entails translocation of glucose transporters from their intracellular compartment to the cell surface membrane. This initial step in glucose metabolism has been shown to control the rate of glucose utilization in healthy individuals, and to be responsible for the impairment of glycogen synthesis in patients with type 2 diabetes mellitus [100, 101].

At the cellular level, insulin-mediated glucose uptake involves a complex network of signalling molecules. Binding of insulin to its receptor causes a conformational change and activation of the tyrosine kinase leading to autophosphorylation of tyrosine residues at the intracellular portion of the b-subunits. This autophosphorylation activates the catalytic sites. The activated insulin receptor is then able to tyrosine phosphorylate intracellular proteins, thereby altering their activity and in this way generating a cascade of activation and deactivation of proteins that gives rise to several signalling pathways. A complex array of substrates has been demonstrated for the insulin receptor tyrosine kinase. The substrates involve docking proteins such as the insulin receptor-substrate molecules (IRS 1-4), which have been linked to insulin-stimulated glucose uptake [102].

One of the earliest events in the insulin-signalling cascade is the activation of phosphoinositol 3 kinase (PI-3 kinase). Besides its catalytic subunit, the PI-3 kinase has a regulatory subunit, which upon insulin stimulation physically associates with IRS-1 and binds to its phosphotyrosine residues. In insulin-sensitive tissues, such as isolated rat adipocytes [103] and intact rat skeletal muscle [104, 105], insulin dramatically increases PI 3-kinase activity. On the other hand, inhibitors of PI 3-kinase block insulin-stimulated glucose transport and GLUT 4 translocation [106, 107].

The entry of glucose into various tissues is facilitated by transmembrane glucose transporter proteins (GLUT). The GLUT transporter isoforms are labelled according to the order in which they were identified. They differ in tissue distribution, in kinetic characteristics and in substrate specificity. GLUT 1 is the most ubiquitously expressed isoform facilitating basal glucose transport. Glucose transport mediated by GLUT 1 is insensitive to insulin, but may contribute to non-insulin mediated glucose transport (glucose effectiveness), which quantitatively plays a major role during low plasma insulin concentrations [16, 20]. GLUT 4 exists exclusively in insulin-sensitive tissue, mainly skeletal and cardiac muscle and adipose tissues, and is thus the major transporter responsible for insulin-mediated glucose uptake. Glucose uptake in hepatocytes is mediated by GLUT 2 transport proteins. Glucose uptake by GLUT 2 is thought to be proportional to the plasma glucose concentration and in contrast to GLUT 4 independent of the prevailing insulin concentration.

The number of GLUT 4 glucose transporters in the cell membrane has proven critical for insulin-stimulated glucose uptake and it is determined by the balance between the appearance and disappearance of GLUT 4 transporters in the cell membrane. Insulin has been shown to increase the number of GLUT 4 glucose transporters in the cell membrane [108, 109]. In both adipocytes and skeletal muscle substantial evidence has documented that activation of PI 3-kinase is necessary for insulin-stimulated glucose uptake and GLUT 4 translocation [106, 110]. However, recent evidence suggests that also PI 3-kinase independent pathways may exist [111]. Like insulin, muscle contraction has been demonstrated to stimulate glucose transport and GLUT 4 translocation in skeletal muscle [106]. Moreover, hyperglycaemia has been demonstrated to induce GLUT 4 translocation through insulin-independent pathways enabling skeletal muscle to rapidly increase glucose uptake in acute hyperglycaemia [112, 113].

A defect in insulin signalling has been associated with insulin resistance, in particular in subjects with type 2 diabetes. These defects include an impairment of insulin binding to its specific receptor [114, 115], defective insulin receptor tyrosine kinase autophosphorylation [114-118], defective insulin receptor substrate-1 (IRS-1) phosphorylation [119], and defective phosphotidylinositol-3 (PI-3) kinase activity [119]. However, while insulin receptor binding defects may explain a right-shifted dose-response curve of insulin action, the presence of spare insulin receptors makes it unlikely that receptor binding defects alone explain the decreased maximal insulin-stimulated glucose disposal rates associated with type 2 diabetes [11].

Recent evidence has pointed to glucose transport, rather than glucose phosphorylation or glycogen synthase activity as the rate controlling step in both normal individuals and in subjects with type 2 diabtes mellitus [100]. In several studies it has been demonstrated that insulin resistant type 2 diabetic subjects are not characterized by lower levels of GLUT 4 expression in crude membranes prepared from skeletal muscles [120-122]. Although conflicting results have been published recent experiments by Ryder et al have shown that patients with type 2 diabetes have markedly reduced insulin induced exposure of GLUT 4 at the cell surface when compared with control subjects suggesting that impaired GLUT 4 translocation is a major contributor to the impaired glucose transport in type 2 diabetic subjects [123]. Moreover, Garvey et al have demonstrated an abnormality in the GLUT 4 subcellular localisation since in the diabetic subjects a greater amount of GLUT 4 is targeted to dense membrane vesicles suggesting that the defects in GLUT 4 trafficing and translocation are a cause of insulin resistance in skeletal muscles [124]. The complexity of the GLUT 4 system has been outlined in a recent set of experiments by Krook et al who studied skeletal muscle cells obtained from type 2 diabetic subjects [125]. In these in vitro studies glucose transport was impaired at different insulin concentrations whereas insulin stimulated tyrosine phosphorylation of IRS-1 and PI3-kinase activity was only reduced at high insulin concentrations indicating that additional defects other than the impaired insulin trasduction are responsible for the reduced insulin stimulated glucose transport seen in skeletal muscles from these individuals. Noteworthy, observations in human as well as in mouse models of lipodystrophy indicate that insulin resistance attributed to decreased insulin-stimulated GLUT 4 activity may result from accumulation of intracellular lipid metabolites [126].

EFFECTS OF GLUCOSE EFFECTIVENESS ON GLUCOSE TOLERANCE

Glucose intolerance is the result of impaired insulin action, inappropriate insulin secretion and/or a defect in glucose effectiveness. Glucose effectiveness is thought to affect glucose tolerance during the early period following carbohydrate ingestion, where insulin concentration is still at a basal level and in conditions where insulin secretion and action are impaired.

The concept of glucose effectiveness dates back to the work of Soskin et al. [127], who demonstrated that after glucose injection, glucose concentrations returned to the pre-injection levels in the absence of an insulin response. These experiments were the first to suggest that glucose is able to stimulate its own disposal via an insulin-independent mechanism. Subsequent experiments by Vranic et al. [94] demonstrated this insulin-independent effect in pancreatectomized dogs at basal insulin concentrations. These experiments demonstrated that plasma glucose returned to baseline level after glucose injection even in the absence of a dynamic insulin response. In rats, Rosetti et al. demonstrated that acute hyperglycaemia suppressed EGP by approximately 50% in the presence of basal insulin and glucagon concentrations [128]. This suppression was accompanied by a marked inhibition of glycogenolysis without a significant change in gluconeogenesis. This inhibitory effect of hyperglycaemia on EGP was confirmed by Rognstad et al. [129] in mice and Sindelar et al. in dogs [130]. In addition, in vitro experiments demonstrated suppressive effects of hyperglycaemia on both glycogenolysis [131] and gluconeogenesis [132]. Extensive experimental data thus implies that hyperglycaemia enjoys the potential both to suppress glucose production and stimulate glucose utilization. While the effects of hyperglycaemia on glycogenolysis seem to be attributable to the

inhibition of glycogen phosphorylase [133], the mechanisms underlying the suppressive effect on gluconeogenesis remains unresolved [20].

Whereas most of the early experiments were conducted in animals, several recent studies have examined the relationship between glucose and insulin in enhancing human glucose uptake. Best et al. [134] and Verdonk et al. [135] utilized the glucose clamp technique to examine dose-response relationships between plasma glucose concentrations and rates of glucose utilization. These studies demonstrated the significant effects of glucose mass action on extra-hepatic glucose uptake. They found that although the increments in glucose utilization produced by an increase in glucose were similar in the glucose range studied, the extrapolated intercept on the ordinate was positive. These studies were therefore the first to challenge the validity of the claim that glucose clearance is independent of plasma glucose concentration.

Evaluation of glucose effectiveness and estimation of its contribution to glucose tolerance has been thoroughly investigated. Studies in dogs by Ader et al. [93] demonstrated that in the absence of a rise in plasma insulin, hyperglycaemia is rapidly normalized following an i.v. glucose bolus injection. These studies were the first to indicate that glucose per se independent of the dynamic insulin response has a significant effect on glucose tolerance. In subsequent experiments by Alzaid et al., glucose effectiveness was measured in type 2 diabetic subjects during insulin infusions that mimicked post-prandial peripheral insulin concentrations [136]. Glucose concentrations were clamped at euglycaemic levels or varied so as to reproduce a pattern resembling that normally observed in non-diabetic individuals after food ingestion. Glucose effectiveness was calculated by subtracting the rates of glucose production and utilization observed during the euglycaemic experiments from those observed during the hyperglycaemic experiments. Insulin action measured in this manner was impaired in the diabetic subjects, while glucose effectiveness was normal. This observation contrasts with minimal model analyses, which in several previous studies have demonstrated a decrease in glucose effectiveness [14, 137]. This discrepancy prompted the speculation that glucose effectiveness could be a function of the prevailing glucose and insulin concentrations, since Alzaid et al. studied glucose effectiveness in the presence of high insulin concentrations, while the minimal model determines glucose effectiveness in the presence of basal insulin concentrations. To address this question Basu et al. conducted a series of experiments in which insulin was maintained constant at individually determined basal levels [13]. Applying this technique, Basu et al. demonstrated that glucose effectiveness was impaired in type 2 diabetes. However, whereas both net glucose effectiveness and glucose-mediated glucose uptake were lower in diabetic than in non-diabetic subjects, hepatic glucose effectiveness was slightly, but not significantly lower in the diabetic subjects.

Taken together the discrepancy in the estimates of glucose effectiveness as reported by Alzaid et al. and Basu et al. appeared to indicate that glucose effectiveness depended on the prevailing insulin concentrations. To address this hypothesis we performed an additional series of experiments in which the effects of hyperglycemia on glucose production and uptake were determined in the presence of low (100 pM) and high (200 pM) insulin concentrations [95]. In these experiments glucose-induced stimulation of its own uptake was impaired in diabetic individuals at both insulin concentrations. However, this was only evident when glucose concentrations exceeded 7.5 mmol/l. In contrast, the ability of glucose to suppress glucose production (both net and total production) did not differ between the two groups. This concentration-dependent defect in glucose uptake is intriguing, since it implies a defect in a glucose responsive rate-limiting step in the diabetic subjects (i.e. glucose transport and/or enzymatic activity). Subsequent experiments have demonstrated that while overnight restoration of euglycaemia in type 2 diabetic subjects improves hepatic but not extra-hepatic insulin action [138], it does not alter glucose effectiveness in these sub-

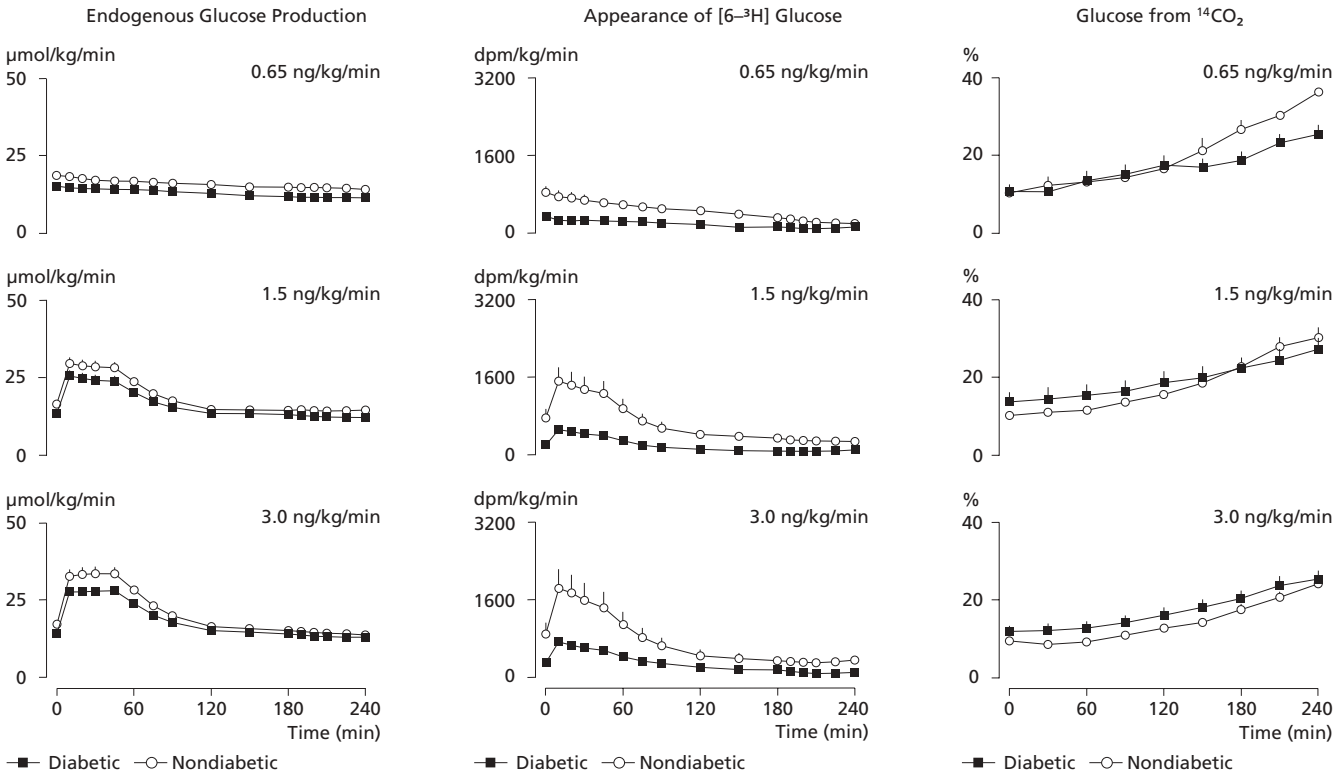

Figure 4. Endogenous glucose production, appearance of [6-^{3}H] glucose from hepatic glycogen and incorporation of $^{14}CO_2$ into glucose in diabetic and non-diabetic subjects, when glucagon was infused at a rate of 0.65 ng/kg/min (130 pg/ml) or when the glucagon infusion rate was increased at time zero to either 1.5 ng/kg/min (180 pg/ml) or 3.0 ng/kg/min (310 pg/ml). Glucagon sensitivity did not differ in control and type 2 diabetic subjects. During glucagon infusion, the rate of release of [6-^{3}H] glucose was 50% lower in diabetic than in non-diabetic subjects, whereas EGP was unaltered in the two groups implying that a greater proportion of the released glycogen was derived from the indirect pathway in the type 2 diabetic subjects. CO_2 incorporation into glucose was unaltered during glucagon infusion. Adapted from Nielsen et al. [141].

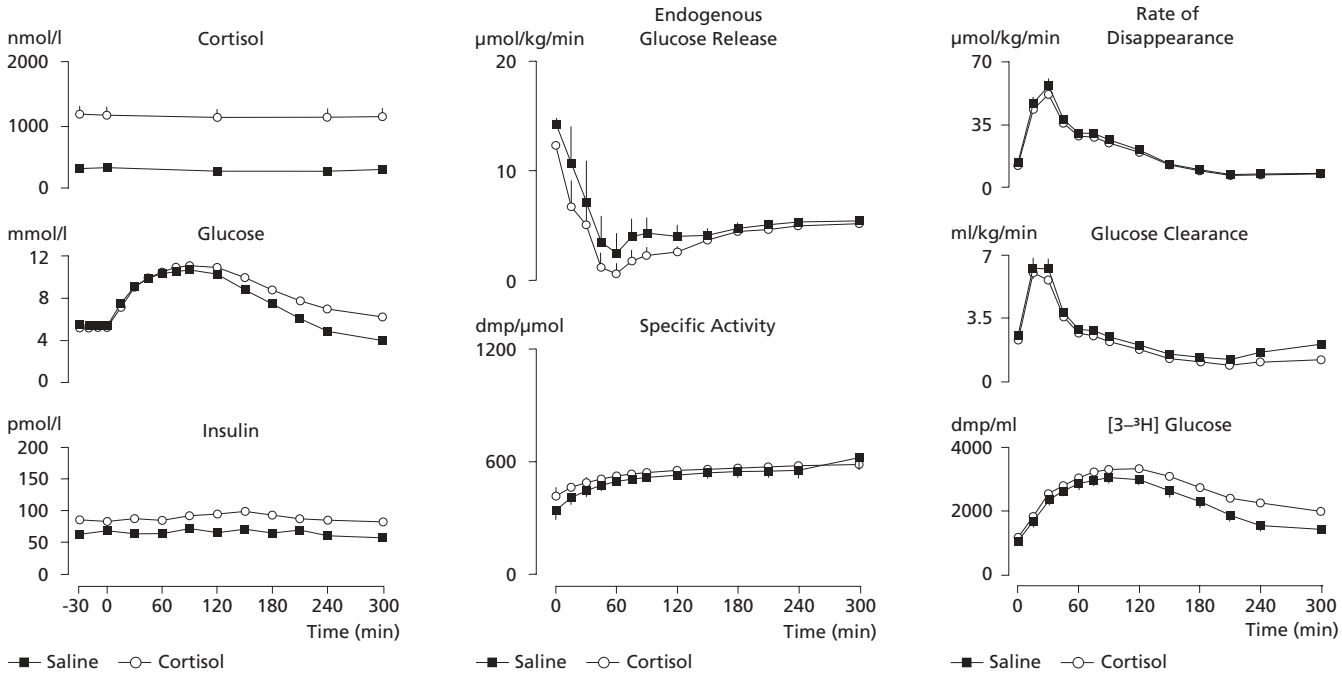

Figure 5. Substrate concentrations and glucose turnover during prandial glucose infusion in the presence of cortisol or saline infusion. The glucose infusion was started at time zero. Adapted from Nielsen et al. [96].

jects [139]. It remains to be determined whether improved long-term normalization of plasma glucose concentration ameliorates glucose tolerance in type 2 diabetes by increasing glucose effectiveness.

PATHOGENESIS OF GLUCOSE INTOLERANCE IN CONDITIONS ASSOCIATED WITH INSULIN RESISTANCE

Glucose intolerance is a common feature in insulin-resistant subjects. Fasting hyperglycaemia and impaired glucose tolerance are well-described metabolic defects in individuals with type 2 diabetes mellitus, but much less attention has been given to the abnormalities of glucose metabolism, which are encountered in a variety of other metabolic states associated with insulin resistance.

In healthy individuals, normal glucose tolerance is achieved by a dynamic interaction between the prevailing glucose, insulin and glucagon concentrations. Glucose intolerance may result from decreased insulin action, but the contribution of and the mechanisms underlying other defects causing glucose intolerance remain ill defined. Such defects include impaired insulin secretion, alteration in glucose effectiveness (defined as the ability of glucose to enhance its own disposal and to suppress its own production) and glucagon secretion. Since new agents are being developed that potentially can influence all of the above parameters, more profound knowledge of these issues have important physiological as well as therapeutic implications.

Type 2 diabetes mellitus is associated with excessive rates of glucose production in both the post-absorptive and the post-prandial state [72]. While these defects may result from impaired hepatic and extra-hepatic insulin action, an increase in hepatic glucagon sensitivity would also contribute to an increae in glucose production. Glucagon excess may trigger an increase in hepatic glucose release. This observation prompted the "bihormonal hypothesis of diabetes" originally introduced by Roger Unger, which states that hyperglycaemia may result from absolute or relative insulin deficiency and/or absolute or relative glucagon excess relative to the prevailing glucose concentration [17, 140]. The effects of glucagon on hepatic glucose production have been studied in both human and animal experiments. However, whether glucagon sensitivity is abnormal in patients with type 2 diabetes mellitus was until recently unknown. To address this question we conducted a series of experiments in which glucagon was infused in the presence of basal insulin concentrations in nondiabetic and type 2 diabetic subjects [141]. In these experiments glucagon-induced stimulation of glucose production did not differ in diabetic and non-diabetic subjects, which suggests that the elevated EGP rates are not due to an increase in hepatic sensitivity to glucagon. This finding has subsequently been confirmed by Matsuda et al. in a comparable set of experiments [142].

Resistance to the effects of insulin but not to glucagon has been reported in obese non-diabetic subjects and lean healthy individuals with hypertriglyceridemia [143, 144]. Furthermore, Baron et al. has observed an unaltered decrement in glucose production in diabetic subjects during somatostatin-induced hypoglucagonaemia, which implies that these individuals are equally sensitive to glucagon when compared to healthy control subjects [145]. In contrast, Orskov et al. reported reduced HGP during glucagon stimulation [146]. These studies were, however, conducted in type 1 diabetic subjects, and the experiments were performed using a euglycaemic clamp design with a fixed and equal basal insulin infusion in the two groups.

The finding of unaltered hepatic glucagon sensitivity in type 2 diabetes does, however, not suggest that pharmacological intervention with glucagon action will be ineffective as a tool for obtaining better glycaemic control. Evidence to this effect has been provided by experiments recently published by Shah et al. In an elegant set of experiments it was demonstrated that an abnormal glucagon suppression can cause hyperglycaemia in the presence of inappropriate insulin secretion [18]. Moreover, it was demonstrated that this lack of suppression contributed to post-prandial hyperglycaemia in subjects with type 2 diabetes mellitus in part by accelerating glycogenolysis [147]. Taken together, these results provide evidence that agents antagonizing glucagon action or secretion may be of value in the treatment of patients with type 2 diabetes. Alternatively, improved glycaemic control could be obtained by suppressing gluconeogenesis. Thus, an improvement in glucose tolerance should be possible by reducing glucose production by lowering the contribution of gluconeogenesis to hepatic glucose output. Recent experiments [141, 148] do, however, suggest that the relative contribution of gluconeogenesis to glycogen synthesis is increased in diabetic subjects. Therefore, while gluconeogenesis inhibitors may improve glucose tolerance in type 2 diabetes by reducing EGP, they may also be associated with a significant risk of hypoglycaemia due to a decrease in hepatic glycogen stores.

Studies performed during the last decade have recognized that

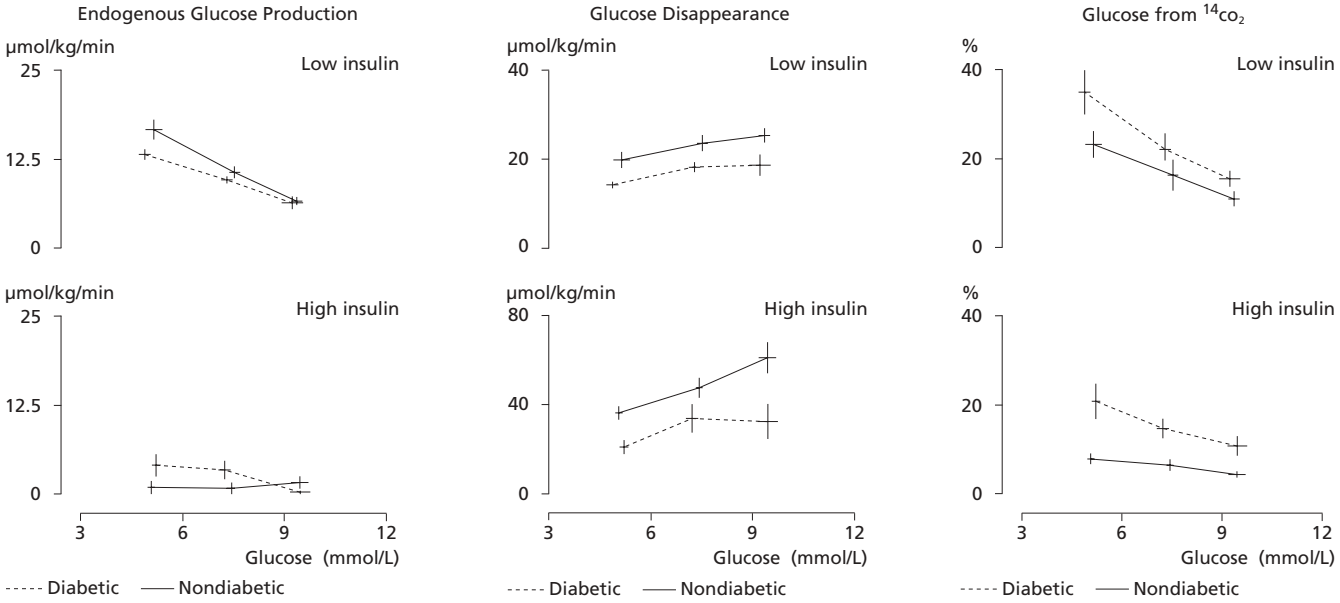

Figure 6. Dose-response curves for suppression of glucose production, stimulation of glucose utilization and for suppression of the incorporation of $^{14}CO_2$ into glucose in type 2 diabetic and non-diabetic subjects. Adapted from Nielsen et al. [95].

glucose effectiveness has a significant impact on glucose tolerance. The minimal model has been used to assess glucose effectiveness. These experiments have consistently reported glucose effectiveness to be decreased in type 2 diabetes. However, whereas the classical minimal model yields a useful estimate of insulin action, it is an open question whether it correctly assesses glucose effectiveness [149]. Several alternative approaches have addressed these limitations. Alzaid et al. studied type 2 diabetic subjects during variable insulin infusion [136]. Using this minimal model independent design glucose effectiveness did not differ in diabetic and non-diabetic subjects. In contrast, Basu et al. conducted a series of experiments in the mid 90s in which glucose effectiveness was measured during basal insulin concentrations [13]. In these experiments net glucose effectiveness was decreased in diabetic subjects due to a defect in glucose mediated glucose uptake whereas the suppressive effect of glucose on EGP was unaltered in the diabetic and non-diabetic subjects.The discrepancy in these reports on glucose effectiveness could be explained if the estimate of glucose effectiveness were depending on the prevailing insulin concentration. In fact, they could indicate that when insulin concentrations were low and glucose-stimulated glucose uptake predominated, glucose effectiveness would be impaired, while on the other hand, when insulin concentrations were high and glucose transiently elevated, the impairment of glucose effectiveness would no longer be present. To address this question we performed a series of glucose clamps designed to determine glucose effectiveness at glucose concentrations throughout the normal glycaemic range in the presence of low or high insulin concentrations [95]. These experiments confirmed the observations previously reported by Verdonk [135] and Best [134] of a linear relationship between glucose uptake and the plasma glucose concentration. The results showed that glucose-induced stimulation of its own uptake is impaired in type 2 diabetes, but the defect could only be determined at glucose concentrations above 7.5 mM. In contrast to glucose uptake, the effect of glucose on glucose production dd not differ in the diabetic and non-diabetic subjects. This concentration-dependent defect glucose uptake is intriguing since it implies a defect in a glucose-responsive rate-limiting step in diabetic subjects that may either be due to a defect in glucose transport and/or a defect in enzymatic activity.

Most clinical studies conducted with the aim of determining defects in carbohydrate metabolism are carried out by infusing insulin throughout the night so as to avoid the confounding effect that would occur if baseline glucose concentrations differed in diabetic and non-diabetic subjects. Moreover, studies have demonstrated that hyperglycaemia per se can cause insulin resistance, a mechanism commonly referred to as glucose toxicity. Studies by Zierath et al. have demonstrated that this insulin resistance can be reversed by incubation of muscle obtained by biopsy from subjects with type 2 diabetes with a glucose concentration of 4 mmol/l for 2 hours [150]. To address the question whether overnight euglycaemia alters glucose production and utilization we conducted a series of experiements in which nocturnal glucose concentrations were either mainted at euglycaemia by the infusion of insulin or allowed to remain elevated during the night. Insulin action was performed the following day using the euglyceamic hyperinsulinaemic clamp. [138]. The results from these experiments demonstrated that overnight normoglycaemia improves hepatic insulin action whereas glucose uptake was unaltered. This finding should be taken into account in studies aiming to examine insulin action in diabetic subjects and may support a therapeutic approach where insulin is deployed to achieve and maintain improved nocturnal glycaemic control. Finally, the results may account for the clinical observation that it is difficult to achieve adequate glycaemic control during the day if the patient is in a hyperglycaemic state in the morning.

Glucose effectivness plays a pivotal role in maintaining a normal glycaemic reponse and a defect in glucose effectiveness is a major determinant of the metabolic defects in subjects with type 2 diabetes. However, it remains unresolved how this impairment in glucose effectiveness contributes to the pathogenesis of type 2 diabetes. This question was first addressed by Henriksen et al. using the classical cold minimal model analysis based on IVGTT data in first-degree relatives of type 2 diabetic subjects [151]. It has been estimated that about 40% of these individuals will develop the disease and this cohort of subjects thus provide an opportunity to study early markers of defects in glucose metabolism in potentially prediabetic subjects. In this study Henriksen et al. were able to confirm the presence of insulin resistance. However in contrast to type 2 diabetic subjects in which glucose effectiveness has been demonstrated to be impaired, net glucose effectiveness was increased in the relative subjects. The authors thus suggested that an increase in glucose effectiveness may provide a physiological mechanism that may compensate for insulin resistance and impaired insulin secretion in maintaining normal glucose tolerance. This conclusion has subsequently been confirmed by the same authors using the euglycaemic pancreatic clamp [152].

In order to confirm these results we applyied a minimal model independent analysis. In this study glucose effectiveness was calculated

during a prandial glucose infusion profile using a combined somatostatin and insulin infusion protocol replacing insulin at near baseline levels [153]. In contrast to the experiments by Henriksen et al. net glucose effectiveness did not differ between the relatives and the control subjects, nor did rates of glucose production and utilization during the prandial glucose infusions. In addition, hot indices of glucose effectiveness were unaltered in the relatives, which implies the presence of normal glucose effectiveness in this group. These data thus contradict the results suggesting increased rates of glucose effectiveness in prediabetic individuals [151, 152] and argue against the hypothesis that an increase in glucose effectiveness may compensate for a defect in insulin action in these individuals.

The maintenance of normal glucose tolerance requires the interaction of insulin and counter-insulin hormones. Cortisol and GH are well-established counter insulin hormones. Numerous studies have established that excessive amounts of cortisol and GH cause insulin resistance and carbohydrate intolerance [154-156]. Both hormones impair insulin-induced suppression of hepatic glucose release and insulin-induced stimulation of glucose uptake. In the absence of a compensatory increase in insulin secretion, both hormones cause hyperglycaemia. Both GH and cortisol are secreted according to a well-defined pattern. In non-stressed humans, cortisol concentrations rarely exceed 15-20 µg/l and such elevations only persist for a few hours. The most marked increase in cortisol in healthy subjects generally occurs from the middle of the night until breakfast, with similar, but less consistent increases also after food ingestion.

Like cortisol, GH concentrations vary considerably throughout the day. GH concentrations fall when glucose concentrations rise, and they rise when glucose concentrations fall. Increases are observed, also in response to stress or exercise. However, the most consistent and pronounced increase in GH secretion occurs during the night. GH generally increases to concentrations of about 5-15 µg/l after the onset of sleep with a second peak normally occurring 1-2 hours later. Several studies have consistently demonstrated that a persistent increase in GH and cortisol results in insulin resistance and glucose intolerance. However, the physiolocal significance of these changes i.e. during conditions of daily living when glucose and insulin concentrations vary has only sparsely been investigated.

Studies performed by Dinneen et al. have shown that the nocturnal rise in cortisol secretion induces glucose intolerance by creating a state of physiologic insulin resistance. In a series of experiments cortisol was infused during the night either at a constant rate or in a pulsatile fashion mimicking the normal nocturnal rise in cortisol secretion [157]. In these experiments the normal nocturnal rise in cortsol was associated with higher pre-and post-prandial glucose concentrations. The augmented glycaemic response arose owing to a combination of an increased rate of hepatic glucose release and reduced rates of tissue glucose uptake. Similar effects have been reported in type 1 diabetics [158]. Following ingestion of a mixed meal, a measure of insulin deficiency was created by means of a computer-driven insulin infusion. In this setting, a greater glycaemic excursion was seen in the presence than in the absence of the normal nocturnal rise in cortisol. This was because of a combination of increased hepatic glucose release (with increased gluconeogenesis) and decreased tissue glucose uptake. Noteworthy defects in glucose production and uptake have also been demonstrated during high-dose cortisol infusion using the hyperinsulinaemic euglycaemic clamp [159].

In contrast to cortisol, little is known about the impact of the physiological changes in GH. It is well-established that a persistent increase in GH to concentration levels within the normal physiological range impairs insulin action, but it is not known whether the transient increase in GH that occur during the night to a similar extent as has been demonstrated for cortisol influence carbohydrate metabolism the following morning. This question was addressed in a series of experiments using the dual isotope technique [160]. However, in controst to cortisol [157, 158], the nocturnal rise in GH had no effect on either glucose tolerance or on the glucose kinetics the following morning. This finding is of importance in relation to the so-called "dawn phenomenon", which refers to an increase in insulin requirements that generally occurs between 0600 and 0900 in most people with type 1 diabetes mellitus. The cause of this increase is not known, but it has been ascribed to the nocturnal increase in both cortisol and GH secretion. Our experiments were not designed to assess the effects of the nocturnal rise in GH on early morning insulin requirements and our data accordingly do not support any effect of the overnight increase in GH secretion on insulin resistance the following morning. In fact, it may very well be that the GH pulses during the night may have had a subtle and temporary effect

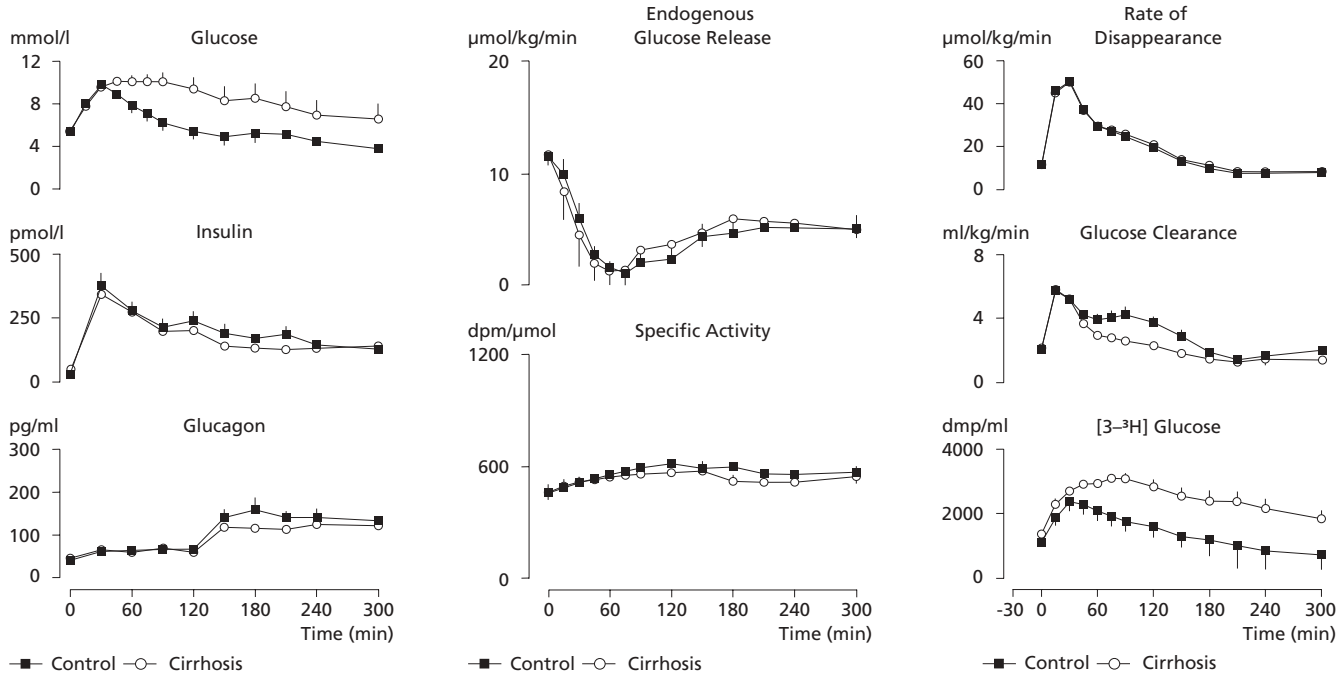

Figure 7. Substrate concentrations and glucose turnover during prandial glucose and insulin infusions in healthy individuals and subjects with liver cirrhosis. Whereas endogenous glucose production was equally suppressed, glucose uptake was impaired in cirrhotic subjects resulting in glucose intolerance. Adapted from Nielsen et al. [168].

on insulin action. If present this effect must, however, have been small and it appears unlikely to have had a significant effect on carbohydrate or FFA metabolism, either during the night or following meal ingestion the following morning. This finding is consistent with previous reports on the effects of GH on insulin action, which suggest that the effects are transient and no longer evident after 5 hours. However, given the variability potentially introduced by concordant changes in insulin, glucagon and GH concentrations during the night, the present experiments cannot totally rule out a small, but undetectable glucoregulatory effects of GH on glucose metabolism.

Excess cortisol induces glucose intolerance but it remains unresolved whether this is a result of an impairment in glucose effectiveness. This question was addressed in a resent study using a minimal model independent design [96]. Net as well as hot indices of glucose effectiveness were calculated during a prandial glucose infusion in the presence of basal insulin concentrations. The results demonstrated an impaired ability of glucose to promote its own metabolism during short-term hypercortisolaemia, i.e. net glucose effectiveness (GE_b) was lower during cortsol than during saline infusion. GE_b is a composite measure of the ability of glucose to suppress its own production (GE_{liver}) and to stimulate its own uptake (GE_b*). In these experiments, GE_{liver} was impaired during cortisol infusion, whereas GE_b*, although higher in absolute numbers, did not reach statistical significance (P=0.15]. The results from these studies therefore suggest that high-dose steroid infusion impairs glucose effectiveness due to a defect in the ability of glucose to suppress its own production and presumably also to promote its own uptake. While this has direct implications for the understanding of the pathogenesis of hyperglycaemia during excess cortisolaemia, the results also have a more profound implication, because they imply the presence of a separate mechanism responsible for the mass action effect of glucose, which is independent of the well-established pathways of insulin action.

Little is known about the mechanisms responsible for glucose effectiveness. It seems likely that the mass action effect is facilitated by GLUT transport proteins. Glucose transport mediated by GLUT-1 is insensitive to insulin and has been suggested to contribute to the non-insulin mediated glucose transport during low plasma insulin concentrations [161]. Moreover, hyperglycaemia has been demonstrated to recruit insulin-independent glucose transporters (GLUT 1 and GLUT 2) to the cell surface via a Ca^{2+}-dependent mechanism, which is mechanistically different from the insulin-dependent mechanism mediated via phosphatidylinositol 3-kinase [113]. Furthermore, hyperglycaemia has been demonstrated to induce translocation of GLUT 4 transporters to the plasma membrane in muscle [112]. In addition, experiments by Petersen et al. have suggested that hyperglycaemia inhibits hepatic glycogenolysis primarily through inhibition of glycogen phosphorylase, whereas insulin inhibits glycogenolysis primarily through stimulation of glycogen synthase [133]. It is worth noticing that while insulin is a major determinant of glucose uptake in skeletal muscle, recent evidence suggests that muscle contraction stimulates transport and GLUT4 translocation in skeletal muscle and that the effects of insulin and contractions on glucose transport and GLUT4 translocation are additive. These findings may suggest the presence of yet undiscovered regulatory pathways responsible for glucose production and uptake. A better understanding of how these mechanisms operate is likely to provide new agents for the treatment of hyperglycaemia, in particular in subjects with type 2 diabetes.

Impaired glucose tolerance is a well-established feature in liver cirrhosis and several studies, using mostly the euglycaemic clamp, have demonstrated a 40-50% reduction in glucose uptake due to defective glucose storage, particularly in skeletal muscle [162-165]. In these studies basal glucose production rates have uniformly been reported to be unaltered with normal suppression of glucose production during insulin infusion, which suggests that hepatic sensitivity to insulin is unaltered. The reduction in extra-hepatic glucose uptake has been attributed to a defect in glycogen synthesis due to lower muscular glycogen synthase activity [166]. Moreover, decreased glucose transport and decreased glycogen synthesis in skeletal muscle have been demonstrated [167]. Finally, both receptor and postreceptor defects have been reported in cirrhotic subjects [162, 163].

In a recently published series of experiments, glucose tolerance was studied using a prandial glucose and insulin infusion profile [168]. This experimental approach was chosen for two reasons. First, it ensures identical insulin concentrations in cirrhotic and control subjects taking into account the confounding effects of higher insulin concentrations in cirrhotic subjects during the OGTT. Second, it allowed us to determine the effects of prandial changes in glucose and insulin concentrations on glucose production and utilization determined in the presence of optimized tracer conditions, i.e. constant specific activities. In this experimental setting, these results are the first to demonstrate that defects in glucose tolerance in liver cirrhosis may be attributed to a defect in glucose uptake and that this defect is present in the face of unaltered rates of glucose production. This may prove to have clinical implications because the results may suggest that agents designed to improve glucose uptake are likely to produce better glycaemic control than agents stimulating insulin secretion. Moreover, agents aiming to improve the hepatic response to insulin are unlikely to improve the glycaemic profile in liver cirrhosis. Of particular interest is the observation that the suppression of glucose production was achieved in the presence of higher glucose concentrations in the cirrhotic subjects. This may imply an inappropriate suppression of EGP by glucose and may thus reveal an undiscovered defect in hepatic glucose regulation. Moreover, it remains unresolved to which extent a potential defect in hepatic insulin extraction may be responsible for extra-hepatic insulin resistance in these individuals.

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