PATHOGENESIS OF TYPE 2 DIABETES MELLITUS

Eugenio Cersosimo, MD PhD Associate Professor of Medicine, Department of Medicine, Division of Diabetes, UTHSCSA, Mail Code 7886 - 7703 Floyd Curl Drive, San Antonio, TX 78229-3900

Curtis Triplitt, PharmD, Clinical Associate Professor of Medicine, Department of Medicine, Dvision of Diabetes, UTHSCSA, Mail Code 7886 – 7703 Floyd Curl Drive, San Antonio , TX 78229-3900

Lawrence J Mandarino, **PhD** Professor of the School of Life Sciences at Arizona State University and Professor of Medicine at the Mayo Clinic Arizona, Arizona State University, Center for Metabolic Biology, School of Life Sciences, Arizona State University , Tempe, AZ 85287-3704

Ralph A DeFronzo, MD Professor of Medicine and ,Chair, Division of Diabetes , Department of Medicine, UTHSCSA, Mail Code 7886 - 7703 Floyd Curl Drive, San Antonio, TX 78229-3900

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ABSTRACT

At least eight distinct pathophysiologic abnormalities have been associated with type 2 diabetes mellitus (T2DM). It is well established that decreased peripheral glucose uptake (mainly muscle) combined with augmented endogenous glucose production are characteristic features of insulin resistance. Increased lipolysis, elevated free fatty acid levels, along with accumulation of intermediary lipid metabolites contributes to further increase glucose output, reduce peripheral glucose utilization, and impair beta-cell function. Compensatory insulin secretion by the pancreatic beta-cells may at first maintain normal plasma glucose levels, but beta-cell function is already abnormal at this stage, and progressively worsens over time. Concomitantly, there is inappropriate release of glucagon from the pancreatic alpha-cells, particularly in the post-prandial period. It has been postulated that both impaired insulin and excessive glucagon secretion in type 2 diabetes are contributed to by the “incretin defect”, defined primarily as inadequate release or response to the gastrointestinal incretin hormones upon meal ingestion. Moreover, hypothalamic insulin resistance (central nervous system) also impairs the ability of circulating insulin to suppress glucose production, and renal tubular glucose reabsorption capacity may be enhanced despite hyperglycemia in T2DM. These pathophysiologic abnormalities should be considered for the treatment of hyperglycemia in patients with type 2 diabetes.

NORMAL GLUCOSE HOMEOSTASIS

In the postabsorptive state (10-12 hour overnight fast), the majority of total body glucose disposal takes place in insulin independent tissues (1). Under basal conditions approximately 50% of all glucose utilization occurs in the brain, which is insulin independent and becomes saturated at a plasma glucose concentration of about 40 mg/dl (2). Another 25% of glucose uptake occurs in the splanchnic area (liver plus gastrointestinal tissues) and also is insulin independent (3). The remaining 25% of glucose metabolism in the postabsorptive state takes place in insulin-dependent tissues, primarily muscle (4,5). Basal glucose utilization averages ~2.0 mg/kg.min and is precisely matched by the rate of endogenous glucose production (1,3-7). Approximately 85% of endogenous glucose production is derived from the liver, and the remaining amount is produced by the kidney (1,8,9). Approximately half of basal hepatic glucose production is derived from glycogenolysis and half from gluconeogenesis (9,10).

Following glucose ingestion, the balance between endogenous glucose production and tissue glucose uptake is disrupted. The increase in plasma glucose concentration stimulates insulin release from the

pancreatic beta cells, and the resultant hyperinsulinemia and hyperglycemia serve (i) to stimulate glucose uptake by splanchnic (liver and gut) and peripheral (primarily muscle) tissues (Table 1) and (ii) to suppress endogenous glucose production (1,3-7,11-14). Hyperglycemia, in the absence of hyperinsulinemia, exerts its own independent effect to stimulate muscle glucose uptake and to suppress endogenous glucose production in a dose dependent fashion (14-16). The majority (~80-85%) of glucose that is taken up by peripheral tissues is disposed of in muscle (1,3-7,11-14), with only a small amount (~4-5%) being metabolized by adipocytes (17). Although fat tissue is responsible for only a fraction of total body glucose disposal, it plays a very important role in the maintenance of total body glucose homeostasis (see below). Insulin is a potent inhibitor of lipolysis and even small increments in the plasma insulin concentration exert a potent antilipolytic effect, leading to a marked reduction in the plasma free fatty acid level (18). The decline in plasma FFA concentration results in increased glucose uptake in muscle (19) and contributes to the inhibition of endogenous glucose production (16,20). Thus, changes in the plasma FFA concentration in response to increased plasma levels of insulin and glucose play an important role in the maintenance of normal glucose homeostasis (21,22).

SITE OF INSULIN RESISTANCE IN TYPE 2 DIABETES

The maintenance of whole-body glucose homeostasis is dependent upon a normal insulin secretory response by the pancreatic beta cells and normal tissue sensitivity to the independent effects of hyperinsulinemia and hyperglycemia (i.e., the mass-action effect of glucose) to augment glucose uptake. In turn, the combined effects of insulin and hyperglycemia to promote glucose disposal are dependent on three tightly coupled mechanisms (Table 1): (i) suppression of endogenous (primarily hepatic) glucose production; (ii) stimulation of glucose uptake by the splanchnic (hepatic plus gastrointestinal) tissues; and (iii) stimulation of glucose uptake by peripheral tissues, primarily muscle (1,4,14). Muscle glucose uptake is regulated by flux through two major metabolic pathways: glycolysis (of which ~90% represents glucose oxidation) and glycogen synthesis.

Hepatic Glucose Production

In the overnight fasted state the liver of healthy subjects produces glucose at the rate of ~1.8-2.0 $mg.kg^{-1}.min^{-1}$ (1,3,4,6,18,54). This glucose flux is essential to meet the needs of the brain and other neural tissues, which utilize glucose at a constant rate of ~1-1.2 $mg.kg^{-1}.min^{-1}$ (2,169). Brain glucose uptake accounts for ~50-60% of glucose disposal during the postabsorptive state and this uptake is insulin independent. Therefore, brain glucose uptake occurs at the same rate during absorptive and postabsorptive periods and is not altered in type 2 diabetes (214). Following glucose ingestion, insulin is secreted into the portal vein and glucagon release is inhibited, and this new hormonal ratio is carried to the liver, where it suppresses hepatic glucose output. If the liver does not perceive this insulin signal and continues to produce glucose, there will be two superimposed inputs of glucose into the body, one from the liver and another from the gastrointestinal tract, and marked hyperglycemia will ensue.

In type 2 diabetic subjects with mild to moderate fasting hyperglycemia (140-200 mg/dl, 7.8-11.1 mmol/L) basal hepatic glucose production [HGP] is increased by ~0.5 mg/kg.min. Consequently, during the overnight sleeping hours (2200 h to 0800 h), the liver of a 80-kg diabetic individual with modest fasting hyperglycemia adds an additional 35 g of glucose to the systemic circulation. The increase in basal HGP is closely correlated with the severity of fasting hyperglycemia (1,3,4,6,18,54,157-159,162). Thus, in type 2 diabetic subjects with overt fasting hyperglycemia (>140 mg/dl, 7.8 mmol/l), an excessive rate of hepatic glucose output is the major abnormality responsible for the elevated fasting plasma glucose concentration. The close relationship between fasting plasma glucose concentration and HGP has been demonstrated in numerous studies (164-166,170-174).

In the postabsorptive state, the fasting plasma insulin concentration in type 2 diabetic subjects is 2-4 fold greater than in nondiabetic subjects. Because hyperinsulinemia is a potent inhibitor of HGP (1,3,4-6,16,18,164,165,175), hepatic resistance to the action of insulin must be present in the postabsorptive state to explain the excessive output of glucose by the liver. Hyperglycemia per se also exerts a powerful suppressive action on HGP (15,167,175-177). Therefore, the liver also must be glucose resistance with respect to the inhibitory effect of hyperglycemia to suppress hepatic glucose output, and this has been well documented (15,167,178,179).

Using the euglycemic insulin clamp technique in combination with tritiated glucose, the dose response relationship between hepatic glucose production and the plasma glucose concentration has been defined by Groop, DeFronzo, et al (18). The following points should be emphasized: (i) first, the dose-response curve relating inhibition of HGP to the plasma insulin concentration is quite steep, with an effective dose for half-maximal insulin concentration (ED50) of ~30-40 μU/ml; (ii) in type 2 diabetic individuals the dose response curve is shifted to the right, indicating the presence of hepatic resistance to the inhibitory effect of insulin on hepatic glucose production. However, at plasma insulin concentrations within the high physiologic range (~100 μU/ml), the hepatic insulin resistance can be largely overcome and a near normal suppression of HGP can be achieved; (iii) the severity of the hepatic insulin resistance is related to the severity of the diabetic state. In type 2 diabetic individuals with mild fasting hyperglycemia, an increment in plasma insulin concentration of 100 μU/ml causes a complete suppression of HGP. However, in diabetic subjects with more severe fasting hyperglycemia, the ability of the same plasma insulin concentration to suppress HGP is impaired (18). These results suggest that there is an acquired component of hepatic insulin resistance and that this defect becomes progressively worse as the diabetic state decompensates over time.

The glucose released by the liver in the postabsorptive state can be derived from either glycogenolysis or gluconeogenesis (6,16,176). Studies employing the hepatic vein catheter technique have shown that the uptake of gluconeogenic precursors, especially lactate, is increased in type 2 diabetic subjects (180). Consistent with this observation, radioisotope turnover studies, using lactate, alanine, and glycerol have shown that ~90% of the increase in HGP above baseline can be accounted for by accelerated gluconeogenesis (181,182). More recent studies employing ^{13}C-magnetic resonance imaging (183) and D_2O (184,185) have confirmed the important contribution of accelerated gluconeogenesis to the increase in HGP. An increased rate of glutamine conversion to glucose also has been shown to contribute to the elevated rate of gluconeogenesis in type 2 diabetic subjects (186). The mechanisms responsible for the increase in hepatic gluconeogenesis include hyperglucagonemia (187), increased circulating levels of gluconeogenic precursors (lactate, alanine, glycerol) (181,188), increased FFA oxidation (18,162,189), enhanced sensitivity to glucagon (190) and decreased sensitivity to insulin (1,4.18,164,165). Although the majority of evidence indicates that increased gluconeogenesis is the major cause of the increase in HGP in type 2 diabetic subjects (181-186), it is likely that accelerated glycogenolysis also contributes to it (181,191).

Because of the inaccessibility of the liver in man, it has been difficult to assess the role of key enzymes involved in the regulation of gluconeogenesis (pyruvate carboxylase, phosphoenol- pyruvate carboxykinase), glycogenolysis (glycogen phosphorylase), and net hepatic glucose output (glucokinase, glucose-6-phosphatase). However, considerable evidence from animal models of type 2 diabetes and some evidence in humans have implicated increased activity of PEPCK and G-6-Pase in the accelerated rate of hepatic glucose production (192-194).

More recent evidence has indicated that changes in hypothalamic insulin signaling may affect endogenous glucose production. The activation of the insulin receptor in the third cerebral ventricle is capable of suppressing glucose production, independent of circulating plasma insulin levels or other counter-regulatory hormones. Conversely, central antagonism of insulin signaling impairs the ability of circulating insulin to inhibit glucose production (6A). These observations have raised the possibility that hypothalamic insulin resistance contributes to hyperglycemia in type 2 diabetes.

The Role of the Kidney

The kidney also has been shown to produce glucose and estimates of the renal contribution to total endogenous glucose production have varied from 5% to 20% (8,9,195). These varying estimates of the contribution of renal gluconeogenesis to total glucose production are largely related to the methodology employed to measure glucose production by the kidney (196). One unconfirmed study suggests that the rate of renal gluconeogenesis is increased in type 2 diabetics with fasting hyperglycemia (197). Arguing against this possibility are studies employing the hepatic vein catheter technique which have shown that all of the increase in total body endogenous glucose production (measured with 3-^{3}H-glucose) in type 2 diabetics can be accounted for by increased hepatic glucose output (measured by the hepatic vein catheter technique) (3). A more relevant aspect on the role of the kidney in the dysregulation of glucose homeostasis in diabetes is the maintenance of hyperglycemia, which results from a maladaptive enhancement of the tubular glucose transport threshold (9A, 9B). In response to an elevated glucose load presented to the proximal tubular lumen, the sodium glucose co-transporter system increases its reabosrptive capacity by upregulating the SGLT-2 expression and kinetics (9C). In patients with type 1 and type 2 diabetes, the augmented tubular glucose transport threshold reaches values between 220-250 mg/dl and is responsible for recycling a substantial amount of glucose back into the peripheral circulation (9D). This contribution of the kidney to hyperglycemia in diabetic patients represents one additional pathogenic mechanism that has been underappreciated.

Peripheral (Muscle) Glucose Uptake

Muscle is the major site of glucose disposal in man (1,3-5,14). Under euglycemic hyperinsulinemic conditions, approximately 80% of total body glucose uptake occurs in skeletal muscle (1,3-5). Studies employing the euglycemic insulin clamp in combination with femoral artery/vein catheterization have examined the effect of insulin on leg glucose uptake in type 2 diabetic and control subjects (3). Since bone is metabolically inert with regards to carbohydrate metabolism and adipose tissue takes up less than 5% of an infused glucose load (17,198,199), muscle represents the major tissue responsible for leg glucose uptake.

In response to a physiologic increase in plasma insulin concentration (~80-100 μU/ml), leg (muscle) glucose uptake increases linearly, reaching a plateau value of 10 mg/kg leg wt per minute (3). In contrast, in lean type 2 diabetic subjects, the onset of insulin action is delayed for ~40 min and the ability of the hormone to stimulate leg glucose uptake is markedly blunted, even though the study is carried out for an additional 60 min in the type 2 diabetic group to allow insulin to more fully express its biological effects (3). During the last hour of the insulin clamp study, the rate of glucose uptake was reduced by 50% in the diabetic group (3). These results provide conclusive evidence that the primary site of insulin resistance during euglycemic insulin clamp studies performed in type 2 diabetic subjects resides in muscle tissue. Using the forearm and leg catheterization techniques (13,153,200,202), a number of investigators have demonstrated a decreased rate of insulin-mediated glucose uptake by peripheral tissues. The use of positron emission tomography (PET) scanning to quantitate leg glucose uptake in type 2 diabetic subjects has provided additional support for the presence of severe muscle resistance to insulin in diabetic subjects (203).

Splanchnic (Hepatic) Glucose Uptake

In humans, it is difficult to catheterize the portal vein, and glucose disposal by the liver has not been examined directly. Using the hepatic vein catheterization technique in combination with the euglycemic

insulin clamp, the contribution of the splanchnic (liver plus gastrointestinal) tissues to overall glucose homeostasis has been examined in lean type 2 diabetic subjects with mild to moderate fasting hyperglycemia (3). In the postabsorptive state, there is a net release of glucose from the splanchnic area (i.e., negative balance) in both control and type 2 diabetic subjects, reflecting glucose production by the liver. In response to insulin, splanchnic glucose output is promptly suppressed (reflecting the inhibition of HGP) and, by 20 min, the net glucose balance across the splanchnic region declines to zero (i.e., there was no net uptake or release) (3). After 2 h of sustained hyperinsulinemia, there is a small net uptake of glucose (~0.5 $mg.kg^{-1}.min^{-1}$) by the splanchnic area (i.e., positive balance). This uptake is virtually identical to the rate of splanchnic glucose uptake observed in the basal state, indicating that the splanchnic tissues, like the brain, are insensitive to insulin at least with respect to the stimulation of glucose uptake (3,5,6,175). There was no difference between diabetic and control subjects in the amount of glucose taken up by the splanchnic tissues at any time during the insulin clamp study (3).

The results of these studies illustrate another important point: namely, that under conditions of euglycemic hyperinsulinemia, very little of the infused glucose is taken up by the splanchnic (and therefore hepatic) tissues (3,5,6,175). During the insulin clamp, the rate of whole body glucose uptake averaged 7 $mg.kg^{-1}.min^{-1}$, and of this, only 0.5 $mg.kg^{-1}.min^{-1}$ or 7%, was disposed of by the splanchnic region. Because the difference in insulin-mediated total body glucose uptake between the type 2 diabetic and control groups during the euglycemic insulin clamp study was 2.5 $mg.kg^{-1}.min^{-1}$, from a purely quantitative standpoint it is obvious that a defect in splanchnic (hepatic) glucose removal never could account for the magnitude of impairment in total body glucose uptake following intravenous glucose/insulin administration. However, after glucose ingestion, the oral route of administration and the resultant hyperglycemia conspire to enhance splanchnic (hepatic) glucose uptake (6,7,11,12,16,26,175) and, under these conditions, diminished hepatic glucose uptake has been shown to contribute to the impairment in glucose tolerance in type 2 diabetes (see discussion below) (6,204,205).

Summary: Whole Body Glucose Utilization

Insulin-mediated whole body glucose utilization during the euglycemic insulin clamp is summarized in Fig. 12. The total height of each bar represents the amount of glucose taken up by all tissues in the body during the insulin clamp in control and type 2 diabetic subjects. Net splanchnic glucose uptake, quantitated by the hepatic venous catheterization technique, is similar in both groups and averaged 0.5 $mg.kg^{-1}.min^{-1}$. Adipose tissue glucose uptake accounts for less than 5% of total glucose disposal (17,198,199). Brain glucose uptake, estimated to be 1.0-1.2 $mg.kg^{-1}.min^{-1}$ in the postabsorptive state (2,169,206), is unaffected by hyperinsulinemia (169). Muscle glucose uptake (extrapolated from leg catheterization data) in control subjects accounts for ~75-80% of the total glucose uptake (1,3,4). In type 2 diabetic subjects, the largest part of the impairment in insulin-mediated glucose uptake is accounted for by a defect in muscle glucose disposal. Even if adipose tissue of type 2 diabetic subjects took up absolutely no glucose, it could, at best, explain only a small fraction of the defect in whole body glucose metabolism.

Glucose Disposal During OGTT

In every day life, the gastrointestinal tract represents the normal route of glucose entry into the body. However, the assessment of tissue glucose disposal following glucose ingestion presents a challenge because of the difficulties in quantitating the rate of glucose absorption, suppression of hepatic glucose production, and organ (liver and muscle) glucose uptake. Moreover, because the plasma glucose and insulin concentrations are changing simultaneously, it is difficult to draw conclusions about insulin secretion or insulin sensitivity.

To address these issues, Ferrannini, DeFronzo, and colleagues (7,11,12,205) administered oral glucose to healthy control subjects in combination with hepatic vein catheterization to examine splanchnic glucose metabolism. The oral glucose load and endogenous glucose pool were labeled with [1-^{14}C]glucose and [3-^{3}H]glucose, respectively, to quantitate total body glucose disposal (from tritiated glucose turnover) and endogenous HGP (difference between the total rate of glucose appearance, as measured with tritiated glucose, and the rate of oral glucose appearance, as measured with [1-^{14}C]glucose).

During the 3.5 h after glucose (68 g) ingestion: (i) 19 g, or 28%, or the oral load was taken up by splanchnic tissues; (ii) 48 g, or 72%, was disposed of by peripheral (non-splanchnic) tissues; (iii) of the 48 g taken up by peripheral tissues, the brain (an insulin-independent tissue) accounted for ~15 g (~1 mg.kg^{-1}.min^{-1}), or 22%, of the total glucose load (12); (iv) basal HGP declined by 53%. Similar percentages for splanchnic glucose uptake (24%-29%) and suppression of HGP (50%-60%) in normal subjects have been reported by other investigators (13,204,207-209). The contribution of skeletal muscle to the disposal of an oral glucose load has been reported to vary from a low of 26% (207) to a high of 56% (208), with a mean of 45% (11,13,207-209). These results emphasize several important differences between oral and intravenous glucose administration. After glucose ingestion: (i) HGP is less completely suppressed, most likely do to activation of local sympathetic nerves that innervate the liver (210); (ii) peripheral tissue (primarily muscle) glucose uptake is quantitatively less important; (3) splanchnic glucose uptake is quantitatively much more important.

In type 2 diabetic individuals (12,204,205,211,212) the disposition of an oral glucose load is significantly altered. The disturbance in glucose metabolism is accounted for by two factors: (i) decreased tissue glucose uptake and (ii) impaired suppression of HGP. Splanchnic glucose uptake is similar in diabetic and control groups. Inappropriate suppression of HGP accounted for approximately one-third of the defect in total-body glucose homeostasis, while reduced peripheral (muscle) glucose uptake accounted for the remaining two-thirds. Since hyperglycemia per se enhances splanchnic (hepatic) glucose uptake in proportion to the increase in plasma glucose concentration (24,175), the splanchnic glucose clearance (SGU/plasma glucose concentration) is markedly reduced in all type 2 diabetic subjects following glucose ingestion. Using a combined insulin clamp/OGTT technique, impairment in glucose uptake by the splanchnic tissues in type 2 diabetics has been demonstrated directly (213).

The gastrointestinal incretin hormones, which are produced in response to nutrient intake and potentiate the stimulus to insulin secretion in the postprandial period have been implicated as additional factors in the pathogenesis of type 2 diabetes (4A,28-30). The combined actions of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) can account for most of the incretin effect in normal subjects (4B). Recent demonstration that in type 2 diabetes the incretin effect is impaired, diminished or absent (4B) has rekindled interest in the potential role of these gastrointestinal peptides in the abnormal handling of glucose by splanchnic tissues and perhaps, in the decline in beta-cell insulin secretion.

When viewed in absolute terms, most studies have demonstrated that the total amount of glucose taken up by all tissues of body over the 4 hour period following the ingestion of an oral glucose load is normal (13) or slightly decreased (204,205,211). However, this occurs at the expense of postprandial hyperglycemia. Thus, the efficiency of glucose disposal, i.e., the glucose clearance (tissue glucose uptake/plasma glucose concentration), is severely reduced. It should be emphasized that it is not the absolute glucose disposal rate, but rather the increment in glucose disposal above baseline that determines the rise in plasma glucose concentration above the fasting value. Every published study (13,204,205,211) has demonstrated that the incremental response in whole-body glucose uptake is moderately to severely reduced in type 2 diabetic individuals. Similar results have been reported for forearm muscle glucose uptake (13,201,202,208,209), pointing out the important contribution of diminished muscle glucose disposal to impaired oral glucose tolerance in type 2 diabetes.

In summary, results of the OGTT indicate that both impaired suppression of HGP and decreased tissue (muscle) glucose uptake contribute approximately equally to the glucose intolerance of type 2 diabetes. The efficiency of the splanchnic (hepatic) tissues to take up glucose (as reflected by the splanchnic glucose clearance) also is impaired in type 2 diabetic individuals.

Summary of Insulin Resistance in Type 2 Diabetes

Insulin resistance involving both muscle and liver are characteristic features of the glucose intolerance in type 2 diabetic individuals. In the basal state, the liver represents a major site of insulin resistance, and this is reflected by overproduction of glucose despite the presence of both fasting hyperinsulinemia and hyperglycemia. This accelerated rate of hepatic glucose output is the primary determinant of the elevated fasting plasma glucose concentration in type 2 diabetic individuals. Although tissue (muscle) glucose uptake in the postabsorptive state is increased when viewed in absolute terms, the efficiency with which glucose is taken up (i.e., the glucose clearance) is diminished. After glucose infusion or ingestion (i.e., in the insulin stimulated state) both decreased muscle glucose uptake and impaired suppression of HGP contribute to the insulin resistance. Following glucose ingestion, the defects in insulin-mediated glucose uptake by muscle and the suppression of HGP by insulin contribute approximately equally to the disturbance in whole-body glucose homeostasis in type 2 diabetes. However, under euglycemic hyperinsulinemic conditions, HPG is largely suppressed and impaired muscle glucose uptake is primarily responsible for the insulin resistance.

DYNAMIC INTERACTION BETWEEN INSULIN SENSITIVITY AND INSULIN SECRETION IN TYPE 2 DIABETES

Type 2 diabetic subjects manifest abnormalities both in tissue (muscle, fat, and liver) sensitivity to insulin and in pancreatic insulin secretion. To understand how these two metabolic disturbances interact to produce the full-blown diabetic condition, it is necessary to quantitate insulin action and insulin secretion in the same individual over a wide range of insulin sensitivity. This dynamic interaction is demonstrated graphically by results obtained in healthy, lean, young normal glucose tolerant women who received a euglycemic insulin clamp (1 $mU.kg^{-1}.min^{-1}$) and were stratified into quartiles based upon the rate of insulin-mediated glucose disposal (49). Insulin secretion was measured independently on a separate day with a +125 mg/dl hyperglycemic clamp. Insulin resistance and insulin secretion were strongly and positively correlated ($r=0.79$, $p<0.001$). Women who were the most insulin resistant (quartile 1) had the highest fasting plasma insulin concentrations and highest early and late phase plasma insulin responses. Similar results relating the plasma insulin response and the severity of insulin resistance have been reported in normal glucose tolerant subjects with the minimal model technique (46,47) and the insulin suppression test/oral glucose tolerance test (214).

A number of groups have examined the dynamic interaction between insulin secretion and insulin sensitivity in type 2 diabetic subjects (1,4,34,35,38,39,42,46-48,58-61,150,162). DeFronzo (4) studied lean (ideal body weight < 120%) and obese (ideal body weight > 125%) subjects with varying degrees of glucose tolerance as follows: Group I-obese subjects (n=24) with normal glucose tolerance; Group II-obese subjects (n=23) with impaired glucose tolerance; Group III-obese subjects (n=35) with overt diabetes, subdivided into those with a hyperinsulinemic response and those with a hypoinsulinemic response during a 100-gram OGTT; Group IV-normal weight type 2 diabetics (n=26); Group V-normal weight subjects (n=25) with normal glucose tolerance. All subjects ingested 100 g of glucose to provide a measure of glucose tolerance and insulin secretion. Whole-body insulin sensitivity was quantitated with the euglycemic insulin (~100 µU/ml) clamp technique, which was performed with indirect calorimetry to

quantitate rates of glucose oxidation and nonoxidative glucose disposal. The later primarily reflects glycogen synthesis (215).

In normal weight type 2 diabetic subjects, insulin-mediated whole-body glucose uptake was reduced by 40-50% and the impairment in insulin action resulted from defects in both oxidative and nonoxidative glucose metabolism (4). Obese nondiabetic individuals were as insulin resistant as the normal-weight diabetic subjects (4). Defects in both glucose oxidation and glucose storage contributed to the insulin resistance in the obese nondiabetic group. From the metabolic standpoint, therefore, obesity and type 2 diabetes closely resemble each other. Similar results concerning reduced whole-body insulin sensitivity in obese and type 2 diabetic individuals have been reported by other investigators (160,161,166,216-218). Despite nearly identical degrees of insulin resistance, normal-weight diabetic subjects manifested fasting hyperglycemia and marked glucose intolerance, whereas the obese nondiabetic individuals had normal or only minimally impaired oral glucose tolerance (4). This apparent paradox is explained by the plasma insulin response during the OGTT. Compared with control subjects, the obese nondiabetic group secreted more than twice as much insulin, and this was sufficient to offset the insulin resistance. In contrast, in normal-weight diabetic subjects, the pancreas, when faced with the same challenge, was unable to augment its secretion of insulin sufficiently to compensate for the insulin resistance. This imbalance between insulin supply by the beta-cells and the insulin requirement by tissues resulted in a frankly diabetic state, with fasting hyperglycemia and marked glucose intolerance.

The fact that plasma insulin response to the development of insulin resistance typically is increased during the natural history of type 2 diabetes does not mean that the beta-cell is functioning normally. To the contrary, recent studies (4C) have demonstrated that the onset of beta-cell failure occurs much earlier and is more severe than previously appreciated. Recognizing that simply measuring plasma insulin response to a glucose challenge does not provide a valid index of beta-cell function, a series of studies were conducted in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes, using an oral glucose tolerance test to evaluate the increment in insulin secretion in response to an increment in plasma glucose. A euglycemic insulin clamp to measure insulin sensitivity was also performed to address the adjustment of the beta-cell to the body's sensitivity to insulin. Thus, the results yielded a better measure of beta-cell function expressed per increment of plasma glucose and corrected for the degree of insulin resistance, the so-called disposition index [$\Delta I/\Delta G \div IR$]. These data revealed a substantial decrease in beta-cell function, most evident in individuals with IGT who had lost anywhere from 60 to 85% of the total insulin secretory capacity.

When obesity and diabetes coexist in the same individual, the severity of insulin resistance is only slightly greater than that in either the normal-weight diabetic or nondiabetic obese groups (4), and the magnitude of the defects in glucose oxidation and nonoxidative glucose disposal are similar in all obese and diabetic groups. Although hyperinsulinemic and hypoinsulinemic obese diabetic subjects were equally insulin resistant, the severity of glucose intolerance is worse in the hypoinsulinemic group, and this was related entirely to the presence of severe insulin deficiency.

An integrated summary of insulin action and insulin secretion in obese, diabetic, and lean groups is presented in figure 4. The plasma insulin response during the OGTT is shown in the top panel, along with the rate of insulin-mediated glucose disposal. In the obese nondiabetic subjects, tissue sensitivity to insulin is markedly reduced, but glucose tolerance remains perfectly normal because the beta-cells are able to augment their insulin secretory capacity appropriately to offset the defect in insulin action. As the obese individual develops impaired intolerance, there is a further reduction in insulin-mediated glucose disposal, which is due primarily to a decrease in glycogen synthesis. However, there is only a small additional impairment in glucose tolerance, because the beta-cells are able to augment further their secretion of insulin to counteract the deterioration in insulin sensitivity. The progression of the obese, glucose intolerant person to overt diabetes is heralded by a decline in insulin secretion without any worsening of insulin resistance. The obese diabetic has tipped over the top of Starling's curve of the pancreas and is now on the descending portion. Even though the plasma insulin response is increased

compared to nondiabetic control subjects, it is not elevated appropriately for the degree of insulin resistance and there is evidence that there is ~80% of beta-cell functional loss by the time of diagnosis in diabetic subjects. In the normal-weight diabetic group, there is a further decline in glucose tolerance, which results from a greater impairment in insulin secretion without any additional deterioration in insulin sensitivity. Lastly, the obese diabetic group with a low insulin response manifests the greatest glucose intolerance, due to the presence of marked insulin deficiency without any further worsening of insulin sensitivity.

The natural history of type 2 diabetes described above is consistent with results in humans and monkeys published by other investigators (33-39,42,43,59-61,98,150). In lean subjects with a wide range of glucose tolerance, Reaven et al (42) demonstrated that the progression from normal to impaired glucose tolerance was marked by the development of severe insulin resistance, which was counterbalanced by a compensatory increase in insulin secretion. The onset of type 2 diabetes was associated with no (or only slight) further deterioration in tissue sensitivity to insulin. Rather, insulin secretion declined and the impairment in beta cell function was paralleled by a decrease in glucose tolerance. A similar sequence of events has been documented prospectively in Pima Indians (34-39,58,60). The sequence of events described in Caucasians (1,4,41,42,44,47,59,162,219), Pima Indians (34-39,58,60,219), and Pacific Islanders (33,62,220) is consistent with the development of type 2 diabetes in the rhesus monkeys (48). As monkeys grow older, they become obese and develop a diabetic condition closely resembling human type 2 diabetes. The earliest detectable abnormality in this primate model is a decrease in tissue sensitivity to insulin. Because of a compensatory increase in insulin secretion, the fasting plasma glucose concentration and glucose tolerance remain normal.

The studies detailed above indicate that insulin resistance is an early and characteristic feature of the natural history of type 2 diabetes in high risk populations. Overt diabetes develops only in those individuals whose beta cells are unable to appropriately augment their secretion of insulin to compensate for the defect in insulin action. It should be recognized, however, that there are well-described type 2 diabetic populations in whom insulin sensitivity is normal at the onset of diabetes, whereas insulin secretion is severely impaired (81-83). How frequently this occurs in typical type 2 diabetic patients remains to be determined. This insulinopenic variety of type 2 diabetes appears to be more common in African-Americans, elderly subjects, and lean Caucasians. In this later group, it is important to exclude type 1 diabetes, since ~10% of Caucasian individuals with older onset diabetes are islet cell antibody and/or GAD positive (220).

ROLE OF THE ADIPOCYTE IN THE PATHOGENESIS OF TYPE 2 DIABETES MELLITUS

The majority (>80%) of type 2 diabetics in the US are overweight (221). Both lean and especially obese type 2 diabetics are characterized by day-long elevations in the plasma free fatty acid concentration, which fail to suppress normally following ingestion of a mixed meal or oral glucose load (222). Free fatty acids (FFA) are stored as triglycerides in adipocytes and serve as an important energy source during conditions of fasting. Insulin is a potent inhibitor of lipolysis, and restrains the release of FFA from the adipocyte by inhibiting the enzyme hormone sensitive lipase. In type 2 diabetics the ability of insulin to inhibit lipolysis (as reflected by impaired suppression of radioactive palmitate turnover) and reduce the plasma FFA concentration is markedly reduced (17). It is now recognized that chronically elevated plasma FFA concentrations can lead to insulin resistance in muscle and liver (1,4,19,21,22,51,162,223,224) and impair insulin secretion (22,225,226) . Thus, elevated plasma FFA levels can cause/aggravate three major pathogenic disturbances that are responsible for impaired glucose homeostasis in type 2 diabetic individuals and the "triumvirate" (muscle, liver, beta cell) was joined by the "fourth musketeer" (227) to form the "disharmonious quartet". In addition to FFA that

circulate in plasma in increased amounts, type 2 diabetic and obese nondiabetic individuals have increased stores of triglycerides in muscle (228,229) and liver (230,231) and the increased fat content correlates closely with the presence of insulin resistance in these tissues. Triglycerides in liver and muscle are in a state of constant turnover and the metabolites (i.e., fatty acyl CoAs) of intracellular FFAs have been shown to impair insulin action in both liver and muscle (1,4,92). This sequences of events has been referred to as "lipotoxicity" (1,4,22,93). Evidence also has accumulated to implicate "lipotoxicity" as an important cause of beta cell dysfunction (22,93) (see earlier discussion).

FFA and Muscle Glucose Metabolism

Four decades ago, Randle (232) proposed that increased FFA oxidation restrains glucose oxidation in muscle by altering the redox potential of the cell and by inhibiting key glycolytic enzymes. The excessive FFA oxidation: (i) leads to the intracellular accumulation of acetyl CoA, a potent inhibitor of pyruvate dehydrogenase (PDH), (ii) increases the NADH/NAD ratio, causing a slowing of the Krebs cycle, and (iii) results in the accumulation of citrate, a powerful inhibitor of phosphofructokinase (PFK). Inhibition of PFK leads to the accumulation of glucose-6-phosphate (G-6-P) which in turn inhibits hexokinase II. The block in glucose phosphorylation causes a build up of intracellular free glucose which restrains glucose transport into the cell via the GLUT4 transporter. The resultant decrease in glucose transport was postulated to account for the impairment in glycogen synthesis, although a direct inhibitory effect of fatty acyl CoAs on glycogen synthase also has been demonstrated (233). This sequence of events via which accelerated plasma FFA oxidation inhibits muscle glucose transport, glucose oxidation, and glycogen synthesis is referred to as the "Randle Cycle" (232). It should be noted that the same scenario would ensue if the FFA were derived from triglycerides stored in muscle (228,229) or from plasma (222).

Felber and coworkers (59,159,162,234,235) were amongst the first to demonstrate that in obese non-diabetic and diabetic humans, basal plasma FFA levels and lipid oxidation (measured by indirect calorimetry) are increased and fail to suppress normally after glucose ingestion. The elevated basal rate of lipid oxidation was strongly correlated with a decreased basal rate of glucose oxidation, as well as with reduced rates of glucose oxidation and non-oxidative glucose disposal (glycogen synthesis) following ingestion of a glucose load. Further validation of the Randle Cycle in man has come from studies employing the euglycemic insulin clamp. In normal subjects, physiologic hyperinsulinemia (80-100 μU/ml) causes a 60-70% decline in plasma FFA concentration and a parallel decline in plasma FFA and total body lipid oxidation (18). When Intralipid is infused concomitantly with insulin to maintain or increase the plasma FFA concentration/oxidation, both glucose oxidation and non-oxidative glucose disposal are inhibited in a dose dependent fashion (223). Using magnetic resonance imaging, it has been shown that the FFA-induced inhibition of non-oxidative glucose disposal reflects impaired glycogen synthesis (236). The inhibitory effect of elevated plasma FFA levels can be observed at all plasma insulin concentrations, spawning the physiologic and pharmacologic range (223).

The inhibitory effect of an acute elevation in plasma FFA concentration on muscle glucose metabolism is time dependent. Thus, the earliest (within 2 hours) observed abnormality is a defect in glucose oxidation (237), as would be predicted by operation of the Randle cycle (232). This is followed (between 2-3 hours) by defects in glucose transport and phosphorylation and eventually (after 3-4 hours) by impaired glycogen synthesis.

Randle Cycle Revisited: Biochemical/Molecular Basis Of FFA-Induced Insulin Resistance

The original description of the Randle cycle was formulated based upon experiments performed in rat diaphragm and heart muscle (232). More recent studies performed in human skeletal muscle suggest that mechanisms in addition to those originally proposed by Randle are involved in the FFA-induced insulin resistance. Thus, several groups (236,238,239) have failed to observe a rise in muscle G-6-P and citrate concentrations when insulin-stimulated glucose metabolism was inhibited by an increase in the plasma FFA concentration. Elevated plasma FFA levels also failed to inhibit muscle phosphofructokinase activity. Thus, while increased FFA/lipid oxidation and decreased glucose oxidation are closely coupled, as originally demonstrated by Randle, mechanisms other than product (i.e., elevated intracellular G-6-P and free glucose concentrations) inhibition of the early steps of glucose metabolism must be invoked to explain the defects in glucose transport, glucose phosphorylation and glycogen synthesis.

Studies in humans and animals have shown a strong inverse correlation between insulin-stimulated glucose metabolism and increased intramuscular lipid pools, including triglyceride (240-242), diacylglycerol (DAG) (243,244), and long chain fatty acyl CoAs (FA-CoA) (245). An acute elevation in plasma FFA concentration leads to an increase in muscle fatty acyl CoA and DAG concentrations. Both long chain fatty acyl CoAs and DAG activate PKC theta (243), which increases serine phosphorylation with subsequent inhibition of IRS-1 tyrosine phosphorylation (246,247). Consistent with this observation, two groups have shown that in human muscle elevated plasma FFA levels inhibit insulin-stimulated tyrosine phosphorylation of IRS-1, the association of the p85 subunit of PI-3 kinase with IRS-1, and activation of PI-3-kinase (248,249). Direct effects of long chain fatty acyl CoAs on glucose transport (250), glucose phosphorylation (251), and glycogen synthase (233) also have been demonstrated in muscle. Lastly, increased muscle ceramide levels (secondary to increased long chain fatty acyl CoAs) have been shown to interfere with glucose transport and to inhibit glycogen synthase in muscle via activation of PKB (252). In summary, elevated plasma FFA concentrations can induce insulin resistance in muscle via multiple mechanisms involving alterations in a variety of intracellular lipid signaling molecules which exert their inhibitory effects on multiple steps (insulin signal transduction system, glucose transport, glucose phosphorylation, glycogen synthase, pyruvate dehydrogenase, Krebs cycle) involved in glucose metabolism.

FFA and Blood Flow

Insulin is a vasodilatory hormone and the stimulatory effect of insulin on muscle glucose metabolism has been shown to result from: (i) a direct action of insulin to augment muscle glucose metabolism, and (ii) increased blood flow to muscle (253,254). The vasodilatory effect of insulin is mediated via the release of nitric oxide from the vascular endothelium (255). In insulin resistant conditions, such as obesity and type 2 diabetes, some investigators have suggested that as much as half of the impairment in insulin-mediated whole body and leg muscle glucose uptake is related to a defect in insulin's vasodilatory action (253,254), although the link between insulin-mediated vasodilation and increased blood flow, as well as the underlying mechanisms have been challenged by others (256, 256A). More recent studies employed contrast-enhanced ultrasonography using 1-methyl-xantine to demonstrate that insulin infusion promotes capillary recruitment in healthy individuals. These data have suggested that there is a time-dependent effect of insulin on regional blood flow redistribution with capillary pre-sphincter relaxation preceding vasodilation and consequent increase in skeletal muscle glucose metabolism (256B). These observations also provided a partial explanation for the discrepant findings reported on the topic of insulin, fatty acids and vasodilatation.

Because type 2 diabetes and obesity are insulin resistant states characterized by day-long elevation in the plasma FFA concentration (222) and impaired endothelium dependent vasodilation (253), investigators have examined the effect of increased plasma FFA levels on limb blood flow and muscle glucose uptake (257,258). In healthy, non-diabetic subjects an acute physiologic increase in plasma FFA concentration inhibited methacholine (endothelium dependent) but not nitroprusside (endothelium independent) stimulated blood flow in association with an impairment in insulin-stimulated muscle glucose disposal. In subsequent studies, the inhibitory effect of FFA on insulin-stimulated leg blood flow was shown to be associated with decreased nitric oxide availability (259). FFA elevation also inhibits nitric oxide production in endothelial cell cultures by decreasing nitric oxide synthase activity (259). Since the IRS-1/PI-3 kinase signal transduction pathway is involved in the regulation of nitric oxide synthase activity (260), one could hypothesize that FFA-induced inhibition of the insulin signal transduction pathway is responsible for the blunted vasodilatory response to the hormone.

FFA and Hepatic Glucose Metabolism

The liver plays a pivotal role in the regulation of glucose metabolism (1,4,6,11,16,205). Following carbohydrate ingestion, the liver suppresses its basal rate of glucose production and takes up approximately one-third of the glucose in the ingested meal (12,24,25,205). Collectively, suppression of hepatic glucose production and augmentation of hepatic glucose uptake account for the maintenance of nearly one-half of the rise in plasma glucose concentration following ingestion of a carbohydrate meal.

Hepatic glucose production is regulated by a number of factors, of which insulin (inhibits HGP) and glucagon and FFA (stimulate HGP) are the most important. In vitro studies have demonstrated that plasma FFA are potent stimulators of HGP and do so by increasing the activity of pyruvate carboxylase and phosphoenolpyruvate carboxykinase, the rate limiting enzymes for gluconeogenesis (261,262). FFA also enhances the activity of glucose-6-phosphatase, the enzyme that ultimately controls the release of glucose by the liver (263).

In normal subjects, increase plasma FFA levels stimulate gluconeogenesis (264,265), while a decrease in plasma FFA concentration reduces gluconeogenesis (264). It has documented that a significant portion of the suppressive effect of insulin on hepatic glucose production is mediated via inhibition of lipolysis and a reduction in circulating plasma FFA concentrations (16,266,267). Moreover, FFA infusion in normal humans under conditions that simulate the diabetic state (268) and in obese insulin-resistant subjects (269) enhances hepatic glucose production, most likely secondarily to stimulation of gluconeogenesis.

In type 2 diabetic subjects, the fasting plasma FFA concentration and lipid oxidation rate are increased and are strongly correlated with both the elevated fasting plasma glucose concentration and basal rate of hepatic glucose production (18,51,59,162,190,270). The relationship between elevated plasma FFA concentration, FFA oxidation, and hepatic glucose production in obesity and type 2 diabetes is explained as follows: (i) increased plasma FFA levels, by mass action, augment FFA uptake by hepatocytes, leading to accelerated lipid oxidation and accumulation of acetyl CoA. The increased concentration of acetyl CoA stimulates pyruvate carboxylase, the rate limiting enzyme in gluconeogenesis (261,262), as well as glucose-6-phosphatase, the rate-controlling enzyme for glucose release from the hepatocyte (263); (ii) the increased rate of FFA oxidation provides a continuing source of energy (in the form of ATP) and reduced nucleotides (NADH) to drive gluconeogenesis; (iii) elevated plasma FFA induce hepatic insulin resistance by inhibiting the insulin signal transduction system (244-248). In type 2 diabetic patients these deleterious effects of elevated plasma FFA concentrations occur in concert with increased plasma glucagon levels (181,190,271), increased hepatic sensitivity to glucagon, and increased hepatic uptake of circulating gluconeogenic precursors.

Summary: FFA and the Pathogenesis of Obesity and Type 2 Diabetes Mellitus

In obese individuals and in the majority (>80%) of type 2 diabetic subjects, there is an expanded fat cell mass and the adipocytes are resistant to the antilipolytic effects of insulin (18). Most obese and diabetic individuals are characterized by visceral adipocity (272) and visceral fat cells have a high lipolytic rate, which is especially refractory to insulin (273). Not surprisingly, both type 2 diabetes and obesity are characterized by an elevation in the mean day-long plasma FFA concentration. Elevated plasma FFA levels, as well as increased triglyceride/fatty acyl CoA content in muscle, liver, and beta cell, lead to the development of muscle/hepatic insulin resistance and impaired insulin secretion.

The OMNIOUS OCTET

The eight principal known causes leading to hyperglycemia through the pathogenesis of type 2 diabetes are summarized in Figure 1. It is already established that decreased peripheral glucose uptake combined with augmented endogenous (hepatic) glucose production are characteristic features of insulin resistance. Increased lipolysis with accumulation of intermediary lipid metabolites contributes to further enhance glucose output while reducing peripheral utilization. Compensatory insulin secretion by the pancreatic beta-cells eventually reaches a maximum and, then it progressively deteriorates. Concomitantly, there is inappropriate release of glucagon from the pancreatic alpha-cells, particularly in the post-prandial period. It has been postulated that both impaired insulin and excessive glucagon secretion in type 2 diabetes are facilitated by the “incretin defect”, defined primarily as inadequate response of the gastrointestinal “incretin” hormones to meal ingestion. Moreover, considering that hypothalamic insulin resistance (central nervous system) also impairs the ability of circulating insulin to suppress glucose production and, the fact that renal tubular glucose reabsorption capacity is enhanced in diabetic patients, the time has arrived to advance the concept from the “triumvirate” to the “omnious octect” (4A). These pathogenetic mechanisms must be taken into account when deciding for the treatment of hyperglycemia in patients with type 2 diabetes.

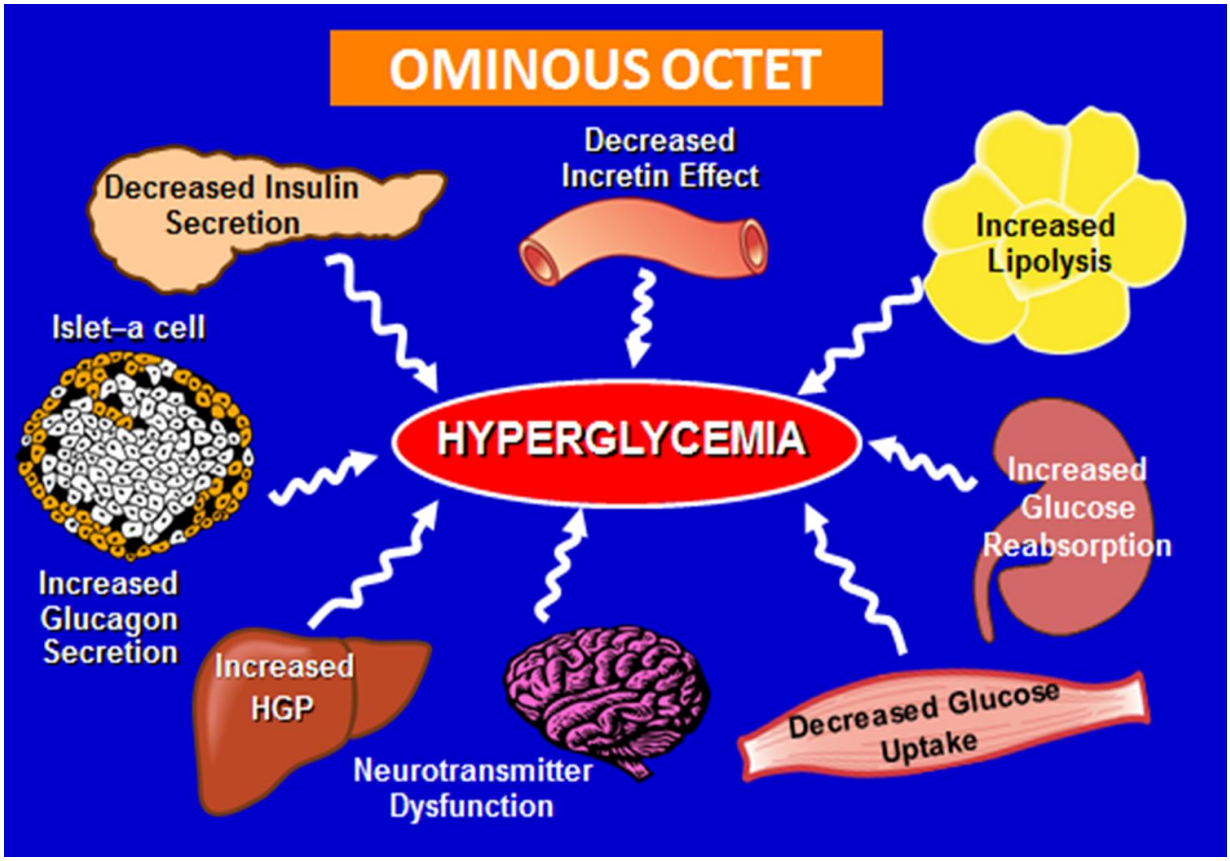

Figure 1. Summary of the eight principal mechanisms contributing to hyperglycemia in patients with type 2 diabetes

CELLULAR MECHANISMS OF INSULIN RESISTANCE

The stimulation of glucose metabolism by insulin requires that the hormone must first bind to specific receptors that are present on the cell surface of all insulin target tissues (1,274-277). After insulin has bound to and activated its receptor, "second messengers" are generated and these second messengers initiate a series of events involving a cascade of phosphorylation-dephosphorylation reactions (1,274-280) that eventually result in the stimulation of intracellular glucose metabolism. The initial step in glucose metabolism involves activation of the glucose transport system, leading to influx of glucose into insulin target tissues, primarily muscle (1,281,282). The free glucose, which has entered the cell, subsequently is metabolized by a series of enzymatic steps that are under the control of insulin. Of these, the most important are glucose phosphorylation (catalyzed by hexokinase), glycogen synthase (which controls glycogen synthesis), and phosphofructokinase (PFK) and PDH (which regulate glycolysis and glucose oxidation, respectively).

Insulin Receptor/Insulin Receptor Tyrosine Kinase

The insulin receptor is a glycoprotein consisting of two alpha subunits and two beta subunits linked by disulfide bonds (1,274-277). The alpha subunit of the insulin receptor is entirely extracellular and

contains the insulin-binding domain. The beta subunit has an extracellular domain, a transmembrane domain, and an intracellular domain that expresses insulin-stimulated kinase activity directed towards its own tyrosine residues (1,274-277). Insulin receptor phosphorylation of the beta subunit, with subsequent activation of insulin receptor tyrosine kinase, represents the first step in the action of insulin on glucose metabolism (274-277). Mutagenesis experiments have shown that insulin receptors devoid of tyrosine kinase activity are completely ineffective in mediating insulin stimulation of cellular metabolism (283,284). Similarly, mutagenesis of any of the three major phosphorylation sites (at residues 1158, 1163, and 1162) impairs insulin receptor kinase activity, resulting in a decrease in the acute metabolic and growth promoting effects of insulin (283,285).

Insulin Receptor Signal Transduction

Following activation, insulin receptor tyrosine kinase phosphorylates specific intracellular proteins, of which at least nine have been identified (282). Four of these belong to the family of insulin-receptor substrate proteins: IRS-1, IRS-2, IRS-3, IRS-4 (the others include Shc, Cbl, Gab-1, p60dok, and APS). In muscle IRS-1 serves as the major docking protein that interacts with the insulin receptor tyrosine kinase and undergoes tyrosine phosphorylation in regions containing amino acid sequence motifs (YXXM or YMXM) that, when phosphorylated, serve as recognition sites for proteins containing src-homology 2 (SH2) domains (where y = tyrosine, M = methionine, and x - any amino acid) (274,275). Mutation of these specific tyrosines severely impairs the ability of insulin to stimulate glycogen and DNA synthesis, establishing the important role of IRS-1 in insulin signal transduction (282). In liver, IRS-2 serves as the primary docking protein that undergoes tyrosine phosphorylation and mediates the effect of insulin on hepatic glucose production, gluconeogenesis and glycogen formation (287). In adipoctes, Cbl represents another substrate which is phosphorylated following its interaction with the insulin receptor tyrosine kinase and which is required for stimulation of GLUT 4 transloctaion. Phosphorylation of Cbl occurs when the CAP/Cbl complex associates with flotillin in caveolae, or lipid rafts, containing insulin receptors (288,289).

In muscle, the phosphorylated tyrosine residues on IRS-1 mediate an association between the two SH2 domains of the 85-kDa regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase), leading to activation of the enzyme (274-284,290,291). PI3-kinase is a heterodimeric enzyme comprised of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. The latter catalyzes the 3-prime phosphorylation of phosphatidylinositol (PI), PI-4-phosphate, and PI-4,5-diphosphate, resulting in the stimulation of glucose transport (274-277). Activation of PI3-kinase by phosphorylated IRS-1 also leads to activation of glycogen synthase (274,275), via a process that involves activation of PKB/Akt and subsequent inhibition of kinases such as GSK-3 (292) and activation of protein phosphatase 1 (PP1) (293). Inhibitors of PI3-kinase impair glucose transport (274-277,294) by interfering with the translocation of GLUT 4 transporters from their intracellular location (281,282) and block the activation of glycogen synthase (295) and hexokinase (HK)-II expression (296). The action of insulin to increase protein synthesis and inhibit protein degradation also is mediated by PI-3 kinase and involves the activation of mTOR (297,298). mTOR controls translation machinery by phosphorylation and activation of p70 ribosomal S6 kinase (p70rsk) (297) and phosphorylation of initiation factors (299). Insulin also promotes hepatic triglyceride synthesis via increasing the transcription factor steroid regulatory element-binding protein (SREBP)-1c (300) and this lipogenic effect of insulin also appears to be mediated via the PI3-kinase pathway (274).

Other proteins with SH2 domains, including the adapter protein Grb2 and Shc, also interact with IRS-1 and become phosphorylated following exposure to insulin (274-276,301). Grb2 and Shc serve to link IRS-1/IRS-2 to the mitogen-activated protein (MAP) signaling pathway, which plays an important role in the generation of transcription factors (274,275). Following the interaction between IRS-1/IRS-2 and Grb2 and Shc, Ras is activated, leading to the stepwise activation of Raf, MEK, and ERK. Activated ERK

than translocates into the nucleus of the cell where it catalyzes the phosphorylation of transcription factors which promote cell growth, proliferation, and differentiation (274-276,301-303). Blockade of the MAP kinase pathway prevents the stimulation of cell growth by insulin but has no effect on the metabolic actions of the hormone (304-306).

Under anabolic conditions insulin stimulates glycogen synthesis by simultaneously activating glycogen synthase and inhibiting glycogen phosphorylase (307-309). The effect of insulin is mediated via the PI3 kinase pathway which inactivates kinases such as glycogen synthase kinase-3 and activates phosphatases, particularly protein phosphatase 1 (PP1). It is believed that PP1 is the primary regulator of glycogen metabolism (307-310). In skeletal muscle, PP1 associates with a specific glycogen-binding regulatory subunit, causing dephosphorylation (activation) of glycogen synthase. PP1 also phosphorylates (inactivates) glycogen phosphorylase. The precise steps that link insulin receptor tyrosine kinase/PI 3-kinase activation to stimulation of PP1 have yet to be defined. Some evidence suggests that p90 ribosomal S6-kinase may be involved in the activation of glycogen synthase (274). Akt also has been shown to phosphorylate and thus inactivate GSK-3 (292). This decreases glycogen synthase phosphorylation, leading to activation of the enzyme (292). A number of studies have convincingly demonstrated that inhibitors of PI3-kinase also inhibit glycogen synthase activity and abolish glycogen synthesis (274,293,310). From the physiological standpoint, it makes sense that activation of glucose transport and glycogen synthase should be linked to the same signaling mechanism in order to provide a coordinated stimulation of intracellular glucose metabolism.

Insulin Signal Transduction Defects in Type 2 Diabetes

Both receptor and postreceptor defects have been shown to contribute to insulin resistance in type 2 diabetic individuals. Some, but not all studies have demonstrated a modest 20-30% reduction in insulin binding to monocytes and adipocytes from type 2 diabetic patients (1,311-316). This reduction is due to a decreased number of insulin receptors without change in insulin receptor affinity. In addition to the decreased number of cell-surface receptors, a variety of defects in insulin receptor internalization and processing have been described (314,315). However, some caution should be employed in interpreting these studies. Muscle and liver, not adipocytes, represent the major tissues responsible for the regulation of glucose homeostasis in vivo and insulin binding to solubilized receptors obtained from skeletal muscle biopsies and liver has been shown to be normal in obese and lean diabetic individuals when expressed per milligram of protein (312,313,316-318). Moreover, a decrease in insulin receptor number cannot be demonstrated in over half of type 2 diabetic subjects (319,320), and it has been difficult to demonstrate a correlation between reduced insulin binding and the severity of insulin resistance (321,322). The insulin receptor gene has been sequenced in a large number of type 2 diabetic patients from diverse ethnic populations using denaturing-gradient gel electrophoresis or single-stranded conformational polymorphism analysis, and, with very rare exceptions (323), physiologically significant mutations in the insulin receptor gene have not been observed (324,325). This excludes a structural gene abnormality in the insulin receptor as a cause of common type 2 diabetes mellitus.

Insulin receptor tyrosine kinase activity has been examined in a variety of cell types (skeletal muscle, adipocytes, hepatocytes, and erythrocytes) from normal-weight and obese diabetic subjects. Most (278,301,312,313,320,326-328), but not all (317,329) investigators have found reduced tyrosine kinase activity that cannot be explained by alterations in insulin receptor number or insulin receptor binding. However, near-normalization of the fasting plasma glucose concentration, (by weight loss) has been reported to correct the defect in insulin receptor tyrosine kinase activity (330). This observation suggests that the defect in tyrosine kinase is acquired and results from some combination of hyperglycemia, defective intracellular glucose metabolism, hyperinsulinemia, and insulin resistance - all of which improved after weight loss. A glucose-induced reduction in insulin receptor tyrosine kinase activity has been demonstrated in rat fibroblast culture in vitro (331). Insulin receptor tyrosine kinase activity assays

are performed in vitro, and the results of these assays could provide misleading information with regard to insulin receptor function in vivo. To circumvent this problem, investigators have employed the euglycemic hyperinsulinemic clamp in combination with muscle biopsies and anti-phosphotyrosine immunoblot analysis (301). Such analysis yields a "snap shot" of the insulin-stimulated tyrosine phosphorylation state of the receptor in vivo. The results of these studies have demonstrated a substantial decrease in insulin receptor tyrosine phosphorylation in both obese nondiabetic and type 2 diabetic subjects (301,328). When insulin-stimulated insulin receptor tyrosine phosphorylation was examined in normal-glucose-tolerant or impaired-glucose-tolerant individuals at high risk of developing type 2 diabetes, a normal increase in tyrosine phosphorylation of the insulin receptor has been observed (332). These observations are consistent with the concept that impaired insulin receptor tyrosine kinase activity in type 2 diabetic patients is acquired secondarily to hyperglycemia or some other metabolic disturbance.

A physiologic increase in the plasma insulin concentration stimulates tyrosine phosphorylation of the insulin receptor and IRS-1 in lean healthy subjects to 150-200% of basal values (280,301,328,332,333). In obese nondiabetic subjects, the ability of insulin to activate these two early insulin receptor signaling events in muscle is reduced, while in type 2 diabetics insulin has no significant stimulatory effect on either insulin receptor or IRS-1 tyrosine phosphorylation (301). The association of p85 protein and PI3-kinase activity with IRS-1 also is greatly reduced in obese non-diabetic and type 2 diabetic subjects compared to lean healthy subjects (301,328-334). Insulin also failed to increase the association of the p85 subunit of PI3-kinase with IRS-2 in muscle, indicating that type 2 diabetes is characterized by a combined defect in IRS-1 and IRS-2 function (301,328). The decrease in insulin stimulation of the association of the p85 regulatory subunit of PI3-kinase with IRS-1 is closely correlated with the impairment in muscle glycogen synthase activity and in vivo insulin-stimulated glucose disposal (301). Defective regulation of PI3-kinase gene expression by insulin also has been demonstrated in skeletal muscle and adipose tissue of type 2 diabetic subjects (335). In animal models of diabetes, an 80% decrease in IRS-1 phosphorylation and a greater than 90% reduction in insulin-stimulated PI3-kinase activity have been reported (336).

In the insulin resistant, normal glucose tolerant offspring of two type 2 diabetic parents, IRS-1 tyrosine phosphorylation and the association of p85 protein/PI3-kinase activity with IRS-1 are markedly decreased despite normal tyrosine phosphorylation of the insulin receptor; these insulin signaling defects are correlated closely with the severity of insulin resistance, measured with the euglycemic insulin clamp technique (332). In summary, a defect in the association of PI3-kinase with IRS-1 and its subsequent activation appears to be a characteristic abnormality in type 2 diabetics, is closely correlated with in vivo muscle insulin resistance, and is unrelated to a disturbance in insulin receptor tyrosine phosphorylation. Several groups (337,338) have reported that a common mutation in the IRS-1 gene (Gly 972 Arg) is associated with type 2 diabetes, insulin resistance, and obesity, but the physiologic significance of this mutation remains to be established (339).

The profound insulin resistance of the PI3-kinase signaling pathway contrasts markedly with the ability of insulin to stimulate MAP kinase pathway activity in insulin-resistant type 2 diabetic and in obese non-diabetics individuals (301,328). Hyperinsulinemia increases MEK1 activity and ERK1/2 phosphorylation and activity to the same extent in lean healthy as in insulin resistant obese nondiabetic and type 2 diabetic patients (301,328). This finding of selective insulin resistance is similar to that recently observed in vasculature of Zucker fatty rats (340). Two possible reasons for this difference are alternate insulin signaling pathways and differential signal amplification. With regard to the former, the MAP kinase pathway can be activated either through Grb2/Sos interaction with IRS-1/IRS-2 or with Shc. Because IRS-1 tyrosine phosphorylation is dramatically reduced in the diabetics, it is possible that insulin activation of the MAP kinase pathway in vivo primarily occurs through Shc activation. There is evidence from in vitro studies to support this concept (341). Like ERK and MEK activity, insulin increased Shc phosphorylation to the same extent in lean and obese nondiabetic and type 2 diabetic subjects (301). These results indicate that, in type 2 diabetes, insulin induces sufficient activation of the insulin receptor

tyrosine kinase to increase Shc phosphorylation normally. It also is possible that differential signal amplification in the PI3-kinase and MAP kinase pathways can explain their differing susceptibilities to the effects of insulin resistance.

Maintenance of insulin stimulation of the MAP kinase pathway in the presence of insulin resistance in the PI3-kinase pathway may be important in the development of insulin resistance. ERKs can phosphorylate IRS-1 on serine residues (342), and serine phosphorylation of IRS-1 and the insulin receptor itself has been implicated in desensitizion insulin receptor signaling (343). Continued ERK activity, when IRS-1 function already is impaired, could lead to a worsening of insulin resistance. Thus, diabetic and obese subjects have inappropriately high MAP kinase activity. One also could postulate that insulin resistance in the metabolic (PI3-kinase) pathway, with its compensatory increase in beta cell function and hyperinsulinemia, leads to excessive stimulation of the MAP kinase pathway in vascular tissue (301,302). This would result in the proliferation of vascular smooth muscle cells, increased collagen formation, and increased production of growth factors and inflammatory cytokines, possibly explaining the accelerated rate of atherosclerosis in type 2 diabetic individuals (340A, 340B).

Glucose Transport

Activation of the insulin signal transduction system in insulin target tissues leads to the stimulation of glucose transport. The effect of insulin is brought about by the translocation of a large intracellular pool of glucose transporters (associated with low-density microsomes) to the plasma membrane (281,282,344). There are five major, different facilitative glucose transporters with distinctive tissue distributions (281,282,345,346) (Table 1). GLUT4, the insulin regulatable transporter is found in insulin-sensitive tissues (muscle and adipocytes), has a Km of ~5 mmol/l, which is close to that of the plasma glucose concentration and is associated with HK-II (347-349). In adipocytes and muscle, its concentration in the plasma membrane increases markedly after exposure to insulin, and this increase is associated with a reciprocal decline in the intracellular GLUT4 pool. GLUT1 represents the predominant glucose transporter in the insulin-independent tissues (brain and erythrocytes), but also is found in muscle and adipocytes. It is located primarily in the plasma membrane, where its concentration changes little after the addition of insulin. It has a low Km (~1 mmol/l) and is well suited for its function, which is to mediate basal glucose uptake. It is found in association with HKI (347-349). GLUT2 predominates in the liver and pancreatic beta-cells, where it is found in association with a specific hexokinase, HKIV (347-350). In the beta-cell, HKIV is referred to as glucokinase (350,351). GLUT2 has a high Km, (~15-20 mmol/l) and, as a consequence, the glucose concentration in cells expressing this transporter rises in direct proportion to the increase in plasma glucose concentration. This characteristic allows these cells to respond as glucose sensors. In summary, each tissue has a specific glucose transporter and associated hexokinase, which allows it uniquely to carry out its specialized function to maintain whole-body glucose economy.

Table 1. Classification of glucose transport and HK activity according to their tissue distribution and functional regulation

Organ	Glucose transporter	HK computer	Classification
Brain	GLUT1	HK-I	Glucose dependent
Erythrocyte	GLUT1	HK-I	Glucose dependent
Adipocyte	GLUT4	HK-II	Insulin dependent
Muscle	GLUT4	HK-II	Insulin dependent

Liver	GLUT2	HK-IVL	Glucose sensor
GK beta-cell	GLUT2	HK-IVB (glucokinase)	Glucose sensor
Gut	GLUT3-symporter	-	Sodium dependent
Kidney	GLUT3-symporter	-	Sodium dependent

Glucose transport activity in type 2 diabetic patints uniformly has been found to be decreased in adipocytes (281,282,320,351,352) and muscle (281,282,354-356). In adipocytes from type 2 diabetic human and rodent models of diabetes, there is a severe reduction in GLUT4 mRNA and protein, and the ability of insulin to elicit a normal translocation response and to activate the GLUT4 transporter after its insertion into the cell membrane is impaired (281,282,320,353,357). In contrast, muscle tissue obtained from lean and obese type 2 diabetic subjects exhibits normal or increased levels of GLUT4 mRNA expression and normal levels of GLUT4 protein (358-361). Moreover, acute (2- 4-h) physiological hyperinsulinemia does not increase the number of GLUT4 transporters in muscle in either healthy or type 2 diabetic subjects (358-361). Several studies have demonstrated an increase in muscle GLUT4 mRNA levels in response to insulin in control subjects (333,360), but not in diabetics (360), suggesting insulin resistance at the level of gene transcription. However, the physiological significance of the blunted increase in muscle GLUT4 mRNA levels in type 2 diabetic subjects is unclear, since both basal and insulin-stimulated GLUT4 protein levels are normal. Large populations of type 2 diabetics have been screened for mutations in the GLUT4 gene (362,363). Such mutations are very uncommon and, when detected, have been of questionable physiologic significance.

The results summarized above indicate that the gene (GLUT4) encoding the major insulin-responsive glucose transporter and its transcriptional/translational regulation are not impaired in type 2 diabetes. However, in contrast to the normal expression of GLUT4 protein and mRNA in muscle of type 2 diabetic subjects, every study that has examined adipose tissue has reported reduced basal and insulin-stimulated GLUT4 mRNA levels, decreased GLUT4 transporter number in all subcellular fractions, diminished GLUT4 translocation, and impaired intrinsic activity of GLUT4 (281,282,353,361,364). These observations demonstrate that GLUT4 expression in humans is subject to tissue-specific regulation. Although insulin does not increase GLUT4 expression in muscle, it stimulates the translocation of GLUT4 transporters from their intracellular location to the cell membrane (354,365,366). In type 2 diabetic humans, the ability of insulin to stimulate GLUT4 translocation in muscle is impaired (354,367). Using a novel triple-tracer technique, the in vivo dose-response curve for the action of insulin on glucose transport in forearm skeletal muscle has been examined in nondiabetic and type 2 diabetic subjects (368-370). Insulin-stimulated inward muscle glucose transport is severely impaired in type 2 diabetic subjects who are studied under euglycemic conditions. The defect in glucose transport cannot be overcome by repeating the insulin clamp at each subject's normal fasting glucose (hyperglycemia) level. Since the number of GLUT4 transporters in the muscle of diabetic subjects is normal (358-361), impaired GLUT4 translocation (281,354,367) and decreased intrinsic activity of the glucose transporter (366,371) must be responsible for the defect in muscle glucose transport. Impaired in vivo muscle glucose transport in type 2 diabetics also has been demonstrated using MRI (372) and PET (373).

Glucose Phosphorylation

Glucose phosphorylation and glucose transport are tightly coupled phenomena (374). Isoenzymes of hexokinase (HKI-HKIV) catalyze the first committed intracellular step of glucose metabolism, the conversion of glucose to glucose-6-phosphate (G-6-P) (347-350,375) (Table 1). HKI, HKII, and HKIII are single-chain peptides that have a number of properties in common, including a very high affinity for

glucose and product inhibition by G-6-P. HKIV, also called glucokinase, has a lower affinity for glucose and is not inhibited by G-6-P. Glucokinase (HKIVB) is believed to be the glucose sensor in the beta-cell, while HKIVL plays an important role in the regulation of hepatic glucose metabolism.

In both rat (375-377) and human (333,348,378-380) skeletal muscle, HKII transcription is regulated by insulin. HKI also is present in human skeletal muscle, but it is not regulated by insulin (378). In response to physiological euglycemic hyperinsulinemia, HKII cytosolic activity, protein content, and mRNA levels increase by 50-200% in healthy non-diabetic subjects (378,380) and this is associated with the translocation of hexokinase II from the cytosol to the mitochondria (381). In contrast, insulin has no effect on HK-I activity, protein content, or mRNA levels (378).

In forearm muscle, insulin-stimulated glucose transport (measured with the triple tracer technique) has been shown to be markedly impaired in lean type 2 diabetics (370). However, since the rate of intracellular glucose phosphorylation was impaired to an even greater extent, insulin caused an increase in the intracellular free glucose concentration. By performing the insulin clamp at each diabetic's normal level of fasting hyperglycemia, normal rates of whole-body glucose disposal and a normal rate of glucose influx into muscle was elicited. However, the rate of intracellular glucose phosphorylation increased only modestly; consequently, there was a dramatic rise in the free glucose concentration within the intracellular space that is accessible to glucose. These observations indicate that in type 2 diabetic individuals, while both glucose transport and glucose phosphorylation are severely resistant to the action of insulin, impaired glucose phosphorylation (HKII) appears to be the rate-limiting step for insulin action. A similar pattern of impaired muscle glucose phosphorylation and transport is present in the insulin-resistant, normal glucose-tolerant offspring of two diabetic parents (382). These results are consistent with dose-response studies using PET to evaluated glucose phosphorylation and transport in skeletal muscle of type 2 diabetics (373). They also are consistent with 31P-NMR studies (383) which demonstrate that, during hyperinsulinemia, muscle G-6-P concentrations decline in type 2 diabetic versus control subjects. However, subsequent studies using ^{31}P-NMR in combination with 1-^{14}C-glucose suggest that the defect in insulin-stimulated muscle glucose transport exceeds the defect in glucose phosphorylation and is responsible for the decline in muscle glucose-6-P concentration (372). Because of methodologic differences, the results of the triple tracer (370) and MRI (372) studies cannot be reconciled at present. Nonetheless, observations from these studies are consistent in demonstrating that the defects in glucose phosphorylation and glucose transport in muscle are established early in the natural history of type 2 diabetes and cannot be explained by glucose toxicity (91). Clear evidence that HKII activity is crucial for glucose uptake derives from studies in transgenic mice who overexpress HKII. In this model, HKII overexpressiion increased both insulin- and exercise-stimulated muscle glucose uptake (384).

In healthy nondiabetic subjects, physiologic hyperinsulinemia for as little as 2-4 hours increases muscle HKII activity, gene transcription, and translation (333,378). In lean type 2 diabetic subjects insulin-stimulated HKII activity and mRNA levels are markedly reduced compared to controls (383,385). Decreased basal muscle HKII activity and mRNA levels (385) and impaired insulin-stimulated HKII activity (379,380,386,387) in type 2 diabetic subjects have been reported by other investigators. A decrease in insulin-stimulated muscle HKII activity also has been described in individuals with IGT (388). Because of its central role in insulin-mediated muscle glucose metabolism, several groups have looked for point mutations in the HKII gene in individuals with type 2 diabetes (388-390). Although several nucleotide substitutions have been found, none have been located close to the glucose and ATP binding sites and none have been associated with insulin resistance. Thus, an abnormality in the HKII gene is unlikely to explain the inherited insulin resistance in common variety type 2 diabetes mellitus.

Glycogen Synthesis

After glucose is phosphorylated by hexokinsae II, it either can be converted to glycogen or enter the glycolytic pathway. Of the glucose that enters the glycolytic pathway, ~90% is oxidized. At low physiologic plasma insulin concentrations, glycogen synthesis and glucose oxidation are of approximately equal quantitative importance. With increasing plasma insulin concentrations, glycogen synthesis predominates (18,391). If the rate of glucose oxidation (determined by indirect calorimetry) is subtracted from the rate of whole-body insulin-mediated glucose disposal (determined from the insulin clamp), the difference represents nonoxidative glucose disposal (or glucose storage) (17,360), which primarily reflects glycogen synthesis (1,4,162,216,392). Glucose conversion to lipid accounts for <5% of total body glucose disposal (18,198,199) and less than 5-10% of the glucose taken up by muscle is released as lactate (5,393,394). Reduced insulin-stimulated glycogen synthesis is a characteristic finding in all insulin-resistant states, including obesity, diabetes, and the combination of obesity plus diabetes (1,4,18,43,59,159,162,218,219,377,393-395). Impaired glycogen synthesis also represents the major cause of insulin resistance in obese subjects with normal or only slightly impaired glucose tolerance (1,4,162,218,393,395,396). Thus, the inability of insulin to promote glycogen synthesis is a characteristic and early defect in the development of insulin resistance in both obesity and type 2 diabetes. The emergence of overt diabetes with fasting hyperglycemia is associated with a major reduction in insulin-mediated nonoxidative glucose disposal (glycogen synthesis) in all ethnic groups (1,4,18,162,377,396). Impaired glycogen synthesis also has been demonstrated in the normal-glucose-tolerant offspring of two diabetic parents (43,397), in the first-degree relatives of type 2 diabetic individuals (41,398,399), and in the normoglycemic twin of a monozygotic twin pair in which the other twin has type 2 diabetes (101).

Using NMR imaging spectroscopy, a decrease in insulin-stimulated incorporation of [1H, ^{13}C]-glucose into muscle glycogen of type 2 diabetic subjects has been demonstrated directly (215). In type 2 diabetics, there was a marked lag in the onset of insulin-stimulated glycogen synthesis that was similar to the delay in insulin-mediated leg muscle glucose uptake. The rate of glycogen synthesis in type 2 diabetic subjects was decreased by ~50%, paralleling the decrease in total glucose uptake by leg muscle (3), and impaired muscle glycogen synthesis accounted for essentially all of the defect in whole body glucose disposal.

In summary, an abundance of convincing evidence demonstrates that impaired glycogen synthesis is the major metabolic defect in normal glucose tolerant obese subjects, in individuals with IGT, and in patients with overt diabetes. Moreover, numerous studies have documented that the earliest detectable metabolic abnormality responsible for the insulin resistance in normal glucose tolerant individuals who are destined to develop type 2 diabetes is impaired glycogen synthesis (4,41,43,101,382,392,399,400).

Glycogen synthase is the key insulin-regulated enzyme which controls the rate of muscle glycogen synthesis (307,308,310,379,401,402). Insulin enhances glycogen synthase activity by stimulating a cascade of phosphorylation-dephosphorylation reactions (307,308,361-363,403) (see above discussion of insulin receptor signal transduction), which ultimately lead to the activation of PP1 (also called glycogen synthase phosphatase) (307,308,310,402). The regulatory subunit (G) of PP1 has two serine phosphorylation sites, called site 1 and site 2. Phosphorylation of site 2 by cAMP-dependent kinase (PKA) inactivates PP1, while phosphorylation of site 1 by insulin activates PP1, leading to the stimulation of glycogen synthase (307,308,402,404). Phosphorylation of site 1 of PP1 by insulin in muscle is catalyzed by insulin-stimulated protein kinase 1 (ISPK-1) (309,405), which is part of a family of serine/threonine protein kinases termed ribosomal S6-kinases. Because of their central role in muscle glycogen formation, considerable attention has focused on the three enzymes glycogen synthase, PP1, and ISPK-1 in the pathogenesis of insulin resistance in individuals with type 2 diabetes.

Glycogen synthase exists in an active (dephosphorylated) and an inactive (phosphorylated) form (307-310). Under fasting conditions, total glycogen synthase activity in type 2 diabetic subjects is reduced and the ability of insulin to activate glycogen synthase is severely impaired (301,384,406-410). An impaired ability of insulin to activate glycogen synthase also has been demonstrated in the normal glucose tolerant relatives of type 2 diabetic individuals (400). Insulin-mediated activation of glycogen synthase and insulin-stimulated glycogen synthase gene expression has been shown to be impaired in cultured myocytes and fibroblasts from type 2 diabetic subjects (411,412). Studies in insulin-resistant nondiabetic and diabetic Pima Indians have documented that the ability of insulin to activate muscle PP1 (glycogen synthase phosphatase) is severely reduced (413). PP1 dephosphorylates glycogen synthase, leading to its activation. Therefore, a defect in PP1 appears to play an important role in muscle insulin resistance (309).

The effect of insulin on glycogen synthase gene transcription and translation in vivo has been studied extensively. Most studies (378,414,415) have shown that insulin does not increase glycogen synthase mRNA or protein expression in human muscle studied in vivo. However, glycogen synthase mRNA expression is decreased in muscle of type 2 diabetic patients (415,416), explaining in part the decreased glycogen synthase activity in this disease. However, the major abnormality in glycogen synthase regulation in type 2 diabetes and other insulin resistant conditions is its lack of dephosphorylation and activation by insulin as a result of insulin receptor signaling abnormalities (see previous discussion). The glycogen synthase gene (417) has been the subject of intensive investigation. An association between glycogen synthase gene markers and type 2 diabetes has been demonstrated in Japanese, French, Finnish, and Pima Indian populations. However, DNA sequencing has revealed either no mutations (418) or rare nucleotide substitutions (419,420) that cannot explain the defect in insulin-stimulated glycogen synthase. Nonetheless, the association between the glycogen synthase gene and type 2 diabetes mellitus (418) suggests that another gene close to the glycogen synthase gene may be involved in the development of type 2 diabetes. The genes encoding the catalytic subunits of PP1 (421) and ISPK-1 (422) have been examined in insulin-resistant Pima Indians and Danes with type 2 diabetes. Several silent nucleotide substitutions were found in the PP1 and ISPK-1 genes in the Danish population; the mRNA levels of both genes were normal in skeletal muscle (422). No structural gene abnormalities in the catalytic subunit of PP1 were detected in Pima Indians (422). Thus, neither abnormalities in the PP1 and ISPK-1 genes nor abnormalities in their translation can explain the impaired enzymatic activities of glycogen synthase and PP1 that have been observed in vivo. Similarly, there is no evidence that an alteration in glycogen phosphorylase plays any role in the abnormality in glycogen formation in type 2 diabetes (423).

In summary, glycogen synthase activity is severely impaired in patients with type 2 diabetes mellitus and in insulin-resistant normal glucose tolerant individuals who are predisposed to develop type 2 diabetes. However, the defect cannot be explained by an abnormality in the genes encoding glycogen synthase or is promoter or by other key genes - PP1 or ISPK-1 - involved in the regulation of glycogen synthase activity.

Glycolysis/Glucose Oxidation

Glucose oxidation accounts for ~90% of total glycolytic flux, while anaerobic glycolysis accounts for the other 10% (393,394). Two enzymes, phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) play pivotal roles in the regulation of glycolysis and glucose oxidation, respectively. In type 2 diabetic individuals the glycolytic/glucose oxidative pathway has been shown to be impaired in many individuals with type 2 diabetes (393,394). Although one study suggested that the activity of PFK is modestly reduced in muscle biopsies from type 2 diabetic subjects (424), the majority of evidence indicates that the activity of PFK is normal (407,412,417). Insulin has no effect on muscle PFK activity, mRNA levels, or protein content in either nondiabetic or diabetic individuals (417). PDH is a key insulin-regulated

enzyme whose activity in muscle is acutely stimulated by a physiological increment in the plasma insulin concentration (415). Three previous studies have examined PDH activity in type 2 diabetic patients. Insulin-stimulated PDH activity is decreased in isolated subcutaneous human adipocytes from patients with type 2 diabetes mellitus (425) and in skeletal muscle from type 2 diabetic subjects undergoing euglycemic hyperinsulinemic clamps (426). However, when type 2 diabetic patients had muscle biopsies during hyperglycemic hyperinsulinemic clamps, activation of PDH by insulin was normal (409), in concert with normalized rates of muscle glucose uptake. These results suggest that insulin stimulation of PDH activity is influenced by glycolytic flux.

Both obesity and type 2 diabetes mellitus are associated with accelerated FFA turnover and oxidation (1,4,18,162), which would be expected, according to the Randle cycle (232), to inhibit PDH activity and consequently glucose oxidation (see prior discussion). Thus, any observed defect in glucose oxidation or PDH activity could be acquired secondarily to increased FFA oxidation and feedback inhibition of PDH by elevated intracellular levels of acetyl-CoA and reduced availability of NAD. Consistent with this observation, the rates of basal and insulin-stimulated glucose oxidation have been shown to be normal in the normal glucose tolerant offspring of two diabetic parents (43) and in the first degree relatives of type 2 diabetic subjects (41,423), while it is decreased in overtly diabetic subjects (1,4,393,394,427). Studies examining PHD activity in muscle tissue from lean diabetic subjects with mild fasting hyperglycemia are needed before the role of this enzyme in the development of insulin resistance in type 2 diabetes can be established or excluded.

In summary, postbinding defects in insulin action primarily are responsible for the insulin resistance in type 2 diabetes. Diminished insulin binding, when present, is small, occurs in individuals with IGT or very mild diabetes, and results secondarily from downregulation of the insulin receptor by chronic sustained hyperinsulinemia. In type 2 diabetic patients with overt fasting hyperglycemia, postbinding defects are responsible for the insulin resistance. A number of postbinding defects have been documented, including diminished insulin receptor tyrosine kinase activity, insulin signal transduction abnormalities, decreased glucose transport, reduced glucose phosphorylation, and impaired glycogen synthase activity. The glycolytic/glucose oxidative pathway appears to be largely intact and, when defects are observed, they appear to be acquired secondarily to enhanced FFA/lipid oxidation. From the quantitative standpoint, impaired glycogen synthesis represents the major pathway responsible for the insulin resistance in type 2 diabetes, and family studies suggests that a defect in the glycogen synthetic pathway represents the earliest detectable abnormality in type 2 diabetes. Recent studies link the impairment in glycogen synthase activation to a defect in the ability of insulin to phosphorylate IRS-1, causing a reduced association of the p85 subunit of PI 3-kinase with IRS-1 and decreased activation of the enzyme (PI 3-kinase).

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