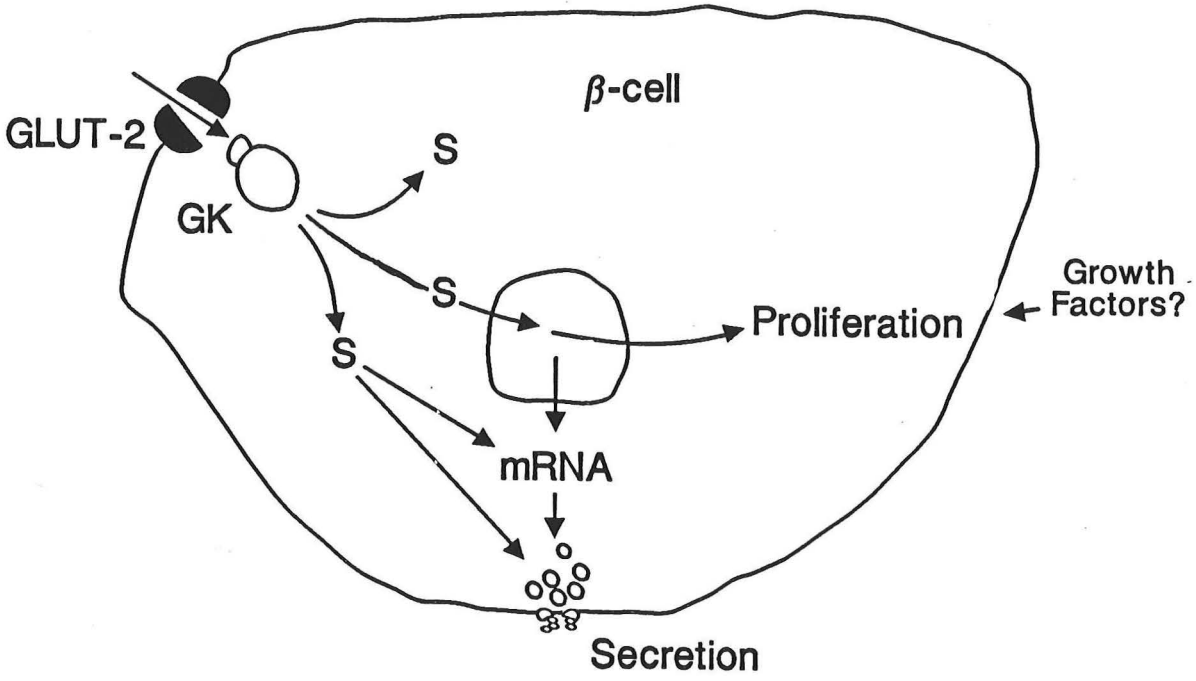
MEDICAL GRAND ROUNDS

"DISORDERS OF THE β-CELL GLUCOSE TRANSPORTER IN THE PATHOGENESIS OF DIABETES"



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Case Reports

Subject 1 - This subject (L.B.) is a 9-year old black female HLA-identical to an older sister with IDDM who is a member of the University of Texas Southwestern Type 1 Diabetes Registry. She had been tested periodically for evidence of β-cell autoimmunity and β-cell functional loss for 4 years. During this time she became islet cell antibody cytoplasmic positive and her 1 + 3 minute insulin response to IV glucose declined from 240 to 117 μU/ml. A diagnosis of Type 1 pre-diabetes was made.

Subject 2 - The subject is an obese 48 year-old Pima Indian housewife who has been followed as part of the NIH Clinical Research Center Program in Phoenix. Both parents, three of her five older siblings and many second degree relatives have been diagnosed as type 2 NIDDM. Her annual oral glucose tolerance tests show decreasing tolerance but her fasting glucose concentrations had been normal until recently, when it was found to be 145 mg/dl with a fasting insulin concentration of 40 μU/ml which did not rise in response to intravenous glucose; an intravenous arginine test elicited 75 μU/ml increase in insulin. A diagnosis of borderline type 2 diabetes was made.

INTRODUCTION

Subjects 1 and 2 exhibit the typical findings of the pre-overt phase of two completely unrelated causes of β-cell dysfunction, type 1 (IDDM) and type 2 (NIDDM) diabetes. As shown in Table 1, the two disorders differ in virtually every significant

Table 1 - Comparison of Type 1 and Type 2 diabetes

	Type 1	vs.	Type 2
β-cell Destruction	yes		no
Monozygotic Twin Discordance	yes		no
HLA-Linkage	yes		no
β-cell Autoimmunity	yes		no
Islet Amyloid Deposition	no		yes
↓ Glucose-Stimulated Insulin Secretion	yes		yes

feature—except that both exhibit a progressive attenuation of the insulin response to intravenous glucose without a parallel loss of the insulin response to arginine prior to the onset of fasting hyperglycemia. In both diseases inability of the β-cells to sense and hence respond to and correct elevated blood glucose concentrations is likely to be the basis for the steady state hyperglycemia upon which the diagnosis of diabetes will be made. Figure 1 provides a simplified diagram of the glucose-stimulatory apparatus of β-cells. Only the proximal, (afferent or stimulatory) portion of the glucose response apparatus is glucose specific; the distal, (efferent or secretory) limb is probably shared by all β-cell secretagogues. The fact that the response to arginine remains unimpaired during progressive attenuation of the glucose-stimulated insulin response strongly points to the afferent glucose-specific pathway as the site of the initial derangement in both type 1 and type 2 diabetes.

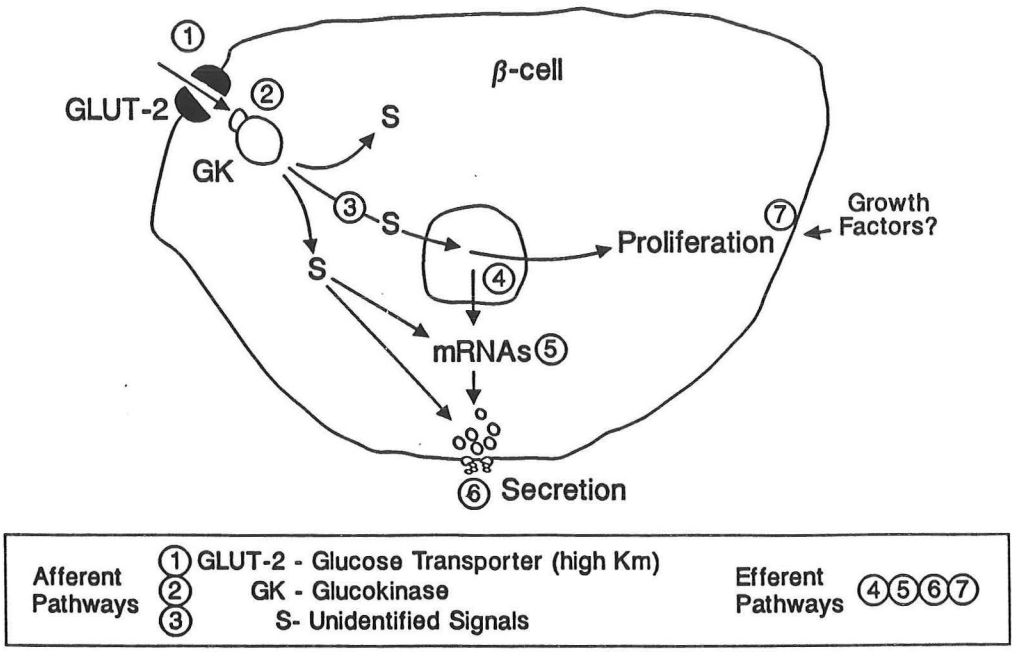

Figure 1 - Major known landmarks in the glucose stimulatory pathway of the β-cell. Proximal or afferent points are presumably glucose-specific and constitute the sensing component, while the distal efferent or secretory segment is probably common to other β-cell secretagogues.

Figure 2 demonstrates progressive loss of glucose-stimulated insulin secretion in the autoimmune diabetes of BB rats (figure 2 - upper panel); on the first day of the diabetes the glucose response is completely absent but the arginine response is intact (1). Figure 2 (lower panel) demonstrates the same phenomenon in a rat model of non-autoimmune type 2 diabetes, the male Zucker diabetic fatty or ZDF rat (2). The fact that the onset of overt diabetes (hyperglycemia > 199 mg/dl; 11 mm) is invariably heralded by selective loss of glucose-stimulated insulin secretion in rodents as in humans indicates that the latter may be the sine qua non of steady state hyperglycemia (if the β-cells cannot sense and respond to hyperglycemia, how can they correct it?).

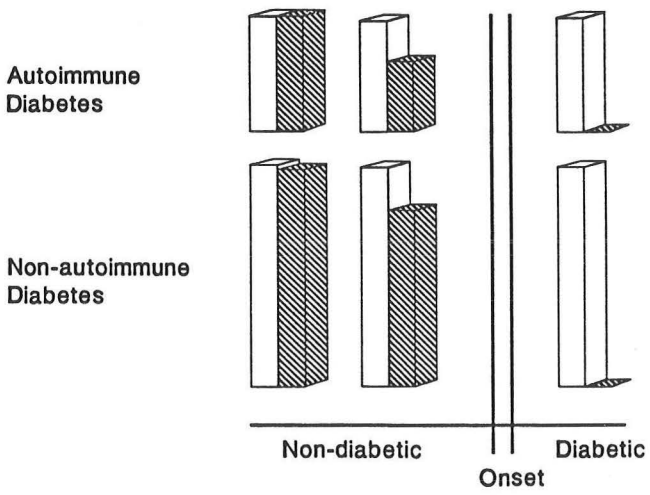

Figure 2 - Prior to the onset of diabetes, both autoimmune (Type 1) or nonautoimmune (Type 2), there is a gradual reduction in the insulin response to glucose ▨ without a parallel reduction in the response to arginine ▭. This has been observed both in humans in vivo and in the perfused pancreata of autoimmune BB rats (1) and non-autoimmune ZDF rats (2). These observations point to a derangement in a glucose-specific locus early in the course of both forms of diabetes.

On the basis of the foregoing evidence the decision was made to explore glucose-specific components of the β-cell glucose response system for abnormalities that would shed light on the pathogenesis of type 1 and type 2 diabetes, beginning with the high K_m facilitative glucose transporter, GLUT-2.

FACILITATIVE GLUCOSE TRANSPORTERS

Facilitative glucose transport across the plasma membranes of cells, the initial event in glucose metabolism, is essential for life. Since the brain relies almost exclusively on glucose as their energy source, the proteins that facilitate the translocation of glucose from the extracellular to the intracellular space are vital for brain life. These proteins, which primarily serve the interest of each individual cell, are structurally and functionally unrelated to the sodium-dependent active glucose transporter (symport) which is expressed only on special cells involved in the translocation of glucose from outside the body (e.g. the lumen of the small bowel and renal tubules) into the body, thus serving the entire organism rather than just the selfish interests of individual cells. Nothing further will be mentioned about active transporters.

Although glucose flux has been studied in vivo for over a century and a half (3) and in vitro glucose uptake by tissues and cells has been extensively examined for 50 years (for review see 4), the proteins that facilitate glucose uptake in cells throughout the body were completely uncharacterized until five years ago. By screening an expression library from hep-G-2 human hepatoma cells with an anti-erythrocyte glucose transporter antibody, Mueckler et al in the Lodish laboratory cloned and sequenced what is now known as GLUT-1 (5). Identification of other facilitative glucose transporter isoforms by Thorens et al in the Lodish lab (6) and by the laboratory of Graeme Bell (7) quickly followed. Today we recognize a family of five facilitative glucose transporter isoforms (8). Although some workers still cling to the nomenclature based on the tissue in which the isoform was first discovered, the most useful nomenclature is the numerical classification introduced by Graeme Bell (GLUT-1 through GLUT-5).

The ancestral facilitative glucose transporter is shown in Figure 3. Topologically similar structures are found throughout phylogeny, from man to trypanosomes, E. coli and yeast strains (8). These molecules all have twelve membranes spanning domains forming six exoplasmic loops with a prominent first loop and five endoplasmic loops with a large hydrophilic central loop. They all have N-terminal and C-terminal intracellular tails.

Figure 4 shows the regions of homology among the five human isoforms. Homologies range from 39 to 65% between tissue isoforms in the same species, compared to as much as 98% homology between a single isoform in rat and man (8). This remarkable isoform conservation between species compared to the divergence of isoforms between tissues within a species implies specialization in different tissues for different purposes. The greatest homology between isoforms is in the membrane spanning domains, suggesting that these may constitute the active glucose transport site. (Perhaps they assemble to form a pore through which glucose can be transported.) The least homologous areas are the first exoplasmic loop and the C-terminal tail. The latter has therefore been proposed as the domain which determines

tissue-specific behavior (8). Whether or not this is true, this domain provides an excellent antigen for the production of isoform-specific antibodies.

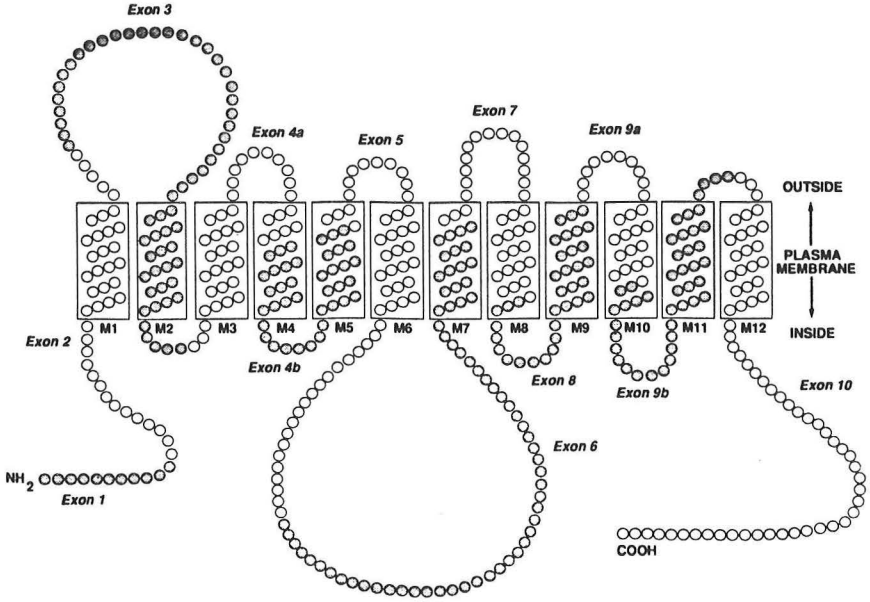

Figure 3 - Model for exon-intron organization of ancestral facilitative glucose-transporter gene. Circles indicate amino acids encoded by each of 12 exons in ancestral gene. Putative membrane-spanning α-helices are numbered M1-M12. (From ref. 8.)

Table 2 lists the tissue localization of the five isoforms, their K_m and certain important and specific features. GLUT-1 is an ubiquitous transporter and is particularly prominent in brain and red cells. Its low K_m assures glucose uptake for the glucose requiring tissues of the brain, even at fasting glucose concentrations below 50 mg/dl (2.8 mM). (The brain also expresses a second low K_m transporter, GLUT-3.) GLUT-1 is expressed in transformed cells (9), even those that normally express another isoform (9), presumably to provide for the increased glucose needs resulting from the Warburg effect in rapidly proliferating malignant tissues.

Table 2 - THE FACILITATIVE GLUCOSE TRANSPORTER FAMILY

Glucose Transporter	Tissue Distribution	Km	Special Features
GLUT-1	Ubiquitous Rbc, brain	1-2	Induced in transformed cells (Hep G2, RIN m5F
GLUT-2	β-cells, liver kidney, small bowel	~17	Glucose homeostatic roles
GLUT-3	Brain, fat	<1	
GLUT-4	Muscle, fat	~5	Insulin recruitable
GLUT-5	Small bowel kidney	1-2	

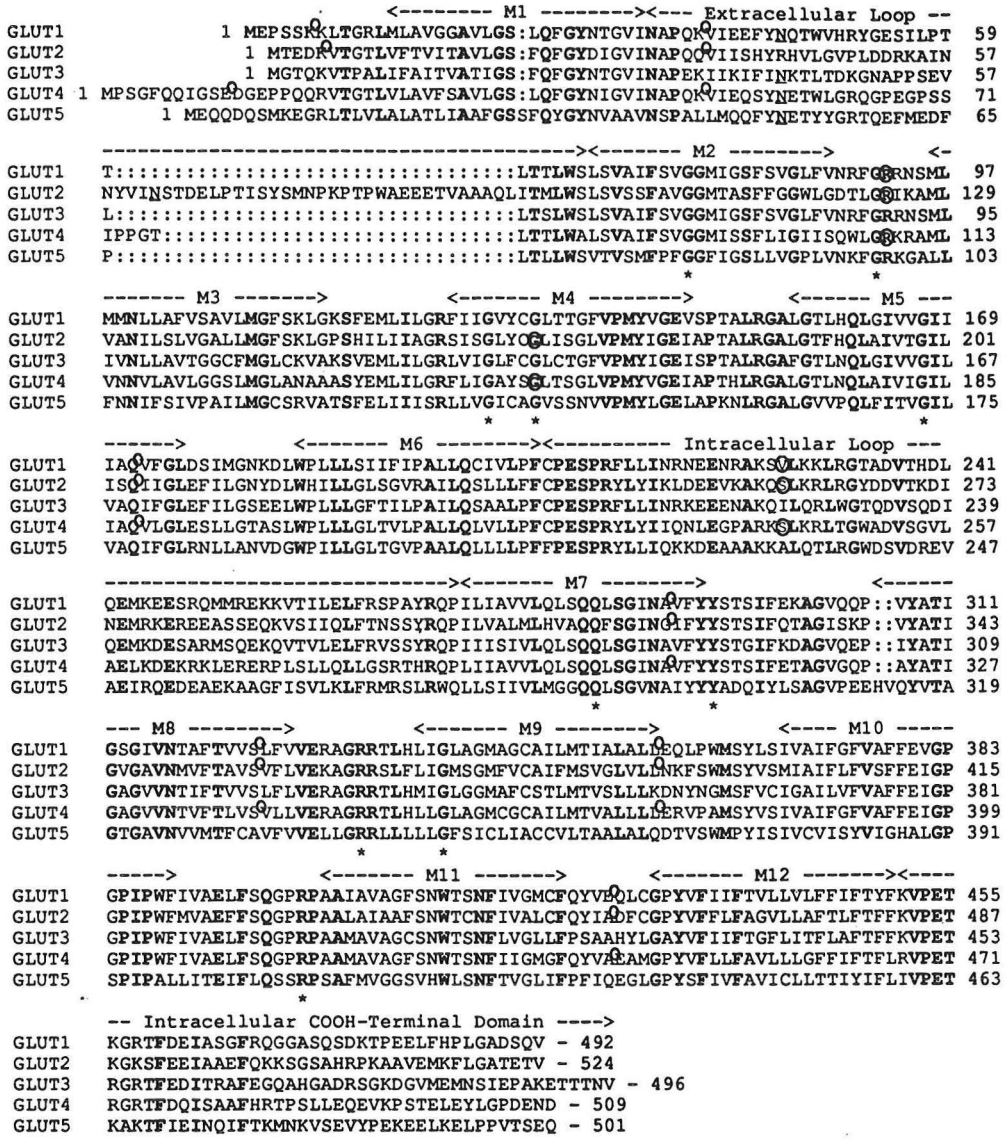

Figure 4 - Comparison of amino acid sequences of human facilitative glucose trasporters. Putative membrane-spanning regions (M1-M12) and other domains of proteins are indicated. Gaps introduced to generate this alignment are noted by colons. Asparagine (N) residue in the extracellular loop that is possible site of oligosaccharide addition is underlined. Identical residues in all 5 isoforms are indicated in bold type. *Invariant amino acids in facilitative transporter superfamily. Circles indicate positions at which introns interrupt gene; circles between residues indicate that intron interrupts gene between codons for adjacent amino acids; encircled amino acids indicate where intron interrupts codon for that residue. Single-letter abbreviations for amino acids are as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methlonine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine. (From ref. 8.)

GLUT-2, the only high K_m isoform thus far described, is expressed in tissues involved in blood glucose homeostasis and glucose-sensing, such as the liver, the β-cells and the basolateral aspect of epithelial cells of the small intestine and renal tubules through which glucose is absorbed (6). The high K_m assures that glucose transport in these cells does not become rate-limiting when glucose concentrations rise above the normal fasting glucose concentration of 5 mM (90 mg/dl) range and into the 10 mM range after a meal (6). *Thus glucose uptake and hence usage by these cells will be*

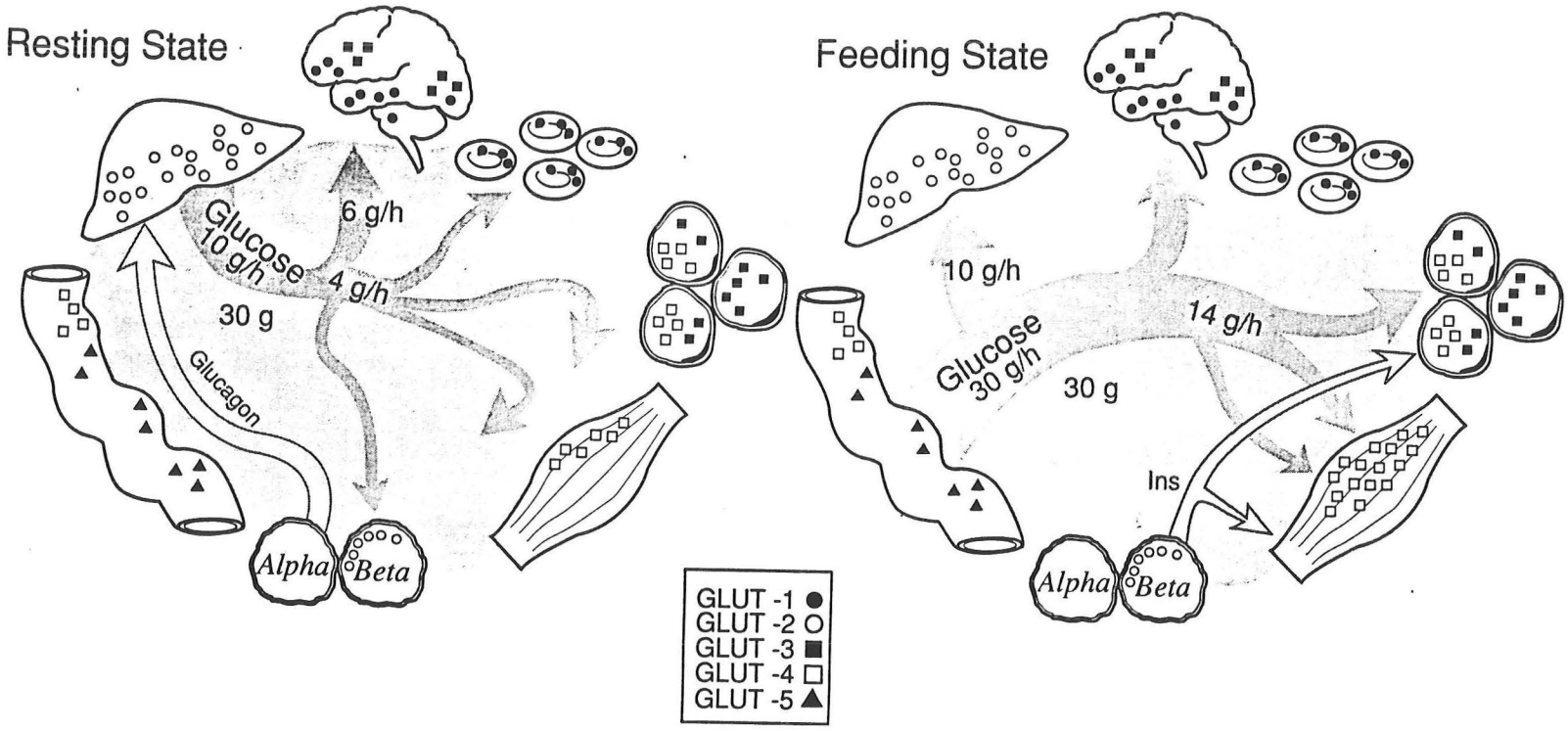

Figure 4A. Tissue distribution of the currently known facilitative glucose transporter isoforms within the framework of regulated glucose flux patterns.

Left hand panel: In the resting or basal state the extracellular glucose pool (shaded area) measures 30 g. The brain expresses GLUT-1 (blood-brain barrier) and GLUT-3, transporters with a K_m in the 1-2 mM range. The low K_m will facilitate delivery of the 6 g/h of glucose required for normal cerebral function at arterial glucose concentrations well below the normal fasting level of 5 mM. In the resting state the other tissues of the body consume glucose at approximately 4 g/h. Insulin secretion (not shown) by GLUT-2 expressing β-cells is very low, thereby excluding glucose from insulin-dependent tissues such as muscle and fat. Under the direction of glucagon, the GLUT-2 expressing liver cells precisely replace the 10 g/h of glucose thereby maintaining the extracellular glucose pool at 30 g.

Feeding state: The ingestion of a large carbohydrate-containing meal could add 30 g/h of new glucose to the extracellular glucose pool of 30 g. The remarkable buffering capacity of the glucoregulatory system minimizes change in the glucose pool by means of an outpouring of insulin. GLUT-4 is recruited from the low density microsomal pool to the surface of fat and muscle cells, which take up approximately half (14 g/h) of the incoming glucose load. Insulin, both by suppressing glucagon secretion and by direct action on hepatocytes, induces net uptake of approximately 10 g/h by the liver which has been converted from an organ of glucose production to one of glucose storage. Thus 24 of the 30 g/h have been shifted to the intracellular compartment through direct or indirect actions of insulin, while the brain continues to extract 6 g/h independently of insulin. Thus, the entire glucose influx is rapidly moved out of the extracellular compartment and hyperglycemia is prevented.

proportional to the extracellular glucose concentration (10). This point is essential to understand the postulated consequences of GLUT-2 disease to be described below.

GLUT-4 is particularly interesting to diabetologists because this is the insulin-recruitable glucose transporter of muscle and adipocytes. In the basal state these transporters are stored in the low density microsomal pool (11). After a meal when insulin levels rise and insulin binds to receptors in these cells. Then, via mechanisms that have not as yet been fully determined, GLUT-4 is translocated via vesicles that fuse with the plasma membrane of cells through the process of exocytosis (12), thereby increasing the number of glucose transporters and thus the V_{max} of glucose transport into these cells (11). As a consequence, the post-prandial rise in glucose is buffered so that hyperglycemia is minimized in magnitude and duration.

Figure 4A places the facilitative glucose transporter isoforms within the framework of the classic physiology of blood glucose homeostasis. While much attention has been focused on the reduced recruitability of GLUT-4 by insulin to explain the insulin resistance that precedes the onset of type 2 diabetes, we will focus only on GLUT-2 of β-cells in the pathogenesis of the common forms of diabetes mellitus.

GLUT-2

GLUT-2 has an apparent molecular weight of about 60 kD (2). Antibodies to the C-terminal hexadecapeptide predicted from the messenger RNA of the liver transporter have been raised by Thorens in the Lodish lab (6) and by Newgard and Johnson in the Center for Diabetes Research at this institution (2). Using the Thorens antibody, Orci confirmed and extended the findings of Thorens that β-cells stain positive with this antibody (13). Figure 5 demonstrates a close-up of a normal islet stained for GLUT-2. Immunofluorescence is confined to the surface of the islet cells. On EM, using protein A-gold immunocytochemistry, Orci demonstrated that most of the transporters are in the microvillar portion of the plasmic membrane facing adjacent endocrine cells, rather than at the capillary poles of the cell (13). Neither alpha-, delta- or PP-cells of the islets are GLUT-2 positive (13).

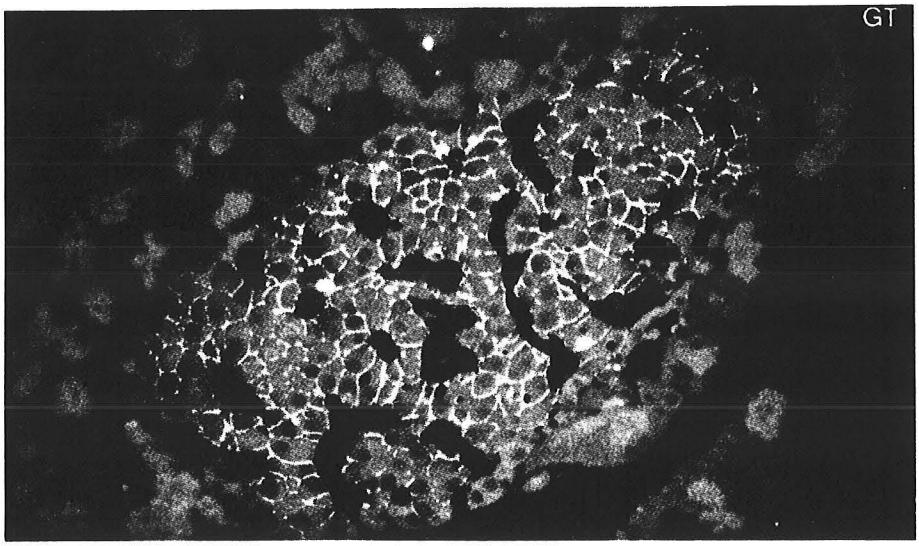

Figure 5 - Localization of the GT on semithin sections of pnacreatic islets by immunofluorescence. Islet labeled with the anti-GT antibody, showing the cell profiles outlined by a discontinuous line of immunofluorescence. (From ref. 13.)

GLUT-2 REGULATION

Studies by Chen and Alam of the Center for Diabetes Research have demonstrated that GLUT-2 mRNA of β-cells is altered by extreme perturbations of glucose homeostasis such as a 12-day insulin infusion and a 5-day infusion of 50% glucose. As shown in Figure 6 and Table 3, the insulin infusion, which caused sustained hypoglycemia of 54 mg/dl, down-regulated both proinsulin mRNA and GLUT-2 mRNA, while the hyperglycemic infusion resulted in up-regulation of both.

Table 3

Effect of insulin-induced hypoglycemia of varying duration and of glucose-induced hyperglycemia upon GLUT-2 mRNA of β-cells. The mRNA density values represent the mean ± S.D. of 20 islets from each group of islets. The blood glucose levels represent the mean ± S.D. of the final determination before sacrifice of the animals.

Infusion	Duration	Blood Glucose (mg/dl)	Proinsulin mRNA density†	P value	GLUT-2 mRNA density†	P value
Control (N = 3)		122 ± 12	0.61 ± 0.03	0.001	0.13 ± 0.05	--
Insulin (N = 4)	12d	54 ± 8	0.30 ± 0.03 (-51%)		not detectable (-100%)	
Control (N = 3)		120 ± 10	0.46 ± 0.11	0.001	0.22 ± 0.07	0.001
Glucose (N = 3)	5d	200 ± 50	0.92 ± 0.18 (+100%)		0.32 ± 0.08 (+46%)	

†Density number is the reciprocal of the exposure time showing on the light meter. Statistical analysis was carried out by the Student t test for two groups using an N of 20. The (N) refers to number of islets in each group.

mRNA density is also expressed as (% change) from the mean of the control values in each group to permit comparison of changes between experimental groups; difference in specific activity of the probes and in the exposure times employed preclude intergroup comparisons of the mRNA density values.

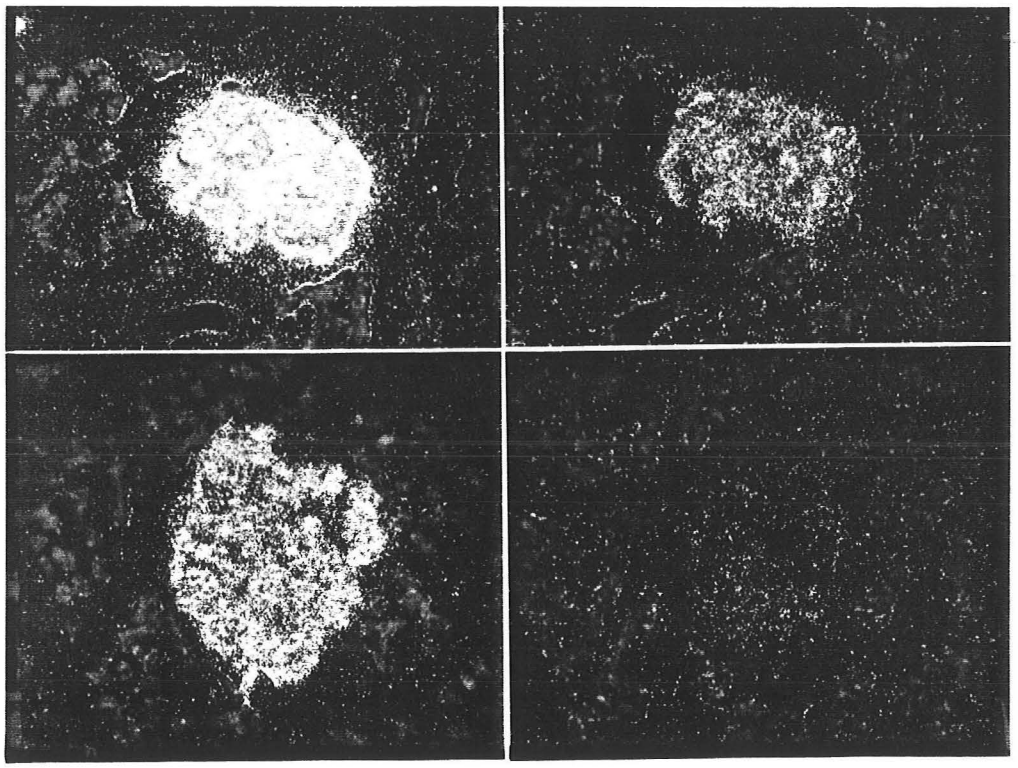

Figure 6 - Dark field photomicrographsof adjacent sections of pancreas hybridized in situ with ^{35}S-labeled antisense RNA probes for insulin (left hand panels) or GLUT-2 (right hand panels). Pancreata were obtained from normo-glycemic control rats (upper panels) and from hypoglycemic rats by a 12d-insulin infusion (lower panels). Bar, 200 μm. (From ref. 14.)

WHAT HAPPENS TO GLUCOSE TRANSPORT IN ISLETS WHEN GLUT-2 HAS BEEN DOWN-REGULATED?

IS GLUT-2 REALLY THE HIGH KM TRANSPORTER?

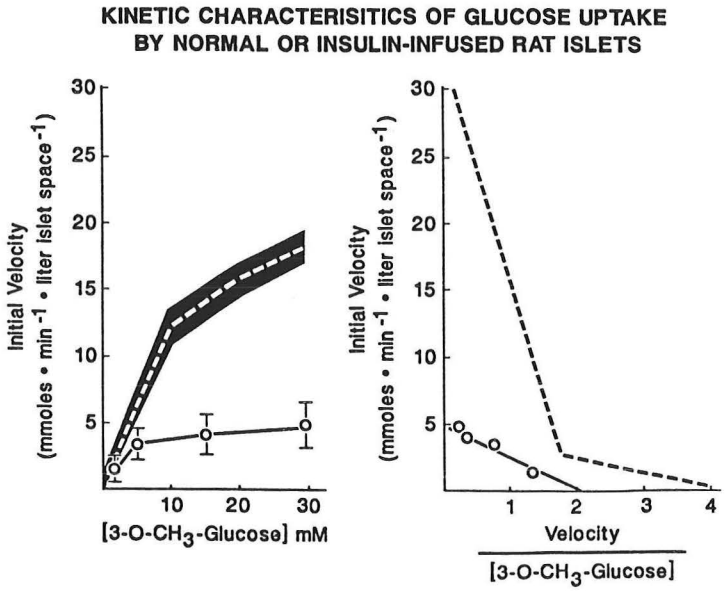

Figure 7 - (Left) Concentration dependence of 3-0-methyl glucose uptake in islets isolated from insulin-infused rats (n=7, solid line) and normal Wistar rats (n=12, dashed line). (Right) Eadie-Hofstee plot of data on the Left for insulin-infused rats (solid line) and normal Wistar rats (dashed line), showing the decrease in high K_m glucose transport in islets from insulin-infused animals. (From ref. 14.)

The assignment of a high K_m role to GLUT-2 is indirect - largely based on studies in hepatocytes which are believed to express only this isoform. To determine more directly if GLUT-2 was a high K_m transporter, Dr. Wick Johnson of the Center for Diabetes Research studied the glucose transport kinetics of islets isolated from normal rats in which β-cell expression of GLUT-2 has been profoundly reduced by the 12-day infusion of insulin (Figure 6)(14). Profound differences in glucose kinetics were observed (14). Whereas in islets from control rats exhibited two glucose transport functions, a high K_m/high V_{max} function and a low V_{max}/low K_m function, in islets from the insulin-infused rats the high K_m/high V_{max} function was profoundly reduced (Figure 7). Whereas in normal islets saturation had not been reached at 30 mM, in the islets of insulin-infused rats saturation occurred at approximately 5 mM glucose. This indicates that GLUT-2 expression is required for unrestricted glucose uptake by β-cells and that *in the absence of GLUT-2 expression glucose transport is rate-limiting at concentrations above 5 mM, i.e. the normal fasting glucose concentration. Loss of GLUT-2 would therefore be expected to cause insensitivity of β-cells and unresponsiveness of insulin secretion to glucose concentrations in excess of 5 mM.*

TYPE 1 DIABETES

Type 1 (IDDM, juvenile onset) diabetes is an autoimmune-mediated destructive disease of β-cells afflicting persons with an inherited susceptibility conferred by or linked to a class II molecule (15). Single amino acid substitutions at the 57 position of the

DQ β-chain (16) and the 52 position of the DQ α-chain (17) are believed to define susceptibility to and protection from β-cell autoimmunity. The primary antigen or antigens presented in eliciting autoimmune β-cell destruction are unknown, but at least four antibodies to β-cell antigens are known to be present in the majority of new-onset type 1 diabetics: cytoplasmic islet cell antibodies (18), unidentified β-cell surface antibodies (19), insulin autoantibodies (20) and antibodies to a 64 kD islet protein (21). None of these antibodies is proven to be destructive to β-cells; rather they are considered to be an immune response to antigens released during β-cell destruction.

The only known functional derangement in the type 1 prediabetic patient is the progressive attenuation in the insulin response to intravenously injected glucose first described by Srikanta et al. of the Joslin Clinic (22). Figure 8 shows the progressive attenuation of glucose-stimulated insulin secretion in a monozygotic twin of a type 1 patient who became islet cell antibody positive years before the appearance of overt diabetes.

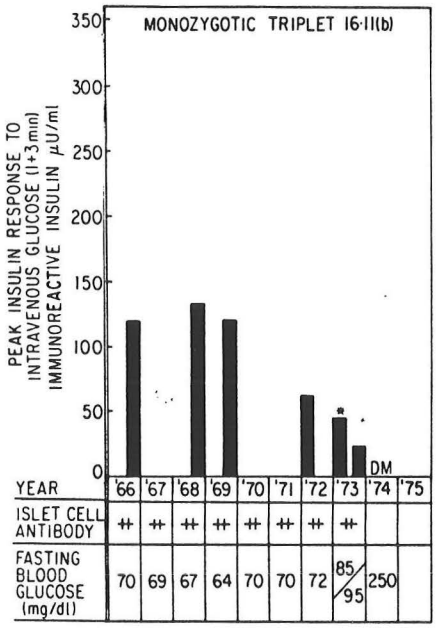

Figure 8 - Monozygotic triplet. Comparison of the results of serial intravenous glucose-tolerance tests and determinations of islet-cell antibodies. The single asterisk indicates tests of cortisone-primed intravenous glucose tolerance. DM denotes a clinical diagnosis of diabetes mellitus. To convert glucose levels to millimoles per liter, multiply by 0.0555. To convert insulin levels to picomoles per liter, multiply by 7.175. (From ref. 22).

The question to be addressed is: do such patients develope antibodies against the high K_m glucose transport of β-cells, GLUT-2, that restrict glucose in flux in β-cells, rendering them unresponsive to glucose? The question is a difficult one to answer by means of commonly available technics. Using Western blotting Dr. Johnson has identified bands in the 60 kD range of GLUT-2 and more recently has found this in the serum of Subject # 1, an islet cell antibody positive pre-type 1 diabetic patient with a declining insulin response to glucose and transport inhibitor activity in her IgG (Figure 9)(23). However, GLUT-2 is not the only protein in this molecular size range.

Although other direct strategies to test antibodies to GLUT-2 are being developed Drs. Wick Johnson and Billy Crider decided to determine by means of a bioassay if immunoglobulins from new-onset type 1 patients inhibited glucose uptake in islets of normal rats. They first scaled up the islet isolation technology so as to provide the tens of thousands of islets required for complete kinetic studies. (Such studies had never before been carried out on normal islets because of difficulties in the enormous numbers of islets required.) As shown in Figure 10 the islets exhibit the typical uptake curve of a facilitative glucose transporter, reaching equilibrium within 60 seconds (24). The stereospecificity of 3-0-methyl-glucose uptake was also established in Figure 10 by the low level of L-glucose uptake. They then compared the initial 3-0-methyl-glucose uptake in islets incubated for 15 minutes in IgG of normal controls, new onset type 1 diabetic patients and patients with NIDDM (Figure 11). An important reduction in glucose uptake occurred only with the diabetic IgG (24). That this effect is glucose-specific is suggested by the fact that no difference in leucine uptake was noted (Figure 12). Only 1 of 28 diabetics fell within 1 standard deviation of the uptake in islets incubated with IgG from controls. If one designates values belos this as positive, the diagnostic sensitivity exceeds that of islet cell antibodies, insulin autoantibodies or 64 k antibodies.

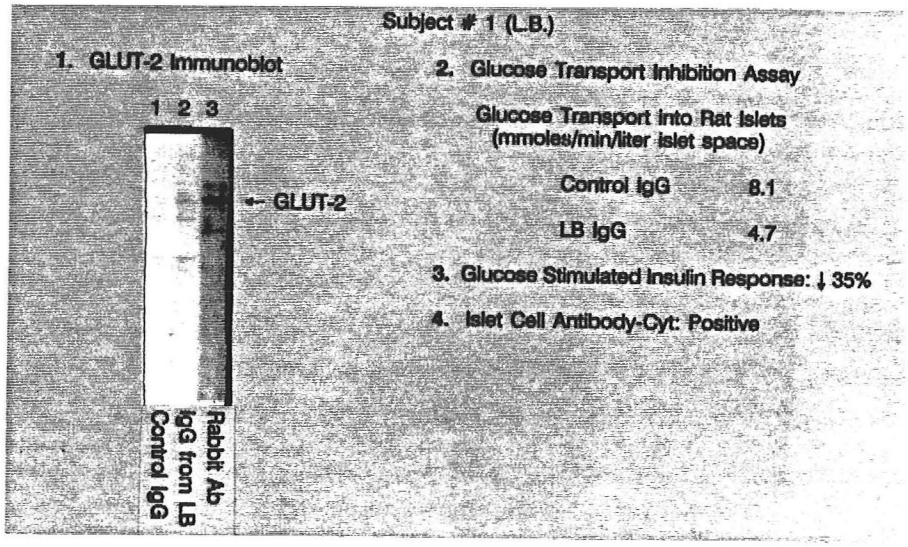

Figure 9 - Immunoblots of rat islet membranes with serum of Subject # 1 (L.B.) in lane 2 and rabbit anti-GLUT-2 serum in lane 3. The IgG of L.B. inhibited 3-0-methyl glucose uptake by normal rat islets by ~50%. At the time the serum specimens were obtained the insulin response to glucose (sum of 1' and 3' values) was 158 μU/ml compared to an earlier response of 240 μU/ml. She was ICA-cyt positive.

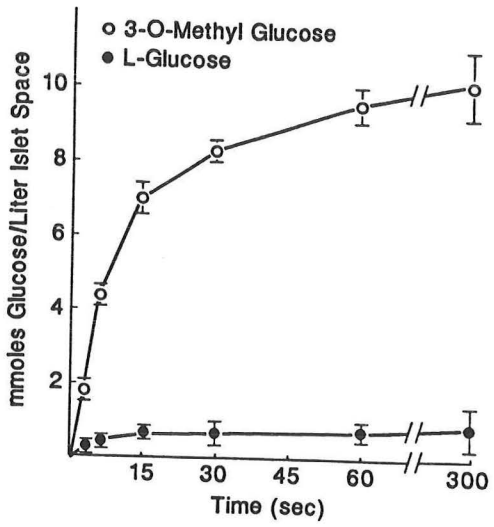

Figure 10 - Time course of 3-0-methyl-D-glucose and L-glucose uptake by rat islets, showing rapid equilibration typical of facilitative glucose transport. The low L-glucose uptake indicates the stereospecificity of the glucose trasnport and the integrity of the permeability barrier of the islet cells. (From ref. 24)

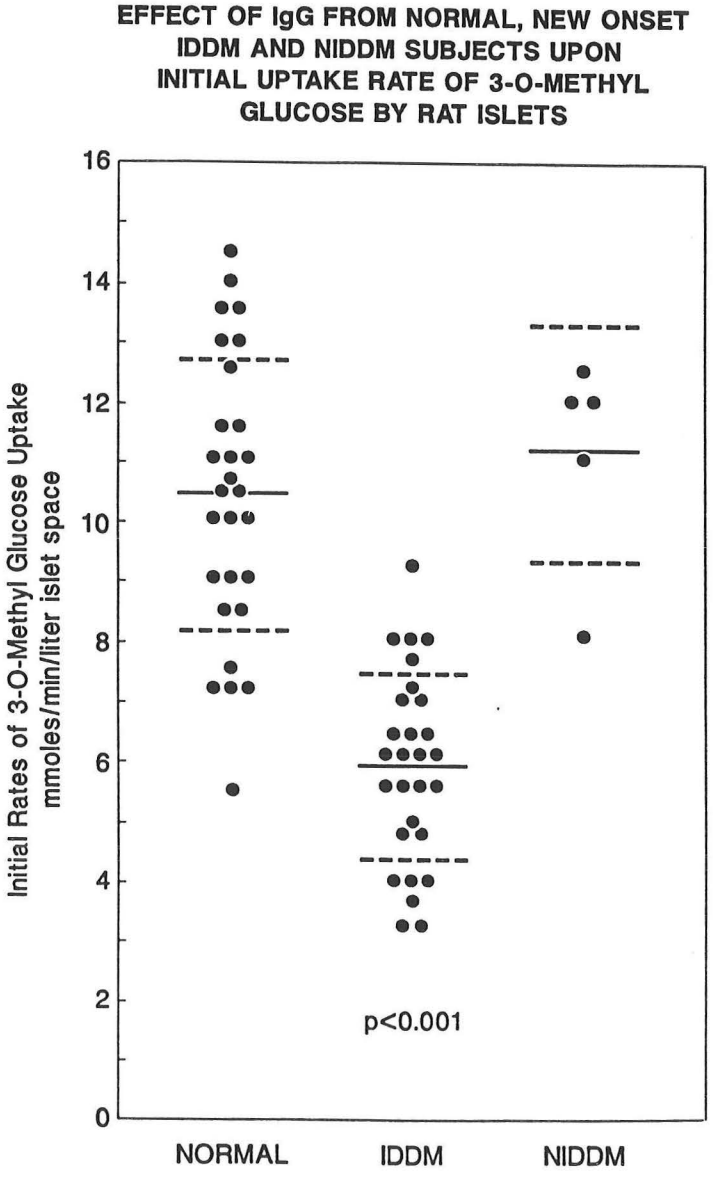

Figure 11 - Initial rates of 3-0-methyl-β-D-glucose uptake by rat islet cells in the presence of IgG from each member of the three study groups. (From ref. 24.)

EFFECT OF IgG FROM NORMAL (●,n=28) AND NEW ONSET TYPE 1 (○,n=27) SUBJECTS ON GLUCOSE AND LEUCINE UPTAKE BY RAT ISLETS

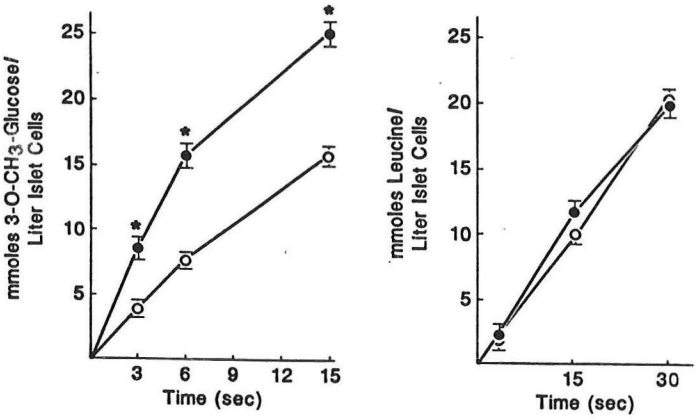

Figure 12 - Effects of IgG fractions from normal subjects (open circles) and patients wtih IDDM (solid circles) on the uptake of 3-0-methyl-β-D-glucose and L-leucine by dispersed rat islet cells. (From ref. 24.)

As mentioned previously, islets have both a low K_m and a high K_m glucose transport function (25). To determine if, as was the case in the islets of rats in which GLUT-2 had been down-regulated by chronic insulin infusion (Figure 6), reduction in high K_m function by diabetic immunoglobulins is the major cause for the reduction in glucose uptake, Dr. Johnson did Eadie-Hofstee transformations of the glucose uptake curves generated with various concentrations of 3-0-methyl glucose in the presence of diabetic IgG. In Figure 13 islets incubated in buffer alone display both the high and the low K_m functions. In the center panel, islets were incubated with 0.4 mM cytochalasin B, a concentration that blocks only low K_m glucose transport; the high K_m function was intact (25). However, in the right hand panel diabetic IgG was preincubated with islets; the V_{max} was profoundly reduced without abolishing the high K_m transport function (24). Low K_m transport was unaffected. This indicates that the IgG inhibited only high K_m GLUT-2.

Dr. Johnson then determined if this inhibitory activity could be specifically adsorbed out by exposing the IgG of diabetic patients to cells or cell membranes that display GLUT-2. As shown in Figure 14 preincubation of tissues such as erythrocytes and kidney brush border, neither of which express GLUT-2, failed to reduce the inhibitory activity of diabetic IgG. On the other hand, preincubation of IgG with either islets or hepatocytes, two of the GLUT-2 expressing tissues, removed all inhibitory reactivity (24).

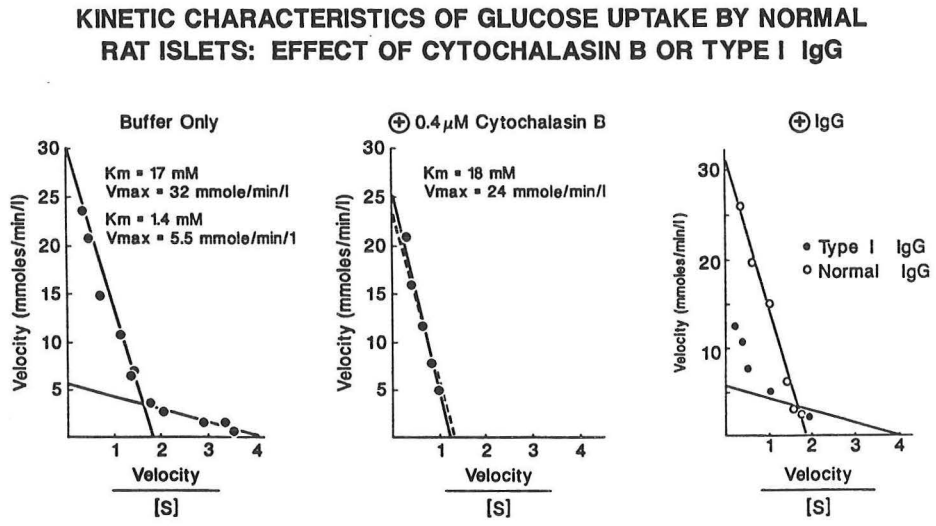

Figure 13 - Kinetic characteristics of glucose uptake by normal rat islets showing the effect of preincubation of islets with cytochalasin B or purified IgG from new onset type 1 diabetics upon the V_{max} of 3-0-methyl glucose transport in normal rat islets. Note that the low dose cytochalasin B completely abolishes low K_m uptake, while the diabetic IgG lowers the V_{max} of high K_m glucose uptake. Diabetic IgG does not influence low K_m glucose uptake.

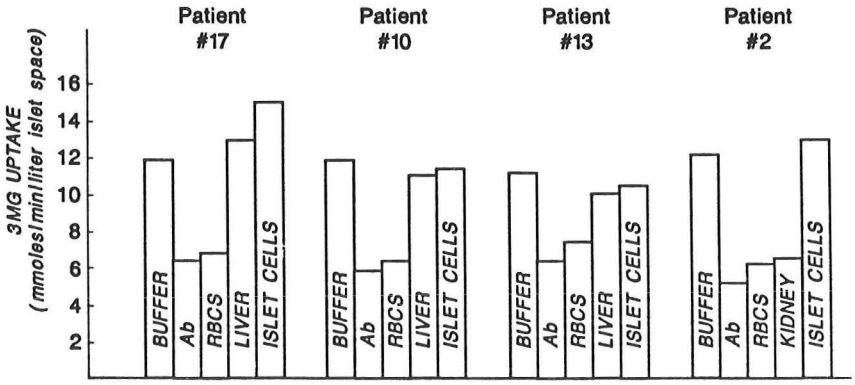

Figure 14 - Effect of preincubation of IgG from four patients with IDDM with cells or cell membranes from islets, liver, erythrocytes, or kidney brush border on 3-0-methyl-β-D-glucose uptake by islet cells. (From ref. 24.)

CONCLUSIONS

Purified IgG from new-onset type 1 diabetics inhibits high K_m glucose transport in islets. This inhibitory activity can be absorbed out by preincubation with GLUT-2 containing membranes but not by plasma membranes or cells devoid of GLUT-2. These findings strongly suggest that antibodies to β-cell GLUT-2 are present in the type 1 diabetic immunoglobulins examined. It is possible that in type 1 prediabetic patients these antibodies impair glucose uptake in a β-cell population shrinking because of ongoing autoimmune destruction and thereby cause a selective attenuation in their insulin response to glucose.

Could GLUT-2 be the primary antigen presented in the pathogenesis of the β-cell destruction? While this cannot be excluded, it seems very unlikely that these antibodies are any more centrally involved in the pathogenesis of the destruction then the antibodies to other β-cell antigens identified previously. Whether or not it will provide a better marker of β-cell autoimmunity in prediabetics remains to be determined.

TYPE 2 (NON-INSULIN DEPENDENT, ADULT ONSET) DIABETES

Type 2 diabetes [non-insulin dependent diabetes (NIDDM)] is the most common form of human diabetes, afflicting from 2 - 6% of all Americans and close to 50% of the natives of the Republic of Nauru and the Pima Indians of Arizona. In contrast to type 1 diabetes there is no reduction in β-cell mass; although late in the disease there may be encroachment on β-cells by amyloid deposits (26), the β-cell mass is normal or even enlarged early on (27,28). The individual β-cells themselves are completely normal at both the light and electron microscopic level (27). And yet, as shown in Figure 15, there is a selective loss of glucose-stimulated insulin secretion.

Inasmuch as the pancreas cannot be studied in living persons, it was necessary to identify an animal model of NIDDM. Fortunately such a model was found in the laboratory of Dr. Richard Peterson, Department of Anatomy, University of Indiana Medical School, Indianapolis, Indiana. By inbreeding glucose-intolerant Zucker fatty (*fa/fa*) rats, he produced in F10 a colony in which all male members became overtly diabetic between 7 and 9 weeks of age; none of the obese females developed diabetes (29). Nor did lean male or female heterozygotes (*fa/+*) develope diabetes. There being no possibility of sex-matched controls, three control groups were employed in all studies: obese non-diabetic females (*fa/fa*), lean male heterozygotes (*fa/+*) and normal Wistar rats.

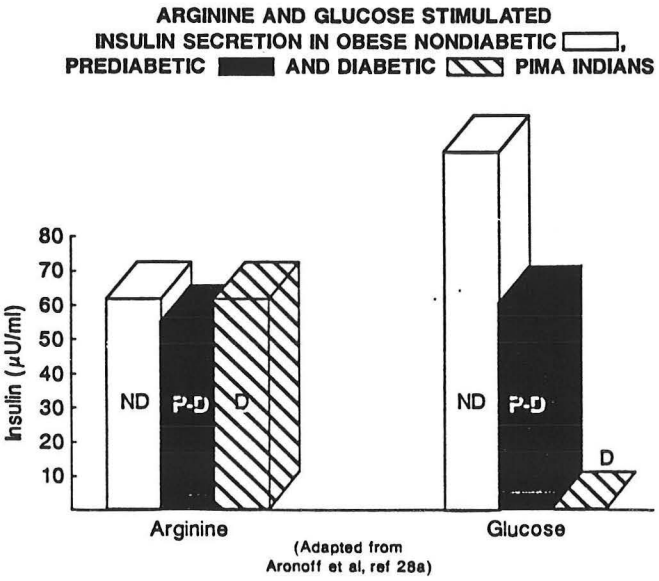

Figure 15. The arginine and glucose response of obese nondiabetic, prediabetic and diabetic Pima Indians. Although the response to arginine is similar in all three groups, the response to glucose is significantly reduced in the prediabetic subjects and is completely absent at the time that fasting hyperglycemia appears. (From ref. 28a.)

To make certain that the ZDF diabetic males, like their human counterparts, exhibited a selective loss of glucose-stimulated insulin secretion, pancreata from these animals were isolated and perfused with 20 mM glucose, 10 mM arginine and a combination of the two by Dr. Atsushi Ogawa, a post-doctoral fellow in my laboratory. As shown in Figure 16, the diabetic animals exhibited no insulin response to glucose, whereas all control groups mounted a robust response. The diabetic rats did however mount a brisk response to arginine, which, although greater than that of the lean animals, was substantially below that of the non-diabetic female controls. The findings are interpreted as evidence of selective loss of glucose-stimulated insulin secretion in overtly diabetic ZDF rats. Perfusion of pancreata from male ZDF animals with early less severe diabetes, revealed that complete disappearance of glucose-stimulated insulin secretion did not occur until fasting hyperglycemia had reached 200 mg or more (11 mM). Thus, as in humans, ZDF NIDDM is characterized by a progressive attenuation of glucose-stimulated insulin secretion.

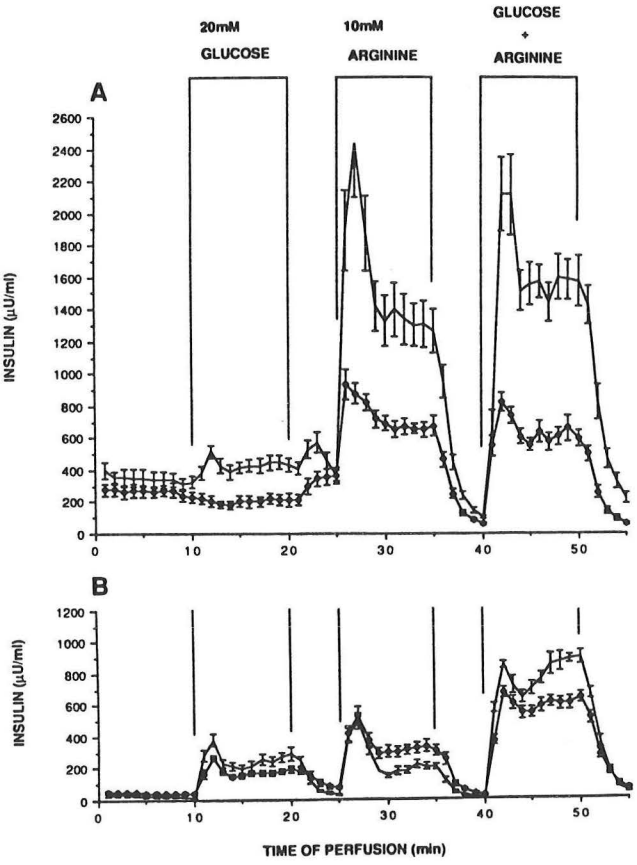

Figure 16 - Insulin response to 20 mM glucose, 10 mM arginine and combination thereof in isolated perfused pancreata of: A. Obese male diabetic ZDF rats (N = 8)(○—○) and nondiabetic obese female Zucker rats (N = 9) (—), and B. Nondiabetic lean male Zucker rats (N = 9)(○—○) and nondiabetic male Wistar rats (—). (From ref. 2)

GLUT-2 ABUNDANCE IN β-CELLS

Quantitation of GLUT-2 was carried out by immunoblotting of plasma membranes of isolated islets, by immunofluorescent staining of pancreatic slices and by protein A-gold immunocytochemistry. The Johnson/Newgard anti-GLUT-2 C-terminal hexadecapeptide was employed for all studies. Dr. Johnson (Figure 17) demonstrated by immunoblotting the absence of detectable GLUT-2 in membranes of severely diabetic

ZDF animal, even when five times as much membrane is loaded (2). The milder diabetic in Figure 16 also has a substantial reduction in GLUT-2 compared to the normal controls. Immunofluorescent staining (30) in Dr. Lelio Orci's Geneva lab similarly revealed a profound reduction in GLUT-2 which was related to the severity of the diabetes (Table 4 and Figure 18). At the electron microscopic level (30), a similarly marked reduction of GLUT-2 per μM of plasma membrane was observed (Figure 18 and Table 5). In addition there was a reduction in the percent of plasma membrane occupied by microvilli, the segment in which β-cell GLUT-2 is normally present in highest abundance (30).

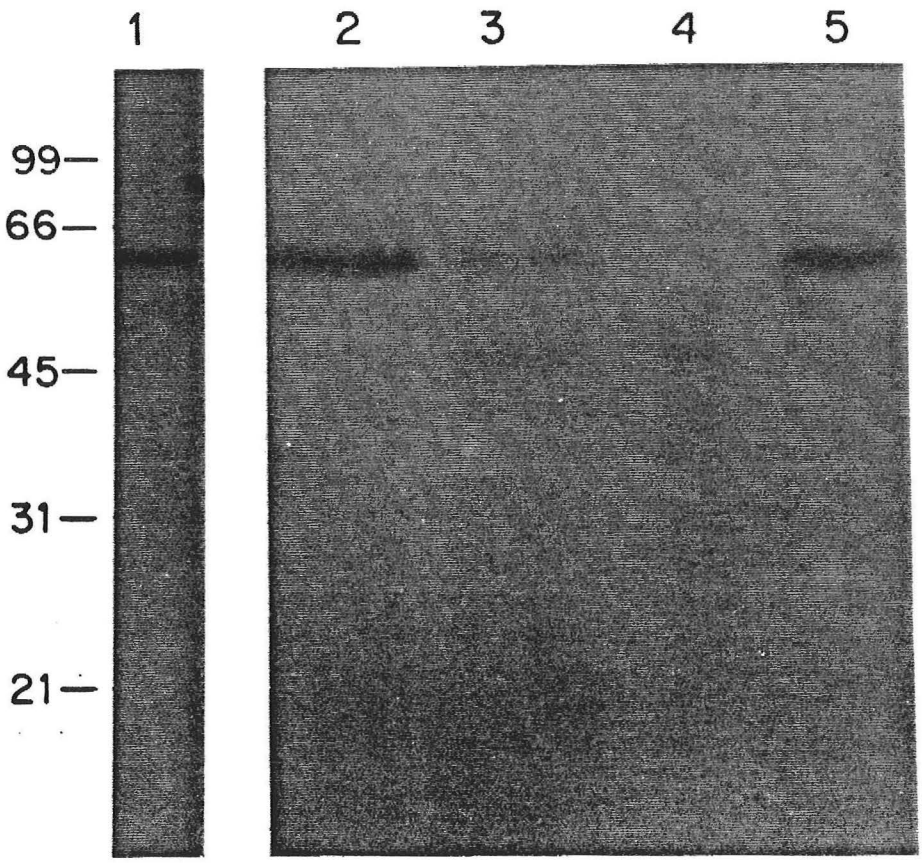

Figure 17 - Immunoblots for GLUT-2 in crude islet membrane preparations (13) from nondiabetic Wistar rats (lane 1), nondiabetic lean male Zucker rats (fa/+)(lane 2), 8 week-old male Zucker diabetic fatty rats (glucose 13.6 mM)(lane 3), 12 week-old male Zucker diabetic fatty rats (glucose 25.3 mM)(lane 4), and 12 week-old female nondiabetic Zucker fatty rats (lane 5). The band with an apparent Mr of 60 kD is blocked by preincubation of the antibody with a 10^3 molar excess of antigen (data not shown). The second band with an apparent Mr 50 kD is not blocked by antigen excess. It is not detected in islet membranes of Wistar rats. (From ref. 2)

TABLE 4

GLUT-2/Insulin Ratio in Islets of ZDF rats with NIDDM

	Morning Plasma Glucose (mg/dl)	Ratio (Vv GLUT-2/Vv insulin)
Nondiabetic lean ♂ (fa/+) (N=9) (8-14 weeks old)	137 ± 4	0.97 ± 0.01
Nondiabetic ♀ ZDF (fa/fa) (N=9) (8-15 weeks old)	147 ± 8	0.94 ± 0.02
Nondiabetic ♂ Wistar (N=5) (8-14 weeks old)	124 ± 6	0.96 ± 0.01
Diabetic ♂ ZDF (Fa/Fa) (N=5) (12 weeks old)	538 ± 45	0.25 ± 0.06
Diabetic ♂ ZDF (fa/fa) (N=5) (35 weeks old)	494 ± 45	0.05 ± 0.01

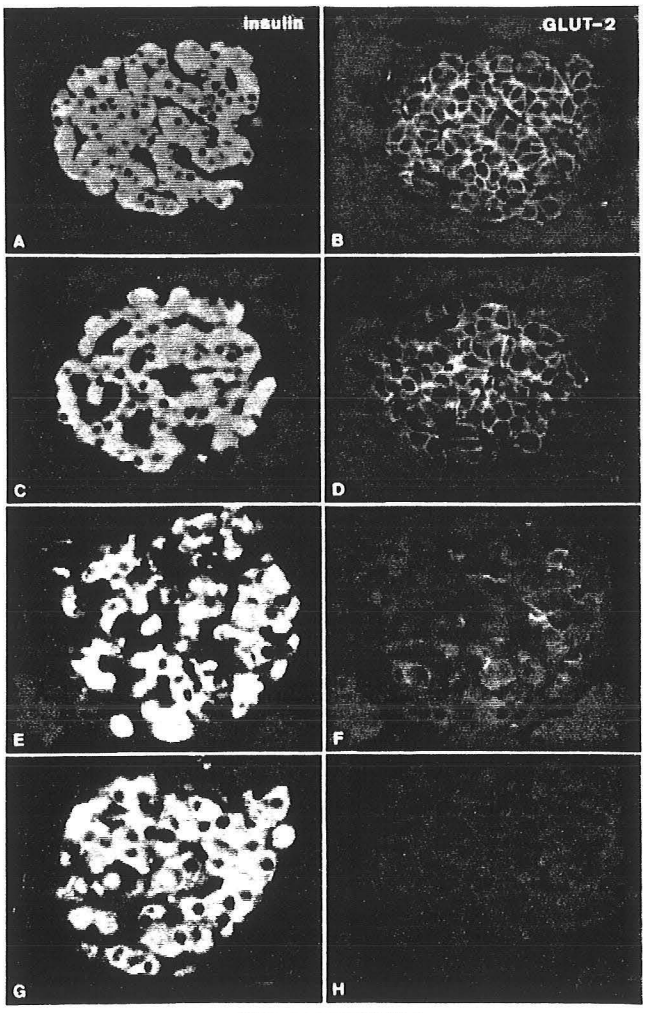

Figure 18 - Pairs of consecutive serial sections stained by immunofluorescence with anti-insulin (left panels) and anti-GLUT-2 (right panels) antibodies.

A-B: An islet of normal appearance in a ZDF nondiabetic female. Virtually all insulin-immunofluorescent cells display GLUT-2 immunostaining. C-D. Minimal diabetes in a ZDF male animal. E-F. More severe reduction of GLUT-2 positive β-cells. G-H. Virtual absence of GLUT-2 positive cells in a severe ZDF male diabetic.

Magnification:X150 (From Ref. 30.)

TABLE 5

	Morning Plasma Glucose (mg/dl)	Flat membrane	Microvilli
Nondiabetic lean ZDF ♂ (fa/+) N=54	133 ± 4	0.69 ± 0.05	2.09 ± 0.13
Nondiabetic ZDF ♀ (fa/fa) N=54	146 ± 7	0.55 ± 0.05	1.77 ± 0.13
Milder diabetic ♂ (fa/fa) N=54	221 ± 23	0.43 ± 0.04	1.07 ± 0.11
Severe diabetic ♂ (fa/fa) N=54	440 ± 56	0.24 ± .04	0.55 ± 0.08

CONCLUSION

Immunodetectable GLUT-2 is profoundly reduced to absent in severely diabetic ZDF rats in proportion with the severity of the diabetes. Prior to hyperglycemia of 200 mg % GLUT-2 is present in normal amounts.

IS LOW GLUT-2 THE CAUSE OR THE CONSEQUENCE OF THE HYPERGLYCEMIA?

The *pari passu* relationship between the GLUT-2 abundance and the hyperglycemia made it impossible to deduce if the dearth of GLUT-2 caused the hyperglycemia or vice versa or if they are two unrelated but parallel occurrences. To determine if hyperglycemia could reduce immunodetectable GLUT-2 [we had already shown that in normal rats hyperglycemic infusion increases GLUT-2 mRNA (Table 3)] we quantitated GLUT-2 in normal rats following a five-day infusion of 50% glucose which maintained blood glucose levels at or near the 200 mg/dl range (Table 6). There was no significant reduction in the percent of β-cells that were GLUT-2 positive. In vitro studies of monolayer cultures of β-cells exposed for 6 weeks to 16.7 mM also revealed no reduction in GLUT-2 at the EM level (Table 7). To exclude the possibility that ZDF rats exhibit a unique and paradoxal response to hyperglycemia, Dr. Orci quantitated GLUT-2 abundance in male ZDF rats in which hyperglycemia had been prevented by the feeding of acarbose, an α-glucosidase inhibitor that prevents glucose absorption. This was begun at six weeks, well before they reached the age of 8 weeks at which diabetes begins (30), and was continued until the age of 26 weeks at which point untreated controls have a mean glucose level of 590 mg/dl (Table 8). By contrast the acarbose-treated animals had an average glucose of 129, even lower than nondiabetic obese female controls. Despite prevention of hyperglycemia, only 20% of β-cells were GLUT-2 positive, compared to over 90% in the female controls (30). Yet in the untreated diabetics only 3% of β-cells were GLUT-2 positive (Table 8). The interpretation is that while poorly controlled NIDDM may in and of itself reduce GLUT-2 positivity in β-cells, the major component of the down-regulation of GLUT-2 is independent of hyperglycemia and occurs even in its complete absence.

TABLE 6

Effect of Hyperglycemia Induced by Glucose Infusion on GLUT-2/Insulin Ratio in β-cells of Normal Rats

	Glycemia (mg/dl)	Ratio
5% glucose (N=2)	116 110	1.00 0.97
50% glucose (N=2)	266 197	0.96 0.98

TABLE 7

Effect of Glucose on GLUT-2 in β-cell Plasma Membranes of Cultured Neonatal Rat ISlets (Protein A-gold)

Culture Time	Glucose Concentration mM	Grains/μm Plasma Membrane
7 days N=20	5.5	1.32 ± 0.14
7 days N=20	33.4	2.02 ± 0.23
5 weeks N=20	33.4	2.42 ± 0.22

TABLE 8

PREVENTION OF HYPERGLYCEMIA WITH ACARBOSE R_x DOES NOT PREVENT UNDEREXPRESSION OF GLUT-2 IN ZDF DIABETIC RATS

	GLUCOSE (mg/dl)	GLUT-2 POS β-cells (%)
Untreated Diabetic ZDF ♂	590	6 ± 1
		p<0.2
Acarbose-Treated Diabetic ZDF ♂	129	20 ± 4
		p<0.001
Nondiabetic ZDF ♀	147	94 ± 2

Is GLUT-2 down-regulation in ZDF rats a pre-translational event? With the possibility that post-translational modification of the GLUT-2 molecule by hyperglycemia accounted for the loss of immunodetectability largely excluded, Drs. Tausif Alam and Ling Chen searched for evidence of pre-translational down-regulation. They employed both in situ hybridization and Northern blotting (Table 9 and Figure 19) using a riboprobe provided by Steve Hughes in the Newgard lab. Both technics showed a significant reduction in GLUT-2 mRNA in the severely diabetic animals compared to all control groups (2). While the reduction in mRNA was less than the reduction of immunodetectable protein, it was sufficient to account for most of the reduction in protein (imprecision of the technics precludes overly literal interpretation of these semiquantitative data).

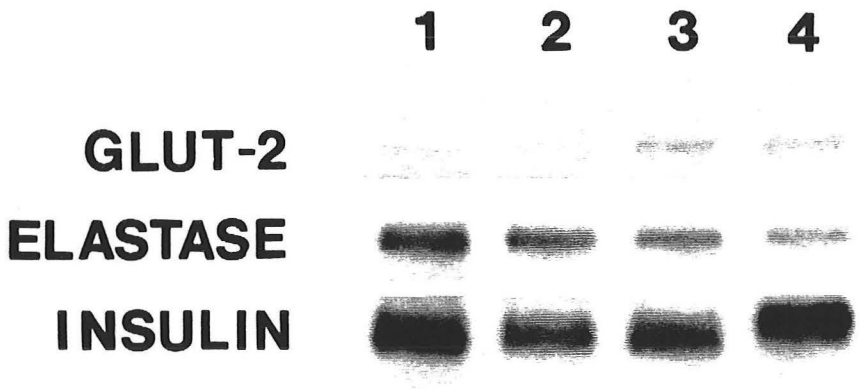

Figure 19 - Northern blots of poly A+ RNA from pancreata of ZDF rats and controls hybridized sequentially with ^{32}P-labeled antisense GLUT-2, proelastase and proinsulin probes under stringent conditions. Lanes 1 and 2 are from male diabetic ZDF rats (blood glucose levels, 33 mM and 24 mM). Lane 3 is from a nondiabetic fatty female and lane 4 is from a lean nondiabetic ZDF heterozygotic male. Proelastase mRNA provides an indication of the amount of pancreatic mRNA loaded. (From ref. 2)

Table 9 - Glut-2 mRNA in β-cells

	In situ	Northern
Obese Diabetic (*fa/fa*) ♂	0.23 ± 0.19*	0.2
Obese Nondiabetic (*fa/fa*) ♀	0.45 ± 0.17	0.9
Lean Nondiabetic (*fa/+*) ♂	0.50 ± 0.06	0.7

*$p<0.0001$

DO GLUT-2 DEFICIENT ISLETS HAVE IMPAIRED GLUCOSE TRANSPORT?

To establish the functional importance of the reduction of GLUT-2 it was necessary for Dr. Johnson to study glucose transport in GLUT-2 deficient islets. Because of the scarcity of diabetic ZDF rats, he could not obtain sufficient islets to do a complete analysis of the concentration dependence of glucose uptake; therefore he chose a 15 mM glucose concentration which is close to the K_m of GLUT-2 and compared glucose uptake of islets isolated from severely diabetic ZDF rats (2). As shown in Figure 20 glucose uptake in these islets was far below that of nondiabetic obese female controls and lean controls. These results explain the inability of β-cells to sense and respond to the 20 mM increment that had been employed in the perfusion experiments (Figure 16).

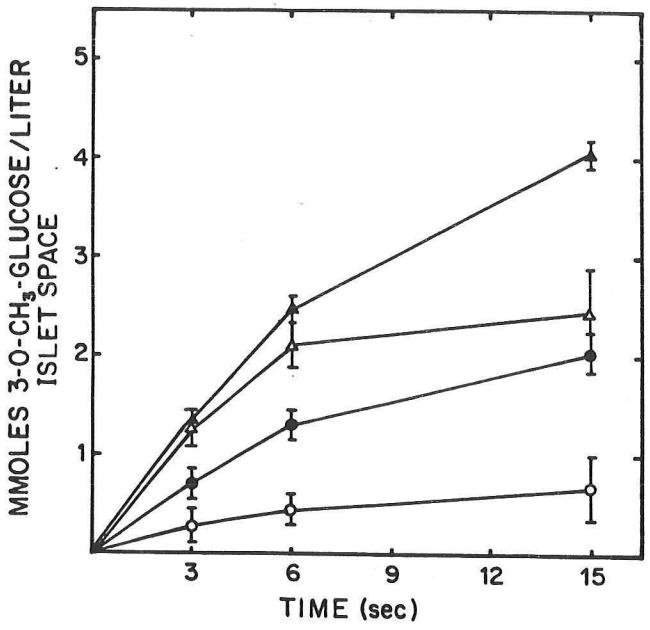

Figure 20 - Time course of uptake of 15 mM 3-0-methyl glucose by dispersed islet cells pooled from 4 nondiabetic Wistar rats (▲), 4 nondiabetic lean male Zucker rats (fa/+) (Δ), 8 nondiabetic obese female Zucker rats (fa/fa)(●) and 8 diabetic obese male Zucker rats (fa/fa)(○)(glucose 24.9-33.2 mM). All Zucker animals were 12 weeks of age. Each data point represents the mean ± SEM of 4 independent experiments in the diabetic and nondiabetic ZDF rats, each of which was carried out in duplicate as described elsewhere. In the Wistar and lean male Zucker rats 2 independent experiments were carried out in duplicate. (From ref. 2)

THE RELATIONSHIPS BETWEEN THE PAUCITY OF GLUT-2 AND THE FUNCTIONAL DERANGEMENTS

As shown in Figure 21 (left panel) the hyperglycemia was inversely correlated with the abundance of GLUT-2. In every rat with hyperglycemia of 200 mg/dl or greater, less than 60% of β-cells were positive for GLUT-2. The 60% mark was also the level at which insulin response to glucose was completely absent (2)(Figure 21, upper right panel). Arginine-stimulated insulin secretion was completely unaffected by the reduction in GLUT-2 and was even at or above normal when GLUT-2 was present on less than 10% of β-cells (Figure 22). It is concluded that fasting hyperglycemia and loss of glucose-stimulated insulin secretion are secondary to the impairment of glucose transport in β-cells resulting from the dearth of high K_m glucose transporter (2).

β-CELL FUNCTION VS. IMMUNOSTAINABLE GLUT-2 IN β-CELLS OF OBESE DIABETIC ZDF RATS AND NONDIABETIC OBESE AND NONOBESE CONTROLS

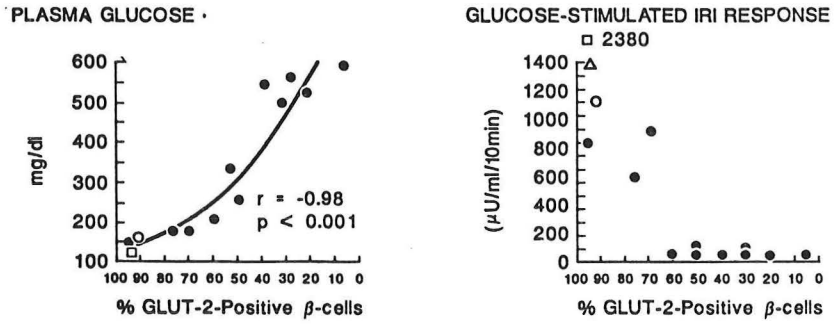

Figure 21 - Blood glucose levels and glucose-stimulated insulin secretion plotted against the percent of β-cells that are GLUT-2 positive by immunofluorescent staining in obese male diabetic ZDF (fa/fa) rats (●) and nondiabetic obese (○) and lean controls (open symbols). Note the strong negative correlation between plasma glucose and percent of GLUT-2 positive β-cells (upper left hand panel). All rats with less than 60% GLUT-2 positive β-cells had glucose concentrations in excess of 200 mg/dl. Glucose stimulated IRI response is absent when the % of GLUT-2-positive β-cells is 60% or less. (From ref. 2.)

β-CELL FUNCTION VS. IMMUNOSTAINABLE GLUT-2 IN β-CELLS OF OBESE DIABETIC ZDF RATS AND NONDIABETIC OBESE AND NONOBESE CONTROLS

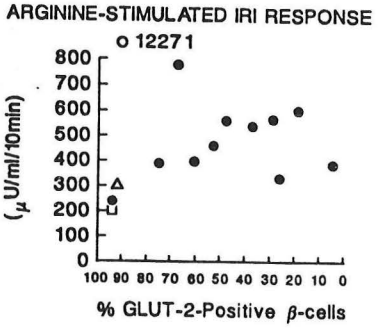

Figure 22 - Arginine-stimulated IRI response as a function of percent of GLUT-2 positive β-cells in diabetic ZDF rats (●). Note the lack of any relationship between the insulin response and the number of GLUT-2 positive β-cells. (From ref. 2.)

CONCLUSIONS CONCERNING THE PATHOGENESIS OF TYPE 2 NIDDM

On the basis of foregoing evidence we conclude that in the NIDDM of Zucker diabetic fatty rats [and of GK and Wistar/Kyoto NIDDM rats (30) in which there is no sex-linked predilection for NIDDM (31)] an unidentified factor results in reduced GLUT-2 mRNA and a reduction in immunodetectable GLUT-2 in β-cells (Table 10). This impairs glucose transport at high concentrations (>5 mM), thereby reducing glucose usage. As a result the diabetic β-cells cannot sense or respond to a rising extracellular concentration of glucose. The lack of an increase in insulin secretion during a rising glucose concentration results in the steady state hyperglycemia upon which the diagnosis of diabetes is based (Table 10)(2,30).

TABLE 10

PUTATIVE PATHOGENESIS OF NIDDM

1. X→↓ β-cell GLUT-2 expression →↓high Km glucose transport
2. ↓ high Km glucose transport→↓insulin response to high glucose
3. ↓ insulin response to high glucose→steady state hyperglycemia = diabetes

INSULIN RESISTANCE

Case Report

Subject 3 - The patient is a markedly obese 39 year-old Pima Indian carpenter who has been overweight as long as he can remember. He is now 100% above his ideal weight. His annual OGTT has always been normal. His fasting insulin level is 86 μU/ml and rises to 220 after glucose. His M value is markedly reduced, indicating severe insulin resistance.

INTRODUCTION

Insulin resistance is said to be present when high insulin levels are associated with a normal glucose concentration. In the United States obesity is by far the most common cause of insulin resistance, but it occurs in nonobese individuals as well. The primary cause of insulin resistance is believed to lie in the target tissues of insulin, primarily muscle and fat. The locus of the abnormality is believed to be at a point distal

to binding of insulin to its receptor, resulting in a reduction in the recruitment of GLUT-4 from the low density microsomal pool of muscle and fat cells to their plasma membranes (32). According to the conventional concept, β-cells compensate for this abnormality by increasing their output of insulin so as to maintain glucose concentrations within a normal range. In some instances, however, compensation for the β-cells begins to wane, resulting in gradually increasing hyperglycemia, which is diagnosed as non-insulin-dependent type 2 diabetes.

Figure 23 (upper portion) depicts the current conventionally accepted scenario in which the development of hyperinsulinemia is secondary to insulin resistance. However there is no evidence that would rule out an alternative scenario (Figure 23, lower portion), namely, that the hyperinsulinemia is the primary defect with insulin resistance a secondary compensation to prevent hypoglycemia.

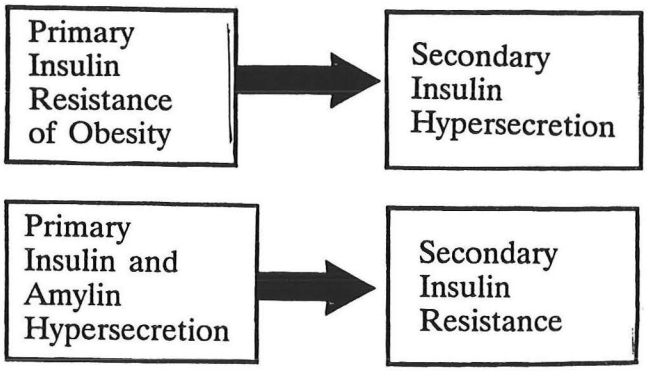

Figure 23 - Conventional (upper) and alternative concepts concerning the primacy of insulin resistance and hyperinsulinemia.

This scenario was suggested by the study in ZDF rats reviewed above. In Figure 16 both the obese nondiabetic and diabetic ZDF rats the baseline insulin levels with a perfusate containing 5.6 mM glucose were 10-15 times those of lean nondiabetic controls. Based on fragmentary data, the relationship between baseline insulin secretion and ambient glucose concentration is depicted in nondiabetic lean animals with normal insulin levels, and in the ZDF obese nondiabetic and diabetic groups (Figure 24). At 5 mM glucose lean animals have an insulin level of 20 μU/ml while the obese animals have levels of 200 μU/ml or greater.

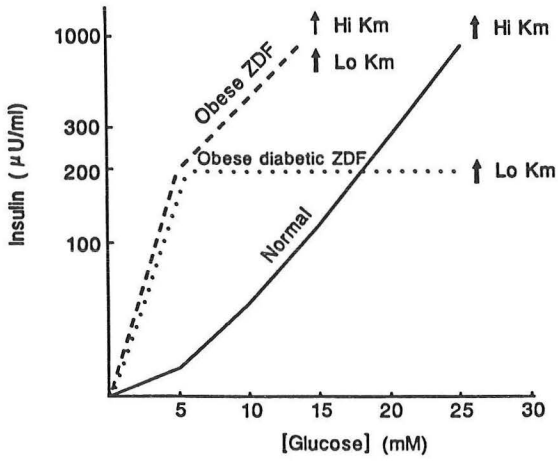

Fig. 24 - Predicted insulin response to glucose in normal rats and in obese diabetic rats with and without noninsulin dependent diabetes. The insulin level secreted by the isolated perfused pancreata of these animals is plotted on the Y-axis as a function of the glucose concentration perfusing the pancreas. In nondiabetic normal and obese animals with a high K_m glucose transport function a maximum responsiveness of glucose occurs well above concentrations of 5 mM, the normal fasting range. However, obese diabetic ZDF animals that lack the high K_m glucose transporter, exhibit no response to glucose above 5 mM. However both obese diabetic and obese nondiabetic animals are hyperinsulinemic at levels between 1 - 5 mM glucose. This strongly suggests the presence of a low K_m glucose transporter driving insulin secretion at fasting levels of glucose.

What is driving the high levels of insulin secretion in both diabetic and nondiabetic obese groups?. We believe it is hypersensitivity to changes in glucose concentrations from 0 to 5 mM. To reduce their glucose level of the obese rats to the 20 μU/ml level maintained by nonobese animals at a 5 mM glucose concentration, one must lower glucose to 1 mM. Thus both obese groups exhibit marked hyperresponsiveness of insulin secretion between 0 and 5 mM. *This must mean that they both express a glucose transporter with a Km below 5 mM.* We can deduce that the β-cells of the diabetic group, which are devoid of GLUT-2, must be expressing some other glucose transporter, in view of their robust response to arginine and morphologic evidence of full viability; if the ZDF males can express an unidentified low Km transporter, the female obese nondiabetic may also be expressing the same transporter, in addition to GLUT-2 which is present on almost all of their β-cells.

What is the putative low Km glucose transporter isoform? This remains to be determined. Our suspicion is that it may be GLUT-5, which is expressed by cells in the small intestine, the tissue from which the pancreas anlage is derived.

Why do β-cells of obese rats express a low Km glucose transporter? One suggestion is that this low Km glucose transporter represents failure of neonatal repression of a transporter normally expressed on fetal islets which are known to be unresponsive to glucose. The result is that β-cells hypersecrete at normal fasting levels of glucose, the target tissues becoming insulin resistant to avoid potentially serious hypoglycemia. Co-secretion of increased levels of amylin may also be a factor in the equation: 1) it may contribute to peripheral insulin resistance and 2) it may preferentially shunt carbohydrate into lipogenic pathways, thereby contributing to obesity.

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