Prolonged Exposure of Human Pancreatic Islets to High Glucose Concentrations In Vitro Impairs the β-Cell Function

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Abstract

The aim of the present study was to clarify whether prolonged in vitro exposure of human pancreatic islets to high glucose concentrations impairs the function of these cells. For this purpose, islets isolated from adult cadaveric organ donors were cultured for seven days in RPMI 1640 medium supplemented with 10% fetal calf serum and containing either 5.6, 11, or 28 mM glucose. There was no glucose-induced decrease in islet DNA content or signs of morphological damage. However, islets cultured at 11 or 28 mM glucose showed a 45 or 60% decrease in insulin content, as compared to islets cultured at 5.6 mM glucose. Moreover, when such islets were submitted to a 60-min stimulation with a low (1.7 mM) followed by a high (16.7 mM) concentration of glucose, the islets cultured at 5.6 mM glucose showed a higher insulin response to glucose than those of the two other groups. Islets cultured at the two higher glucose concentrations showed increased rates of insulin release in the presence of low glucose, and a failure to enhance further the release in response to an elevated glucose level. Islets cultured at 28 mM glucose showed an absolute decrease in insulin release after stimulation with 16.7 mM glucose, as compared to islets cultured at 5.6 mM glucose. The rates of glucose oxidation, proinsulin biosynthesis, and total protein biosynthesis were similar in islets cultured at 5.6 or 11 mM glucose, but they were decreased in islets cultured at 28 mM glucose. These combined results suggest that lasting exposure to high glucose concentrations impairs the function of human pancreatic islets. (*J. Clin. Invest.* 1992. 90:1263–1268.) Key words: pancreatic islets • glucose • insulin release • glucose oxidation • proinsulin biosynthesis

Introduction

There are several studies showing that intensive insulin therapy in the early stages of insulin-dependent diabetes mellitus (IDDM)[1] can improve and preserve β-cell function (1–3). These observations suggest that the increased glucose levels prevailing in early IDDM are somehow deleterious for the β-cells.

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1. *Abbreviation used in this paper:* IDDM, insulin-dependent diabetes mellitus.

Indeed, the possibility of glucose-induced pancreatic β-cell damage has been much debated in the literature (for recent reviews see 4, 5). According to this idea, the defective insulin response to glucose observed in the early phases of IDDM, and in non–insulin-dependent diabetes mellitus, would reflect a chronic hyperglycaemic stimulation of a reduced β-cell mass (4, 5). Experimental support for this concept relies mainly on animal models, like the neonatal injection of streptozotocin, partial pancreatectomy, and in vivo infusion of glucose (4–6). However, it has been difficult to demonstrate these glucose effects under tissue culture conditions. Thus, while some groups reported that culture of rodent islets in the presence of high glucose concentrations impairs β-cell function (7–11), we and others were unable to observe any deleterious effects of glucose in vitro (12–18). Possible explanations for these discrepancies are the use of different culture conditions, and genetic differences between the experimental models. Indeed, recent findings suggest that hyperglycemia may be injurious to pancreatic islets obtained from C57BL/Ks mice, but not to islets obtained from C57BL/6 mice (19).

These observations raise the question to what extent human islets are sensitive to the potential deleterious effects of high glucose concentrations. Due to major problems in obtaining well-characterized human islets under culture conditions, it has been difficult to address this issue properly.

In the current study human islets were isolated from cadaveric donors. Following a detailed characterization of the islet cell populations, they were cultured at different glucose concentrations (5.6, 11, or 28 mM) for one week. After this period the islet glucose-stimulated insulin release, the number of islets retrieved after culture, the islet DNA and insulin content, and the rates of glucose oxidation and proinsulin biosynthesis were evaluated. The combined data suggest that lasting exposure to high glucose impairs the function of human islets.

Methods

Islet isolation. 14 human pancreata were excised from adult cadaveric organ donors at European hospitals affiliated with the β-Cell Transplant—European Concerted Action for the Treatment of Diabetes, Brussels, Belgium. The mean age of the donors (±SEM) was 38±3 yr (range of 18–54 yr). Each gland was flushed and cooled (4°C) in situ with University of Wisconsin or Eurocollins solutions. The glands were then removed and transported to the Central Unit of the β-Cell Transplant (Medical Campus, Vrije Universiteit Brussel, Brussels, Belgium). At the Central Unit the glands were processed by ductal distension with collagenase, gentle dissociation, and Ficoll gradient purification of islets, as previously described (20). Aliquots of the purified suspension were examined by electron microscopy, which indicated < 10% dead cells in all preparations, and the presence of 6.6±2.3% exocrine cells (mean±SEM; n = 14). The prevalence of insulin-positive cells, as evaluated by light microscopical examination of immunocytochemically stained islets, varied between 23 and 61% in the different preparations

($35\pm4\%$, $n = 14$), and the islet insulin content was 1.3 ± 0.2 ng insulin/ng DNA.

Islet culture. After isolation, the islets were cultured in Ham F10 medium (Flow Laboratories, Irvine, Strathclyde, UK) containing 6.1 mM glucose, and supplemented with 0.5% bovine serum albumin, 0.08 mg/ml penicillin, and 0.1 mg/ml streptomycin. The culture period in Brussels varied between 22 and 168 h (mean±SEM, 75.7 ± 12.9 h, $n = 14$), and the islets were subsequently sent by air to Uppsala, Sweden, in the same medium as described above, supplemented with 2% newborn calf serum and 10 mM Hepes (transport time: 4–8 h). Functional and morphological examinations (see below) of the islets immediately upon arrival in Uppsala showed no signs of islet damage induced by the transport procedure (data not shown). The first three preparations received in Uppsala were used to elaborate optimal conditions for tissue culture over a 7-d period. The variables examined were: type of culture medium (RPMI 1640 versus Ham F10, both from Flow Laboratories); type of serum (FCS versus pooled human serum); and serum concentration (1% versus 10%). The data (not shown) suggested that the best functional outcome (as evaluated by islet retrieval, islet insulin and DNA contents, insulin release in response to glucose, and rates of glucose oxidation) was obtained with islets maintained free-floating in RPMI 1640 medium containing 5.6 mM glucose and supplemented with 10% FCS (Flow Laboratories), benzylpenicillin (100 U/ml), and streptomycin (0.1 mg/ml) (culture conditions as in reference 21). Islets maintained for seven days in Ham F10 medium supplemented with 10% FCS presented a similar insulin and DNA content as observed in islets maintained in RPMI 1640. However, the absolute rates of islet insulin release at 16.7 mM glucose after culture in Ham F10 (insulin release studied as described below) was only half of that in islets cultured in RPMI 1640 (data not shown). After these initial experiments, all subsequent batches of human islets received in Uppsala were cultured in groups of 100–150 islets/dish in RPMI 1640 medium containing 10% FCS, as described above. The medium was supplemented either with 5.6, 11, or 28 mM glucose, and was changed every two days. At every medium change aliquots of the medium were frozen for subsequent insulin determination by radioimmunoassay (22). In some experiments, groups of islets were transferred for a further 2-d culture at 5.6 mM glucose after the initial 7-d culture at the different glucose concentrations. Light microscopical examinations were performed of paraffin sections of Bouin-fixed islets immunocytochemically stained for insulin and counterstained with hematoxylin.

Islet insulin release, DNA and insulin contents, glucose oxidation, proinsulin, and total protein biosynthesis. For the insulin release experiments, the islets were divided into triplicate groups of seven islets each and placed in sealed glass vials containing 0.25 ml bicarbonate buffer (23) supplemented with 10 mM Hepes and 2 mg/ml albumin (KRBH buffer). During the first hour of incubation at 37°C (O_2:CO_2; 95:5) the KRBH medium contained 1.7 mM glucose. The medium was then gently removed and replaced by 0.25 ml KRBH supplemented with 16.7 mM glucose, followed by incubation for a second hour. The insulin concentration in the incubation medium was determined by radioimmunoassay (22). After the insulin release experiments, the islets were pooled and disrupted by sonication in 0.2 ml bidistilled water. A 50-μl aliquot of the aqueous homogenate was mixed with 125 μl acid ethanol and the insulin extracted overnight at 4°C. DNA was measured fluorophotometrically in another fraction of the water homogenate (24). To measure islet glucose oxidation rates, triplicate groups of 10 islets were transferred to glass vials containing D-[U-^{14}C] glucose (Amersham International, Amersham, UK) and nonradioactive glucose to a final concentration of 1.7 or 16.7 mM glucose (sp act 0.5 mCi/mM) in medium KRBH, without albumin. The determination of the glucose oxidation rates were performed as previously described (25).

For the study of proinsulin and total protein biosynthesis, groups of 20 islets were placed in multiwell plates (well capacity 0.4 ml; Linbro Chemical Co., Hamden, CT), containing 100 μl KRBH supplemented with either 1.7 or 16.7 mM glucose and L-[4,5-^{3}H] leucine (Amer-

sham; final concentration of 50 μCi/ml) and incubated in 95% air + 5% CO_2 for 2 h at 37°C. The islets were subsequently washed with Hanks' solution containing nonradioactive leucine (10 mM) and sonicated in redistilled water. Proinsulin and insulin were separated from other proteins in the islet homogenates by means of an immunobinding technique (26). Total protein biosynthesis was estimated by measurements of the radioactivity after trichloroacetic acid precipitation of a fraction of the homogenate. Another fraction of the homogenate was used to determine DNA contents, as described above.

Statistical analysis. Data are presented as means±SEM, and groups of data were compared using the paired Student's *t* test. In all experimental series each islet preparation (i.e., islets obtained from one donor) was considered as one individual observation. When experiments were performed in duplicate or triplicate, a mean was calculated and considered as one separate observation. The coefficients of correlation were obtained by simple linear regression, and the statistical significance of the correlations were evaluated by analysis of variance.

Results

The loss of islets after the 7-d culture period was ~ 10% in all groups (Table I). Exposure of the islets to high glucose concentrations did not modify retrieval of islets or the islet DNA content. Moreover, light microscopical examination revealed well-preserved islets, with no evidence of cell necrosis in any of the studied groups (a detailed account of the islet morphology will be published elsewhere). However, islets cultured at 11 or 28 mM glucose had a much lower insulin content than islets cultured at 5.6 mM glucose (Table I).

During the whole period of observation, culture of human islets at both 11 or 28 mM glucose induced an increased insulin accumulation in the medium, as compared to islets cultured in the presence of 5.6 mM glucose (Table II). In each of the three experimental groups there was a progressive decrease in insulin accumulation into the medium over the six days of culture. This was especially marked in the islets cultured at 28 mM glucose. Thus, in these islets the insulin release into the medium decreased by 75% over the culture period, while in islets cultured at 5.6 mM glucose there was a 40% decrease over the same period of time. After seven days in culture at 5.6 mM glucose, the human islets showed a 6.1-fold increase in insulin release in response to an acute glucose stimulation (Table III). Islets cultured at 11 mM glucose had a higher insulin release at 1.7 mM glucose than islets cultured at 5.6 mM glucose. How-

Table I. Islet Retrieval, DNA, and Insulin Contents of Human Islets after Seven Days in Culture at Different Glucose Concentrations

Medium glucose	Islet retrieval	DNA content	Insulin content
mM	*%*	*ng/10 islets*	*ng/10 islets*
5.6	88±3	300±28	229±52
11	88±3	304±32	125±29*
28	90±2	277±37	88±29*

Human islets were maintained in tissue culture at different glucose concentrations for seven days. The islet retrieval was expressed as the percentage of islets remaining in culture on day 7 as compared to day 0. The results are means±SEM of 9–11 observations. * $P < 0.01$ when compared to islets cultured at 5.6 mM glucose.

Table II. Insulin Accumulation in a Medium Containing Human Islets over a Six-Day Period in Culture at Different Glucose Concentrations

Medium glucose	Insulin accumulation in the medium		
	Days 1–2	Days 3–4	Days 5–6
mM	ng/100 islets × 48 h		
5.6	920±146	676±135	576±60
11	2,378±568*	1,430±249‡	1,152±223‡
28	4,660±982‡	1,791±336‡	1,186±112§

Human islets were maintained in culture as described in Table I. Medium was collected for insulin determinations at 48-h intervals. The results are means±SEM of 9–10 observations, each performed in duplicate. * $P < 0.02$; ‡ $P < 0.01$; § $P < 0.001$ when compared to islets cultured at 5.6 mM glucose.

ever, these islets failed to increase further their insulin release in response to a glucose stimulus (increase ratio = 1). Islets cultured at 28 mM glucose also presented a high insulin release at 1.7 mM glucose. As observed in islets cultured at 11 mM glucose, islets cultured at 28 mM glucose failed to increase the insulin release in response to 16.7 mM glucose (increase ratio < 1). However, during short-term incubations at 16.7 mM glucose islets cultured at 11 mM glucose secreted similar amounts of insulin in response to glucose as islets cultured at 5.6 mM glucose, whereas in these experiments islets maintained at 28 mM glucose showed an absolute decrease in insulin secretion.

Culture in the presence of high glucose concentrations also impaired the insulin release in response to 16.7 mM glucose + 5 mM theophylline. Thus, islets previously cultured at 5.6 mM glucose released 41.9±14.2 ng insulin/10 islets × 60 min (experimental conditions as described in Table III; the basal insulin release at 1.7 mM glucose was 3.2±0.3 ng insulin/10 islets × 60 min and the increase ratio in response to glucose plus theophylline, 18.0±5.4, $n = 5$), while islets cultured at 11

Table III. Glucose-stimulated Insulin Release of Human Islets Maintained for Seven Days in Culture at Different Glucose Concentrations

Medium glucose	Insulin release		
	1st h (1.7 mM glucose)	2nd h (16.7 mM)	Increase ratio (vs. 1.7 mM)
mM	ng/10 islets × 60 min		
5.6	3.1±0.3	17.5±3.6	6.1±1.4
11	20.7±6.3*	17.0±4.3	1.0±0.2‡
28	11.8±3.3§	8.1±1.8§	0.9±0.3‡

Human islets were cultured as described in Table I. Islet insulin release experiments were performed by incubating groups of seven islets in KRBH containing 1.7 mM glucose. After 60 min at 37°C the medium was removed and the islets incubated for another 60 min in KRBH containing 16.7 mM glucose. The increase ratio was determined in each experiment by dividing the insulin release in the second hour by that in the first hour of incubation. The results are means±SEM of nine observations, each performed in triplicate. § $P < 0.05$; * $P < 0.02$; ‡ $P < 0.01$ when compared to islets cultured at 5.6 mM glucose.

Table IV. Glucose Oxidation of Human Islets after Seven Days in Culture at Different Glucose Concentrations

Medium glucose	Glucose oxidation	
	1.7 mM glucose	16.7 mM glucose
mM	pmol/10 islets × 90 min	
5.6	115±21	380±50
11	104±23	353±60
28	79±15*	279±57‡

Human islets were maintained in culture as described in Table I. Rates of glucose oxidation were measured in groups of 10 islets for 90 min in KRBH buffer (without albumin) supplemented with D-[U-^{14}C]glucose and 1.7 or 16.7 mM nonradioactive glucose (sp act 0.5 mCi/mmol). The results are means±SEM of seven observations, each performed in triplicate. * $P < 0.01$ ‡ $P < 0.05$; whem compared to islets cultured at 5.6 mM glucose.

mM glucose released 31.0±10.9 ng insulin/10 islets × 60 min (the basal insulin release at 1.7 mM glucose was 12.9±3.5 ng/10 islets × 60 min and the increase ratio, 2.4±0.4; $n = 5$; $P < 0.05$ when compared to the increase ratio of islets cultured at 5.6 mM glucose) and islets cultured at 28 mM glucose released 10.3±2.6 ng insulin/10 islets × 60 min (the basal insulin release at 1.7 mM glucose was 5.4±0.9 ng/10 islets × 60 min and the increase ratio, 1.9±0.2; $n = 5$; $P < 0.05$ when compared to the increase ratio of islets cultured at 5.6 mM glucose). The pooled DNA content of islets studied in this experimental series was 193±29 ng/10 islets ($n = 15$), and there were no differences between the DNA contents of islets cultured at 5.6, 11, or 28 mM glucose (data not shown).

There was a positive correlation ($P < 0.02$) between the islet insulin content and the islet insulin release in response to 16.7 mM glucose in all groups studied. The coefficients of correlation between insulin release and insulin content were, respectively, 0.70, 0.61, and 0.93 for the islets cultured at either 5.6, 11, or 28 mM glucose.

The glucose oxidation rates were similar in islets cultured at 5.6 and 11 mM glucose (Table IV). However, islets cultured at 28 mM glucose showed decreased rates of glucose oxidation in the presence of both 1.7 or 16.7 mM glucose, as compared to islets maintained at 5.6 mM glucose.

The contribution of proinsulin to the total pool of labeled proteins in the human islets was in the range of 7–10%, independently of the glucose concentration in the medium (Table V). There were no differences in the proinsulin or total protein biosynthesis rates between islets cultured at 5.6 or 11 mM glucose. However, islets cultured at 28 mM glucose presented a decreased proinsulin and total protein biosynthesis when exposed to 16.7 mM glucose. Moreover, while islets cultured at 5.6 mM glucose doubled their rate of proinsulin biosynthesis in response to 16.7 mM glucose, islets cultured at 28 mM glucose failed to increase the insulin biosynthesis in response to the hexose.

To assess if the functional impairment induced by high glucose was reversible, islets from the three culture groups were exposed to an additional 2-d culture at 5.6 mM glucose (Table VI). After this period, there was still a 20–40% decrease in insulin content in islets precultured at 11 or 28 mM glucose

Medium glucose	Proinsulin biosynthesis		Total protein biosynthesis	
	1.7 mM	16.7 mM	1.7 mM	16.7 mM
mM	10^2 dpm/10 islets $\times$ 2 h		10^2 dpm/10 islets $\times$ 2 h	
5.6	35±3	89±20	571±71	954±168
11	47±8	86±22	607±98	936±137
28	43±9	40±5*	598±98	615±97[‡]

Human islets were maintained in culture as described in Table I. Rates of proinsulin biosynthesis were measured by an immune absorption technique, and total protein biosynthesis was estimated by measuring the incorporation of L-[4,5-^{3}H] leucine (50 μCi/ml) into trichloroacetic-precipitable proteins. The islets in groups of 20 were incubated for 2 h in KRBH medium supplemented with 1.7 or 16.7 mM glucose, as indicated. The results are means±SEM of eight observations. * $P < 0.05$; [‡] $P < 0.01$ when compared to islets cultured at 5.6 mM glucose.

Medium glucose	Insulin content	Insulin release		
		1st h (1.7 mM)	2nd h (16.7 mM)	Increase ratio (vs. 1.7 mM)
mM	ng/10 islets	ng/10 islets $\times$ 60 min		
5.6–5.6	294±74	2.7±0.3	18.5±6.3	6.5±1.8
11–5.6	225±58*	3.7±0.5	15.0±4.4	3.9±0.7
28–5.6	164±40	4.8±1.5	10.3±2.0	2.7±0.5

Human islets were precultured at different glucose concentrations (5.6, 11, or 28 mM glucose) for seven days and then exposed to an additional 2-d culture at 5.6 mM glucose. At the end of this 9-d period, the sequential islet insulin release in response to 1.7 mM and 16.7 mM glucose was measured as described in Table III. The results are means ± SEM of seven to eight observations, each performed in triplicate. * $P < 0.05$ when compared to islets cultured during the whole period at 5.6 mM glucose (group 5.6–5.6).

and then transferred to culture at 5.6 mM glucose, but this reduction was less marked than that observed immediately after seven days exposure to high glucose (Table I). The 2-d culture at 5.6 mM glucose also improved the insulin increase ratio in the islets previously cultured at 11 or 28 mM glucose (Table VI; compare with values in Table III). The higher values for the increase ratio in the 11-5.6 and 28-5.6 groups were mainly due to a decrease in the insulin release at 1.7 mM glucose, which was now around 3.5-5.0 ng insulin/10 islets × 60 min in both groups, similar to the values observed in islets cultured at 5.6 mM glucose during the whole period of observation.

Discussion

The data obtained in the present study suggest that exposure to high glucose concentrations affects the function of human islets in a dose-dependent way. Thus, culture at 11 mM glucose mainly impaired the ability of the β-cells to increase their insulin output when sequentially challenged with a low (1.7 mM) and a high (16.7 mM) glucose concentration. On the other hand, culture at a higher glucose concentration (28 mM) not only decreased insulin release at 16.7 mM glucose, but also affected glucose metabolism and protein biosynthesis, suggesting a more widespread impairment in islet function. The data on proinsulin biosynthesis, however, should be interpreted with caution, due to the possibility that culture at different glucose concentrations may modify the rate of conversion of proinsulin to insulin, and thus affect the immunoprecipitation of labelled proteins.

It is noteworthy that culture conditions identical to those used in the present study did not induce deleterious effects after exposure to 16.7 or 28 mM glucose of islets isolated from mice (15, 16, 18) and rats (27). Moreover, culture at high glucose in most cases tended to increase, rather than decrease, islet function (15, 16, 18, 27). Only in one study, in which rat islets were exposed to extremely high glucose concentrations (56 mM) for one week (28), was it possible to observe an effect similar to that of 11 mM glucose in human islets, i.e., a failure of the islets

to further increase the insulin release in response to glucose. These combined data suggest that the sensitivity of β-cells to glucose may be species specific (19), and that human islets are more affected by the deleterious effects of hyperglycemia than islets isolated from rodents. This is in line with the in vivo observations that prolonged exposure to hyperglycemia may cause permanent diabetes in cats (29) and dogs (30), but not in rats (31).

Another possibility is that the function of human islets may be more affected by the in vitro conditions than that of islets obtained from rodent species, thus inducing a special sensitivity to further functional loads, like high glucose. The islet isolation procedure is probably more traumatic to the human β-cells than to the β-cells isolated from rats and mice. This may be due to the higher content of fibrous tissue in the human pancreas, and the longer periods of warm and cold ischemia inherent in the procurement of human organs. These difficulties in human islet isolation may also explain the presence of just 30–60% insulin-positive cells in most preparations. However, the observations that human islets maintained in culture at 5.6 mM glucose show a well-preserved insulin release and proinsulin biosynthesis in response to glucose (present data), and that manifest glucose "toxicity" cannot be shown in rodent islets even when culture at high glucose is superimposed on previous toxic- or autoimmune-mediated damage (16, 18), reinforce the possibility of relevant differences among species in the sensitivity to the hexose. Finally, in a recent study in which human islets were isolated by another procedure, and cultured in a different medium (CMRL 1066), it was also observed that culture of the islets at high glucose concentrations (16.7 mM glucose) for 48 h impaired glucose-induced insulin release (32).

The mechanisms behind the deleterious effects of glucose on human islets remain to be clarified. The observation that culture in the presence of 28 mM glucose induced a marked decrease in islet insulin content, and a decrease in islet glucose metabolism, suggests that depletion of secretory insulin pools and a defective hexose metabolism contribute to a deficient

glucose-induced insulin release. Indeed, the close correlation between the islet insulin content and insulin release observed in the different experimental groups reinforce the possibility that a glucose-induced decrease in insulin content may contribute to the impaired insulin release. The decrease in islet insulin content probably reflects a discrepancy between insulin release and proinsulin biosynthesis, leading to islet insulin depletion.

It is conceivable that other mechanisms are also relevant for the observed glucose-induced impairment in β-cell function. Thus, it has been previously suggested that exposure of rodent islets to hyperglycemia may decrease insulin release by impairing phosphoinositide hydrolysis (33), by inducing islet glycogen accumulation (34), or by directly exhausting membrane components related to the final steps of glucose-induced insulin release (35, 36). These possible mechanisms now need to be studied in human islets.

A main objective for the development of techniques for human islet isolation and culture is the possibility of islet transplantation as a cure for IDDM. Indeed, in recent years successful islet transplantations have been achieved in diabetic patients (37, 38). In light of the current data, it can be envisaged that a tight control of the glycemic levels with aggressive insulin therapy would be beneficial for the function of the graft after transplantation.

It has been previously observed that intensive insulin therapy and tight control of serum glucose improves β-cell function in early IDDM (1–3). Two main explanations for this observation have been put forward: first, that high glucose by itself may contribute to β-cell dysfunction (4, 5), and second, that increased functional activity would increase expression of membrane antigens by the β-cells, rendering them more vulnerable to the autoimmune aggression (39, 40). The present observations, suggesting that glucose directly impairs β-cell function, and recent data showing that exposure of human islets in vitro to high glucose increase the expression of the 64-kD/GAD antigen (Björk, E., O. Kämpe, F. A. Karlsson, D. Pipeleers, A. Andersson, C. Hellerström, and D. L. Eizirik, submitted for publication), suggest that both mechanisms may be operative in the early stages of IDDM.

Acknowledgments

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