# **Evaluation of relationship between dental caries, diabetes mellitus and oral microbiota in diabetics**

# <sup>1</sup>Mahesh Shenoy, <sup>2</sup>Nishath Sayed Abdul, <sup>3</sup>Pushpraj Singh, <sup>4</sup>GC Shivakumar, <sup>5</sup>S Sahana

<sup>1</sup>Assistant Professor in Oral Pathology, College of Dentistry, Riyadh Elm University, Riyadh, Saudi Arabia. <sup>2</sup>Assistant Professor in Oral Pathology, College of Dentistry, Riyadh Elm University, Riyadh, Saudi Arabia. <sup>3</sup>Senior Lecturer, Department of Dentistry, Government Medical College, Shahdol. India.

<sup>4</sup>Professor, Department of Oral Medicine and Radiology, People's College of Dental Sciences and Research Centre, Bhopal, Madhya Pradesh, India

<sup>5</sup>Professor and Head, Department of Public Health Dentistry, People's College of Dental Sciences and Research Centre, Bhopal, Madhya Pradesh, India

### Abstract

Background: Diabetes is a metabolic disorder characterised by improper lipid, carbohydrate, and protein metabolism. Hyperglycaemia causes a decrease in salivary flow rate, which is common during periods of poor diabetes metabolic control, allowing aciduric bacteria to thrive and caries to form. The aim of the research was to see how diabetes mellitus affected the microorganisms that cause caries in the mouth. Methods: This research involved 60 people divided into two groups. The study group (Group A) included 30 people with diabetes and dental caries, while the control group (Group B) included 30 people with dental caries but no systemic disease. All subjects had their DFS index analysed. The levels of Streptococcus mutans were measured in unstimulated salivary flow. Results: In comparison to Group B, the fasting blood sugar of Group A individuals was shown to be higher, resulting in a higher streptococcus mutans count and thus a higher caries index. Conclusion: It was concluded from the research that as diabetics' age, blood sugar levels, and DMFT values rise, dental caries rises faster than in normal (control) participants, indicating that a link exists between diabetes, oral microbiota, and dental caries.

**Keywords**: Biochemical tests, culture media, dental caries, diabetes mellitus, microbiological tests, oral microbiota, Robertson's cooked-meat medi.

#### INTRODUCTION

Diabetes is a metabolic disorder characterised by improper lipid, carbohydrate, and protein metabolism. Insulin-dependent diabetes (IDDM; type 1) and non-IDDM (type 2) are the two basic kinds of primary diabetes. (1) Several studies have shown that some caries markers, such as lower salivary flow and mutans streptococci counts, can be linked to metabolic control and thus influence the caries process in diabetes mellitus (DM) sufferers. Hyperglycaemia causes a decrease in salivary flow rate, which is common during periods of poor diabetes metabolic management. During this time, glucose leakage into the oral cavity is possible, enabling the growth of aciduric and acidogenic bacteria as well as the development of caries lesions. (2)

One in six people with diabetes in the world is from India. The numbers place the country among the top 10 countries for people with diabetes, coming in at number two with an estimated 77 million diabetics. China leads the list with over 116 million diabetics. The International Diabetes Foundation Diabetes (IDF) Atlas makes it clear India needs to pause and re-evaluate its strategy to combat diabetes. According to various estimates, 10% of global health expenditure is being spent on diabetes.

The IDF Diabetes Atlas (3) also offers projections that continue to put India at the second slot right up to 2045, and frankly speaking the numbers are quite staggering just over 134 million Indians will be diabetics in the next 25 years. India is on the top of the table of a clutch of countries in from Southeast Asia — Bangladesh, Sri Lanka, Nepal and Mauritius. Bangladesh, which is second on the list of top five countries with diabetes (20-79 years), however, has only 8.4 million diabetics. The fact cannot be stressed enough that there is an urgency to develop and implement multisectorial strategies to combat the growing epidemic, because diabetes, being a lifestyle disorder with multidimensional causative factors, definitely needs a multidimensional approach. Table 1 evidently shows the position of India as the world's second largest country in terms of the number of diabetes sufferers across the age group of 20-79 years.

Table 1: Different country with number ofdiabetes sufferers across the age group

2021						
Number of people with diabetes (in millions)						
Rank Country/Territory						
1.	China	140.9				
2.	India	74.2				
3.	Pakistan	33				
4.	USA	32.2				
5.	Indonesia	19.5				
6.	Brazil	15.7				
7.	Mexico	14.1				
8.	Bangladesh	13.1				
9.	Japan	11				
10.	Egypt	10.9				

#### Diabetes in India- At a glance

The number of individuals undiagnosed with diabetes in India (in the age group of 20-79 years old) is around 39.4 million, which

accounts for nearly 53% of the total individuals. This happens to be the 2nd highest rate in the whole world. Another worrying statistic is that India ranks as the number one nation in terms of children aged between 0-19 years that are suffering from type 1 diabetes.

All current known studies point to the fact that diabetes is fast gaining the status of a potential epidemic in India with more than 74 million diabetic individuals currently diagnosed with the disease. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2045 diabetes mellitus may afflict up to 174.4 million individuals in India, while China (124.9 million) and the United States (36.3 million) will also see significant increases in those affected by the disease as can be seen by table 2. India currently faces an uncertain future in relation to the potential burden that diabetes may impose upon the country. Many influences affect the prevalence of disease throughout a country, and identification of those factors is necessary to facilitate change when facing health challenges. (3)

Table 2: Number of people with diabetes

2045		
Rank	Country/ Territory	Number of people with diabetes (in millions)
1.	China	174.4
2.	India	124.9
3.	Pakistan	62.2
4.	USA	36.3
5.	Indonesia	28.6
6.	Brazil	23.2
7.	Banglades	22.3
	h	
8.	Mexico	21.2
9.	Egypt	20
10.	Turkey	13.4

The study's primary goal was to investigate and connect the number of Streptococcus mutans (SM) with dental caries and consequently determine the impact of DM on diabetic patients' dental caries.

### Materials and methods

The patients' medical history was noted, as well as their decaying, missing, and filled teeth (DMFT). Before sample collection, the patient were pre-informed and made duly aware of the research and were given an informed consent form for their signature.

Group A included 30 diagnosed cases of controlled diabetes mellitus, while the control group (Group B) included 30 healthy nondiabetic participants of all ages. This study comprised type 2 diabetes patients with and without habits. The study excluded patients with other systemic illnesses.

Participants were instructed to rinse their mouths with water. By touching the saliva of the mouth cavity with a sterile swab stick, unstimulated saliva was collected and immediately put into Robertson's cooked-meat media, where it was incubated at 37°C for 24 hours.

The medium became turbid the next day, therefore it was inoculated on sterile blood agar and MacConkey agar plates using the sterile inoculating loop. The plates were then incubated overnight at 37°C. If there was any growth, it was thoroughly investigated. The colony properties were studied the next day, and Gram staining was performed. Standard biochemical assays were used to identify the organisms.

When growth-containing material was plated, the bacteria appeared to grow as independent colonies, each of which was usually a pure culture descended from a single infected cell. Isolation is usually accomplished through meticulous culturing from a plate culture with well-separated colonies. An isolated colony was chosen with an inoculating wire loop and subcultured in a tube or plate of new sterile culture medium, suspected of being that of an important or harmful organism based on its appearance. The organism's physical and cultural traits were then employed to conduct decisive testing.

Streptococci either produced -haemolysis or haemolysis. The streptococci colonies were then incubated for 24 hours after being passed in mannitol sugar for fermentation. It's streptococcus if the sugar changes colour from blue to yellow following fermentation.

### Results

The age and gender distribution of the cases under investigation revealed that the average age in years for males was 59.47 and for females was 55.91 for Group A (study group). Table 3 represents the fact that the average age in years for males in Group B (control group) was 50.79 and for females was 45.91, with a male: female ratio of 1.19:1.

Table 3: Average age in years

	Diabetic	study	Non-diabetic		
Age of	group (n=	=30)	control	group	
individua			(n=30)		
ls (in					
years)				-	
	Male	Female	Male	Female	
30-40	3	0	4	4	
40-50	7	1	4	3	
50-60	4	8	7	3	
60-70	5	2	4	1	
Total	19	11	19	11	
	(63.33	(36.67	(63.33	(36.67	
	%)	%)	%)	%)	

By using the Z-test for difference between two proportions, it was discovered that there was a highly significant difference between proportions of organisms SM, Pseudomonas aeruginosa (PA), and Staphylococcus aureus (SA) in study and control groups (i.e., P <0.01), and significant for organisms Klebsiella (K) and non-hemolytic streptococci (i.e., P >0.05), depicted clearly by table 4.

Organisms	Study group number and percentage	Control group number and percentage	Z-test	P	Result
SM	14 (46.67%)	27 (90%)	4.08	<i>P</i> <0.01	Particularly important
PA	6 (20%)	0	2.73	<i>P</i> <0.01	Particularly important
Κ	2 (6.67%)	0	1.96	<i>P</i> <0.05	Important
Е	2 (6.67%)	3 (10%)	0.46	<i>P</i> <0.05	Not important
NHS	2 (6.67%)	0	1.96	<i>P</i> <0.05	Important
SA	4 (13.32%)	0	2.15	<i>P</i> <0.01	Particularly important
Total	30 (100%)	30 (100%)			

Table 4.	Different	oroanisms	in	dental	caries
1 auto 4.	Different	or gunisms	in	ueniui	curies

By using Student's t-test to compare mean values of DMFT index in the study and control groups, it was discovered that there was a very significant difference between mean values of DMFT score in the study and control groups (i.e., P < 0.01). As a result, the study group has higher dental cavities than the control group.

The Chi-square test revealed a significant relationship between fasting blood sugar level and organisms in the study group (i.e., P < 0.05). As can be seen in table 5, as fasting blood sugar levels rise, so do organisms.

Table 5: Fasting blood sugar level

Blood	Organisms						
sugar	SM	PA	Κ	Е	SA	NHS	Total
levels							
<100	3	0	0	0	0	0	3 (10%)
100-150	7	0	0	0	2	2	11 (36.66%)
150-200	4	4	2	2	0	0	12 (40%)
200-250	0	2	0	0	2	0	4 (13.64%)
Total	14 (46.66%)	6 (20%)	2 (6.66%)	2 (6.66%)	4 (13.33%)	2 (6.66%)	30 (100%)

The application of Student's t-test also revealed that there was a highly significant difference between mean values of DMFT in organism types SM and E in study and control groups (i.e., P < 0.01).

# Discussion

Diabetes mellitus is a term used to describe a set of illnesses characterised by high blood glucose levels. This rise is caused by a lack of insulin secretion or increased cellular resistance to insulin's activities, resulting in a variety of metabolic disorders involving carbs, lipids, and proteins.

Diabetes is a serious disruption in glucose metabolism characterised by severe hyperglycemia and insulin insufficiency. Dental caries, gingivitis, periodontitis, salivary dysfunction, changed taste, oral mucosal illnesses, and infections such as lichen planus, recurrent aphthous stomatitis, and candidiasis have all been linked to DM. Individual education is the most important factor in preventing periodontal deterioration in diabetic patients. (4-6)

Patients should be informed about the importance of dental health for diabetics, as well as the fact that gingival bleeding is the most common symptom of periodontal disease. (7) Candidiasis is a symptom of an underlying immune system problem, and a decrease in salivary flow is another risk factor for oral candidiasis. (4)

The link between dental caries and diabetes mellitus is convoluted. Children with type 1 diabetes are often put on diets that limit carbohydrate-rich, cariogenic foods, whereas children and adults with type 2 diabetes – which is commonly linked to obesity and a high-calorie, carbohydrate-rich diet – should expect to be exposed to more cariogenic foods. Furthermore, among diabetics with neuropathy, a decrease in salivary flow has been documented. (8)

The DMFT index was used to determine the influence of diabetes on dental caries in diabetic and control groups, and it was discovered that the average score in diabetics (10.66) was higher than in the control (5.6) group, and the results were statistically significant (P < 0.01). There is no consistent pattern in the research on the relationship between dental caries and diabetes. (9-13)

However, some sources (11) have indicated an increased risk of caries owing to DM, which is consistent with the findings of the study, which demonstrated a very significant difference in mean DMFT score between the study and control groups (i.e., P < 0.01). As a result, the study group had higher dental cavities than the control group. Increased blood sugar levels were also observed to produce an increase in SM count in the study group, which was statistically significant (P < 0.05). As a result, the higher the SM count, the greater the risk of caries.

High caries levels in diabetics were linked to age, plaque score, and a reduced unstimulated salivary flow rate, according to Siudikiene et al. (2) Reduced salivary secretion increases the risk of tooth decay, but good metabolic control prevents the most dangerous salivary changes, such as high glucose content and lower pH, and a good diabetic diet, rich in fibre and low in carbohydrates, simple can slow plaque formation and the proliferation of acidogenic bacterial microflora. (14-18) Twetman et al. found that diabetes patients with poor metabolic control developed three times more lesions over the course of the study than those with better metabolic control, which matched our findings, in which higher blood sugar levels resulted in higher SM counts that were statistically significant (P < 0.05). (19) In another study, according to Orbak et al., type 1 diabetes mellitus affects the dentition and oral

health of children and adolescents. Infections of the connective tissues are more common in children with type 1 DM than in children without the disease. This is because infection contributes to tooth loss in children with type 1 diabetes. (1)

Through their research findings, Miko et al. discovered in their study that poor glycaemic control and early start of DM may increase the risk of dental caries, but that good oral hygiene combined with good metabolic control may prevent dental caries in adolescents with type 1 diabetes. Study participants had less decaying and more filled teeth, according to the findings. (20)

Plaque, calculus, pocket formation, increased tooth mobility, and tooth loss were observed to be more common in patients with impaired glucose tolerance, according to Sheridan et al. (19-25)

The mean DMFT index in the study group was greater than in the control group, according to Seethalakshmi et al., whose findings are similar to the study's. (26) This is due to the lack of the saliva's protective mechanism in diabetics. Saliva's cleaning and buffering abilities are also compromised. Low salivary pH encourages the growth of aciduric bacteria, which in turn encourages the growth of acidogenic bacteria, providing an unfavourable environment for the protective oral bacteria. This causes a shift in the oral environmental balance in favour of cariogenic bacteria, which lowers salivary pH even further, and the cycle repeats. (27)

Dental caries is more common in diabetic people, according to studies by Jawed et al. and Akpata et al., (28) and in yet another study, Sri Kenneth et al. discovered in their study that patients with uncontrolled diabetes had lower salivary pH and a higher incidence of dental caries than the control group. (29)

Satish et al. discovered that glycosylated haemoglobin A1c was measured in both type 2 diabetic patients and the control group, and that there was a substantial association between HbA1c and serum glucose concentrations in both groups. (30) According to Singh et al., persons with type 2 diabetes have a high rate of dental caries and are at a high risk of developing caries. (31)

The etiology of diabetes in India is multifactorial and includes genetic factors coupled with environmental influences such as obesity associated with rising living standards, steady urban migration, and lifestyle changes. Yet despite the incidence of diabetes within India, there are no nationwide and few multicentric studies conducted on the prevalence of diabetes and its complications. The studies that have been undertaken are also prone to potential error as the heterogeneity of the Indian population with respect to culture, ethnicity, and socio- economic conditions, mean that the extrapolation of regional results may give inaccurate estimates for the whole country.

Although the Indian urban population has access to reliable screening methods and antidiabetic-medications, such health benefits are not often available to the rural patients. There is а disproportionate allocation of health resources between urban and rural areas, and in addition poverty in rural areas may be multifaceted. Food insecurity, illiteracy, poor sanitation, and dominance of communicable diseases may all contribute, which suggests that both policy makers and local governments may be undermining and under-prioritizing the looming threat of diabetes. Such inadequacies contribute to an infrastructure that may result in poor diabetes screening and preventive services, non-adherence to diabetic management guidelines, lack of available counselling, and a far-fetched travel to health services which further increases the difficulty in maintaining optimal time-in ratio (TIR) targets for the individual. Aged care facilities in rural areas report disparity in the diabetes management compared with their urban counterparts, with these populations more likely to suffer from diabetic complications compared to their urban counterparts. More needs to be done to address the rural-urban inequality in diabetes intervention. (32)

An upsurge in number of early-onset diabetes cases is also responsible for the development of

various diabetic complications due to longer duration, however data on the disease prevalence on diabetic complications across the whole of India is scarce. A recent international study reported that diabetes control in individuals worsened with longer duration of the disease (9.9 years), with neuropathy the most common complication (24.6 per cent) followed by cardiovascular complications (23.6 per cent), renal issues (21.1 per cent), retinopathy (16.6 per cent) and foot ulcers (5.5 per cent). These results were closely in line with other results from the South Indian population, however further data from different sections of India is required to be able to assess whether patterns of complications rates vary across the country. Poor glycemic control, a factor that has been observed in the Indian diabetic population, is responsible for microand macrovascular changes that present with diabetes, and can predispose diabetic patients to complications other such as diabetic myonecrosis and muscle infarction. Developing countries like sub-Saharan African countries have noted rise in Plasmodium falciparum cases in patients with diabetes mellitus, and the convergence of two such diseases provide for complications that not only limit the available treatment options but also increase the morbidity, mortality and financial burden on a resource limited country like India.

# Conclusion

To reduce the disease burden that diabetes creates in India, appropriate government interventions and combined efforts from all the stakeholders of the society are required. Clinicians may be targeted to facilitate the implementation of screening and early detection programs, diabetes prevention, selfmanagement counselling, and therapeutic management of diabetes in accordance with the appropriate local guidelines form the backbone of controlling the predicted diabetes epidemic. Thus, upon further introspection of the above study, it can be summarised that dental caries increases in the diabetic group as age, blood sugar levels, and DMFT values rise higher than in the control group and, as a result, a diabetic

patient should constantly ensure that he or she maintains adequate dental hygiene by brushing their teeth properly. They should also get their teeth fixed as soon as possible if they find any sort of decay or carious lesions that might be visible to them. And most importantly, they should follow the physician's or dietician's directions for adopting a noncariogenic diet.

#### Reference

- [1] Orbak R, Simsek S, Orbak Z, Kavrut F, Colak M. The influence of type-1 diabetes mellitus on dentition and oral health in children and adolescents. Yonsei Med J. 2008 and 49:357–65.
- [2] Siudikiene J, Machiulskiene V, Nyvad B, Tenovuo J, Nedzelskiene I. Dental caries and salivary status in children with type 1 diabetes mellitus, related to the metabolic control of the disease. Eur J Oral Sci. 2006 and 114:8–14.
- [3] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JC, Mbanya JC, Pavkov ME. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes research a.
- [4] Lamster IB, Lalla E, Borgnakke WS, Taylor GW. The relationship between oral health and diabetes mellitus. J Am Dent Assoc. 2008 and Suppl):19S–24S, 139(10.
- [5] Siudikiene J, Maciulskiene V, Nedzelskiene I. Dietary and oral hygiene habits in children with type I diabetes mellitus related to dental caries. Stomatologija. 2005 and 7:58–62.
- [6] Guggenheimer J, Moore PA, Rossie K, Myers D, Mongelluzzo MB, Block HM, et al. Insulin-dependent diabetes mellitus and oral soft tissue pathologies: II. Prevalence and characteristics of Candida and Candidal lesions. Oral Surg Oral Med Oral Pathol Oral Rad.
- [7] Iughetti L, Marino R, Bertolani MF, Bernasconi S. Oral health in children and adolescents with IDDM – A review. J Pediatr Endocrinol Metab. 1999 and 12:603–10.
- [8] Moore PA, Guggenheimer J, Etzel KR, Weyant RJ, Orchard T. Type 1 diabetes mellitus, xerostomia, and salivary flow

rates. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001 and 92:281–91.

- [9] 2003, Ship JA. Diabetes and oral health: An overview. J Am Dent Assoc. and 134:4S–10S.
- [10] Collin HL, Uusitupa M, Niskanen L, Koivisto AM, Markkanen H, Meurman JH, et al. Caries in patients with noninsulin-dependent diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998 and 85:680–5.
- [11] Jones RB, McCallum RM, Kay EJ, Kirkin V, McDonald P. Oral health and oral health behaviour in a population of diabetic outpatient clinic attenders. Community Dent Oral Epidemiol. 1992 and 20:204–7.
- [12] Sampaio N, Mello S, Alves C. Dental caries-associated risk factors and type 1 diabetes mellitus. Pediatr Endocrinol Diabetes Metab. 2011 and 17:152–7.
- [13] Syrjälä AM, Niskanen MC, Ylöstalo P, Knuuttila ML. Metabolic control as a modifier of the association between salivary factors and dental caries among diabetic patients. Caries Res. 2003 and 37:142–7.
- [14] Ciglar I, Sutalo J, Sjaljac-Staudt G, Bozikov J. Saliva as a risk factor for caries in diabetic patients. Acta Stomatol Croat. 1991 and 25:143–9.
- [15] Got I, Fontaine A. Teeth and diabetes. Diabete Metab. 1993 and 19:467–71.
- [16] Karjalainen KM, Knuuttila ML, Käär ML. Relationship between caries and level of metabolic balance in children and adolescents with insulin-dependent diabetes mellitus. Caries Res. 1997 and 31:13–8.
- [17] Karjalainen KM, Knuuttila ML, Käär ML. Salivary factors in children and adolescents with insulin-dependent diabetes mellitus. Pediatr Dent. 1996 and 18:306–11.
- [18] Taylor GW, Manz MC, Borgnakke WS. Diabetes, periodontal diseases, dental caries, and tooth loss: A review of the literature. Compend Contin Educ Dent. 2004 and 25:179.
- [19] Twetman S, Petersson GH, Bratthall D. Caries risk assessment as a predictor of metabolic control in young type 1 diabetics. Diabet Med. 2005 and 22:312– 5.

- [20] Miko S, Ambrus SJ, Sahafian S, Dinya E, Tamas G, Albrecht MG, et al. Dental caries and adolescents with type 1 diabetes. Br Dent J. 2010 and 208:E12.
- [21] 1971, Wegner H. Dental caries in young diabetics. Caries Res. and 5:188–92.
- [22] Goteiner D, Vogel R, Deasy M, Goteiner C. Periodontal and caries experience in children with insulin-dependent diabetes mellitus. J Am Dent Assoc. 1986 and 113:277–9.
- [23] Hatun S, Tezic T. Insulin-dependent diabetes mellitus prevalence in ankaradaki school children. Child Sagligiv Journal of Diseases. 1996 and 39:465–71.
- [24] Sheridan RC, Jr, Cheraskin E, Flyn AC. Epidemiology of diabetes mellitus II 100 dental patients. J Periodontol. 1959 and 30:298–323.
- [25] Swanljung O, Meurman JH, Torkko H, Sandholm L, Kaprio E, Mäenpää J, et al. Caries and saliva in 12-18-year-old diabetics and controls. Scand J Dent Res. 1992 and 100:310–3.
- [26] Seethalakshmi C, Reddy RC, Asifa N, Prabhu S. Correlation of salivary pH, incidence of dental caries and periodontal status in diabetes mellitus patients: A cross-sectional study. J Clin Diagn Res. 2016 and 10:ZC12–4.
- [27] Deepak G, Harshaminder K, Manveen KJ, Sonika V, Swati P. Salivary pH and dental caries in diabetes mellitus. Int J Oral Maxillofac Pathol. 2012 and 3:13–6.
- [28] Akpata ES, Alomari Q, Mojiminiyi OA, Al-Sanae H. Caries experience among children with type 1 diabetes in Kuwait. Pediatr Dent. 2012 and 34:468–72.
- [29] Sri Kenneth JA, Sanjay R, Peramachi P. Evaluation of correlation between salivary pH and prevalence of dental caries in subjects with and without diabetes mellitus. Res J Recent Sci. 2014 and 3:224–6.
- [30] Satish BN, Srikala P, Maharudrappa B, Awanti SM, Kumar P, Hugar D, et al. Saliva: A tool in assessing glucose levels in diabetes mellitus. J Int Oral Health. 2014 and 6:114–7.
- [31] Singh I, Singh P, Singh A, Singh T, Kour R. Diabetes an inducing factor for dental caries: A case control analysis in Jammu. J Int Soc Prev Community Dent. 2016 and 6:125–9.

[32] Jawed M, Shahid SM, Qader SA, Azhar A. Dental caries in diabetes mellitus: Role of salivary flow rate and minerals. J Diabetes Complications. 2011 and 25:183–6.