

Plasma anhydro-D-glucitol (1,5-AG) as an indicator of hyperglycaemic excursions in pregnant women with diabetes

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Abstract

Aims To evaluate the use of the plasma 1,5-anhydro-D-glucitol (1,5-AG) level as a possible marker for glucose excursions in pregnant women with diabetes.

Methods The study group consisted of 55 pregnant women with diabetes (gestational diabetes mellitus—GDM, $n = 28$ or pre-gestational diabetes mellitus—PGDM, $n = 27$), without hepatic or renal insufficiency, gestational age range 5–38 weeks. In each patient, 24-h glucose profile, glycated haemoglobin and 1,5-AG plasma levels were measured. Mean blood glucose (MBG) and M-value (by Schlichtkrull) were calculated. MBG, M-value and maximal daily glycaemia (MxG) were used as indexes of daily glycaemic excursions.

Results A significant correlation was found between the 1,5-AG plasma level and MxG [$r = (-0.3)$] and between the 1,5-AG level and M-value [$r = (-0.36)$]. There was no association between the 1,5-AG level and gestational age. Multivariate regression analysis, with 24-h glucose profile, gestational age and MxG as independent variables, showed that MxG was the main parameter determining the 1,5-AG plasma level [$\beta = (-0.68)$]. The M-value, the coefficient of glucose fluctuations, also determined the 1,5-AG level but with lower statistical power [$\beta = (0.41)$]. No statistical differences were found in the group with $HbA_{1c} < 6\%$ or $> 6\%$ for 1,5-AG and M-value, while MBG was higher in poorly controlled patients ($HbA_{1c} > 6\%$).

Conclusions The plasma 1,5-AG level may be a useful marker of daily glucose excursion in pregnant women with diabetes, as an adjunct to HbA_{1c} monitoring.

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Keywords diabetes mellitus, pregnancy, acute hyperglycaemia, 1,5-anhydro-D-glucitol

Abbreviations 1,5-AG, 1,5-anhydro-D-glucitol; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; MBG, mean blood glucose; MxG, maximal daily glycaemia; PGDM, pre-gestational diabetes mellitus

Introduction

It has been well documented that disturbances in carbohydrate metabolism observed during pregnancy result in numerous

serious complications of fetal development. Fetal hyperglycaemia, which is a direct consequence of maternal hyperglycaemia, stimulates beta cell hyperfunction resulting in hyperinsulinaemia which in turn leads to fetal macrosomy, in addition to neonatal hypoglycaemia. Moreover, maternal hyperglycaemia, especially during the first trimester of pregnancy, is considered to be a teratogenic factor. Hyperglycaemia in later periods of pregnancy is responsible for abnormalities in fetal growth and central

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nervous system development, organomegaly or immaturity of the respiratory system. Inadequate treatment of diabetes leads to increased rates of perinatal mortality and morbidity in mothers and neonates [1,2]. Therefore, methods of monitoring the serum glucose level during pregnancy are required to be particularly sensitive to any hyperglycaemic episodes.

It is well established that good metabolic control, defined as normal HbA_{1c} or fructosamine levels, is necessary to diminish the risk of hyperglycaemia-dependent fetal malformations [2]. Parameters such as HbA_{1c} or fructosamine are routinely used as long-term markers of the overall glycaemic state. Unfortunately, they do not reveal every acute hyperglycaemic spike [3]. In the same respect, even frequent self-monitoring of blood glucose fails to reveal short-term glucose fluctuations that may occur between measurements [4].

It has been shown that abnormal fetal growth depends not only on chronic hyperglycaemia but also on postprandial glucose levels. Control of postprandial hyperglycaemia has been indicated as a therapeutic goal to protect against fetal macrosomy [5–7].

1,5-Anhydroglucitol (1,5-AG) (1-deoxyglucose) is one of the major polyols in human fluids. It has been reported that 1,5-AG concentration in plasma is significantly decreased in poorly controlled subjects with diabetes [8]. The reference values of 1,5-AG plasma levels in healthy human subjects range from 14.4 to 30.2 mg/l, while in patients with diabetes the plasma 1,5-AG levels are markedly reduced. For example, in patients with Type 2 diabetes the plasma 1,5-AG concentration varies between 0.9 and 26.6 mg/l [4].

1,5-AG re-absorption is competitively inhibited by glucosuria, resulting in plasma 1,5-AG reduction. Therefore, the plasma 1,5-AG level indirectly reflects episodes of hyperglycaemia which stimulate an increase in renal glucose filtration and elimination. At the onset of glucosuria due to hyperglycaemic episodes, the plasma 1,5-AG level promptly falls [9]. For several years, the 1,5-AG plasma level has been proposed as an adjunct to routinely used parameters of glycaemic control in diabetic patients [4,10]. Unfortunately, the plasma 1,5-AG level is significantly decreased by renal failure and it can not serve as indicator of hyperglycaemia in patients with renal insufficiency [11].

In view of the current varied opinions regarding the plasma 1,5-AG level in pregnant women with diabetes [12,13], we focused our interest on the changes in serum levels of this compound during pregnancy in diabetic women. The aim of the present study was to evaluate whether the 1,5-AG plasma level could be a useful marker of carbohydrate metabolism in this population.

Patients and methods

The study group consisted of 55 pregnant women [mean age 26.0 years, range 17.0–42.0 years, mean gestational age 28.0 weeks (range 5.0–38.0 week)] with gestational diabetes (GDM) (28 patients) or with pre-gestational diabetes (PGDM) (27 patients).

Pre-gestational and gestational diabetes were diagnosed according to WHO criteria [14]. Twenty-six patients with GDM were treated with diet only and two patients required treatment with insulin.

In all groups, treatment regimens aimed to maintain normoglycaemia throughout pregnancy, i.e. FPG < 5.6 mmol/l and postprandial glucose level < 6.7 mmol/l. If glucose levels exceeded target values in the GDM group, insulin therapy was started.

All patients self-monitored blood glucose levels four times a day. Once a month patients were admitted to hospital in order to obtain a diurnal glucose profile.

Patients with renal failure and/or hepatic insufficiency, and/or severe anaemia were excluded from the study.

In each patient, 24-h serum glucose profile (12 glycaemic points, every 2 h), HbA_{1c} and 1,5-AG plasma level were assayed. Blood samples for 1,5-AG were collected into vials containing heparin, plasma was obtained by centrifugation immediately after collection of the blood and stored in –80°C until estimation.

The study protocol was approved by the Poznan University of Medical Sciences Ethics Committee and each participant gave written informed consent.

Measurements

Venous plasma glucose was measured by the glucose oxidase method and analysed immediately using a Cormay analyser (PZ Cormay, Lublin, Poland).

HbA_{1c} (normal range: 4.8–6.0%) was assayed by turbidimetric inhibition immunoassay (TINA) using a Tina-quant [a] HbA_{1c} II assay standardized according to DCCT/NGSP [15].

The plasma concentration of 1,5-AG was measured using a modified column enzymatic method described previously [16,17]. Briefly, 100 µl plasma was deproteinized with trichloroacetic acid and passed through a two-layer micro-column packed with ion-exchange resins (cationite Dowex 50WX8; anionite Dowex 1×8, Sigma, Steinheim, Germany) to remove glucose. 1,5-AG was efficiently recovered in the flow-through fraction. Hydrogen peroxide formed in the enzymatic oxidation of 1,5-AG with pyranose oxidase was detected by a standard method utilizing an enzymatic colour-developing system. The intra-assay CV was 4.9%, inter-assay CV –3.2% and the mean recovery was 96.6% when estimated in our laboratory using plasma samples from 80 non-diabetic persons aged 20–60 years. Reference range of 1,5-AG was 14.4–30.2 mg/l [4]. The mean value for non-diabetic pregnant women established by Tetsuo *et al.* [12] was 10.2 ± 4.6 mg/l.

Based on the 24-h glucose profile, MBG (mean blood glucose) and M-value by Schlichtkrull [18] were calculated. The M-value is especially sensitive for hyperglycaemic spikes, much more than for hypoglycaemic troughs. The mean maximal daily glycaemia (MxG) was established as the mean of the maximum daily plasma glucose values of all patients.

Statistical analysis

Results were expressed as means ± SD or medians. The Shapiro-Wilks' test was used to assess the distribution of 1,5-AG and the W-value was 0.88763 (*P* < 0.05). Thus we regarded 1,5-AG as following a non-Gaussian distribution in the range observed in

this study. Data of the two groups were compared using the Mann–Whitney *U*-test. Multivariate regression analysis was used to determine if MxG, M-value or MBG predicted the 1,5-AG plasma level independently of fasting or postprandial glucose values. Multiple regression analysis was conducted three times. The 1,5-AG level in plasma was the dependent value in every analysis while independent values were as follows: (i) in the first analysis: all the 11 glycaemic points from the 24-h glucose profile MxG, week of pregnancy; (ii) in the second analysis: all the 11 glycaemic points from the 24-h glucose profile, M-value, week of pregnancy; (iii) in the third analysis: all of the 11 glycaemic points from the 24-h glucose profile, MBG, week of pregnancy.

Regression analysis was performed to account for the interaction between 1,5-AG concentrations and MxG, M-values and MBG. Statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). A *P*-value < 0.05 was considered statistically significant.

Results

Relation between 1,5-AG and gestational age

There was no correlation between 1,5-AG level and the week of pregnancy.

Associations between 1,5-AG plasma level and glycaemia

We did not find a linear correlation between any glycaemic point in the glucose profile and 1,5-AG or between MBG and 1,5-AG. There was a significant association between the 1,5-AG plasma level and MxG ($r = -0.3$), and between 1,5-AG and M-value ($r = -0.36$) (Fig. 1).

Multivariate regression analysis, with 24-h glucose profile, gestational age and MxG, showed MxG as the main parameter determining 1,5-AG plasma level [$\beta = (-0.68)$, $P = 0.01$]. M-value also determined the 1,5-AG level, but with lower statistical power [$\beta = (-0.41)$] (Table 1).

1,5-AG as a complement to HbA_{1c} for assessing diabetic control

To demonstrate that plasma 1,5-AG is a complementary, but not an alternative parameter for glucose level monitoring, we analysed HbA_{1c}, MBG, M-value and plasma 1,5-AG level.

In the groups with HbA_{1c} less than or greater than 6.0%, the plasma levels of 1,5-AG were low and did not differ significantly. As a result, the plasma level of 1,5-AG was indicative of past episodes of acute hyperglycaemia in both groups. M-values were high in both groups and did not differ significantly, whereas MBG was significantly higher in the poorly controlled group (Table 2).

Discussion

It has been demonstrated in numerous studies that the 1,5-AG plasma level has a sensitive and rapid response to serum glucose

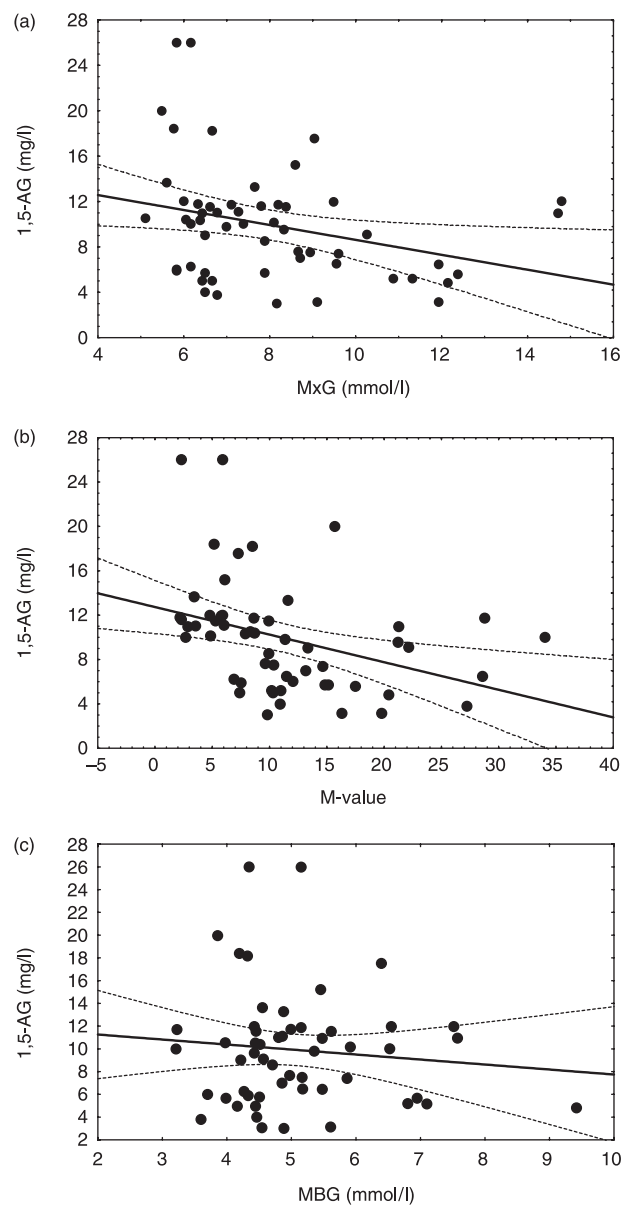


Figure 1 Correlation between plasma 1,5-AG concentration and MxG [$Y = 15.197 - 0.6576X$, $r = (-0.3)$] (a) and M-values (by Schlichkrull) [$Y = 12.733 - 0.2487X$, $r = (-0.36)$] (b) in pregnant women with diabetes. There was no significant correlation between plasma 1,5-AG concentration and MBG [$r = (-0.11)$] (c).

levels. In humans, 1,5-AG is an abundant polyol of which 90% is derived from ingested food. The metabolic transformation of 1,5-AG is negligible [19], and a large internal pool of 1,5-AG produces a stable baseline. Elimination of 1,5-AG is mainly by the kidneys. 1,5-AG is completely filtered in the renal glomeruli, and subsequently reabsorbed in the proximal tubules. Glucosuria competitively inhibits 1,5-AG re-absorption and results in a reduction in plasma 1,5-AG. Thus, the plasma 1,5-AG level reflects episodes of hyperglycaemia that increase renal glucose filtration and cause glucosuria. Hyperglycaemic episodes result in a prompt drop in the plasma 1,5-AG level [9,10].

Table 1 Linear multiple regression analyses with the 1,5-AG plasma level as a dependent value

β	$R^2 = 0.37$	β	$R^2 = 0.37$	β	$R^2 = 0.28$
MxG	-0.68*	M-value	-0.41*	MBG	1.35
G _{8am}	0.29	G _{8am}	0.08	G _{8am}	-0.25
G _{10am}	0.17	G _{10am}	-0.002	G _{10am}	-0.21
G _{12am}	-0.23	G _{12am}	-0.36	G _{12am}	-0.42
G _{02pm}	0.15	G _{02pm}	-0.10	G _{02pm}	-0.38
G _{04pm}	0.13	G _{04pm}	0.03	G _{04pm}	-0.27
G _{06pm}	0.33	G _{06pm}	0.24	G _{06pm}	0.15
G _{08pm}	0.07	G _{08pm}	0.01	G _{08pm}	-0.25
G _{10pm}	-0.25	G _{10pm}	-0.26	G _{10pm}	-0.53
G _{12pm}	-0.07	G _{12pm}	-0.24	G _{12pm}	-0.28
G _{02am}	0.01	G _{02am}	-0.001	G _{02am}	-0.04
G _{G04am}	0.16	G _{G04am}	0.04	G _{G04am}	-0.03
G _{06am}	0.22	G _{06am}	0.28	G _{06am}	-0.21
Week of pregnancy	0.25	Week of pregnancy	0.16	Week of pregnancy	0.25

*, statistically significant; β , standardized coefficient for independent values; R, multiple regression coefficient.

G_{8am}, G_{10am}, G_{12am}, G_{02pm}, G_{04pm}, G_{06pm}, G_{08pm}, G_{10pm}, G_{12pm}, G_{02am}, G_{04am}, G_{06am}, respectively: glucose levels at 08.00, at 10.00, at 12.00, at 14.00, at 16.00, at 18.00, at 20.00, 22.00, 24.00, at 02.00, at 04.00, and at 06.00 hours.

Table 2 1,5-AG plasma level, M-value and MBG in pregnant women with diabetes with HbA_{1c} \leq 6.0% or $>$ 6.0%

	HbA _{1c} \leq 6% (5.7 \pm 0.2)	HbA _{1c} $>$ 6% (7.4 \pm 1.7)
Age (years)	25.8 \pm 6.7	26.0 \pm 3.3
BMI (kg/m ²)	25.5 \pm 5.0	26.3 \pm 5.0
MBG (mmol/l)	4.6 \pm 0.7	5.4 \pm 1.3*
M-value	9.1 \pm 5.4	12.8 \pm 7.7
1,5-AG (mg/l)	10.3 \pm 5.4	9.5 \pm 5.4

* $P < 0.006$.

Renal function during pregnancy is characterized by significant changes in filtration and tubular re-absorption. Glucosuria increases up to 10-fold during pregnancy dependent on gestational age and the time of day. Great variability of glucosuria day to day or within days is common. In diabetic patients, glucosuria depends mainly on GFR, tubular Na⁺ re-absorption capacity/extracellular fluid volume and the general status of tubules [20,21].

Doubts about the suitability of the 1,5-AG plasma level as a marker of metabolic control stem from concerns regarding the mode of 1,5-AG elimination and presence of glucosuria in pregnant women with diabetes. Although Tetsuo *et al.* [12] suggested that a change in 1,5-AG plasma level during pregnancy may reflect a mild alteration of carbohydrate metabolism, other authors concluded that differences in renal glucose threshold between patients limit the usefulness of 1,5-AG estimation when monitoring or screening for diabetes [13].

In our study, we analysed which factor is the most significant predictor of 1,5-AG in plasma among diabetic pregnant women, bearing in mind that multidirectional changes in the renal glucose threshold may affect the 1,5-AG plasma level. No association was found between gestational age and the concentration of 1,5-AG in plasma. However, the 1,5-AG plasma level was correlated with MxG measured 24 h prior to 1,5-AG assay, with no simultaneous associations between 1,5-AG and each individual glucose serum level from this profile.

Multivariate regression analysis revealed that MxG was strongly predictive of a low plasma 1,5-AG concentration independent of glucose profile. Similar results were obtained when M-value was used instead of MxG in analysis.

Our study confirmed that in pregnant women with diabetes, the 1,5-AG level is primarily determined by hyperglycaemic peaks. Interestingly, a similar correlation was revealed between 1,5-AG and MxG and M-value in non-pregnant patients with Type 2 diabetes [4,22]. This indirectly supports the usefulness of 1,5-AG as an index of metabolic control in diabetic pregnant women.

In our opinion, the variation in renal threshold for glucose is the important limitation for the use of 1,5-AG for diabetes screening, but not for diabetes monitoring. Yamanouchi *et al.* [23] revealed that although 1,5-AG and glucose concentrations in urine are strongly correlated, these relations vary between individuals. Hyperglycaemic spikes induce a prompt fall in plasma 1,5-AG, but recovery of plasma 1,5-AG to normal levels requires about 5 weeks [24]. These data indicate that in practice the 1,5-AG concentration should be interpreted on an individual basis.

HbA_{1c} is a marker of overall glycaemic status. Because the HbA_{1c} values represent changes over the past 90 days, considerable lag times exist between actual glycaemic changes and HbA_{1c} response. Previous studies revealed that HbA_{1c} is not sensitive enough to reflect acute hyperglycaemic spikes [3,4]. Our results also suggest that a routinely used long-term parameter such as HbA_{1c} is not sufficient for adequate assessment of metabolic control in diabetic pregnant women as groups with HbA_{1c} $<$ 6.0% and \geq 6.0% had similar values of 1,5-AG and M-value. Self-monitoring of blood glucose is not performed sufficiently frequently to calculate reliable M-values. In the group with HbA_{1c} $<$ 6.0%, women with poor short-term glucose control were revealed by changes in plasma 1,5-AG level. Thus it could be concluded that assessment of metabolic control of pregnant women with diabetes requires measurement of 1,5-AG in addition to HbA_{1c}.

In conclusion, 1,5-AG is a monitoring tool to evaluate glucose levels of pregnant women with diabetes which may serve as a useful adjunct to HbA_{1c} and can provide clinicians with valuable information regarding effectiveness of treatment.

References

- 1 Nold JL, Georgieff MK. Infants of diabetic mothers. *Pediatr Clin North Am* 2004; 3: 619–637.

- 2 Banerjee S, Ghosh US, Banerjee D. Foetomaternal complications in pregnancies with diabetes mellitus: association with the amount of insulin requirement, mean terminal blood glucose and HbA_{1c} levels. *J Indian Med Assoc* 2003; **12**: 728, 730–732, 740.
- 3 Bonora E, Calceterra F, Lombardi S, Bonfante N, Formenti G, Bonadonna RC *et al*. Plasma glucose levels throughout the day and HbA_{1c} interrelationships in type 2 diabetes. *Diabetes Care* 2001; **24**: 2023–2029.
- 4 Dworacka M, Winiarska H, Szymańska M, Kuczyński S, Szczawińska K, Wierusz-Wysocka B. 1,5-Anhydro-D-glucitol: a novel marker of glucose excursions. *Int J Clin Prac* 2002; **129**: 40–44.
- 5 De Veciana M, Major CA, Morgan MA, Asrat T, Toohey JS, Lien JM *et al*. Postprandial versus preprandial blood glucose monitoring in women with gestational diabetes mellitus requiring insulin therapy. *N Engl J Med* 1995; **333**: 1237–1241.
- 6 Jovanovic-Peterson LJ, Peterson CMH, Reed GF, Metzger BE, Mills JL, Knopp RH *et al*. Maternal postprandial glucose levels and infants birth weight. The Diabetes in Early Pregnancy Study. The National Institute of Clinical Health and Human Development—Diabetes in Early Pregnancy Study. *Am J Obstet Gynecol* 1991; **164**: 103–111.
- 7 Leguizamón G, Krupitzki H, Glujovsky D, Olivera Ravasi M, Reece EA. Blood glucose monitoring in gestational diabetes mellitus: 1- versus 2-h blood glucose determinations. *J Matern Fetal Neonatal Med* 2002; **6**: 384–388.
- 8 Akanuma H, Ogawa K, Lee Y, Akanuma Y. Reduced levels of plasma 1,5-anhydroglucitol in diabetic patients. *J Biochem* 1981; **90**: 157–160.
- 9 Akanuma Y, Morita M, Fukuzawa N. Urinary excretion of 1,5-anhydro-D-glucitol accompanying glucose excretion in diabetic patients. *Diabetologia* 1988; **31**: 831–835.
- 10 Yamanouchi T, Akanuma Y. Serum 1,5-anhydro-D-glucitol: new clinical marker for glycemic control. *Diabet Res Clin Pract* 1994; **24**: 261–268.
- 11 Shimizu H, Shouzu A, Nishikawa M, Omoto S, Hayakawa T, Miyake Y *et al*. Serum concentration and renal handling of 1,5-anhydro-D-glucitol in patients with chronic renal failure. *Ann Clin Biochem* 1999; **36**: 749–754.
- 12 Tetsuo M, Hamada T, Yoshimatsu K, Ishimatsu J, Matsunaga T. Serum levels of 1,5-anhydro-D-glucitol during the normal and diabetic pregnancy and puerperium. *Acta Obstet Gynecol Scand* 1990; **69**: 479–485.
- 13 Kilpatrick ES, Keevil BG, Richmond KL, Newland P, Addison GM. Plasma 1,5-anhydroglucitol concentrations are influenced by variations in the renal threshold for glucose. *Diabet Med* 1999; **16**: 496–499.
- 14 World Health Organization Department of Noncommunicable Disease Surveillance. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Report of WHO Consultation*. [WWW document]. WHO/NCD/MCS/992 URL http://whqlibdoc.who.int/hq/1999/WHO_NCD_NCS_99.2.pdf.
- 15 Jarausch J, Lotz J, Hafner G. Reference values for the Tina-quant HbA1c Assay. *Clin Chem* 1996; **42**: 1717–1723.
- 16 Yabuuchi M, Masuda M, Katoh K. Simple enzymatic method for determining 1,5-anhydro-D-glucitol for diagnosis of diabetes mellitus. *Clin Chem* 1984; **35**: 2039–2943.
- 17 Chusney GD, Philippa M, Pickup JC. Comparison of micro-enzymatic and high performance liquid chromatographic methods for the assay of serum 1,5-anhydroglucitol. *Clin Chim Acta* 1995; **235**: 91–99.
- 18 Schlichtkrull J, Munck O, Jersild M. The M-value, an index of blood-sugar control in diabetics. *Acta Med Scand* 1965; **177**: 95–102.
- 19 Yamanouchi T, Tachibana Y, Akanuma K, Minoda S. Origin and disposal of 1,5-anhydro-D-glucitol a major polyol in the human body. *Am J Physiol* 1992; **263**: 268–273.
- 20 Davison JM, Hytten FE. The effect of pregnancy on the renal handling of glucose. *Br J Obstetr Gynaecol* 1975; **82**: 374–381.
- 21 Davison JM, Dunlop W. Renal hemodynamics and tubular function in normal human pregnancy. *Kidney Int* 1980; **18**: 152–161.
- 22 Kubota M, Arai K, Morishima T, Kawamori R, Kamada T. 1,5-Anhydro-D-glucitol evaluates daily glycemic excursions in well-controlled NIDDM. *Diabetes Care* 1995; **8**: 1156–1159.
- 23 Yamanouchi T, Ogata N, Kawasaki T, Funato H, Yoshimura T *et al*. Relationship between serum 1,5-anhydroglucitol and urinary excretion of N-acetylglucosaminidase and albumin determined at onset of NIDDM with 3-year follow-up. *Diabetes Care* 1998; **4**: 619–623.
- 24 Yamanouchi T, Minoda S, Yabuuchi M, Akanuma Y, Akanuma H, Miyashita H. Plasma 1,5-anhydro-D-glucitol as a new clinical marker of glycemic control in NIDDM patients. *Diabetes* 1989; **38**: 723–729.