



Glycemic instability is an underestimated problem in Type II diabetes

Stephan F.E. Praet, Ralph J.F. Manders, Ruth C.R. Meex, A. G. Lieveverse, Coen D.A. Stehouwer, Harm Kuipers, Hans A. Keizer, Luc J.C. van Loon

► To cite this version:

Stephan F.E. Praet, Ralph J.F. Manders, Ruth C.R. Meex, A. G. Lieveverse, Coen D.A. Stehouwer, et al.. Glycemic instability is an underestimated problem in Type II diabetes. *Clinical Science*, 2006, 111 (2), pp.119-126. <10.1042/CS20060041>. <hal-00479322>

HAL Id: hal-00479322

<https://hal.science/hal-00479322v1>

Submitted on 30 Apr 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Glycemic instability is an underestimated problem in type 2 diabetes

Praet S.F.E., MD¹, Manders R.J.F., MSc², Meex R.C.R., MSc², Lieveise A.G., MD PhD³, Stehouwer C.D.A., MD PhD⁴, Kuipers H., MD PhD¹, Keizer H.A., MD PhD¹, van Loon L.J.C., PhD^{1,2}

¹Department of Movement Sciences, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, Maastricht, The Netherlands

²Department of Human Biology, NUTRIM, Maastricht University, Maastricht, The Netherlands

³Department of Internal Medicine, Máxima Medical Center, Eindhoven, The Netherlands

⁴Department of Internal Medicine, University Hospital Maastricht, Maastricht, The Netherlands

Address for correspondence:

Stephan F.E. Praet, MD.

Department of Movement Sciences

Maastricht University

PO Box 616

6200 MD Maastricht, the Netherlands

Tel: +31 6 17066506

Fax: +31 43 3670976

e-mail S.Praet@mmc.nl

ABSTRACT

OBJECTIVE: To assess the level of glycemic control by the measurement of 24 h blood glucose profiles and standard blood analyses under identical nutritional and physical activity conditions in type 2 diabetes patients and healthy, normoglycemic controls.

RESEARCH DESIGN AND METHODS: A total of 11 male, type 2 diabetes patients and 11 healthy, matched controls participated in a 24 h continuous subcutaneous glucose monitoring (CGMS) assessment trial under strictly standardized dietary and physical activity conditions. In addition, fasting plasma glucose, insulin and HbA_{1c} concentrations were measured, and an oral glucose tolerance test was performed to calculate indices of whole-body insulin sensitivity, oral glucose tolerance and/or glycemic control.

RESULTS: In the healthy control group, hyperglycemia (blood glucose concentration >10 mmol/l) was hardly present ($2\pm 1\%$ or 0.4 ± 0.2 / 24 h). However, in the type 2 diabetes patients hyperglycemia was experienced for as much as $55\pm 7\%$ of the time (13 ± 2 h / 24 h) while using the same standardized diet. Breakfast-related hyperglycemia contributed most ($46\pm 7\%$, ANOVA, $P<0.01$) to the total amount of hyperglycemia and postprandial glycemic instability. In the diabetes patients, blood HbA_{1c} contents correlated well with the duration of hyperglycemia and the postprandial glucose responses ($P<0.05$).

CONCLUSIONS: CGMS measurements show that standard measures for glycemic control underestimate the amount of hyperglycemia prevalent during real-life conditions in type 2 diabetes. Given the macro- and microvascular damage caused by postprandial hyperglycemia, CGMS provides an excellent tool to evaluate alternative therapeutic strategies to reduce hyperglycemic blood glucose excursions.

Running title: Glycemic instability in type 2 diabetes

Key words: CGMS, hyperglycemia, CONGA, type 2 diabetes, HbA_{1c}

INTRODUCTION

Over the last 15 years, improvements in microdialysis and biosensor technology have enabled clinicians to reliably monitor plasma and/or interstitial glucose concentrations in an ambulatory and continuous way [1, 2]. These, so-called, continuous subcutaneous glucose-monitoring systems (CGMS) have proven quite useful to optimize individual exogenous insulin administration in diabetes patients [3], since they provide information on ambulatory postprandial [4] and/or nocturnal glucose excursions [5]. Moreover, both in children and adults with type 1 diabetes it has been shown that average 24 h blood glucose concentrations strongly correlate with HbA_{1c} concentrations [6, 7]. However, the inter- and intra-individual day-to-day variation in glycemic load, meal composition [8] and daily physical activity [9] can complicate therapeutic decision-making based on these 24 h blood glucose profiles [10, 11]. Therefore, in order to compare CGMS results between normoglycemic and diabetic subjects, standardization of both diet [8] and physical activity [9] is essential.

Epidemiological studies and preliminary intervention studies have shown that postprandial hyperglycemia is a direct and independent risk factor for the development of cardiovascular disease (CVD) [12]. Importantly, the postprandial rapid increase in blood glucose concentrations or ‘hyperglycemic spikes’ seem to be even more relevant to the onset of cardiovascular complications than merely elevated fasting plasma glucose [13]. Therefore, therapeutic targets should be aimed at reducing postprandial blood glucose excursions. Although scientific studies on the prevalence of hyperglycemic spikes in type 2 diabetes are still scarce [13], recommendations on proper glycemic control have recently been redefined [14, 15]

To define abnormal postprandial blood glucose excursions and relate this to the pathogenesis of diabetic vascular complications, it is important to have more detailed information on normal postprandial blood glucose profiles in a non-insulin resistant population under exactly the same dietary ambulatory conditions. Therefore, in the present study, we investigated 24 h blood glucose profiles in type 2 diabetes patients on oral blood glucose lowering medication and healthy, normoglycemic controls under strictly

standardized, but free-living conditions. As such, this study provides a frame of reference for future studies on the role of real-life postprandial hyperglycemia in the pathogenesis of diabetic complications.

SUBJECTS AND METHODS

Subjects

A total of 11 long-term diagnosed male type 2 diabetes patients and 11 healthy, age and BMI matched, normoglycemic control subjects were selected to participate in this study. Subjects' characteristics are presented in Table 1. Exclusion criteria were impaired renal or liver function, severe obesity (BMI>35 kg/m²), cardiac disease, hypertension, diabetic complications, and exogenous insulin therapy. All type 2 diabetes patients were treated with oral plasma glucose lowering medication (metformin only (n=3), or in combination with sulfonylureas (n=8)). All medication was continued during the trials. All subjects were informed about the nature and the risks of the experimental procedures before their written informed consent was obtained. The study was approved by the local Medical Ethical Committee.

Screening

Before inclusion, all subjects first performed an oral glucose tolerance test (OGTT). Blood glucose lowering medication was withheld prior to the screening. After an overnight fast, subjects reported at the laboratory at 8.00 a.m. A catheter (Baxter BV, Utrecht, the Netherlands) was inserted into an antecubital vein and a resting blood sample was drawn after which a bolus of 75 g glucose (dissolved in 250 ml water) was ingested (t= 0 min). After the bolus was consumed, blood was sampled every 30 min until t=120 min. Plasma glucose concentrations were measured to determine glucose intolerance and/or type 2 diabetes according to the World Health Organization criteria of 1999 [16]. In addition, plasma glucose and insulin concentrations were used to assess insulin sensitivity (IS) using the oral glucose insulin sensitivity (OGIS)-index for a 2 h OGTT as described by Mari *et al* [17] and whole-body insulin resistance using the homeostasis model assessment insulin resistance index (HOMA-IR) [18].

Blood sample analysis

Blood (10 ml) was collected in EDTA containing tubes and centrifuged at 1,000 g and 4°C for 10 min. Aliquots of plasma were immediately frozen in liquid nitrogen and stored at -80°C until analyses. Glucose concentrations (Uni Kit III, Roche, Basel) were analyzed with the COBAS FARA semi-automatic analyzer (Roche). Plasma insulin was determined in duplicate and averaged by radioimmunoassay (HI-14K, Linco research Inc, St. Charles, USA). To determine HbA_{1c} content a 3 ml blood sample was collected in EDTA containing tubes and analyzed by high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany).

Study protocol

All experimental trials described in this study are part of a greater project investigating the effects of nutritional interventions to improve glycemic control in type 2 diabetes patients. On the first day subjects reported to the laboratory in the afternoon and were instructed about their diet, and on the use of the food intake and physical activity diaries. Next, subjects received instructions in the use of the capillary blood sampling method (Glucocard Memory PC, A. Menarini Diagnostics, Firenze, Italy) used for the calibration of the continuous glucose monitoring system. All subjects were instructed to measure capillary blood glucose concentrations before every meal. After the subjects were fully instructed, a microdialysis fiber (Medica, Medolla, Italy) with an internal diameter of 0.17 mm and a cut-off weight of 18 kD was inserted in the peri-umbilical region, without anesthesia, using an 18-gauge Teflon catheter as a guide, as described previously [19]. For the measurements the micro-fiber was then connected to a portable CGMS (GlucoDay[®]S, A. Menarini Diagnostics, Firenze, Italy), which consists of a peristaltic pump that pumps Dulbecco's solution at 10 µL/min through the microdialysis fiber. A more detailed description of the device has been published earlier [1]. Briefly, the subcutaneous interstitial fluid is taken up by the microdialysis fiber and is transported to the measuring cell. The glucose sensor, consisting of immobilized glucose oxidase, measures the glucose concentration every sec and stores an average value every 3 min for

a total of 48 h. The entire device weighs about 250 g and is worn in a pouch under the subjects' clothes. After the CGMS was checked for proper function, subjects were provided with their diet (pre-weighed and packaged meals, drinks and snacks) and were allowed to return home and resume all their normal activities. CGMS data of the second test day (from 7.00 am to 7.00 am) were used for data analysis. The first period was used to familiarize subjects with the equipment and, therefore, not used in the data analyses.

Diet and physical activity

All subjects maintained their normal physical activity patterns throughout the entire experimental period. Subjects refrained from heavy physical labor and exercise training for at least 3 d prior to and on the day of the trial. Subjects were asked to keep a comprehensive record of time spent performing all activities (to the nearest 10 min) including sleeping, eating sitting, standing, watching television, occupational activity and household tasks, as well as information on the duration and relative intensity (e.g. light, moderate) of all structured activities. The rate of energy expenditure for each activity was then determined using the Compendium of Physical Activities [20]. Daily energy expenditure did not differ between groups and averaged 13.6 ± 0.7 and 13.3 ± 0.6 MJ day⁻¹ in the type 2 diabetes patients and normoglycemic controls, respectively. All meals, snacks and beverages were provided in pre-weight packages and ingested at pre-determined time points to ensure fully standardized dietary modulation. On the evening prior to the 24h analyses period, all subjects received the same standardized meal (43.8 kJ kg⁻¹ BW; consisting of 60 Energy % (En%) carbohydrate, 28 En% fat and 12 En% protein). The following day the subjects were instructed to ingest their designated meals, drinks and snacks at set time-points. Throughout this 24 h test period subjects received a standardized diet (3 meals and 3 snacks per day) representing an energy intake of 121 kJ kg⁻¹ BW per day consisting of 64 En% carbohydrate, 25 En% fat and 11 En% protein. Before and after consuming a meal (i.e. breakfast, lunch and dinner) subjects were asked to obtain a capillary blood glucose sample (Glucocard Memory PC). The following day the subjects reported back to our laboratory to obtain a non-fasting venous blood glucose measurement and to remove the CGMS. The

acquired data were then downloaded from the device to a personal computer with GlucoDay[®] software (V3.0.5). Values reported by the CGMS were converted into glucose values using the capillary glucose measurements as calibration values.

Statistics and data analyses

Data are expressed as means \pm SEM. Glucose responses were calculated as mean glucose area under the curve (AUC) up to 6 h after each meal. Since the CGMS device provides an average glucose value every 3 min, AUC is expressed as mmol/L * 3 min. To quantify and compare the glucose excursions in the control and diabetes population, AUC and the amount of time during which glucose concentrations were above 10.0 mmol/L or below 3.9 mmol/L were calculated. On the first and second study day, fasting glucose was determined from the calibrated CGMS curves 10 min before breakfast and averaged. The non-fasting venous blood glucose measurement was used to calculate the coefficient of variation (CV) of the CGMS data. Relationships between CGMS parameters and standard measures of IS were calculated using linear regression models.

To assess intra-day glycemic variability, continuous overall net glycemic action (CONGA), a novel method recently described by McDonnell *et al*, was used [21]. CONGA n has been defined as the standard deviation of the differences in glucose concentration using varying time differences of n hours. We used CONGA1, CONGA2 and CONGA4, indicating intra-day glycemic variability based on 1 h, 2 h and 4 h time differences, respectively. In normal non-diabetic subjects CONGA values vary between 0.4 and 1.2, while values above 1.5 indicate glycemic lability [21].

Before pooling data from all 22 subjects, homogeneity of regression was tested using ANCOVA in order to exclude significant interaction. Time dependent variables were tested using repeated-measures ANOVA with a Tukey-Kramer post-hoc test when applicable. For non-time dependent variables, a Student's t-test for unpaired observations was applied. Significance was set at the

0.05 level of confidence. All statistical calculations were performed using the SPSS 12.0.1 software package (SPSS Inc, Chicago, IL, USA).

RESULTS

Baseline and postprandial blood glucose responses are provided in Table 2. Total 24 h blood glucose responses in both diabetes patients and healthy controls are illustrated in Fig. 1. Basal and mean glucose concentrations were significantly greater in the type 2 diabetes patients vs the normoglycemic controls (t-test, $p<0.05$). In the type 2 diabetes patients, the prevalence of hyperglycemia (>10.0 mmol/L) was $55\pm7\%$ of the 24 h period. In contrast, in the normoglycemic controls, hyperglycemia was evident in $1.6\pm1\%$. As such, hyperglycemia was present for 13.3 ± 1.7 h and 0.38 ± 0.2 h, respectively.

The postprandial AUCs above 10.0 mmol/L following breakfast, lunch and dinner contribute, respectively $46\pm7\%$, $29\pm3\%$, $11\pm3\%$ to the total amount of hyperglycemia in our type 2 diabetes patients present during the 24 h monitoring period. This breakfast-related hyperglycemia was significantly greater (ANOVA, $p<0.01$) compared to the amount of hyperglycemia during the evening or during the night.

Both in type 2 diabetes patients and healthy controls the average CONGA1 values following breakfast were significantly raised compared to the 6 h following lunch and dinner (Table 2, ANOVA, $p<0.01$). CONGA1 values were lowest during the night (ANOVA, $p<0.01$) and did not differ between groups from 01.00 – 06.00 h (ANOVA, $p>0.05$, Table 2).

In this study the coefficient of variation (CV) between interstitial CGMS glucose values and venous blood glucose was on average $8.0\pm1.3\%$

Correlations between CGMS parameters and our standard measures for glycemic control are presented in Table 3. In the diabetes patients, HbA_{1c}-levels correlated well with the average 24 h blood glucose concentrations ($R=0.81$, $P<0.01$), the time during which blood glucose levels were >10 mmol/L ($R=0.70$, $P<0.05$), and postprandial AUC following lunch ($R=0.80$, $P<0.01$) and dinner ($R=0.87$, $P<0.01$). In a subgroup of diabetes patients with apparent acceptable glycemic control ($\text{HbA}_{1c} \leq 7.0$, $n=6$), hyperglycemia was present for $46\pm8\%$ of the day (11.0 ± 1.9 h).

Both in the diabetes and control group, mean 24 h and nocturnal blood glucose concentrations correlated strongly with fasting plasma glucose levels (R between 0.61-0.86, $P<0.05$). In both groups, no significant correlations were reported between the 24 h CONGA indices and HbA_{1c} content, however, in the diabetes patients a significant correlation was found between postprandial CONGA1 values and AUC in the 6 h following a meal ($R=0.47$, $p<0.01$). When pooling the data from both groups, the 24 h CONGA_n values correlated significantly with blood HbA_{1c} content ($R=0.53$ -0.66, $P<0.01$), mean 24 h glucose concentrations ($R=0.73$ -0.77, $P<0.001$) and to a lesser extent with mean fasting plasma glucose concentrations ($R=0.50$ -0.52, $P<0.05$). Also, a significant correlation was found between postprandial CONGA1 values and AUC 6 h following a meal ($R=0.60$, $p<0.001$)

DISCUSSION

The present study shows that under normal, standardized dietary conditions, type 2 diabetes patients using oral blood glucose lowering medication experience a substantial amount of hyperglycemia for more than 13 h within a 24 h period. This disturbance in blood glucose homeostasis is predominantly present following breakfast. After comparing 24 h blood glucose profiles between healthy, normoglycemic controls and type 2 diabetes patients under usual medical care by a general practitioner, it seems clear that standard treatment schemes with oral blood glucose lowering drugs appear to have insufficient therapeutic strength to normalize postprandial hyperglycemia. Given the clinical relevance of the hyperglycemic spikes [13], CGMS provides an excellent tool to evaluate the level of glycemic stability in type 2 diabetes patients.

The concept that oral blood glucose lowering therapy provides inadequate protection against hyperglycemia is not new [22-24]. Epidemiological studies and preliminary intervention studies have shown that postprandial hyperglycemia is a direct and independent risk factor for the development of cardiovascular disease [12]. However, the postprandial rapid increase in blood glucose concentrations seems to be more relevant to the onset of cardiovascular complications than merely elevated fasting plasma glucose concentrations [13]. Therefore, more detailed information on 24 h blood glucose profiles in a diabetic state is essential to increase our understanding of the relationship between hyperglycemia, glucotoxicity and cardiovascular morbidity. In an attempt to assess postprandial glycemic instability in type 2 diabetes, we applied CGMS in diabetes patients and compared this with blood glucose profiles of normoglycemic subjects under strict nutritional and exercise standardization, but otherwise free-living conditions. In most of our normoglycemic subjects, hyperglycemia or glycemic instability was not detectable. In contrast, despite healthy dietary conditions and continued use of oral blood glucose lowering medication according to standard primary care [25] and international guidelines [15], the type 2 diabetes patients were hyperglycemic during more than 13 h per day, while using exactly the same diet as the normoglycemic controls. In accordance with earlier observations by Monnier et al [26], this study

shows that postprandial hyperglycemia was most prominent following breakfast, and less evident during the night. As we provided a healthy, balanced diet (43.8 kJ/kg BW; consisting of 60 En% carbohydrate, 28 En% fat and 12 En% protein), it could be speculated that the total amount of hyperglycemia may even be worse under normal, unrestricted dietary conditions. The observed levels of hyperglycemia during the day (13 ± 2 h / 24 h) are unacceptable and likely cause the excess formation of advanced glycation end-products [27], causing the macro- and microvascular damage [28].

In line with earlier studies [29-31], our findings emphasize the need for different types of interventional strategies in type 2 diabetes patients. It should be noted that there is a weak, non-significant, correlation between fasting blood glucose and the percentage of hyperglycemia ($R^2=0.25$, $p>0.05$, Table 3). The latter indicates that FPG is unlikely to be of sufficient sensitivity to successfully evaluate new treatment strategies that focus on reducing postprandial hyperglycemia. For more long-term evaluation purposes, changes in blood HbA_{1c} concentrations have generally been assessed, since blood HbA_{1c} content correlates relatively well with both mean 24 h [32, 33] and postprandial glucose levels [6, 7]. In accordance, in the present study we observed strong correlations between HbA_{1c} and mean 24 h glucose and postprandial glucose levels following lunch and dinner (Table 3). It should be mentioned here that even under clinically acceptable HbA_{1c} levels (i.e. $\text{HbA}_{1c} \leq 7.0$ in 6 out of 11 diabetes patients) hyperglycemia can still be unacceptably large at 11 ± 2 hours of blood glucose excursion >10 mmol/L per 24 h. Therefore, these results extend on earlier findings [24, 34, 35], and strongly suggest that the ability of HbA_{1c} to monitor postprandial hyperglycemia is debatable. Moreover, the measurement of prospective changes in blood HbA_{1c} content only has sufficient sensitivity to detect changes in glucose homeostasis during middle to long-term interventions [36]. Therefore, the present study underlines the notion that CGMS is a promising tool when evaluating short-term (<3 months) changes in blood glucose homeostasis following pharmacological, dietary and/or exercise interventions [1].

Another benefit of the CGMS approach, that has potential clinical application as well, is the possibility to calculate the level of glycemic instability in insulin resistant states. This so-called Continuous Overall Net Glycemic Action (CONGA_n) is probably a more appropriate measure to assess short-term changes in

glucose homeostasis throughout the day [21]. This CGMS measure reflects the standard deviation of the differences in glucose concentration using varying time windows [21]. Therefore, we determined CONGA_n values in both our diabetes patients and normoglycemic controls (Table 2). The proposed sensitivity of CGMS to detect subtle variations in glycemic control was confirmed in our normoglycemic control group. Interestingly, 2 of our control subjects appeared to have rather high postprandial CONGA1 values that almost approached values observed in the type 2 diabetes patients (i.e. average postprandial CONGA1 >2.1). These 2 subjects also showed the highest insulin values during the oral glucose tolerance test, and were the only 'normoglycemic' persons who showed some hyperglycemia throughout the day (data not shown). Altogether, our results suggest that more advanced CGMS analyses techniques provide promising measures to assess glycemic instability in diabetes patients [21]. Research is warranted to investigate the diagnostic value of CGMS in other diabetes related populations, like patients in a pre-diabetic and/or insulin resistant state.

In conclusion, detailed analyses of 24 h blood glucose profiles show that standard measures for glycemic stability grossly underestimate the amount of hyperglycemia during real-life conditions in type 2 diabetes patients. Given the macro- and microvascular damage caused by postprandial hyperglycemia, CGMS provides an excellent tool to more directly evaluate additional therapeutic strategies to reduce the amount of glycemic instability and risk of cardiovascular complications in type 2 diabetes patients.

ACKNOWLEDGEMENTS

The authors would like to thank *A. Menarini Diagnostics BENELUX* and Hanneke van Milligen for the technical support. We gratefully acknowledge our volunteers for participating in the experimental trials.

REFERENCES

- 1 Maran, A., Crepaldi, C., Tiengo, A., et al. (2002) Continuous subcutaneous glucose monitoring in diabetic patients: A multicenter analysis. *Diabetes Care*. **25**, 347-352
- 2 Jungheim, K., Wientjes, K. J., Heinemann, L., Lodwig, V., Koschinsky, T. and Schoonen, A. J. (2001) Subcutaneous continuous glucose monitoring: Feasibility of a new microdialysis-based glucose sensor system. *Diabetes Care*. **24**, 1696-1697
- 3 Schaepelynck-Belicar, P., Vague, P., Simonin, G. and Lassmann-Vague, V. (2003) Improved metabolic control in diabetic adolescents using the continuous glucose monitoring system (cgms). *Diabetes Metab*. **29**, 608-612
- 4 Hay, L. C., Wilmschurst, E. G. and Fulcher, G. (2003) Unrecognized hypo- and hyperglycemia in well-controlled patients with type 2 diabetes mellitus: The results of continuous glucose monitoring. *Diabetes Technol Ther*. **5**, 19-26
- 5 Bolinder, J., Hagstrom-Toft, E., Ungerstedt, U. and Arner, P. (1997) Self-monitoring of blood glucose in type i diabetic patients: Comparison with continuous microdialysis measurements of glucose in subcutaneous adipose tissue during ordinary life conditions. *Diabetes Care*. **20**, 64-70
- 6 Salardi, S., Zucchini, S., Santoni, R., et al. (2002) The glucose area under the profiles obtained with continuous glucose monitoring system relationships with hba(1c) in pediatric type 1 diabetic patients. *Diabetes Care*. **25**, 1840-1844
- 7 Sharp, P. and Rainbow, S. (2002) Continuous glucose monitoring and haemoglobin a(1c). *Ann Clin Biochem*. **39**, 516-517
- 8 Wolever, T. M. (1990) The glycemic index. *World Rev Nutr Diet*. **62**, 120-185
- 9 Burstein, R., Epstein, Y., Shapiro, Y., Charuzi, I. and Karnieli, E. (1990) Effect of an acute bout of exercise on glucose disposal in human obesity. *J Appl Physiol*. **69**, 299-304
- 10 Bantle, J. P. and Laine, D. C. (1988) Day-to-day variation in glycemic control in type i and type ii diabetes mellitus. *Diabetes Res*. **8**, 147-149
- 11 Kerksen, A., De Valk, H. W. and Visser, G. H. (2004) Day-to-day glucose variability during pregnancy in women with type 1 diabetes mellitus: Glucose profiles measured with the continuous glucose monitoring system. *BJOG*. **111**, 919-924
- 12 Wautier, M. P., Massin, P., Guillausseau, P. J., et al. (2003) N(carboxymethyl)lysine as a biomarker for microvascular complications in type 2 diabetic patients. *Diabetes Metab*. **29**, 44-52
- 13 Ceriello, A. (2005) Postprandial hyperglycemia and diabetes complications: Is it time to treat? *Diabetes*. **54**, 1-7
- 14 De Backer, G., Ambrosioni, E., Borch-Johnsen, K., et al. (2003) European guidelines on cardiovascular disease prevention in clinical practice. Third joint task force of european and other societies on cardiovascular disease prevention in clinical practice. *Eur Heart J*. **24**, 1601-1610
- 15 Amerian Diabetes Association, (2006) Standards of medical care in diabetes-2006. *Diabetes Care*. **29**, S4-S34
- 16 WHO, World Health Organisation (1999) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Geneva: World Health Organisation
- 17 Mari, A., Pacini, G., Murphy, E., Ludvik, B. and Nolan, J. J. (2001) A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care*. **24**, 539-548

- 18 Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F. and Turner, R. C. (1985) Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. **28**, 412-419
- 19 Meyerhoff, C., Bischof, F., Sternberg, F., Zier, H. and Pfeiffer, E. F. (1992) On line continuous monitoring of subcutaneous tissue glucose in men by combining portable glucosensor with microdialysis. *Diabetologia*. **35**, 1087-1092
- 20 Ainsworth, B. E., Haskell, W. L., Whitt, M. C., et al. (2000) Compendium of physical activities: An update of activity codes and met intensities. *Med Sci Sports Exerc*. **32**, S498-504
- 21 McDonnell, C. M., Donath, S. M., Vidmar, S. I., Werther, G. A. and Cameron, F. J. (2005) A novel approach to continuous glucose analysis utilizing glycemic variation. *Diabetes Technol Ther*. **7**, 253-263
- 22 Roy, R., Navar, M., Palomeno, G. and Davidson, M. B. (2004) Real world effectiveness of rosiglitazone added to maximal (tolerated) doses of metformin and a sulfonylurea agent: A systematic evaluation of triple oral therapy in a minority population. *Diabetes Care*. **27**, 1741-1742
- 23 Davies, M. (2004) The reality of glycaemic control in insulin treated diabetes: Defining the clinical challenges. *Int J Obes Relat Metab Disord*. **28 Suppl 2**, S14-22
- 24 Del Prato, S. (2002) In search of normoglycaemia in diabetes: Controlling postprandial glucose. *Int J Obes Relat Metab Disord*. **26 Suppl 3**, S9-17
- 25 Wiersma, T. J., Heine, R. J. and Rutten, G. E. (1999) [summary of the practice guideline 'diabetes mellitus type 2' (first revision) of the dutch college of general practitioners]. *Ned Tijdschr Geneesk*. **143**, 1688-1691
- 26 Monnier, L., Colette, C., Rabasa-Lhoret, R., et al. (2002) Morning hyperglycemic excursions: A constant failure in the metabolic control of non-insulin-using patients with type 2 diabetes. *Diabetes Care*. **25**, 737-741
- 27 Aronson, D. and Rayfield, E. J. (2002) How hyperglycemia promotes atherosclerosis: Molecular mechanisms. *Cardiovasc Diabetol*. **1**, 1
- 28 Spijkerman, A. M., Henry, R. M., Dekker, J. M., et al. (2004) Prevalence of macrovascular disease amongst type 2 diabetic patients detected by targeted screening and patients newly diagnosed in general practice: The hoorn screening study. *J Intern Med*. **256**, 429-436
- 29 Shorr, R. I., Franse, L. V., Resnick, H. E., Di Bari, M., Johnson, K. C. and Pahor, M. (2000) Glycemic control of older adults with type 2 diabetes: Findings from the third national health and nutrition examination survey, 1988-1994. *J Am Geriatr Soc*. **48**, 264-267
- 30 (1998) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (ukpds 33). Uk prospective diabetes study (ukpds) group. *Lancet*. **352**, 837-853
- 31 Shichiri, M., Kishikawa, H., Ohkubo, Y. and Wake, N. (2000) Long-term results of the kumamoto study on optimal diabetes control in type 2 diabetic patients. *Diabetes Care*. **23 Suppl 2**, B21-29
- 32 Lerman-Garber, I., Lopez-Ponce, A., Murcio Flores, R. A., et al. (2001) Comparing easy and accessible parameters of glycemic control in type 2 diabetes. *Rev Invest Clin*. **53**, 518-525

- 33 Murata, G. H., Hoffman, R. M., Duckworth, W. C., Wendel, C. S. and Shah, J. H. (2004) Contributions of weekly mean blood glucose values to hemoglobin a1c in insulin-treated type 2 diabetes: The diabetes outcomes in veterans study (doves). *Am J Med Sci.* **327**, 319-323
- 34 Jeffcoate, S. L. (2004) Diabetes control and complications: The role of glycated haemoglobin, 25 years on. *Diabet Med.* **21**, 657-665
- 35 Bonora, E., Calcaterra, F., Lombardi, S., et al. (2001) Plasma glucose levels throughout the day and hba(1c) interrelationships in type 2 diabetes: Implications for treatment and monitoring of metabolic control. *Diabetes Care.* **24**, 2023-2029
- 36 Miyazaki, Y., Mahankali, A., Matsuda, M., et al. (2001) Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. *Diabetes Care.* **24**, 710-719

Table 1 Subjects' characteristics

	Controls	Diabetes
Age (yrs)	59 ± 2	58 ± 1
Body Mass Index (kg/m ²)	27.8 ± 1.4	27.9 ± 1.2
Years type 2 diabetes	NA	8.1 ± 2.1
HbA _{1c} (%)	5.5 ± 0.1	7.4 ± 0.3 [*]
Fasting glucose (mmol/L)	5.7 ± 0.2	10.6 ± 1.0 [*]
HOMA-IR	3.5 ± 0.5	8.0 ± 1.4 [†]
OGIS	374 ± 18.	256 ± 19 [*]

BMI, body mass index; HbA_{1c}, glycated hemoglobin; Fasting glucose was determined after 48 hour abstinence of blood glucose lowering medication and 15 min before the oral glucose tolerance test (OGTT); HOMA-IR, homeostasis model assessment insulin resistance index as described by Matthews et al ; OGIS, oral glucose insulin sensitivity-index for a 2 h OGTT as described by Mari et al [14]. *: significantly different between groups; P<0.01, †: P<0.001.

Table 2 CGMS measurements

	<i>Control subjects (n=11)</i>	<i>Diabetes patients (n=11)</i>
24 h analysis (7-7 am)		
Mean 24h glucose (mmol/L)	6.3 ± 0.2	10.8 ± 0.5*
Hyperglycemic episodes (h)	0.4 ± 0.2	13.3 ± 1.7*
Hypoglycemic episodes (h)	0.5 ± 0.2	0.1 ± 0.05
FPG (mmol/L)	5.9 ± 0.4	8.6 ± 0.6*
Mean nocturnal glucose (mmol/L)	7.0 ± 0.9	9.3 ± 0.8*
CONGA1	1.5 ± 0.1	2.5 ± 0.1*
CONGA2	1.7 ± 0.1	3.4 ± 0.1*
CONGA4	1.8 ± 0.2	4.2 ± 0.2*
Post-prandial analyses		
AUC PP breakfast	778 ± 38	1559 ± 75*
AUC PP lunch	713 ± 20	1419 ± 80*
AUC PP dinner	736 ± 39	1158 ± 82*
Glycemic variability		
CONGA1 PP breakfast	1.8 ± 0.3	3.4 ± 0.3 [#]
CONGA1 PP lunch	1.5 ± 0.2	2.2 ± 0.2 ^{\$}
CONGA1 PP dinner	1.5 ± 0.2	2.0 ± 0.3 ^{\$}
CONGA1 nocturnal Fasting	1.0 ± 0.2	1.2 ± 0.3

Data presented are means±SEM.; * significant group difference, P<0.001, # = P<0.01, \$ = P<0.05, ANOVA; Hyperglycemic episodes, total time during which [glucose] levels are above 10.0 mmol/L¹; Hypoglycemic episodes, total time during which [glucose] levels are below 3.9 mmol/L¹; FPG, fasting glucose was determined from the calibrated CGMS curves 10 min before breakfast on the the first and second day; Mean Noct glucose, average glucose concentration between 24:00 and 07:00 h; CONGA1,2,4: continuous overall net glycemic action describing intra-day glycemic variability between respectively 1, 2 and 4 h time periods over 24 h ; AUC PP, area under the curve 6 h postprandial (mmol/L* 3 min); CONGA1 glycemic variability between 1 h time periods. CONGA1 PP breakfast: 07:00 –12:00; PP lunch:13:00-18:00 ; PP dinner:19:00 – 24:00; Nocturnal fasting: from 01:00 till 6:00 h under fasting conditions

Table 3 Pearson's correlation matrix between standard insulin sensitivity measures and CGMS measures in both diabetes patients and control subjects

Variable	mean 24 h glucose	% hyper- glycemia	mean noct. Glucose	AUC PP breakfast	AUC PP lunch	AUC PP dinner
<i>Diabetes patients (n=11)</i>						
FPG (mmol/L)	0.61 [*]	0.50	0.70 [*]	0.01	0.35	0.41
HbA _{1c} (%)	0.81 [†]	0.70 [*]	0.30	0.37	0.80 [†]	0.87 [†]
<i>Control Subjects (n=11)</i>						
FPG (mmol/L)	0.84 [†]	0.37	0.86 [†]	0.45	0.40	0.71 [*]
HbA _{1c} (%)	0.05	-0.18	0.03	-0.17	0.32	0.01

%Hyperglycemia., percentage of time glucose concentration above 10.0 mmol/L; FPG, fasting glucose was determined from the calibrated CGMS curves 10 min before breakfast on the second day; HbA_{1c}, glycated hemoglobin; %Hyperglycemia, percentage of time [glucose] above 10.0 mmol/L; mean noct glucose, average glucose concentration between 00:00 am and 07:00 h; AUC PP, area under the curve 6h postprandial (mmol/L* 3 min); *significant correlations P<0.05, † P<0.01;

Figure legends:

Figure 1

Mean \pm SEM glucose concentrations from 07:00 till 07:00 h using CGMS in respectively, eleven healthy, control subjects (lower curve) and eleven type 2 diabetes patients (upper curve). The SEM is indicated by the gray bars. The vertical dash lines indicate the time that subjects were consuming their standardized dietary components, consisting of breakfast (07:00-07:30), morning snack (10:30-11:00), lunch (12:30-13:00), afternoon Snack (15:30-16:00), dinner (18:30-19:00) and evening snack (20:30-21:00), respectively.

Figure 1

