Nocturnal differences in subcutaneous tissue glucose between forearm and abdominal sites during continuous glucose monitoring in normal subjects

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Summary

Objective: A number of short-term studies using the continuous glucose monitoring system (CGMS) indicate that improved metabolic control can be observed in patients with type 1 diabetes when CGMS is applied in clinical practice. Data have also accumulated to suggest that spot measurements of glucose performed four times a day would not detect as much as 70% of all hypoglycaemic episodes registered by CGMS. When more frequent reference values were obtained however it was inferred that nighttime hypoglycaemia reported by CGMS may be spurious. As most assessments with CGMS have been utilizing abdominal subcutaneous tissue, we were interested to evaluate whether differences between blood glucose and sensor readings obtained from different sites exist.

Research design and methods: Two viscometric affinity glucose sensors, applicable to subcutaneous tissue of both forearm and abdomen, were inserted subcutaneously in 12 non-diabetic subjects. Sensors generated glucose data at 3 min intervals and venous blood glucose was determined in duplicates by HemoCue at 15-90 min intervals for 24 hours. Each subject consumed three carbohydrate-rich meals, performed an exercise test, and was observed during nocturnal bed-rest at the research center.

Results: The initial decrease of blood glucose during exercise was not fully detected by the sensors. Otherwise, no significant differences between sensor values and blood glucose were observed during day-time. During nocturnal bed-rest abdominal sensor values came approximately 20% lower than blood glucose (P < 0.001) and forearm sensor readings.

Conclusion: It is concluded that a difference between glucose values obtained from abdominal and forearm subcutaneous fat can be observed during nocturnal bed-rest in non-diabetics.

Key-words: Glucose sensors · Hypoglycaemia · Nocturnal · CSII.

Résumé

Objectifs : De nombreuses études à court terme utilisant le système de mesure du glucose en continu (CGMS) montrent qu’une amélioration du contrôle métabolique est obtenue chez les patients atteints d’un diabète de type 1 quand le CGMS est appliqué en clinique. Bon nombre de données suggèrent que des mesures isolées de la glycémie avec une fréquence de 4 fois par jour parviennent à détecter moins de 70% de tous les épisodes hypoglycémiques mesurés par CGMS. Cependant l’augmentation de la fréquence des mesures de la glycémie a permis de mettre en doute l’hypoglycémie nocturne détectée par CGMS. Comme la plupart des études réalisées avec CGMS sont fondées sur des mesures à partir des tissus sous-cutanés de l’abdomen, nous avons vérifié si les résultats de sensors placés à des endroits différents sont en accord avec la mesure de la glycémie veineuse.


Résultats : La diminution initiale de la glycémie pendant un exercice ne fut pas détectée de façon aussi marquée par les sensors. En outre il n’y avait pas de différences significatives entre les mesures des sensors et la glycémie pendant la journée. Enfin pendant un repos nocturne les mesures des sensors abdominaux se révélerent d’environ 20% inférieures à celles de la glycémie et des sensors de l’avant-bras.

Conclusion : Chez des non-diabétiques en repos nocturne, les résultats de la mesure des concentrations de glucose des tissus sous-cutanés de l’abdomen sont significativement inférieurs à ceux du glucose sanguin et du tissu sous-cutané de l’avant bras.

Mots-clés : Sensors de glucose · Hypoglycémie · Nocturne · Pompe à insuline.

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The goal of the management of type 1 diabetes is to maintain blood glucose and HbA1c levels as close to normal as possible. Tight control of blood glucose increases the risk of hypoglycaemia considerably as was demonstrated in the Diabetes Control and Complications Trial (DCCT) in which intensification of insulin therapy was associated with a threefold increase in the incidence of severe hypoglycaemia when compared to conventional insulin therapy [1]. Many severe attacks of hypoglycaemia occur during sleep and such events are particularly troublesome since they are difficult to recognize and therefore to treat promptly. A device for continuous in vivo monitoring of glucose concentrations in people with diabetes has thus been a clinical and research priority for many years, the main object being the detection of hypoglycaemia. Recently, glucose sensors have been developed that can be inserted subcutaneously in order to continuously monitor glucose concentrations over several days [2]. It has been assumed that continuous glucose monitoring during the nocturnal period may reveal previously unrecognized hypoglycaemic episodes as well as hyperglycemic excursions, and that subsequent adjustments of insulin usage may decrease the incidence of such swings of blood glucose which in turn may lead to reduction in the risk of long-term complications. A number of short-term studies using the continuous glucose monitoring system (CGMS) indicated that better management can be obtained, with reductions of HbA1c levels, when CGMS is being used in clinical practice [3-6].

When continuous glucose monitoring systems have been applied in clinical practice data have accumulated to suggest that spot measurements of glucose performed four or seven times per day by finger-pricking would not detect 71% or 58% respectively of all hypoglycaemic episodes registered by continuous glucose monitoring [7]. Reference blood glucose values were too infrequently obtained during nocturnal monitoring however to confirm this notion, and skepticism arose in our own research team, and was supported by reports from others [8], when we experienced recordings not seldom generated glucose values below 3 mmol/l in healthy subjects. Those recordings were performed in subcutaneous fat of the abdomen and we therefore asked ourselves whether such low readings could be related to measurement site rather than reflecting blood glucose levels, and whether recordings from other subcutaneous sites would generate similar signals throughout a 24 hour period. We chose the forearm for sensor application as it has been previously evaluated with respect to alternative site testing and is easily accessible. Using a recently developed viscometric affinity sensor system [9], which is applicable to subcutaneous fat of both forearm and abdomen, we aimed at investigating this issue in non-obese non-diabetic subjects.

The study was approved by the Ethics Committee of Karolinska Institutet.

Research design and methods

Subjects

Twelve non-obese, none-diabetic young volunteers, six of each sex, without a family history of diabetes, were recruited to participate in the study. Their age was 26.0 ± 2.8 years, BMI 21.6 ± 0.3, and fasting blood glucose 4.1 ± 0.4 mmol/l.

Methods

In this investigation we utilized a viscometric affinity glucose sensor not yet launched on the market. This prototype sensor (GlucOnline, developed by Disetronic Medical Systems AG, Burgdorf, Switzerland) based on microdialysis uses a sensitive liquid containing the carbohydrate binding protein concanavalin A, whose binding of the polymer glycoligand dextran is reversibly weakened by glucose. The characteristics of this sensor has previously been presented elsewhere [9], and in vitro it shows a linear and long-term stable dependence on the glucose concentration without detectable drift. Furthermore, in vivo studies have demonstrated that it is an efficient tool for research regarding glucose monitoring in subcutaneous tissue. We chose this sensor for the current project since it had been developed to be applicable to subcutaneous tissue of the forearm and the abdomen. Altogether, 24 sensors were being used in our experiments. HemoCue was used as a reference for blood glucose determinations in duplicates.

Subjects were admitted to the clinical research laboratory of the Department of Medicine at Danderyd Hospital at 8 AM. An intravenous catheter was inserted in a distal arm vein for blood sampling, one sensor was inserted into the subcutaneous fat of the abdomen, in the paraumbilical region, and another in the right forearm subsequent to transdermal perforation by a single use 20G needle under dermal anesthesia by Emla creme. During and following this procedure the subcutaneous position of each probe was ascertained by physical examination. Local temperature within each sensor was registered and a glucose value produced every 3 min throughout the study. Following a run-in period of approximately two hours each subject ingested a carbohydrate rich meal in the research laboratory followed by a 40 min ergometer exercise test at approximately 60% of maximal oxygen uptake as estimated by a reference table. Dinner was consumed between 6 and 7 PM, and subjects took to their beds in the metabolic ward at 11 PM. The following morning, breakfast was ingested between 7 and 8 AM. All sensors were removed at noon.

Reference blood glucose was assessed at 15-90 min intervals, the shortest intervals being in association with meals and physical exercise, and the longest during the night. Over-night surveillance of study subjects and glucose sensors was performed by research nurses, one physician and one technician.
Intravenous blood glucose values obtained at onset of dinner and breakfast and during the following two hours were used for a multi-point calibration. Later, data were recalculated with a new single-point calibration provided by the manufacturers.

Data analysis

Mean reference blood glucose values were calculated from each duplicate HemoCue determination. These values were compared to means of the 5-7 sensor glucose levels closest in time to each reference. Sensor temperature readings were handled accordingly.

All results are expressed as mean ± SD. Area under the curve (AUC) was calculated using the trapezoidal method.

Statistical analysis

Students T-test was used in the statistical analysis since values were normally distributed.

Results

The present study was conducted over 24 hours and during this time one out of 24 sensors failed to function. Data generated from the subject using this sensor were not included in our analysis, neither were data produced with 3 sensors during a period of 36-78 mins during the exercise test, when perspiration affected the attachment of the fixing tape resulting in temporary disturbances of signals. Moderate changes in sensor temperature were registered throughout the study. The mean difference between abdominal and arm sensor temperature was 0.13 ± 1.87 °C. During lunch the difference was at an average 0.63 ± 1.68 °C, during night 0.36 ± 1.88 °C, and during the exercise test – 0.18 ± 1.57 °C. Mean venous blood glucose values and calculated means of sensor glucose values during lunch, exercise and nocturnal bed-rest are illustrated in Fig. 1-3. Mean sensor values were calculated during daytime using the five sensor values closest in time to each venous blood glucose, and during nighttime seven sensor values were used accordingly.

In association with the lunch meal sensing in forearm and abdominal fat, with the exception of the first half hour, generated similar results not differing from reference values (Fig 1). During exercise, the initial rapid blood glucose lowering was not fully detected by the sensors. Apart from that, sensing in forearm and abdominal fat yielded similar results (Fig 2).

Sensing in forearm fat during nocturnal bed-rest yielded results similar to reference blood glucose.

Sensing in abdominal fat, however, generated lower values. Two hours after dinner forearm sensor readings were 5.0 ± 0.7 mmol/l, abdominal sensor readings 4.8 ± 0.5 mmol/l and reference blood glucose 5.1 ± 0.5 mmol/l (Fig 3). One hour later, abdominal values (4.6 ± 0.6 mmol/l) were lower than blood glucose (5.0 ± 0.4 mmol/l, P = 0.037) in contrast to forearm values (5.2 ± 0.5 mmol/l), which were not significantly different (P = 0.65) from the reference. At 11:30 PM abdominal sensors values were 4.1 ± 0.5 mmol/l in contrast to a reference value of 5.2 ± 0.6 mmol/l (P < 0.001). The abdominal sensor values remained low throughout the night, with readings about 20% or 1 mmol/l lower than those of the reference.

Figure 1
Mean glucose values obtained in forearm subcutaneous adipose tissue (white bars), venous blood (grey bars) and abdominal subcutaneous tissue (black bars) in association with a meal. Time 0 represents onset of meal.
lower than blood glucose reference and arm sensor readings (AUC \( P < 0.001 \)). During this period none of the reference values were below 3.5 mmol/l. The corresponding values for arm sensor readings was 3%, and 32% for abdominal sensor readings. There was no detectable difference in blood or sensor glucose values between men and women.

**Conclusions**

The present study demonstrates that significant differences between glucose-sensor values obtained from abdominal as compared to forearm subcutaneous fat can be registered during nocturnal bed-rest in non-diabetic subjects. This has, to our knowledge, previously not been
reported. Interestingly, during day-time glucose sensing, we did not consistently obtain significant differences between abdominal and forearm subcutaneous fat tissue in association with a meal or moderate exercise. When comparing venous blood glucose and sensor readings our observations during day-time seem to be very much in line with what has previously been documented in studies using other types of glucose sensors [3-4, 10-11].

In the present study we applied a visometric affinity sensor technique to monitor tissue glucose while the vast majority of published reports on subcutaneous glucose sensing concern data generated with amperometric sensors with immobilized glucose oxidase [12-14]. A well recognized problem with subcutaneously implanted glucose-oxidase sensors has been their considerable drift of sensor current by time [15]. It has been assumed that the bio-instability of subcutaneous sensors is largely attributed to changes in the environmental surrounding of the probe since function of explanted sensors often appeared unaffected [16]. So far no long-term data have been published on this sensor but up to now no detectable reduction of sensor sensitivity during periods up to 44 hours has been recognized [9]. The present study was conducted over 24 hours only and during this time period only one out of 24 sensors used gave evidence of considerable instability. Even though nocturnal abdominal sensor readings were lower than arm sensor readings and reference blood glucose no difference was seen between abdominal readings and reference values the following day. Neither were the arm sensor readings lower than reference blood glucose during the night, suggesting that the low abdominal values were not attributed to sensor drift. In addition, the calibration procedures were undertaken in the same manner with all sensors. Data were re-analyzed with a new one-point calibration method provided by the manufacturers yielding almost identical outcome.

Concanavalin A based affinity sensors are less glucose-specific than glucose-oxidase sensors on the one hand, but on the other, they are independent from oxygen pressure and electrode poison and their glucose receptor protein exhibits an extremely high stability [17]. One particular matter of concern with such a sensor is its possible dependence on temperature. Previous experiences suggest that it is independent of moderate changes in temperature [18]. In the present study such changes in temperature were registered during nocturnal bed rest. In our statistical evaluation, however, these differences in temperature did not explain the variation in interplay between sensor signals and reference venous blood glucose values.

In a recently published study by Kulcu and co-workers it was inferred that differences between interstitial glucose and blood glucose in both magnitude and timing may reflect physiological variation in glucose uptake, utilization and elimination in blood and interstitial space and cells [19]. On the basis of our present study we can only speculate upon mechanisms to explain the differences between abdominal and forearm subcutaneous glucose during nocturnal bed-rest. It is generally held that abdominal and omental fat has a higher metabolic activity than non-abdominal subcutaneous fat [20-21], and it is possible that our findings could be explained by a higher nocturnal tissue utilization of glucose to support re-esterification of free fatty acids. Another factor to consider is that of the adipose tissue blood flow (ATBF) since a sufficient blood supply is of importance for the performance of a tissue glucose sensor. It has been demonstrated that ATBF is of importance in regulating metabolic processes as demonstrated e.g. by the enhanced triglyceride extraction across the tissue in response to an increase in ATBF [22]. Concerning differences between various subcutaneous regions it has been demonstrated that ATBF in the abdominal region is higher than in the femoral region in non-obese subjects and that fasting for 7 days increased abdominal ATBF by 45% while femoral ATBF remained unchanged [23].

The present study was undertaken in normoglycaemic non-diabetic subjects and therefore the variation of blood glucose, as expressed by the results from duplicate HemoCue analyses, was quite small. The systematically lower sensor-values from abdominal tissue overnight, when expressed in relative terms, were as much as 20% lower than sensor-values from the forearm and reference blood glucose. McGowan and colleagues evaluated glucose data within a similar blood glucose range by comparing glucose values obtained by CGMS with plasma glucose analyses achieved with a Beckman instrument in tightly controlled patients with type 1 diabetes [24]. Interestingly, overnight they found systematically lower glucose sensor values by approximately 40% as compared to plasma glucose and concluded that "reports of asymptomatic nighttime hypoglycaemia may be spurious and should be interpreted with caution in subjects with tightly controlled diabetes".

We believe that our present observation adds a potentially important piece of information on the possible impact of compartment-specific differences of glucose measurements obtained from subcutaneous adipose tissue.

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References


