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Glucose content in human skin: relationship with blood glucose levels

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In order to acertain the dynamic relationship between the extracellular glucose in upper skin layers and blood glucose, skin suction blisters were raised in six Type 1 diabetic patients during a three-step glucose clamp. Blister glucose closely paralleled venous glucose (mean of $r=0.998$). However, in three patients blister glucose was constantly lower than plasma glucose and this appeared to be related to their slower formation of skin blisters. A substantial difference in skin blister suction time was noted among patients and it was found that suction time was linearly correlated to glycosylated haemoglobin (HbA_{1c}) ($n=6$, $r=0.865$, $p=0.026$). It is concluded that a non-invasive blood glucose monitoring system could be successfully based on measurement of alterations in skin glucose contents.

Key words: glucose; glucose-clamp; skin; suction-blister

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Monitoring of blood glucose concentration is the cornerstone in the management of diabetes mellitus. Recent studies have confirmed that maintaining blood glucose concentrations close to normal levels effectively delays the onset and slows the progression of diabetic long-term complications [1–3]. This goal can at present only be approached on the basis of multiple daily blood glucose determinations and frequent adjustments of therapy. Therefore more convenient methods for blood glucose monitoring, preferably without blood sampling, are needed.

Great efforts have been applied to developing minimally invasive methods for blood glucose measurement, to avoid multiple skin incisions and possibly, in future to control insulin delivery systems [4–9]. The designs used have employed electronic measurements of penetrating or reflecting light from the skin. Some reports have postulated excellent agreement between the results of these measurements and blood glucose, but most of the studies comprise a very limited number of patients and are in abstract form [6, 7].

One obstacle to understanding how and why the light intensity might change in concert with blood glucose is that it is not totally clear what is behind these changes, but it is generally believed that changes in light absorption and scattering are important. Also it is not known which tissue is primarily responsible for the changes in light intensity. Since changes in light reflectance from the upper cell layers of the skin may be as important as those occurring in blood, it is important to know the relationship between glucose concentrations in blood and skin.

The aim of our study was, therefore, to obtain information about glucose concentration in human skin, measured as the glucose content in the dermal interstitial space.

Interstitial fluid can be attained by microdialysis or estimated by the skin blister technique. The latter technique has been used and investigated by several authors, who found that skin blister fluid correlates very well with dermal interstitial fluid [10–13].

In the present study blister fluid was obtained and blister glucose concentration (BlG) was measured and compared to plasma glucose concentrations (PG) during a glucose clamp where variations in PG (three steps) were induced by intravenous infusion of glucose.

MATERIALS AND METHODS

Subjects

Six Type 1 diabetic men with a mean age of 32.5 years (range 18–49) and duration of diabetes of 12.2 years (range 3–33) participated in the study.

Body mass index (BMI) ranged between 22.1 and 25.2 kg m^{-2} and glycosylated haemoglobin (HbA_{1c}) was 8.6% (range 6.4–12.8). Four of the patients were given intensive conventional insulin therapy (i.e. preprandial subcutaneous injections of short-acting insulin and a bedtime injection of intermediate-acting insulin) while two were given conventional therapy (i.e. two daily injections of intermediate-acting insulin).

None of the patients had diabetic complications except simplex retinopathy (one patient).

All patients gave written informed consent, and the study was approved by the regional ethical committee.

Skin suction blisters

A total of 10 suction blisters were raised on the volar aspect of the forearm. The suction blisters were raised succesively according to the blood glucose levels (1×2 blisters at 4 mmol l^{-1}, 3×2 at 8 mmol l^{-1} and 1×2 at 16 mmol l^{-1}). The blisters were 6 mm in diameter and the vacuum was 320 mmHg [10]. The development of each blister was monitored by visual examination. When a blister filled the suction cup, the vacuum was released, and a sample of blister fluid was collected in an insulin syringe.

Blister suction time was defined as the period from application of the vacuum until the start of skin blistering, whereas blister production time was defined as the period from the start of skin blistering until the vacuum was released.

Glucose clamp

A stepwise variation in PG was attained by a manual glucose clamp procedure [14–16]. A fixed amount (0.04 mU kg^{-1} min^{-1}) of short-acting insulin was infused together with variable amounts of glucose (20%) solution, and sodium chloride (0.9%), in an antecubital vein. Venous blood samples were drawn from an opposite wrist vein at 10-min intervals, and PG concentration was determined immediately. According to the glucose measurements venous glucose concentration was fixed at values of approximately 4, 8 and 16 mmol l^{-1}.

All procedures were carried out at constant room temperature without arterialization of venous blood.

Glucose analysis

Glucose concentrations in blister fluid and plasma were measured using a Beckmann Glucose Analyzer (Beckmann Instruments, Fullerton, California, USA). All measurements were done in duplicate. Blood samples were centrifuged and measurements were performed immediately.

Statistics

BlG is given as means of measurements from two blisters (simultaneously performed). Mean PG is calculated as mean of plasma glucose concentrations during blister production at each PG level.

Linear regression analysis was performed by the least-squares method. The paired *t*-test was performed for corresponding BlG and PG means.

RESULTS

Six patients were clamped with venous PG concentration starting at fasting values, mean 13.0 mmol l^{-1}, $\pm$4.2 mmol l^{-1}. Glucose clamping resulted in PG levels of means 4.3, 7.9 and

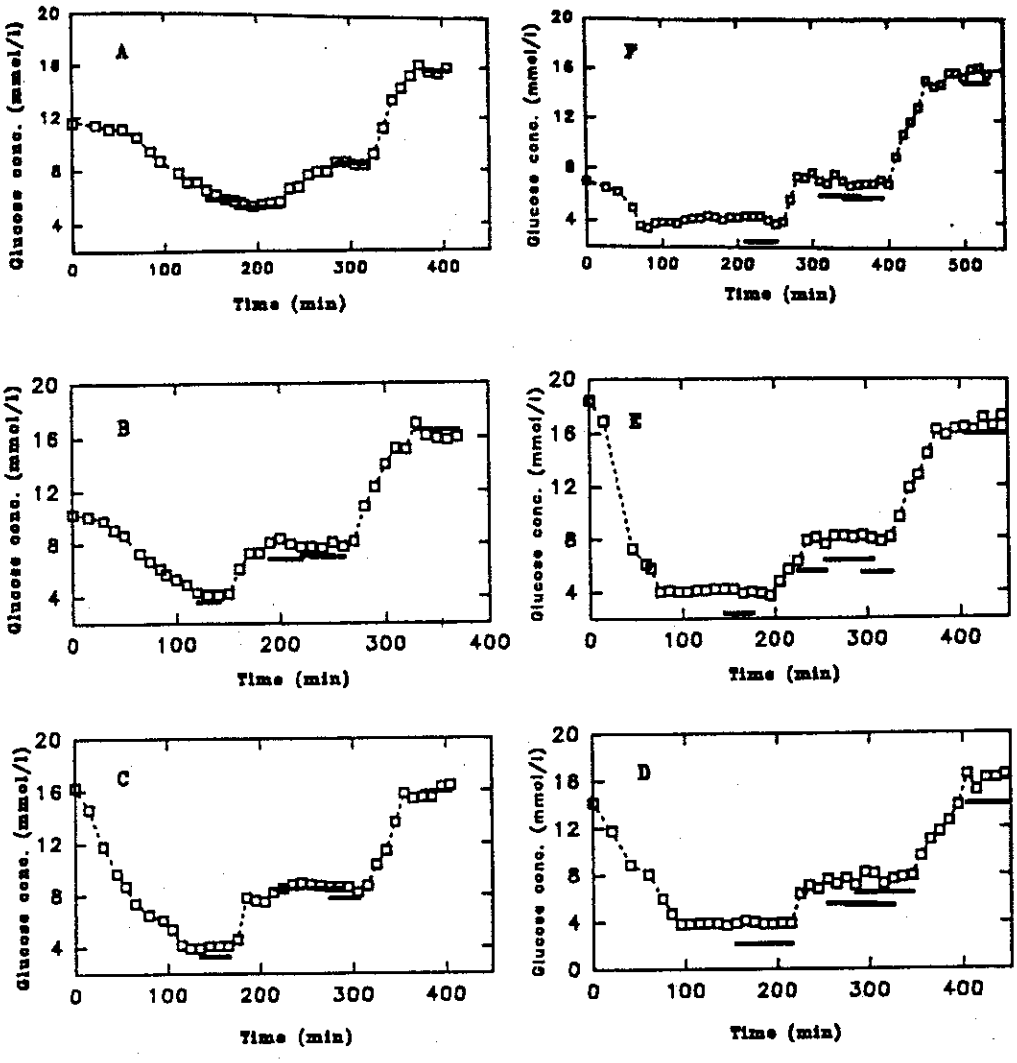


FIG. 1. Plasma glucose (PG) concentrations (□) and blister glucose (BlG) concentrations (—) for each clamp situation (A–F). BlG are mean of each set of blisters and illustrated as horizontal bars giving the blister production time. PG are mean of two measurements done at 10-min intervals. The course of the six clamp investigations comparing BlG with PG is illustrated.

16.1 mmol l^{-1}. These levels were kept constant during skin blister production (Fig. 1). In Figure 1 the mean of each set (2 skin blisters) of BlG is illustrated as horizontal bars, giving the blister production time and compared to the variations in PG. As mentioned earlier, our aim was to create 5 sets (2 blisters each) of skin suction blisters (1×2 blisters at 4 mmol l^{-1}, 3×2 at 8 mmol l^{-1} and 1×2 at 16 mmol l^{-1}), but because of variations in suction time some variation in timing of blisters according to PG levels resulted. All BlG means were below or equal to PG (Fig. 1 and Table 1).

The linear regression between PG and BlG means, for each patient, document the linear relationship (mean of correlation, $r = 0.998$, SEM $= 0.002$, $n = 6$) (Fig. 2). Fitting a regression line between PG and skin BlG resulted in negative intercepts, again demonstrating that BlG means were below PG means. Summarizing the data, there was a statistically significant difference between PG and BlG (paired *t*-test, $n = 6$,

TABLE I. Patient characteristics.

Patient	Age, years	HbA_{1c}, %	Duration of diabetes, years	Blister suction time (SD), min	Mean PG−BIG (SD), mmol l^{-1}
A	45	8.0	4	30 (10.0)	0.15 (0.14)
B	37	7.3	33	48 (8.4)	0.65 (0.59)
C	21	6.4	7	56 (11.4)	0.45 (0.41)
D	49	8.3	7	72 (11.0)	1.22 (0.33)
E	25	8.7	19	64 (11.4)	1.67 (0.66)
F	18	12.8	3	122 (22.0)	1.96 (0.39)

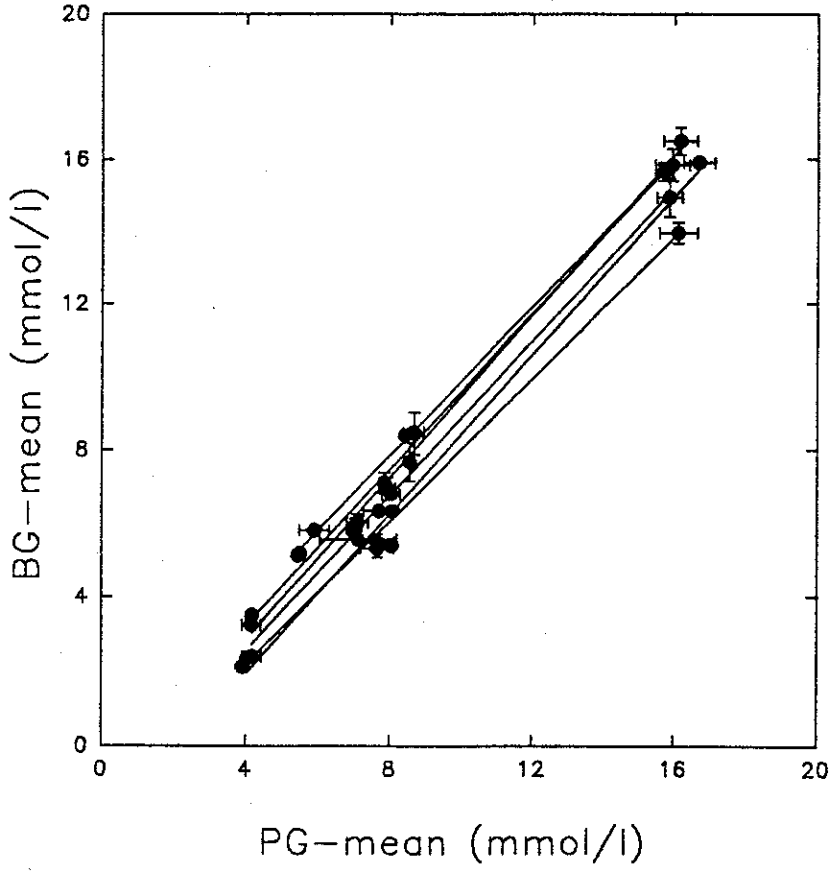
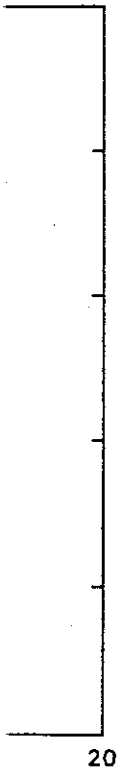


FIG. 2. Blister glucose concentrations (mean ± SEM) against plasma glucose concentrations (mean ± SEM) for five sets of blisters for each patient (A–F).

Mean PG − BIG (SD), mmol l^{-1}
0.15
(0.14)
0.65
(0.59)
0.45
(0.41)
1.22
(0.33)
1.67
(0.66)
1.96
(0.39)



ons (mean ± SEM) for

p=0.018). We found no relationship between the difference (PG − BIG) and PG level.

A substantial difference in skin blister suction time was noted among patients (Table I). We found that suction time increased linearly with increasing HbA_{1c} (n=6, r=0.865, p=0.026) and the difference between PG and BIG was found to increase with increasing suction time (n=6, r=0.859, p=0.029). Suction time was not correlated to age, duration of diabetes or insulin requirements.

DISCUSSION

The present results agree with the data presented by Petersen *et al.* [9], who, using a microdialysis technique, found a highly significant correlation between skin glucose and PG. However, the data of Petersen *et al.* were obtained during steady state glucose levels in healthy non-diabetic patients. Our study demonstrate that this correlation still exists over a wide span of PG levels and in individual diabetic patients.

Our results are also in agreement with a study in which changes in transcutaneous glucose flux were studied after partial removal of stratum corneum. In that study a linear relationship between glucose flux and blood glucose concentration during a glucose tolerance test was found [8].

It is worthy of mention that the concurrent results between PG and BIG were independent of the direction in which PG was changing: PG was decreased before production of the first set of blisters and increased before production of the following sets.

Although the blister technique does not allow precise conclusions about the dynamic relationship between BIG and PG, it seems that there is only a short delay, lasting merely a few minutes, in time between PG and BIG, as BIG parallels PG independently of preceding changes (direction) in PG. Furthermore, three sets of blisters are almost identical even though they have been produced at different times in proportion to PG changes.

Assuming that the contents of blister fluid are an acceptable estimate of contents in interstitial fluid [11–13], we would expect that BIG should be equal to or slightly above PG (venous). An explanation for the finding of lower BIG than PG could relate to the consumption of glucose by the filtrating barrier (dermal tissue). This hypothesis is supported by the observation of a correlation between the suction time and the difference in plasma and blister glucose concentration (greater difference with longer suction time).

Our data strongly support the concept that glucose is not concentrated in the skin as previously accepted (see ref. 17 for review).

It is noticable that the three patients (D–F) who had the greatest differences between BIG and PG were patients with a long run-in period. The duration of this run-in period was mostly determined by blister suction time.

The differences in suction time may indicate differences in the mechanical strength of the epidermal–dermal junction. We found a positive correlation between HbA_{1c} and suction time, which could be explained by increasing stability with increasing glycosylation of dermal/epidermal proteins, but our study is small. Other authors have found that the content of non-enzymatic cross-links in skin collagen correlates with HbA_{1c} and duration of diabetes [18–20]. Another explanation could be that suction time is variable according to differences in skin thickness. This explanation is supported by Collier *et al.*, they had shown that skin thickness was significantly positively correlated to previous glycaemic control [21].

We conclude that BIG parallels PG, although BIG is slightly below PG at all levels of PG. Our findings indicate that efforts to develop a non invasive glucose monitoring system based on skin measurements could be succesfully based on alterations in skin glucose concentrations. Our data concerning skin blister suction times could be explained by differences between patients in the mechanical strength of the epidermal–dermal junction; a parameter that might be of prognostic value in the monitoring of diabetic patients.

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