Journal of Innovative Optical Health Sciences
Vol. 3, No. 2 (2010) 81–90

DOI: 10.1142/S1793545810000903

DIABETIC INTERSTITIAL GLUCOSE IN THE SKIN TISSUE BY ATR-FTIR SPECTROSCOPY VERSUS CAPILLARY BLOOD GLUCOSE

NATALJA SKREBOVA EIKJE*
MC Professional OÜ, Võistluse 23/25-35, Tallinn, Estonia
mcproffou@gmail.com
neikje@yahoo.co.uk

Recently introduced horizontal attenuated total reflectance (HATR) Fourier transform infrared (FTIR) spectroscopy for real-time assessment and continuous monitoring of glucose biomolecules in the skin tissue directly on the patients might appear a promising alternative to interpret the activity of interstitial glucose metabolism *in vivo* by means of evaluating the dynamics of changes of glucose concentrations in interstitial fluid (IF). In the present study, *in vivo* spectra by ATR-FTIR spectroscopy were obtained post-prandially during a 120–180-minute continuous monitoring in three patients with type 2 diabetes and compared to pre-prandial spectra. In all patients with diabetes interstitial glucose levels at 1030 and 1041 cm^{-1} reflected the best relationship with blood glucose. The lag time (LT) required for glucose to diffuse from the capillary to epidermal skin tissue was calculated between 0 and 60 minutes at all measured glucose biomolecules. Data showed intra- and inter-subject variations of each glucose biomolecule, pointing to similarities and differences among interstitial glucose metabolism of the patients. Finally, the findings suggest that HATR-FTIR spectroscopy might have the potential for clinical interpretation of activity of glucose metabolism for diagnosis, management, and treatment of patients with diabetes.

Keywords: ATR-FTIR spectroscopy; skin interstitial glucose; diabetes.

1. Introduction

Measuring the concentrations of interstitial glucose in the skin tissue might challenge the dominance of glucose meters in the management of patients with diabetes. Although there are many unsolved questions about the relationships between interstitial and blood glucose levels, it can be argued that not only accurate and precise measurements of interstitial glucose levels, but also measurements of glucose activity dynamics may be more important clinically. A prime example could be persistence of impaired cognition for prolonged periods after correction of hypoglycemia, possibly related to delays in the correction of glucose concentrations in interstitial fluid (IF).[1]

Clinicians are used to interpret changes in plasma glucose, providing for them an earlier warning signal in respect of evolving hypo- and hyperglycemia, and which, of course, precede changes in interstitial glucose. Nevertheless, developing a new set of metrics to evaluate normal, as well as clinically relevant high and low, interstitial glucose levels might open for clinicians a new way in the interpretation of activity of glucose metabolism for treatment and management of the patients.[1]

*Hasleveien 26, L-702, Oslo 0571, Norway; Anne 92-44, Tartu 50705, Estonia.

Recently introduced horizontal attenuated total reflectance (HATR) Fourier transform infrared (FTIR) spectroscopy for real-time assessment and continuous monitoring of glucose biomolecules in the skin tissue directly on the patients might appear a promising alternative to interpret the activity of interstitial glucose metabolism *in vivo* by means of evaluating the dynamics of changes of glucose concentrations in IF.[2,3]

In the present study, *in vivo* spectra by ATR-FTIR spectroscopy were obtained after a meal within a 120–180-minute continuous monitoring in three patients with type 2 diabetes mellitus (DM), in comparison to spectra measured before a meal and analyzed as "fasting" (pre-prandial) of an individual. Then, differences between pre-prandial and post-prandial glucose absorbance peak levels were estimated in each patient in relationship to capillary blood glucose levels; the lag time (LT) between changes in blood and interstitial glucose levels was calculated.

2. Experimental

2.1. *ATR-FTIR spectrometer*

To perform measurements on the patients with diabetes *in vivo*, Shimadzu FTIR spectrometer (Shimadzu IRPrestige — 21/FTIR-8400S, Japan) was used with the PIKE Technologies HATR (ATR-8200 HA) accessory, commercially available for the analysis of pliable solid films. This ATR crystal is of a trapezoid shape and is 80 mm long, 10 mm wide, and 4 mm thick. The thickness of the crystal and its dimensions were carefully chosen by the manufacturer in order to maximize the signal-to-noise in the resulting spectra. The crystal was placed on the base unit by means of two-dowel location pins.[4]

The compact design of ATR-FTIR spectrometer employs a pair of transfer lenses to direct the IR beam to one end of an IR transmitting ATR crystal. A similar pair of optics directs the beam emitted from the other end of the ATR crystal to the spectrometer detector.[4]

2.2. *Spectra acquisition*

The absorbance spectra were obtained in the 700–4000 cm^{-1} region at a resolution of 4 cm^{-1}. Twenty frames of accumulation were used for the collection of each interferogram. For every subject before each group of measurements a background spectrum was obtained, so as to acquire each spectrum by ratio analysis of a scan measured from the skin area to a background scan. Left inner wrist area on the volar forearm, used as a site of measurement for all subjects, was not pre-treated before spectral acquisition.

2.3. *Spectra treatment*

Each absorbance spectrum was treated in the following sequence: (1) normalization to amide I (about 1645 cm^{-1}); (2) multiple baseline correction; (3) assignment of glucose-specific peaks at about 1030 cm^{-1}, 1041 cm^{-1}, 1080 cm^{-1}, 1118 cm^{-1}, and 1153 cm^{-1}.[2–7] (The peaks at about 1030 and 1041 cm^{-1} were always found bounded in ATR-FTIR spectra *in vivo*; therefore, in this study, they were mentioned together as 1030–1041 cm^{-1}.)

2.4. *Blood glucose test meter*

The glucose meter (SKK GluTestS, Sanwa Chemical Institute, Nagoya, Japan) was used to read a blood sample from a fingertip on a test strip inserted into a glucose meter. It digitally displays the glucose level as a number in milligrams per deciliter (mg/dL) that can be recorded for glucose monitoring.

In this study, interpretation of capillary blood glucose units obtained from the fingertip before and after a meal was similar to those used clinically (pre-prandial levels: <110 mg/dL for healthy and <126 mg/dL for diabetes; post-prandial levels (2 h): <140 mg/dL for healthy and ≥200 mg/dL for diabetes), according to WHO Diabetes Criteria in 1999.[2]

Literature reports an agreement between capillary blood glucose levels at the fingertip and from the forearm during times of blood glucose stability; therefore, these sites were comparatively used in the study.[1]

2.5. *Subjects with type 2 diabetes*

Three subjects with type 2 diabetes were measured before and after a meal (from one to three hours post-prandially) in the same time of the day, avoiding diurnal differences in blood flow in subjects.

Subject 1 (S1, age 67, male) was on insulin therapy. Subject 2 (S2, age 70, female) had had a history of type 2 diabetes for several years treated orally with metformin, changing to a diet control. Subject 3 (S3, age 69, male) had had a recurrent or persistent hyperglycemia due to type 2 diabetes,

treated with insulin injections for the last two years.

Informed consents were obtained from all measured subjects in this study. Two subjects with type 2 diabetes, S1 and S2, were measured within the same day. S3 was measured on two different days.

2.6. *Control group of healthy subjects*

Pre-prandial levels (i.e., when refrained from eating from 10 to 16 hours) of four healthy volunteers (three males (23, 24, and 60 y.o.) and one female (35 y.o.)) were used as healthy control levels versus pre-prandial levels of two patients with diabetes (S2 and S3).

Differences in post-prandial levels at two-hour assessment were studied among four healthy volunteers (three males (24, 59, 60 y.o.) and one female (35 y.o.)) and three patients with diabetes.

One healthy male of 59 years old was monitored within two hours after a meal in parallel with two patients with diabetes (S2 and S3) measured post-prandially on the same day.

3. Results

3.1. *Visual assessment of continuous monitoring of interstitial glucose versus blood glucose*

Curves of a 120-minute continuous monitoring of interstitial glucose levels of three subjects with type 2 diabetes in comparison to monitored interstitial levels of a healthy subject, considered as a control, are shown in Figs. 1(a)–1(d).

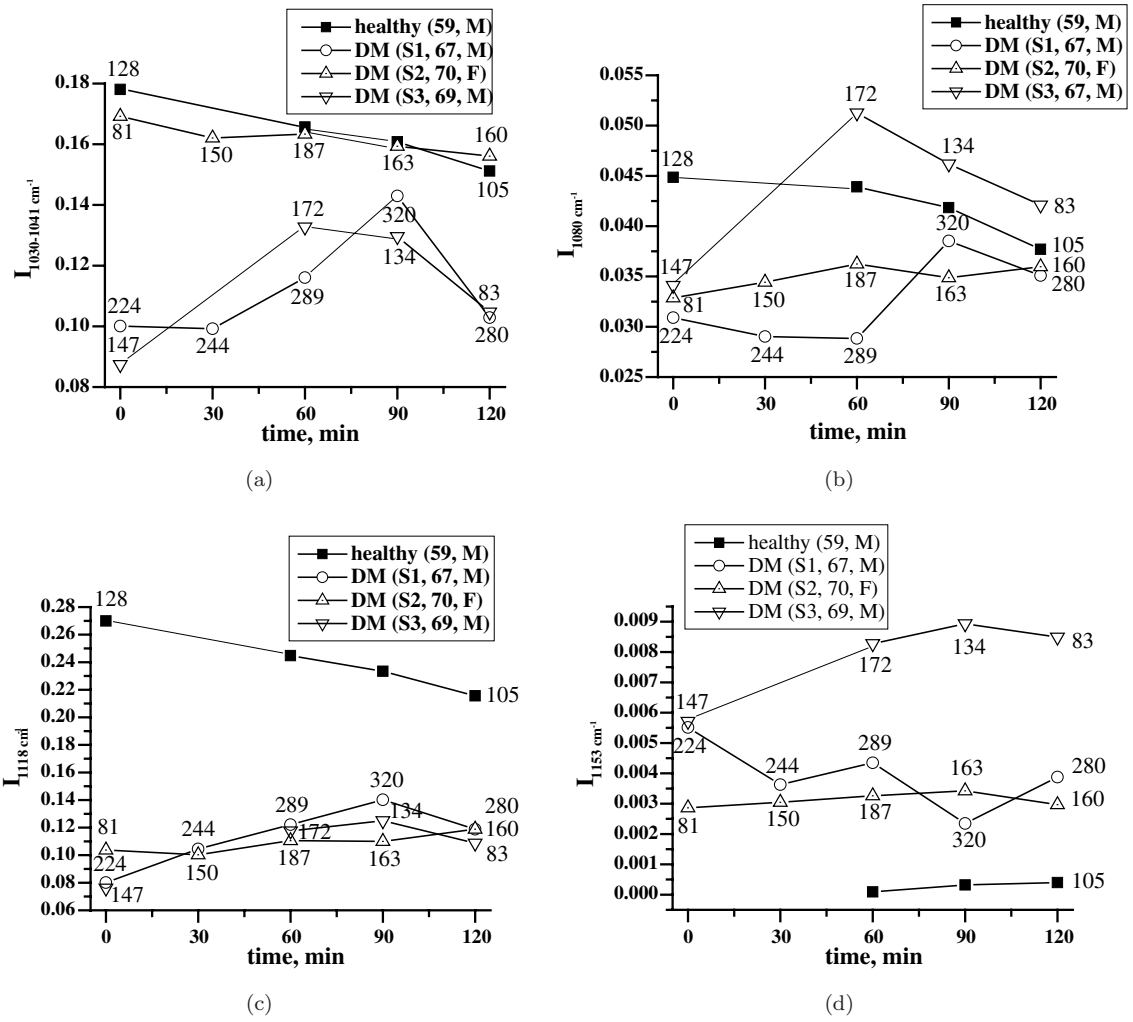

Fig. 1. A 120-minute post-prandial continuous monitoring of interstitial glucose levels at about 1030–1041, 1080, 1118, and 1153 cm^{-1} in three subjects (S1, S2, and S3) with type 2 DM and one healthy control subject ("0"-point is pre-prandial). Capillary blood glucose levels in mg/dL are shown in numbers along curves for each measured subject.

At visual assessment among all measured glucose-specific peaks interstitial glucose levels at the peak at about 1080 cm^{-1} reflected the best relationship with capillary blood glucose levels (Figs. 1(a)–1(d)). Moreover, the intensities of post-prandial interstitial glucose levels at this peak could be clearly evaluated as low for S2 (70, F, diet control), as medium for S1 (67, M, insulin therapy), and as high for S3 (69, M, insulin therapy) (Fig. 1(b)).

During a 120-minute post-prandial monitoring, peak absorbancy changes at about 1080 and 1118 cm^{-1} were constantly above pre-prandial levels in all subjects with diabetes (Fig. 1(c)), and at 1030–1041 cm^{-1} post-prandial levels were above pre-prandial levels only in two subjects with diabetes that were on insulin therapy (S1 and S3) (Fig. 1(a)).

Before a meal interstitial glucose levels at about 1030–1041, 1080, and 1118 cm^{-1} of a healthy subject were always the highest in comparison to diabetic ones (Figs. 1(a)–1(c)), but the lowest at about 1153 cm^{-1} (Fig. 1(d)). Furthermore, during a 120-minute post-prandial continuous monitoring interstitial glucose levels at about 1030–1041, 1080, and 1118 cm^{-1} of a healthy subject were gradually decreasing at all times, except at about 1153 cm^{-1} (Figs. 1(a)–1(d)).

Not unexpectedly, apparent similarity between a healthy subject and a subject with diabetes on a diet control was observed in curvatures and levels at about 1030–1041 cm^{-1} (Fig. 1(a)) and in curvatures at about 1153 cm^{-1} (Fig. 1(d)). Two subjects with diabetes being on insulin therapy (S1 and S3) showed similar curve patterns at about 1030–1041 and 1118 cm^{-1} (Figs. 1(a) and 1(c)).

3.2. *The LT between changes in blood and interstitial glucose levels*

The time required for glucose to diffuse from the capillary to the tissue plays an important role in the LT between changes in plasma and interstitial glucose levels. Therefore, the LT of interstitial glucose values was studied in relationship with the LT of capillary blood glucose values during a 120–180-minute post-prandial monitoring by ATR-FTIR spectroscopy in each subject with type 2 diabetes.

3.2.1. *Subject* 1 (67*, M, insulin therapy*)

A steep increase in interstitial glucose values at about 1030–1041, 1080, and 1118 cm^{-1} took place within 90 minutes, similar to the time of increment shown by capillary blood glucose values (Figs. 2(a)–2(c)). A two-level (biphasic) increase in interstitial glucose values at about 1153 cm^{-1} was observed at 60 and 120 minutes (Fig. 2(d)). Accordingly, calculated LT between changes in blood and interstitial glucose levels was 0 minutes at 1030–1041, 1080, and 1118 cm^{-1}, 30′ minutes at about 1153 cm^{-1} (Figs. 2(a)–2(d), Table 1).

3.2.2. *Subject* 2 (70*, F, diet control*)

A 60-minute increment for capillary blood glucose values was similar to the time for slightly increased interstitial glucose levels only at 1030 cm^{-1} (Fig. 3(a)). At about 1153 cm^{-1}, the time of increment was 90 minutes (Fig. 3(d)). A biphasic increase at 60 and 120 minutes was observed at about 1180 and 1118 cm^{-1} (Figs. 3(b) and 3(c)). Estimated LT between changes in blood and interstitial glucose levels was 0 minutes at about 1030 cm^{-1}, 0 and 60 minutes at about 1080 and 1118 cm^{-1}, and 30 minutes at about 1153 cm^{-1} (Table 1).

3.2.3. *Subject* 3 (69*, M, insulin therapy*)

3.2.3.1. Day 1 measurement

A medium increase in interstitial glucose levels was observed within 60 minutes at 1030 cm^{-1} (Fig. 4(a)), within 120 minutes at about 1080 and 1153 cm^{-1} (Figs. 4(b) and 4(d)). A biphasic increase in interstitial glucose values at about 1118 cm^{-1} was observed at 60 and 180 minutes (Fig. 4(c)).

Revealed LT between changes in blood and interstitial glucose levels was 0 minutes at 1030 cm^{-1}, 60 minutes at about 1080 and 1153 cm^{-1}, and 0 and 120 minutes at about 1118 cm^{-1} (Figs. 4(a)–4(d), Table 1).

3.2.3.2. Day 2 measurement

A steep increase in interstitial glucose levels was observed within 60 minutes at about 1030 and 1080 cm^{-1} (Figs. 5(a) and 5(b)) and within 120 minutes at about 1118 cm^{-1} (Fig. 5(c)). Estimated LT between changes in blood and interstitial glucose levels was 0 minutes at about 1030 and 1080 cm^{-1} and 60 minutes at 1118 cm^{-1} (Figs. 5(a)–5(c), Table 1).

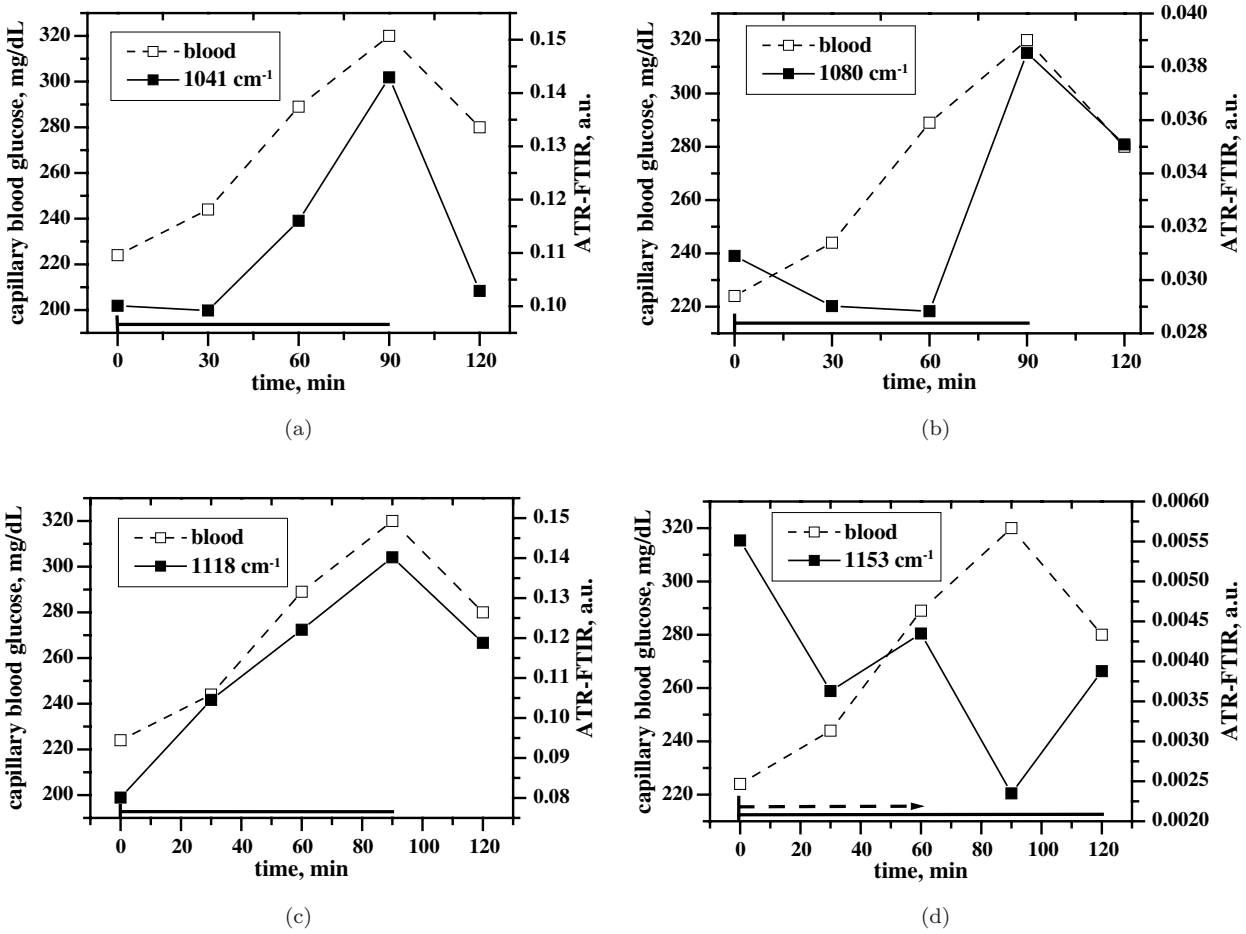

Fig. 2. A 120-minute post-prandial continuous monitoring at glucose-specific peaks at about 1041, 1080, 1118, and 1153 cm^{-1} for S1 with type 2 diabetes is shown by solid lines. Curves of capillary blood glucose levels are shown by dashed lines. "0"-point is pre-prandial. The time of increment for each interstitial glucose value is indicated by a solid line along x-axis. In case of a biphasic increase, a first phase is indicated by a dashed line along x-axis.

Table 1. Estimated LT between changes in capillary blood glucose and interstitial glucose values within a 120-minute post-prandial continuous monitoring in three subjects with type 2 diabetes (S1, S2, and S3) by HATR-FTIR spectroscopy *in vivo*.

Subjects	1030–1041 cm^{-1}	1080 cm^{-1}	1118 cm^{-1}	1153 cm^{-1}
S1	$0'$	$0'$	$0'$	$30'$
S2	$0'$	[$0'$ & $60'$]	[$0'$ & $60'$]	$30'$
S3 (D1)	$0'$	$60'$	$0'$	$60'$
S3 (D2)	$0'$	$0'$	$60'$	—

3.2.3.3. Day-to-day measurements

Figures 6(a)–6(c) demonstrates day-to-day measurements for S3 with diabetes. Curves of interstitially monitored glucose during 120 minutes on days 1 and 2 showed similarity at about 1080 and 1030–1041 cm^{-1}. On different days of measurements, a 60-minute increment for interstitial glucose levels at 1030–1041 cm^{-1} was similar to capillary blood glucose levels.

Calculated LT between changes in blood and interstitial glucose levels can be seen in Table 1.

3.3. *Control group of healthy subjects*

Only at about 1080 cm^{-1}, the pre-prandial levels of randomly measured healthy volunteers were low in comparison to high pre-prandial levels of two subjects with DM (S1 and S2) and showed the best difference among all interstitially measured glucose biomolecules. The same tendency was seen in subjects with impaired glucose tolerance (pre-diabetes) (unpublished data).[7]

At two-hour assessment post-prandial differences between randomly measured four healthy volunteers and three patients with diabetes differed only and a little at about 1080 cm^{-1}.

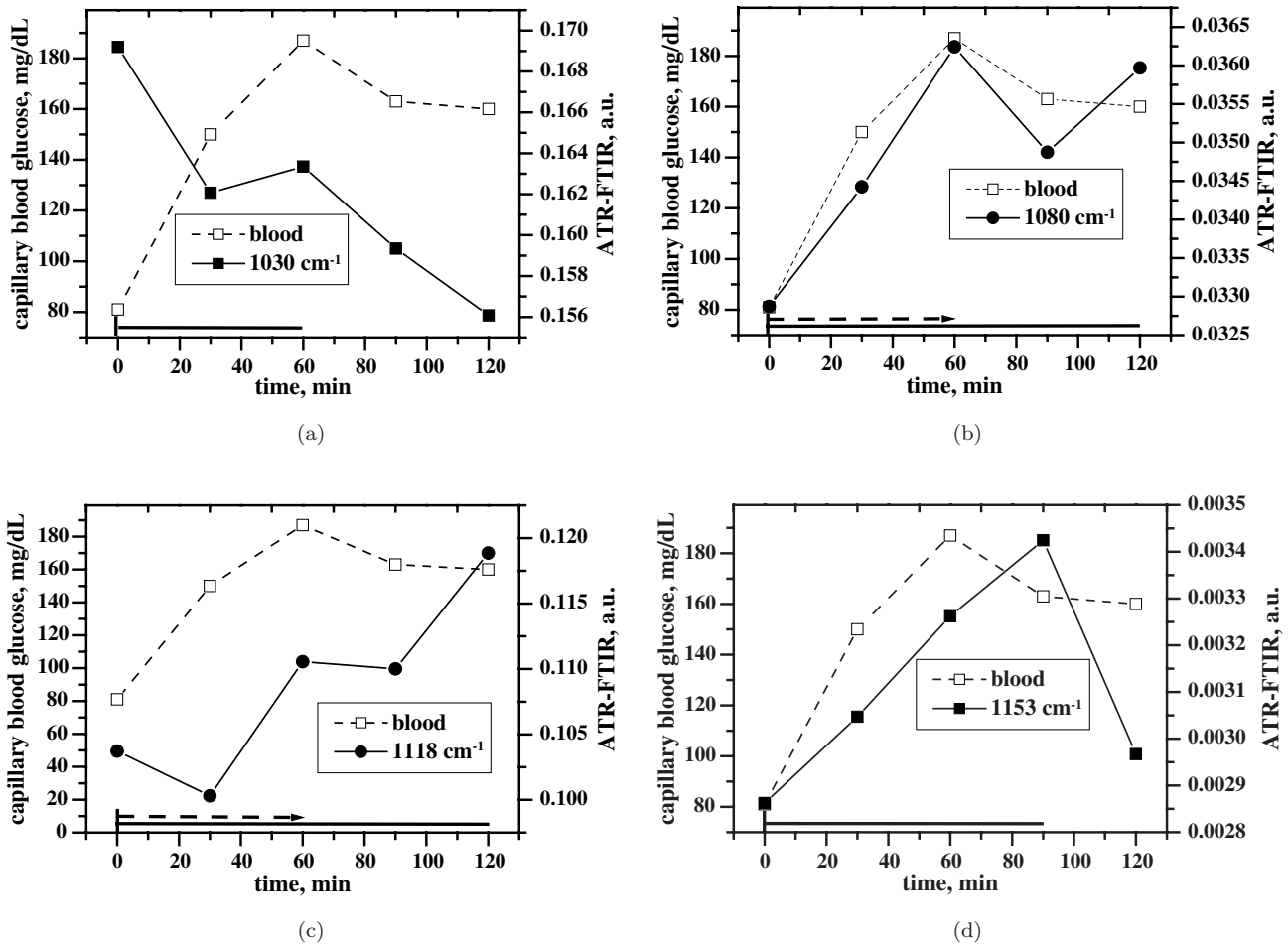

Fig. 3. A 120-minute post-prandial continuous monitoring at glucose-specific peaks at about 1030, 1080, 1118, and 1153 cm^{-1} for S2 with type 2 diabetes is shown by solid lines. Curves of capillary blood glucose levels are shown by dashed lines. "0"-point is pre-prandial. The time of increment for each interstitial glucose value is indicated by a solid line along x-axis. In case of a biphasic increase, a first phase is indicated by a dashed line along x-axis.

4. Discussion

In the present study, ATR-FTIR spectroscopy was used to comparatively investigate post-prandial spectra in the glucose-specific region obtained *in vivo* in three patients with type 2 diabetes in relationship with capillary blood glucose. The results on pre- and post-prandial measurements by this spectroscopy method provided insight to the glucose dynamics between the blood and IF compartments in the upper layer of skin tissue by means of measuring the LT. In all subjects during a 120-minute continuous monitoring, the best relationship with blood glucose was reflected by interstitial glucose levels at 1030–1041 cm^{-1} with the LT of 0 minute.

The range of LT between changes in blood and epidermal interstitial glucose levels in three measured patients with DM was in agreement with that reported in the literature, i.e., the LT range of 0–45 minutes in subcutaneous tissue measured by other methods.[1] The LT described by HATR-FTIR spectroscopy here is longer and ranges from 0 to 60 minutes (see Table 1), which can be explained by slower kinetics in the epidermis than in subcutaneous tissue.

A biphasic increase in interstitial glucose values was noticed in all subjects with type 2 diabetes; however, at the spectral peaks, only at about 1080 and 1118 cm^{-1} with the LT of 0 minute and/or 60 minutes that possibly characterize the physiological LT and the delayed LT due to other reasons (unpublished data).[7]

The results also demonstrate variations in the LT between glucose-specific molecules within one subject, except at 1030–1041 cm^{-1}, suggesting individuality of the activity of interstitial glucose metabolism and related characteristic performance

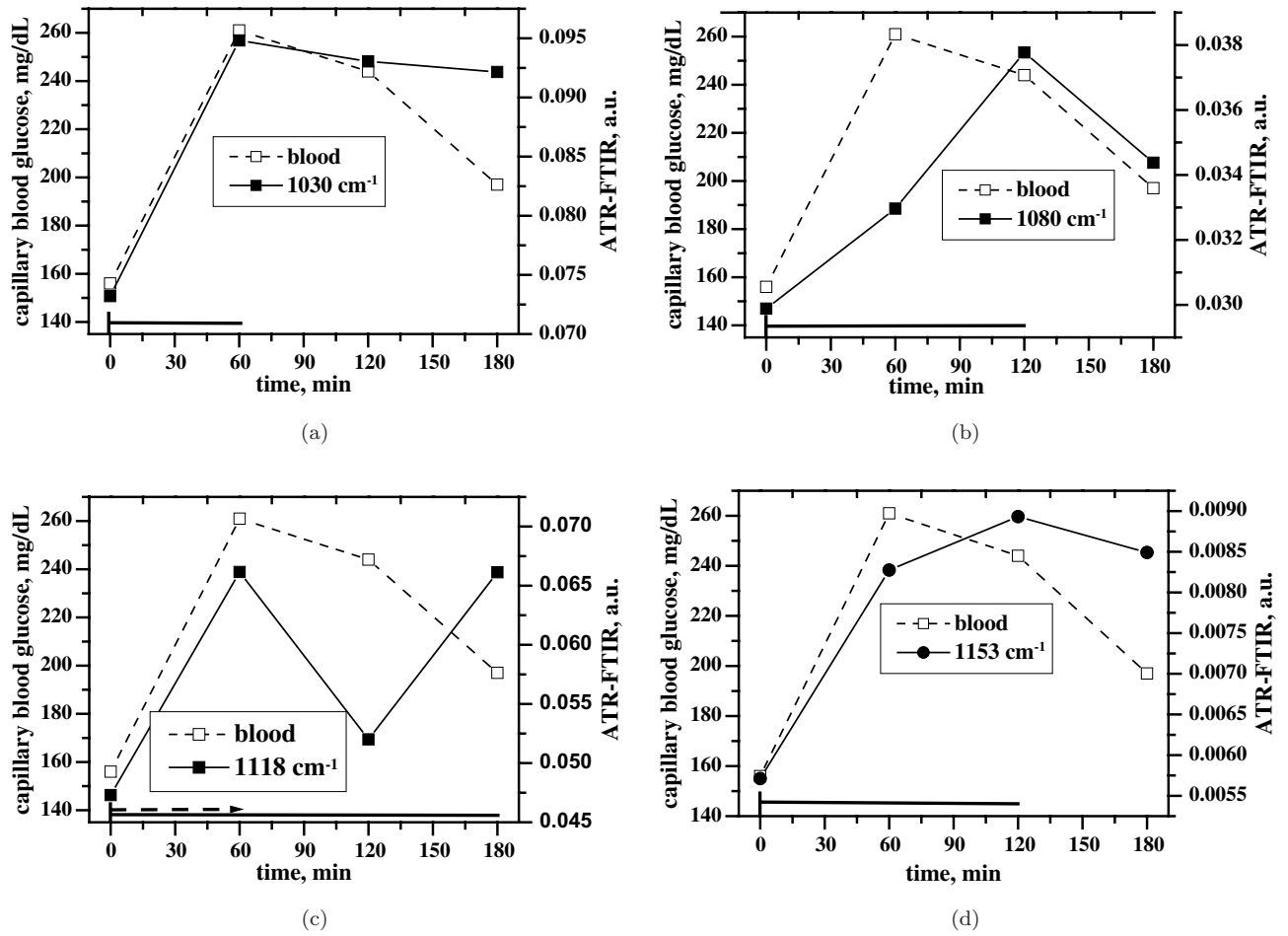

Fig. 4. A 180-minute post-prandial continuous monitoring at glucose-specific peaks at about 1030, 1080, 1118, and 1153 cm^{-1} for S3 with type 2 diabetes (day 1) is shown by solid lines. Curves of capillary blood glucose levels are shown by dashed lines. "0"-point is pre-prandial. The time of increment for each interstitial glucose value is indicated by a solid line along x-axis. In case of a biphasic increase, a first phase is indicated by a dashed line along x-axis.

of each glucose biomolecule. Moreover, observed differences in the LT between glucose biomolecules when measured on different days in the same subject may be further important in the interpretation of physiological (meals, physical activity, emotional state, etc.) and pathophysiological (therapy and stability) variations of glucose metabolism. Additionally, a time of increment of interstitial glucose levels varied for each glucose biomolecule within one subject, being only at 1030–1041 cm^{-1} similar to a time of increment of capillary blood glucose levels.

What is unclear is whether such variability is due to individual tissue glucose utilization, prevailing insulin levels, device specifics, or anything else. Interstitial glucose concentrations are known to be distributed in a heterogeneous concentration throughout extracellular fluid. *In vivo* glucose uptake differs from *in vitro* in that blood flow can determine the rate of glucose delivery to tissue. Unknown and unrecognized factors not present *in vitro* may modify glucose uptake and output, and *in vivo* experiments have their own artifacts.

Blood flow to the skin is controlled by many factors, including autonomic nervous system, temperature, hormonal changes during menstrual cycle for females, and chemical inputs.

Dermis comprises many arterioles, venules, and capillaries, including a deep vascular plexus interfacing dermis and the subcutaneous tissue. Another vascular plexus important for interpretation of data in this study is located 0.3–0.6 mm from the skin surface and is formed by the feeding vessels arising from the deep vascular plexus. It supplies the blood flow to the dermis and epidermis with the help of small capillary loops branching from the superficial plexus.

IF constitutes approximately 45% of the volume fraction of human skin, with blood vessels

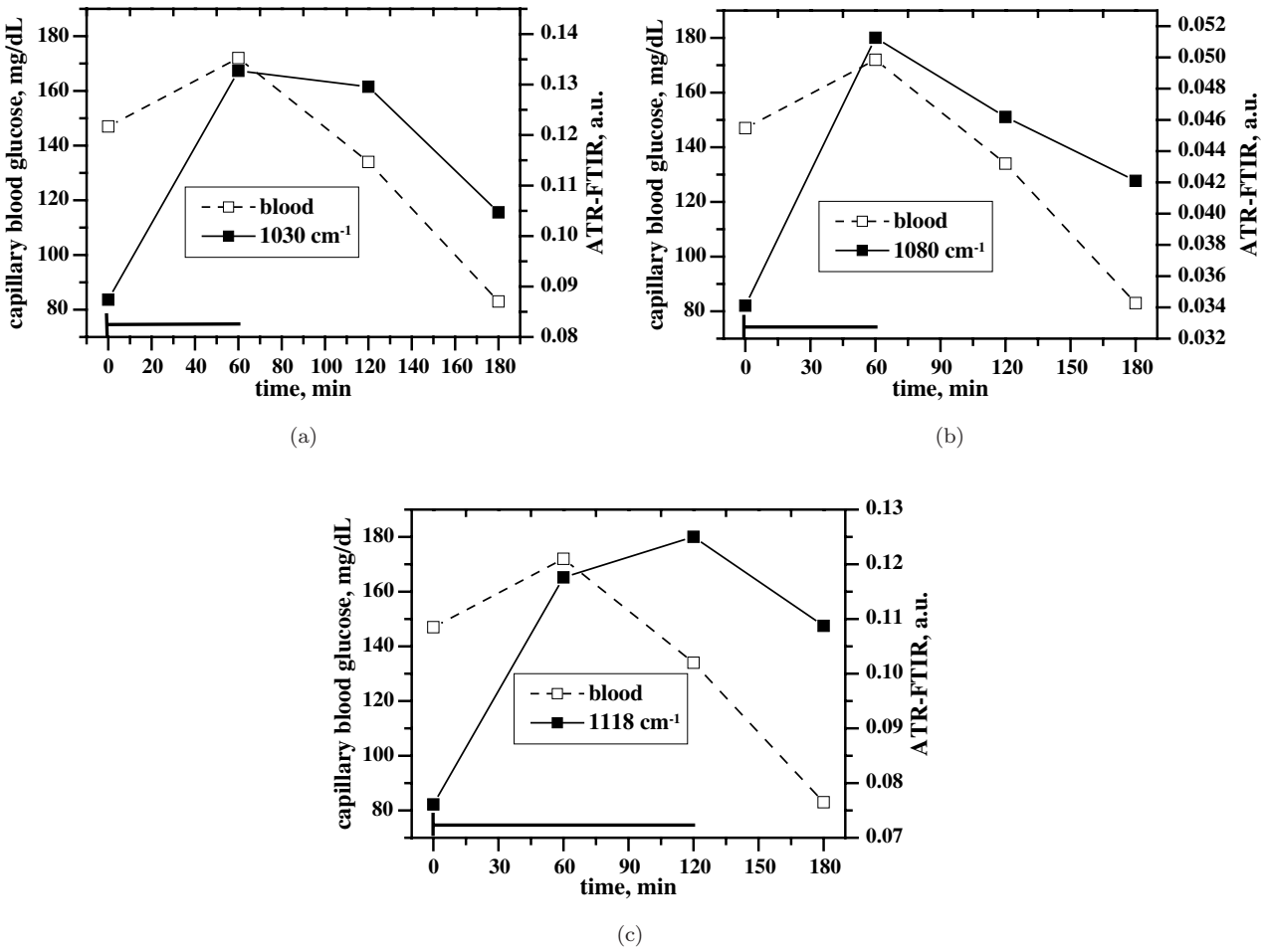

Fig. 5. A 180-minute post-prandial continuous monitoring at glucose-specific peaks at about 1030, 1080, and 1118 cm^{-1} for S3 with type 2 diabetes (day 2) is shown by solid lines. Curves of capillary blood glucose levels are shown by dashed lines. "0"-point is pre-prandial. The time of increment for each interstitial glucose value is indicated by a solid line along x-axis. In case of a biphasic increase, a first phase is indicated by a dashed line along x-axis.

contributing approximately 5% of the skin volume. IF bathes the cells and feeds them with nutrients, including glucose, by providing a corridor between the capillaries and the cell. Glucose is transferred from the capillary endothelium to the IF by simple diffusion across a concentration gradient without the need of an active transporter. Blood flow to the area dictates the amount of glucose delivered. Interstitial glucose values are determined by the rate of glucose diffusion from plasma to the IF and the rate of glucose uptake by subcutaneous tissue cells. Thus, the metabolic rate of the adjacent cells and other factors, such as insulin, affecting glucose uptake by cells, the glucose supply from the blood vessel, blood flow to the area, and the permeability of the capillary that can be altered by many factors, including nerve stimulation, influence the interstitial glucose levels.

Findings of this study show that the metabolic activity of glucose uptake in the epidermal skin tissue can be monitored by HATR-FTIR spectroscopy. Although the epidermis is an avascular epithelial membrane, it has enzymes with glucose-metabolizing effect. Moreover, glucose is formed from the breakdown of ceramide at the stratum granulosum–corneum interface.

Literature describes almost no data on epidermal interstitial glucose levels, because of the complexity of direct sampling methods, including insertion of wicks, blister formation, lymph sampling, and ultrafiltration.[1] Current HATR-FTIR spectroscopy method has the advantage due to its non-invasiveness to evaluate real-time dynamical changes of IF glucose concentrations.

Data showed intra- and inter-subject variations of each glucose biomolecule, pointing to similarities and differences between patients' interstitial glucose

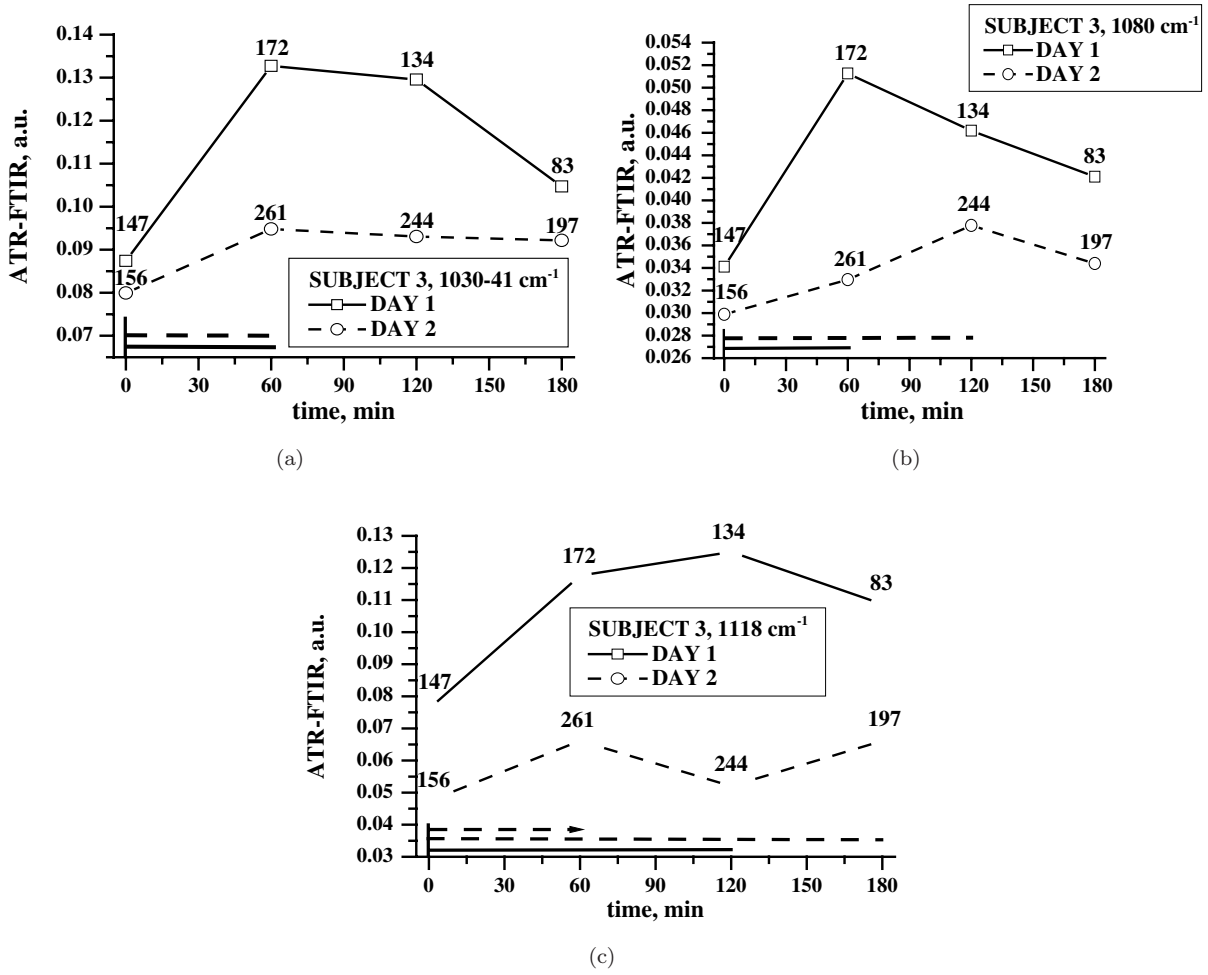

Fig. 6. A 180-minute post-prandial continuous monitoring at glucose-specific peaks at about 1030, 1080, 1118, and 1153 cm^{-1} for S3 with type 2 diabetes on days 1 and 2. Capillary blood glucose levels in mg/dL are shown in numbers along curves for each measured subject. "0"-point is pre-prandial. The time of increment for each interstitial glucose value is indicated by a solid line along x-axis. In case of a biphasic increase, a first phase is indicated by a dashed line along x-axis.

metabolism. Finally, findings suggest that HATR-FTIR spectroscopy might have a potential for clinical interpretation of activity of glucose metabolism for diagnosis, management, and treatment of patients with diabetes.

Acknowledgments

The author is grateful to Prof K. Aizawa and Prof T. Sota from Department of Electrical Engineering at Waseda University and to Prof M. Kanazawa from the Third Department of Internal Medicine at Tokyo Medical University in Japan for being volunteers and for their help with subjects for this study. The author is also grateful to JSPS (Japanese Society for Promotion and Science) for financial support as a postdoctoral fellow for a period between 2006 and 2008.

The author is very much thankful to Prof V. V. Tuchin from Saratov State University in Russia for the invitation to present these data at Saratov Fall Meeting on September 21, 2009.

References

1. E. Cengiz, W. V. Tamborlane, "A tale of two compartments: Interstitial versus blood glucose monitoring," *Diabetes Technol. Ther.* **11**(1), S11–S15 (2009).
2. N. S. Eikje, K. Aizawa, T. Sota, Y. Ozaki, S. Arase, "Identification and characterization of skin biomolecules for drug targeting and monitoring by vibrational spectroscopy," *TOMCJ.* **2**, 38–48 (2008).
3. N. S. Eikje, T. Sota, K. Aizawa, "Cutaneous approach towards clinical and pathophysiological aspects of hyperglycemia by ATR-FTIR spectroscopy," in *Diagnostic Optical Spectroscopy in Biomedicine IV*, D. Schweitzer, M. Fitzmaurice, Eds., Proceedings of

SPIE, the International Society for Optical Engineering, Washington, USA **6628**, 66281M (2007).
4. Pike Technologies Inc., Instrumentation (from Installation and User Guide).
5. C. Inada, M. Kanazawa, K. Aizawa, "Measurement of glucose concentrations by infrared spectroscopy method," *Tokyo Medical University Journal* **61**(2), 146–153 (2003).
6. R. K. Dukor, Vol. 5 in *Handbook of Vibrational Spectroscopy*; J. M. Chalmers, P. R. Griffiths, Eds., John Wiley & Sons Ltd., pp. 3335–3361 (2002).
7. N. S. Eikje, "*In vivo* interstitial glucose characterization and monitoring by ATR-FTIR Spectroscopy," to be published.