Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2902003 A1 2013/09/19

(21) 2 902 003

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2013/02/21
(87) Date publication PCT/PCT Publication Date: 2013/09/19
(85) Entrée phase nationale/National Entry: 2015/08/20
(86) N° demande PCT/PCT Application No.: EE 2013/000002
(87) N° publication PCT/PCT Publication No.: 2013/135249
(30) Priorité/Priority: 2012/02/21 (US61/596,250)

(51) Cl.Int./Int.Cl. *A61B 5/145* (2006.01),
A61B 5/00 (2006.01)

(71) Demandeurs/Applicants:
SKREBOVA, IRINA, EE;
MC PROFESSIONAL OU, EE;
EIKJE, NATALJA, NO

(72) Inventeur/Inventor:
EIKJE, NATALJA, NO

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : FORMULE ET PROCEDE POUR SURVEILLER UNE REPONSE METABOLIQUE INDIVIDUELLE ET POUR GENERER UNE METRIQUE MEDICALE PREDICTIVE

(54) Title: A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE AND FOR GENERATING PREDICTIVE MEDICAL METRICS

(57) Abrégé/Abstract:
Provided is a formula and method for monitoring individual metabolic response that involve calculation of the lag/latency time (LT) for the peak levels of measured a variety of glucose values by HATR-FTIR (horizontally attenuated total reflection Fourier transform infrared) spectroscopy and the LT for the peak level of capillary blood glucose (CBG), with subsequent calculation of the LT changes between them. Obtained meaningful time-dependent and dose-dependent glucose values and their LT changes characterize glycemic variability (GV) in a qualified subject, that can be used to predict the patient's risk of hyperglycemia, to stage Type II diabetes and, in general, to be considered as a new metrics of assessing the quality of metabolic control.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 September 2013 (19.09.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/135249 A3

(51) **International Patent Classification**:
A61B 5/145 (2006.01) *A61B 5/00* (2006.01)

(21) **International Application Number**:
PCT/EE2013/000002

(22) **International Filing Date**:
21 February 2013 (21.02.2013)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
61/596,250 21 February 2012 (21.02.2012) US

(71) **Applicants**: **MC PROFESSIONAL OÜ** [EE/EE]; Võistluse 23-35, Tallinn 10132, EE (EE). **SKREBOVA, Irina** [EE/EE]; Võistluse 23/25-35, Tallinn 10132 (EE).

(72) **Inventor; and**
(71) **Applicant** : **EIKJE, Natalja** [EE/NO]; Urnesveien 11, Tysvaervaag 5565 (NO).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) **Date of publication of the international search report**:
6 March 2014

(54) **Title**: A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE AND FOR GENERATING PREDICTIVE MEDICAL METRICS

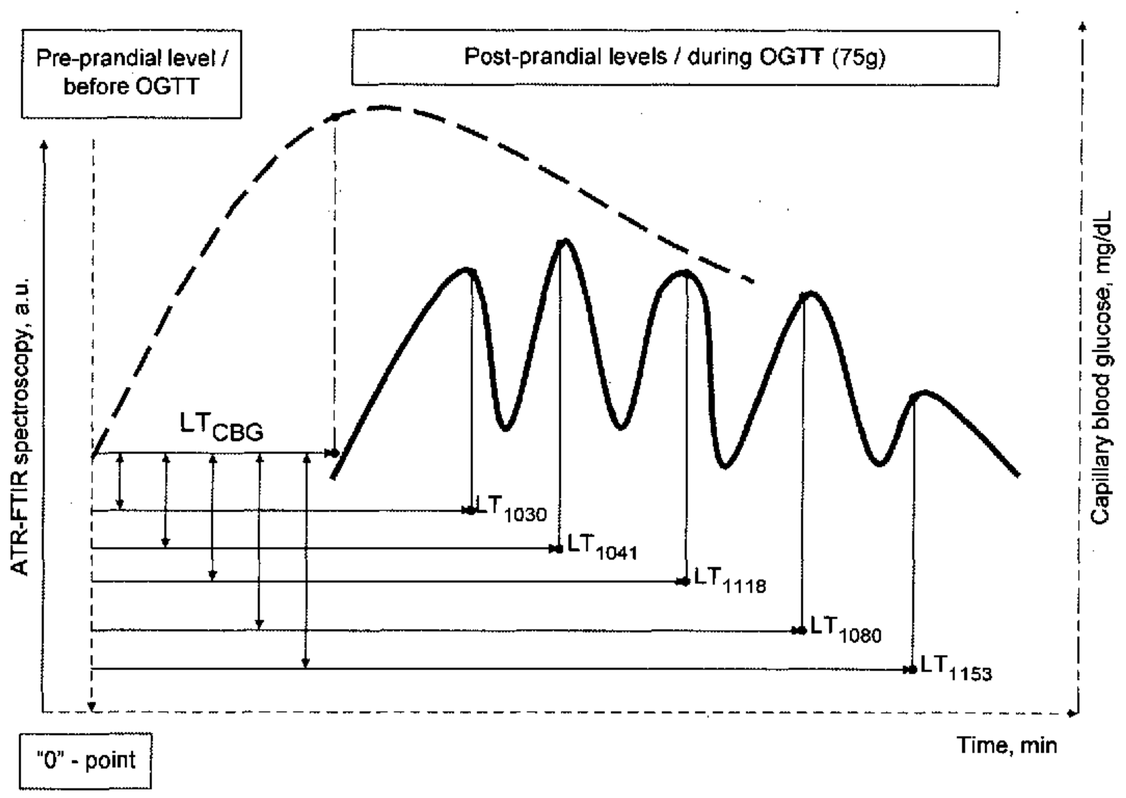

Fig. 1

(57) **Abstract**: Provided is a formula and method for monitoring individual metabolic response that involve calculation of the lag/latency time (LT) for the peak levels of measured a variety of glucose values by HATR-FTIR (horizontally attenuated total reflection Fourier transform infrared) spectroscopy and the LT for the peak level of capillary blood glucose (CBG), with subsequent calculation of the LT changes between them. Obtained meaningful time-dependent and dose-dependent glucose values and their LT changes characterize glycemic variability (GV) in a qualified subject, that can be used to predict the patient's risk of hyperglycemia, to stage Type II diabetes and, in general, to be considered as a new metrics of assessing the quality of metabolic control.

WO 2013/135249 A3

A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE AND FOR GENERATING PREDICTIVE CLINICAL METRICS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The invention relates generally to monitoring metabolic response in a qualified subject, that comprises the step of consecutive performance of a plurality of measurements and further calculations of glucose levels, obtained by HATR-FTIR (horizontally attenuated total reflection Fourier transform infrared) spectroscopy. More particularly, the invention further relates to a formula for calculating the lag/latency time (LT) changes between measured in capillary blood the peak glucose level and by HATR-FTIR spectroscopy the peak levels of glucose values after achieved response in test subjects' from the ingestion of quantified amount of pure glucose and post-prandially, allowing to assess stability of metabolic control in healthy, prediabetic and diabetic subjects. Furthermore, qualitative and quantitative interpretation of glycemic variability (GV) by using a formula for calculation of the LT changes, based on a method of simultaneous assessment of 5 glucose-specific values by HATR-FTIR spectroscopy, helps to generate parameters, their characteristics, patterns and, thus, to establish suitable metrics for GV in the clinical practice.

DESCRIPTION OF THE RELATED ART

There are numerous published metrics to quantify different aspects of GV, but still there is no method of GV that is accepted in the clinical practice of diabetes care.

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 the activity of glucose metabolism for diagnosis, treatment and management of the patients with diabetes mellitus and carbohydrate metabolism disorders.

The technique of using ATR-FTIR spectroscopy has been long known for non-invasive glucose measurement, but through oral mucosa. The drawbacks of such measurements included glucose contamination of the measurement site by food and a highly variable rate of saliva.

Attempts have been made to demonstrate a proof of HATR-FTIR spectroscopy technique to detect, characterize and verify interstitial origin of glucose-specific signals at about 1030, 1041, 1080, 1118 and 1153 cm^{-1} in the skin of healthy, prediabetic and diabetic subjects during OGTT (Oral Glucose Tolerance Test) and post-prandially, randomly and on mornings, i.e. fasting measurements.

Carbohydrate intolerance is one of the major criteria for a diagnosis of diabetes mellitus. OGTT employs ingested carbohydrate in a predetermined form and an amount to quantify a test subject's response to a resulting glucose challenge. However, this test is only concerned with

the peak blood level of glucose, but not with the rate of change in glucose levels or the amount of time it takes for glucose levels to fluctuate from a high point to a lower point.

The medical significance of blood glucose fluctuations, i.e. frequency and magnitude, has been a controversial topic and the subject of extensive research, proving that GV can be used to describe a general risk of hyperglycemia over long periods of time, or when focused on events of short duration, such as meals or overnight.

Since glucose on the surface of the skin and within the stratum corneum has been considered as a source for extraneous glucose contamination during testing of invasive glucose monitoring devices, there was not found any direct method for in vivo glucose molecule(s) characterization and monitoring directly on healthy, prediabetes and diabetes subjects.

SUMMARY OF THE INVENTION

An object of the present invention is to provide the user with in vivo method for monitoring individual metabolic response and a formula for qualitative and quantitative characterization of GV, in order to further recognize patterns from the patients data for establishing predictive clinical metrics.

Specifically, the invented method is based on a formula for calculating the LT changes between the LT measured for the peak of CBG and the LT for the peaks of epidermally measured interstitial glucose levels by HATR-FTIR spectroscopy on healthy, prediabetic and diabetic subjects under OGTT with different doses (low, medium, high) and post-prandially, applicable for staging Type 2 diabetes and assessing diabetes control, including estimation of intra-day and intra-week risks, as well a general risk of hyperglycemia. More, the invented formula can be further applied for screening of disorders of glucose metabolism, such as impaired glucose tolerance and diabetes mellitus by means of evaluation of the time required for glucose to diffuse from the capillary to the living skin tissue. In addition, the invented formula can be also served for individual calibration of obtained glucose profiles by HATR-FTIR spectroscopy in a dynamic time-dependent manner in a qualified subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 schematically presents a formula and method for calculation of the LT changes between the LT for the peak of CBG and for the peak of each epidermally measured interstitial glucose level at about 1030, 1041, 1080, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy in a qualified subject.

Figure 2 A-D presents differences between GV obtained between CBG values (shown by dashed lines) and each glucose value (shown by solid lines) at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in Subject 1 with Type 2 diabetes. The time of increment for each

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.

Figure 3 A-D presents differences between GV obtained between CBG values (shown by dashed lines) and each glucose value (shown by solid lines) at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in Subject 2 with Type 2 diabetes. The time of increment for each 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.

Figure 4 A-D presents differences between GV obtained between CBG values (shown by dashed lines) and each glucose value (shown by solid lines) at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in Subject 3 with Type 2 diabetes. The time of increment for each 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.

Figure 5 A-C presents a comparative dose-dependent GV during monitoring of metabolic response by HATR-FTIR spectroscopy at about 1030, 1041, 1118 and 1153 cm^{-1} during OGTT at different doses, at 5 g (low), 20 g (medium) and 75 g (high), in a healthy subject (Subject 4).

Figure 6 A-C presents a comparative dose-dependent GV during monitoring of metabolic response by HATR-FTIR spectroscopy simultaneously shown at about 1030, 1041, 1118 and 1153 cm^{-1} during OGTT at different doses, at 5 g (low), 20 g (medium) and 75 g (high), in Subject 3 with Type 2 diabetes.

Figure 7 A-C illustrates examples of characteristic differences in the levels of glucose values and wavenumber shifts between a healthy subject (Subject 4) and a subject with Type 2 diabetes (Subject 3) under OGTT with different doses.

Figure 8 A-F illustrates a variety of examples of GV simultaneously shown at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy in 2 Type 2 diabetes (Subject 3) under OGTT with 75 g and 20 g; in a prediabetic subject (Subject 5) under OGTT with 75 g and under OGTT with 75 g in 3 healthy subjects (Subject 1, Subject 3, Subject 4).

Figure 9 A-D presents GV simultaneously shown at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in 3 patients (Subjects 1-3) with Type 2 diabetes, in comparison to a healthy subject (Subject 5).

Figure 10 A-C presents day-to-day variations on GV during a 120-minute post-prandial monitoring of individual metabolic response in Subject 3 with Type 2 diabetes on 2 consecutive days, comparatively shown at about 1030, 1041, 1080 and 1118 cm^{-1} by HATR-FTIR spectroscopy.

Figure 11 A-D comparatively presents GV during monitoring of individual metabolic response in

3 healthy subjects (Subjects 1, 2, 4), in 1 subject as a suspect of having impaired glucose tolerance, i.e. prediabetes (Subject 3), and in Subject 3 with Type 2 diabetes by HATR-FTIR spectroscopy.

BRIEF DESCRIPTION OF THE TABLES

Table 1. LT changes estimated within a 120-minute post-prandial monitoring of metabolic response in 3 subjects with Type 2 diabetes (S1, S2 and S3) by HATR-FTIR spectroscopy.

Table 2. LT changes between the LT for the peak of CBG and the LT peak for each measured glucose level at about 1030, 1041, 1118 and 1153 cm^{-1}, obtained in a healthy subject during OGTT at different doses (5 g, 20 g, 75 g).

Table 3. LT changes between the LT for the peak of CBG and the LT peak for each measured glucose level at about 1030, 1041, 1118 and 1153 cm^{-1}, obtained in a diabetic subject during OGTT at different doses (5 g, 20 g, 75 g).

Table 4. LT changes under OGTT with 75 g., i.e. clinical, a healthy subject vs. a subject with Type 2 diabetes.

Table 5. LT changes under OGTT with 20 g., i.e. clinical, a healthy subject vs. a subject with Type 2 diabetes.

Table 6. LT changes estimated for the same subject with Type 2 diabetes on 2 consecutive days.

Table 7. OGTT (75 g)., i.e. clinical OGTT, demonstrate estimated LT changes for healthy, prediabetic and diabetic subjects.

DETAILED DESCRIPTION OF THE EMBODIMENT

As described further herein, in vivo glucose spectral measurements from the skin surface of the inner wrists of measured subjects have been performed on a commercially available FT-IR spectrometer (Shimadzu IRPrestige - 21/8400S, Japan), that measures the absorbance spectra in the 700-4000 cm^{-1} region at a resolution of 4 cm^{-1}, using 20 frames of accumulation to collect interferogram. Non-invasive glucose monitoring is achieved by tight contact of the measured site with a specially designed flat-plated prism with a mounted ATR crystal for the PIKE Technologies Horizontal accessory (ATR-8200 HA). This horizontal accessory is of a trapezoid shape with carefully chosen dimentions by a manufacturer (80 x 10 x 4 cm), in order to maximize S/N ratio in the measured spectra.

A portable glucosemeter (SKK GluTestS, Sanwa Chemical Institute, Nagoya, Japan) has been used for CBG determinations in mg/dL.

Noninvasive spectroscopic interstitial glucose monitor to an individual necessitates a calibration. Generating such a calibration requires reference CBG values that are uncorrelated to sampling factors. The invented formula provides a method to calibrate dynamic measurements of

interstitial glucose values in vivo by HATR-FTIR spectroscopy, based on referenced CBG values, that are described in Figure 1.

A test subject's CBG values are controlled or manipulated through the oral ingestion of carbohydrate, i.e. meals, and/or through oral consumption of dissolved in water pure glucose at different determined doses (f.e. 5 g, 20 g, 75 g) in such a way that the changes of the targeted glucose profiles of Figures 1-4 are reproduced by the subject's own glucose profiles. Thus, since the subject's CBG is under active control, the influence of other sampling factors are eliminated.

Steps of the invented formula and method:

- performing reference CBG measurements at pre-determined intervals prior to spectral acquisition by HATR-FTIR spectroscopy
- gathering *in vivo* HATR-FTIR spectra from the forearm of a tested subject at predetermined intervals in the 700-4000 cm^{-1} region with further spectra normalization to amide I, at about 1650 cm^{-1}, always after a background scan collection by HATR-FTIR spectroscopy
- multiple baseline correction of the 1000-1180 cm^{-1} region with assignment of glucose-specific peaks at about 1030 cm^{-1}, 1041 cm^{-1}, 1118 cm^{-1} and 1153 cm^{-1}, where the peaks at about 1030 cm^{-1} and 1041 cm^{-1} are always mentioned together
- subject's forearm repositioning after each measured spectrum for avoidance of hydration effect
- manipulating a subject's capillary blood glucose levels in order to obtain meaningful time-dependent LT's and their changes by subject himself/herself under OGTT with different doses (5 g, 20 g, 75 g), post-prandially, or under any other screening metabolic test
- manipulating a subject's glucose values by HATR-FTIR such those produce meaningful time-dependent changes in the levels of CBG and in the levels of glucose values at about 1030, 1041, 1118 and 1153 cm^{-1} by subject himself/herself under OGTT with different doses (5 g, 20 g, 75 g), post-prandially, or under any other screening metabolic test
- calculation of the LT changes between the estimated LT for the peak of CBG and the estimated LT for the peaks of each glucose value at about 1030, 1041, 1118 and 1153 cm^{-1} measured by HATR-FTIR spectroscopy

The invention utilizes the targeted glucose profiles by HATR-FTIR spectroscopy schematically presented in Figure 1. "0"- point is pre-prandial, i.e. fasting, and/or before OGTT.

The CBG values and the spectral measurements furnish a data set upon which a calculation of the LT changes is made by using Equation 1, and which a calibration is also based in a qualified subject.

Equation 1 utilizes only parameters described in Figure 1:

LT changes=LT(CBG peak level) - LT(HATR-FTIR peak level(s) (1030/1041/1080/1118/1153 cm^{-1})

The various aspects of the invention are described in greater detail below.

Examples of inducing levels fluctuations/shifts in the subject's CBG values and epidermal glucose values by HATR-FTIR, in order to obtain meaningful time-dependent and dose-dependent glucose values, their LT's for maximum peaks and their LT changes towards monitored characterization of GV in a qualified subject, with or without hyperglycemia, stable or unstable, that can be used to predict the patient's risk of hyperglycemia, to stage Type II diabetes and, in general, to be considered as a new metrics of assessing the quality of metabolic control, are presented in Tables 1-5 and in Figures 2-6.

SUBJECTS

Type 2 diabetes (totally 3 subjects):

Subject 1, Male, age 67, insulin therapy because of recurrent/persistent hyperglycemia, due to no response to orally prescribed tabletes of Metformin for the last 7 years

Subject 2, Female, age 70, orally prescribed tabletes of Metformin has been changed to a diet control

Subject 3, Male, age 69, insulin therapy because of recurrent/persistent hyperglycemia, due to no response to orally prescribed tabletes of Metformin for the last 2 years

Impaired glucose tolerance, i.e. prediabetic subjects, and subjects as suspects of having impaired glucose tolerance due to occasional post-prandial hyperglycemia, based on accepted in the clinical practice interpretation of blood glucose units obtained under different conditions (mg/dL) and according to WHO Diabetes Criteria (totally 3 subjects):

Subject 1, Male, age 49, occasional post-prandial hyperglycemia

Subject 2, Male, age 58, occasional post-prandial hyperglycemia

Subject 3, Male, age 49, prediabetes

Healthy (totally 5 subjects):

Subject 1, Male, age 23

Subject 2, Male, age 24

Subject 3, Male, age 60

Subject 4, Female, age 35

Subject 5, Male, age 59

RESULTS

1. A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE IN A QUALIFIED SUBJECT

(i) individually at each glucose value

(ii) simultaneously at all glucose values

2. A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE IN A QUALIFIED SUBJECT

(i) in one subject

(ii) between the subjects

3. A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE IN A QUALIFIED SUBJECT

(i) within one group of disease/condition

(ii) between different groups/conditions

4. A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE IN A QUALIFIED SUBJECT

(i) under one screening test

(ii) comparatively, under different screening tests

5. A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE IN A QUALIFIED SUBJECT

(i) for qualitative interpretation

(ii) for quantitative interpretation

6. A FORMULA AND METHOD FOR MONITORING INDIVIDUAL METABOLIC RESPONSE AND FOR GENERATING PREDICTIVE CLINICAL METRICS IN A PATIENT

(i) for generating a pattern and parameters characterizing GV in the patients with Type 2 diabetes

(ii) for generating a pattern and parameters characterizing GV in the patients with impaired glucose tolerance, i.e. the patients with prediabetes

(iii) for generating a pattern and parameters characterizing GV in healthy subjects

Tables 1-5 describe that:

the LT changes show dose-dependency in healthy, prediabetic and diabetic subjects, that differ at duration

the LT changes are the shortest in a diabetes subject and the longest in a healthy subject, independent of intaken dose of glucose under OGTT

the LT changes differ at single, bi-phasic and a cascade appearance between healthy subjects, subjects as suspects of having impaired glucose tolerance, i.e. prediabetes, and subjects with Type 2 diabetes

the LT changes show pattern recognition for healthy subjects, prediabetic subjects and subjects with Type 2 diabetes, based on the assessed LT changes (in minutes)

the LT changes show day-to-day variations, connected to a pattern recognition

Figures 2-11, in addition to the results, shown in Tables 1-7, show differences in GV between healthy, prediabetic and diabetic subjects towards their pattern recognition, based on specific characteristics:

recognized to healthy and prediabetic/diabetic subjects wavenumber changes, i.e. shifts of glucose-specific peaks to the left or to the right, accordingly

recognized glucose levels as low, medium and high, for healthy, prediabetic and diabetic subjects, assessed at determined time intervals under OGTT with different doses

recognized meaningful time-dependent fluctuations of GV assessed at each glucose-specific value, having common and specific features for healthy, prediabetic and diabetic subjects

EXAMPLES

EXAMPLE 1

Figure 2 A-D presents differences between GV obtained between CBG values (shown by dashed lines) and each glucose value (shown by solid lines) at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in Subject 1 with Type 2 diabetes. The time of increment for each 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.

Figure 3 A-D presents differences between GV obtained between CBG values (shown by dashed lines) and each glucose value (shown by solid lines) at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in Subject 2 with Type 2 diabetes. The time of increment for each 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.

Figure 4 A-D presents differences between GV obtained between CBG values (shown by dashed lines) and each glucose value (shown by solid lines) at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in Subject 3 with Type 2 diabetes. The time of increment for each 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 displays LT changes estimated within a 120-minute post-prandial monitoring of metabolic response in 3 subjects with Type 2 diabetes (S1, S2 and S3) by HATR-FTIR spectroscopy.

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	0'	60'	0'	60'

EXAMPLE 2

Figure 5 A-C presents a comparative dose-dependent GV during monitoring of metabolic response by HATR-FTIR spectroscopy at about 1030, 1041, 1118 and 1153 cm^{-1} during OGTT at different doses, at 5 g (low), 20 g (medium) and 75 g (high), in a healthy subject (Subject 4).

Table 2 dislays LT changes between the LT for the peak of CBG and the LT peak for each measured glucose level at about 1030, 1041, 1118 and 1153 cm^{-1}, obtained in a healthy subject during OGTT at different doses (5 g, 20 g, 75 g).

Healthy	LT	OGTT (5 g)	OGTT (20 g)	OGTT (75 g)
1030-1041 cm^{-1}	$LT_{changes}$	15' & 30'	10'	5' & 30'
1080 cm^{-1}	$LT_{changes}$	15' & 30'	0' & 10' & 20'	5'
1118 cm^{-1}	$LT_{changes}$	10' & 25'	10'	5 '& 25'
1153 cm^{-1}	$LT_{changes}$	35' & 50'	10' & 15'	10'

Figure 6 A-C presents a comparative dose-dependent GV during monitoring of metabolic response by HATR-FTIR spectroscopy simultaneously shown at about 1030, 1041, 1118 and 1153 cm^{-1} during OGTT at different doses, at 5 g (low), 20 g (medium) and 75 g (high), in Subject 3 with Type 2 diabetes.

Table 3 displays LT changes between the LT for the peak of CBG and the LT peak for each measured glucose level at about 1030, 1041, 1118 and 1153 cm^{-1}, obtained in a diabetic

subject during OGTT at different doses (5 g, 20 g, 75 g).

Type 2 diabetes	LT	OGTT (5 g)	OGTT (20 g)	OGTT (75 g)
1030-1041 cm^{-1}	$LT_{changes}$	0' & 10'	0'	0'
1080 cm^{-1}	$LT_{changes}$	10'	15'	0'
1118 cm^{-1}	$LT_{changes}$	0' & 10 & 25'	5'	0'
1153 cm^{-1}	$LT_{changes}$	15' & 25'	25' & 40'	30'

EXAMPLE 3

Figure 7 A-C illustrates examples of characteristic differences in the levels of glucose values and wavenumber shifts between a healthy subject (Subject 4) and a subject with Type 2 diabetes (Subject 3) under OGTT with different doses.

Table 4 displays LT changes under OGTT with 75 g., i.e. clinical, a healthy subject vs. a subject with Type 2 diabetes.

Subjects	healthy	Type 2 diabetes
1030-1041 cm^{-1}	15' & 30'	10'
1080 cm^{-1}	15' & 30'	10'
1118 cm^{-1}	10' & 25'	0' & 10' & 25'
1153 cm^{-1}	35' & 50'	15' & 25'

Table 5 displays LT changes under OGTT with 20 g., a healthy subject vs. a subject with Type 2 diabetes.

Subjects	healthy	Type 2 diabetes
1030-1041 cm^{-1}	10'	0'
1080 cm^{-1}	0' & 10' & 20'	15'
1118 cm^{-1}	10'	5'
1153 cm^{-1}	10' & 15'	25' & 40'

EXAMPLE 4

Figure 8 A-F illustrates a variety of examples of GV simultaneously shown at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy in 2 Type 2 diabetes (Subject 3) under OGTT with 75 g and 20 g; in a prediabetic subject (Subject 5) under OGTT with 75 g and under OGTT with 75 g in 3 healthy subjects (Subject 1, Subject 3, Subject 4).

EXAMPLE 5

Figure 9 A-D presents GV simultaneously shown at about 1030, 1041, 1118 and 1153 cm^{-1} by

HATR-FTIR spectroscopy during a 120-minute post-prandial monitoring of individual metabolic response in 3 patients (Subjects 1-3) with Type 2 diabetes, in comparison to a healthy subject (Subject 5). A 120-minute post-prandial monitoring of metabolic response in 3 subjects (S1, S2, and S3) with Type 2 DM and 1 healthy control subject ("0"-point is pre-prandial). CBG levels in mg/dL are shown in numbers along curves for each measured subject.

EXAMPLE 6

Fig. 10 A-C presents day-to-day variations on GV during a 120-minute post-prandial monitoring of individual metabolic response in Subject 3 with Type 2 diabetes on 2 consecutive days, comparatively shown at about 1030, 1041, 1080 and 1118 cm^{-1} by HATR-FTIR spectroscopy.

Table 6 displays LT changes estimated for the same subject with Type 2 diabetes on 2 consecutive days.

Subjects	1030-1041 cm^{-1}	1080 cm^{-1}	1118 cm^{-1}	1153 cm^{-1}
S3 (Day 1)	0'	60'	0'	60'
S3 (Day 2)	0'	0'	60'	-

EXAMPLE 7

Figure 11 A-D comparatively presents GV during monitoring of individual metabolic response in 3 healthy subjects (Subjects 1, 2, 4), in 1 subject as a suspect of having impaired glucose tolerance, i.e. prediabetes (Subject 3), and in Subject 3 with Type 2 diabetes by HATR-FTIR spectroscopy.

Table 7 displays OGTT (75 g)., i.e. clinical OGTT, demonstrate estimated LT changes for healthy, prediabetic and diabetic subjects.

Subjects	healthy	prediabetes	Type 2 diabetes
1030-1041 cm^{-1}	5' & 30'	5' & 20' & 30' & 45'	0'
1080 cm^{-1}	5'	5' & 20' & 45'	0'
1118 cm^{-1}	5' & 25'	5' & 20' & 30' & 40'	0'
1153 cm^{-1}	10'	0' & 15' & 25' & 55'	30'

CLAIMS

1. A formula and a method for monitoring individual metabolic response, comprising of steps:
(i) induction of glucose excursions in the blood glucose, influenced by food and/or consumption of pure glucose at different doses (low, medium, high) in a qualified subject
(ii) performance of reference CBG measurements at predetermined intervals, thus generating the first data in a qualified subject
(iii) gathering non-invasive glucose spectral measurements at assigned 5 glucose-specific peaks at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy
(iv) calculation of the LT, i.e. CBG peak level for CBG measurements
(v) calculation of the LT, i.e. HATR-FTIR peak level(s) at about 1030, 1041, 1118 and 1153 cm^{-1} for each glucose value by device
(vi) calculation of the LT changes between the estimated LT for CBG and the estimated LT for each glucose value at about 1030, 1041, 1118 and 1153 cm^{-1} by HATR-FTIR spectroscopy

2. A formula and method for qualitative interpretation of GV, based on individual and/or simultaneous assessment of glucose values, in a qualified subject under any performed screening test, comprising of steps in Claim 1

3. A formula and method for quantitative interpretation of glycemic variability, based on individual and/or simultaneous assessment of glucose values, in a qualified subject under any performed screening test, comprising of steps in Claim 1

4. A formula and method for screening of disorders of glucose metabolism by means of establishing suitable metrics for glycemic variability in the clinical practice, such as impaired glucose tolerance and diabetes mellitus in a qualified subject, comprised of the steps:
(i) generation of patterns
(ii) generation of parameters
(iii) generation of characteristics
based on:
recognized to healthy and prediabetic/diabetic subjects wavenumber changes, i.e. shifts of glucose-specific peaks to the left or to the right, accordingly
recognized glucose levels as low, medium and high, for healthy, prediabetic and diabetic subjects, assessed at determined time intervals under OGTT with different doses
recognized meaningful time-dependent fluctuations of GV assessed at each glucose-specific value, having common and specific features for healthy, prediabetic and diabetic subjects

5. A formula and a method, as of Claims 1-4, for generating a pattern, parameters and

characteristics for type II diabetes mellitus in a qualified subject, comprising of:
(i) based on dose-dependent duration of the LT changes
(ii) based on duration of the LT changes: fasting vs. post-prandial
(iii) based on time-dependent characterization of the LT changes for each glucose molecule by HATR-FTIR spectroscopy

6. A formula and method, as of Claims 1-4, for generating a pattern, parameters and characteristics for impaired glucose tolerance in a qualified subject, comprising of:
(i) based on dose-dependent duration of the LT changes
(ii) based on duration of the LT changes: fasting vs. post-prandial
(iii) based on time-dependent characterization of the LT changes for each glucose molecule by HATR-FTIR spectroscopy

7. A formula and method, as of Claims 1-4, for generating a pattern, parameters and characteristics for healthy individual, comprising of:
(i) based on dose-dependent duration of the LT changes
(ii) based on duration of the LT changes: fasting vs. post-prandial
(iii) based on time-dependent characterization of the LT changes for each glucose molecule by HATR-FTIR spectroscopy

8. A formula and method for monitoring interstitial glucose metabolism in a qualified subject, based on the calculation of the LT changes in a qualified individual under OGTT and post-prandially, but within the range of 0' and 60', as of Claims 1-7

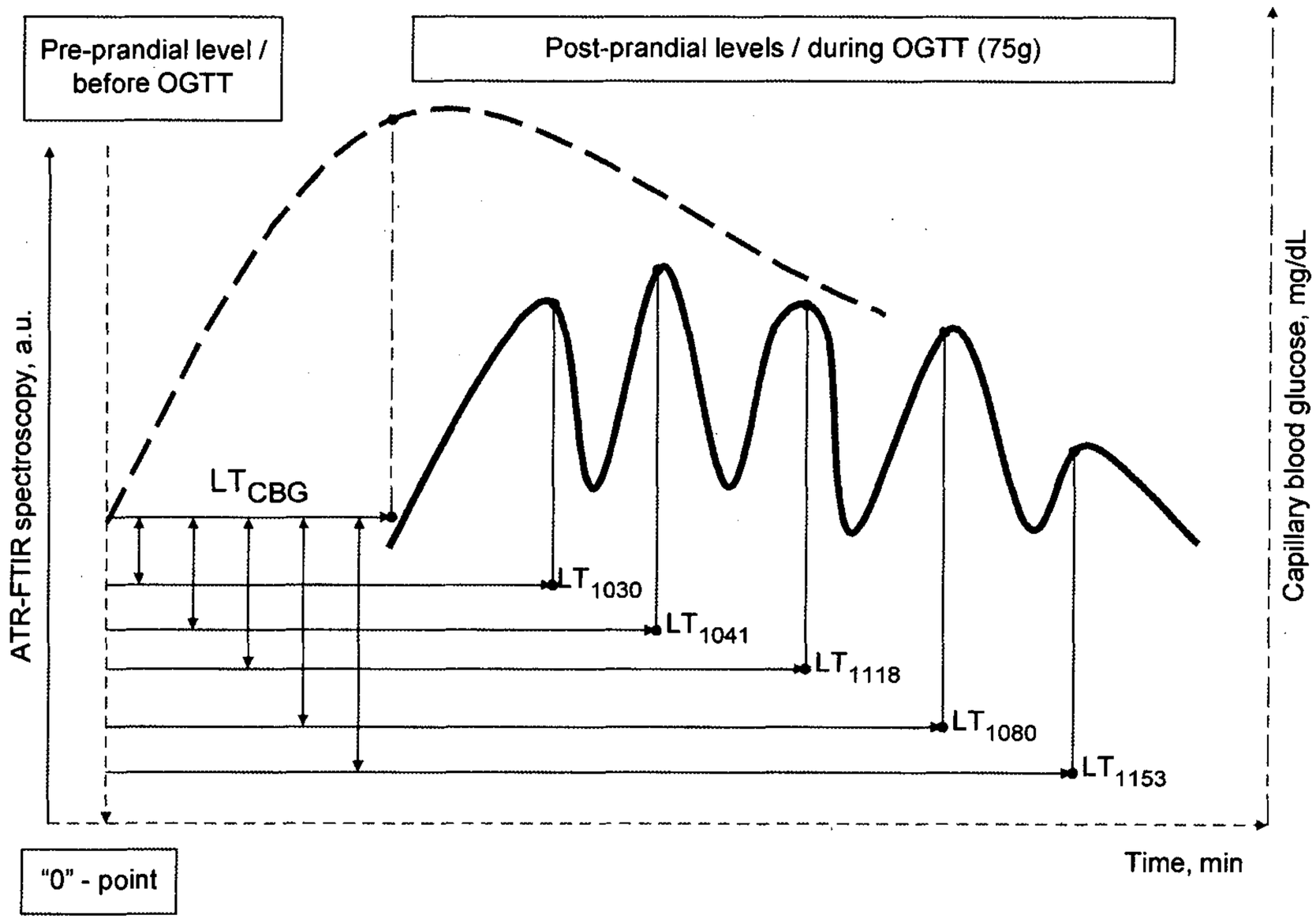
Pre-prandial level / before OGTT
Post-prandial levels / during OGTT (75g)
ATR-FTIR spectroscopy, a.u.
Capillary blood glucose, mg/dL
LT_{CBG}
LT_{1030}
LT_{1041}
LT_{1118}
LT_{1080}
LT_{1153}
"0" - point
Time, min

Fig. 1

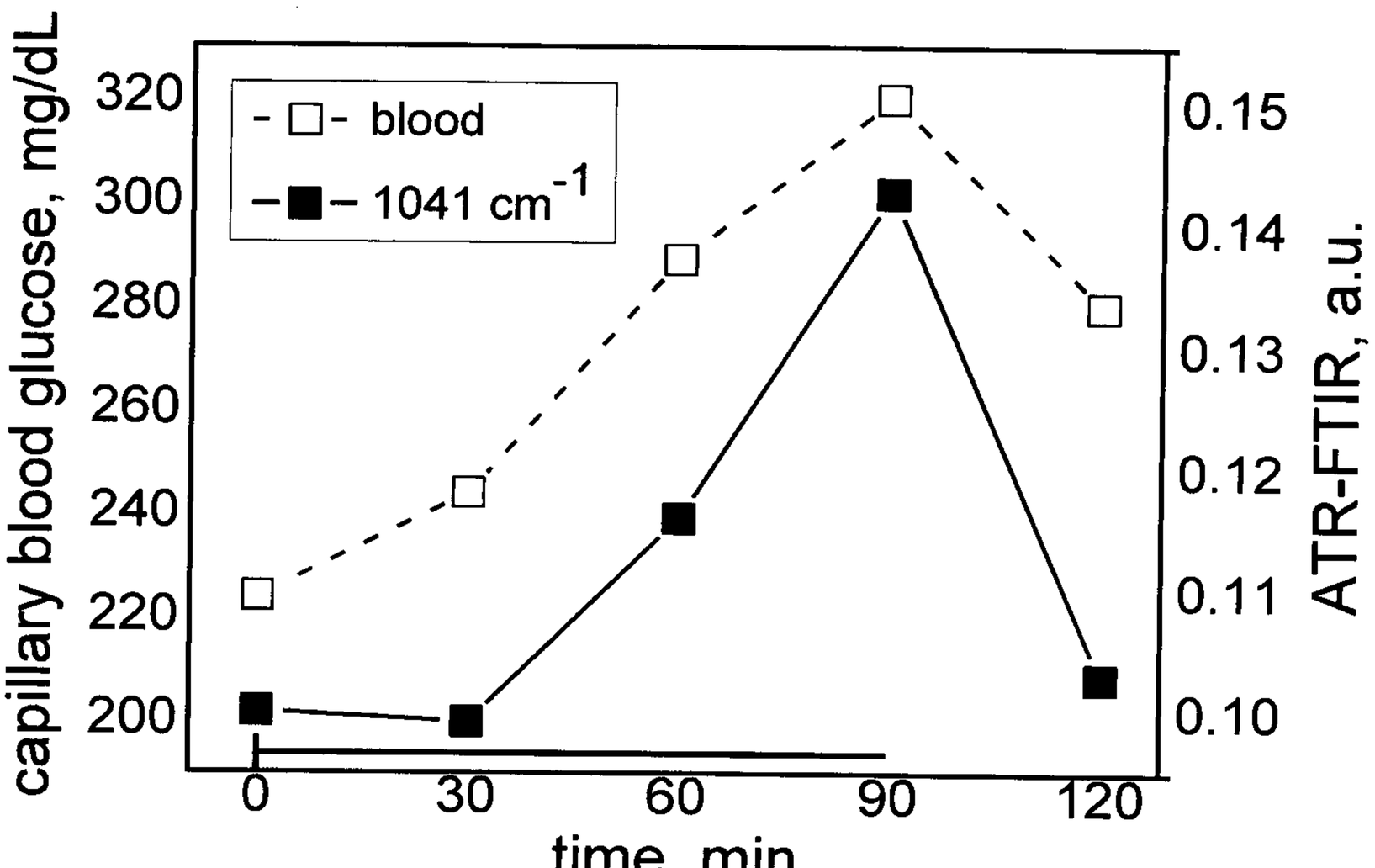
capillary blood glucose, mg/dL
320
300
280
260
240
220
200
blood
1041 cm-1
0.15
0.14
0.13
0.12
0.11
0.10
ATR-FTIR, a.u.
0
30
60
90
120
time, min

Fig. 2A

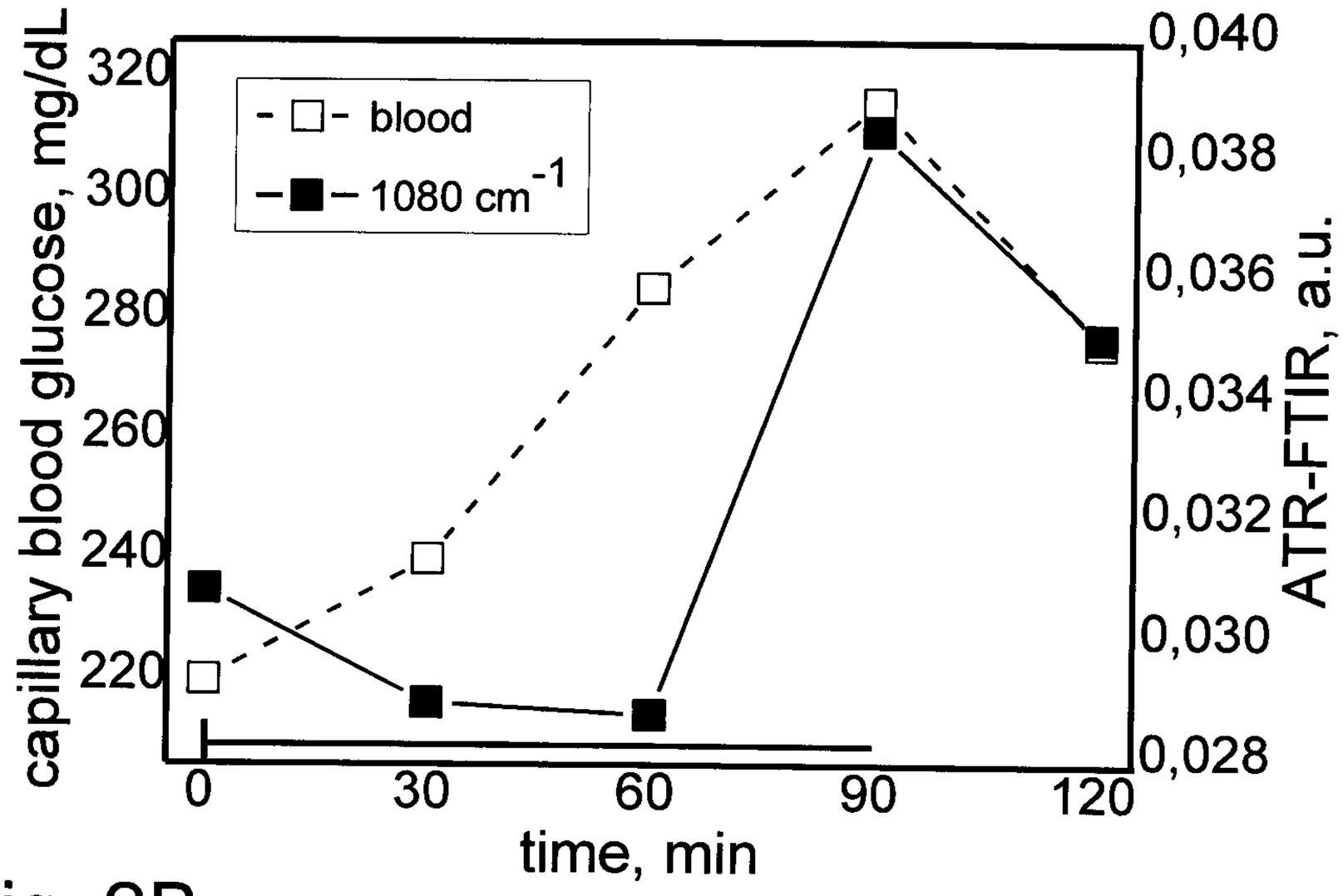
capillary blood glucose, mg/dL
320
300
280
260
240
220
blood
1080 cm-1
0,040
0,038
0,036
0,034
0,032
0,030
0,028
ATR-FTIR, a.u.
0
30
60
90
120
time, min

Fig. 2B

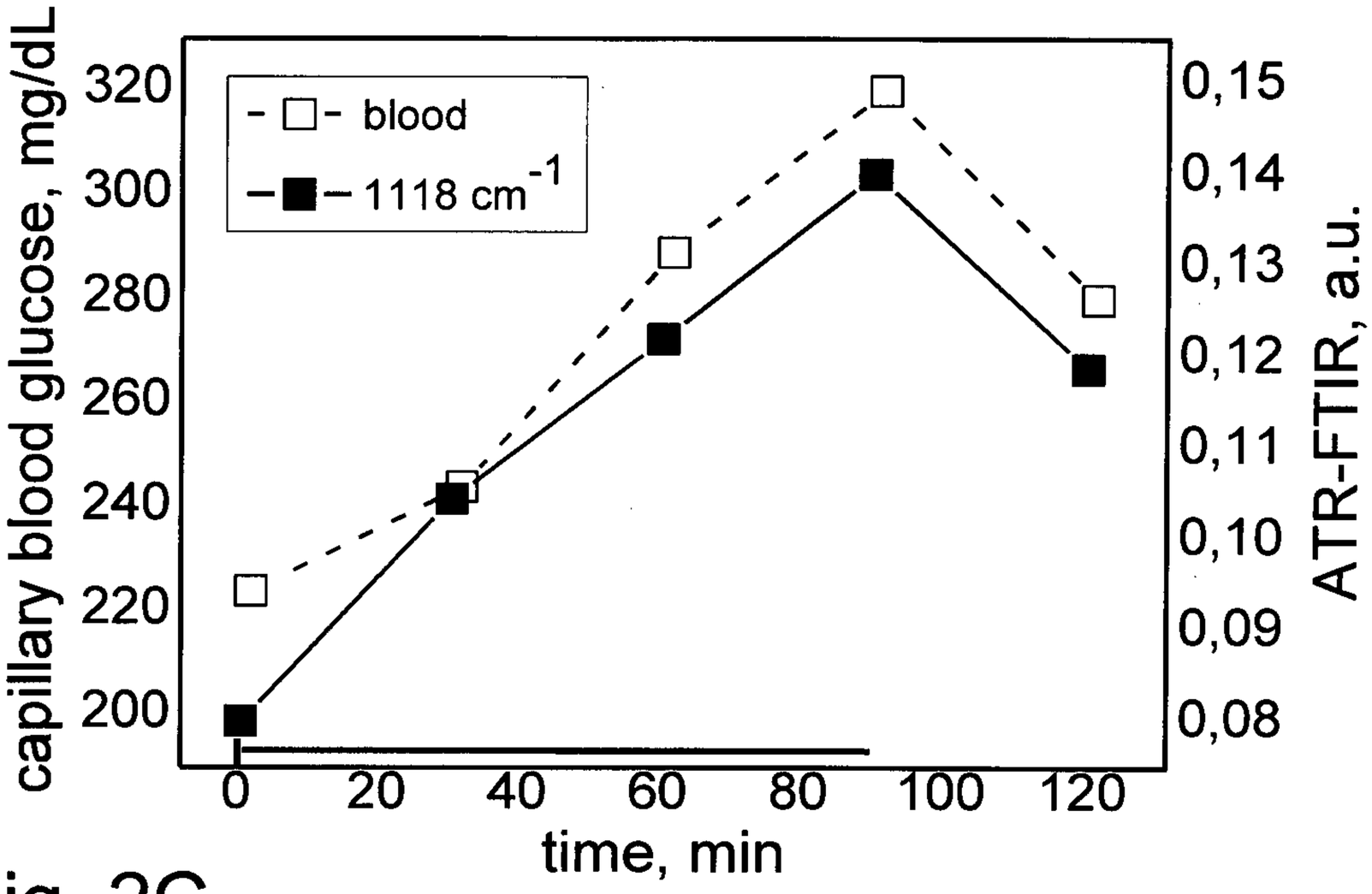
capillary blood glucose, mg/dL
320
300
280
260
240
220
200
blood
1118 cm-1
0,15
0,14
0,13
0,12
0,11
0,10
0,09
0,08
ATR-FTIR, a.u.
0
20
40
60
80
100
120
time, min

Fig. 2C

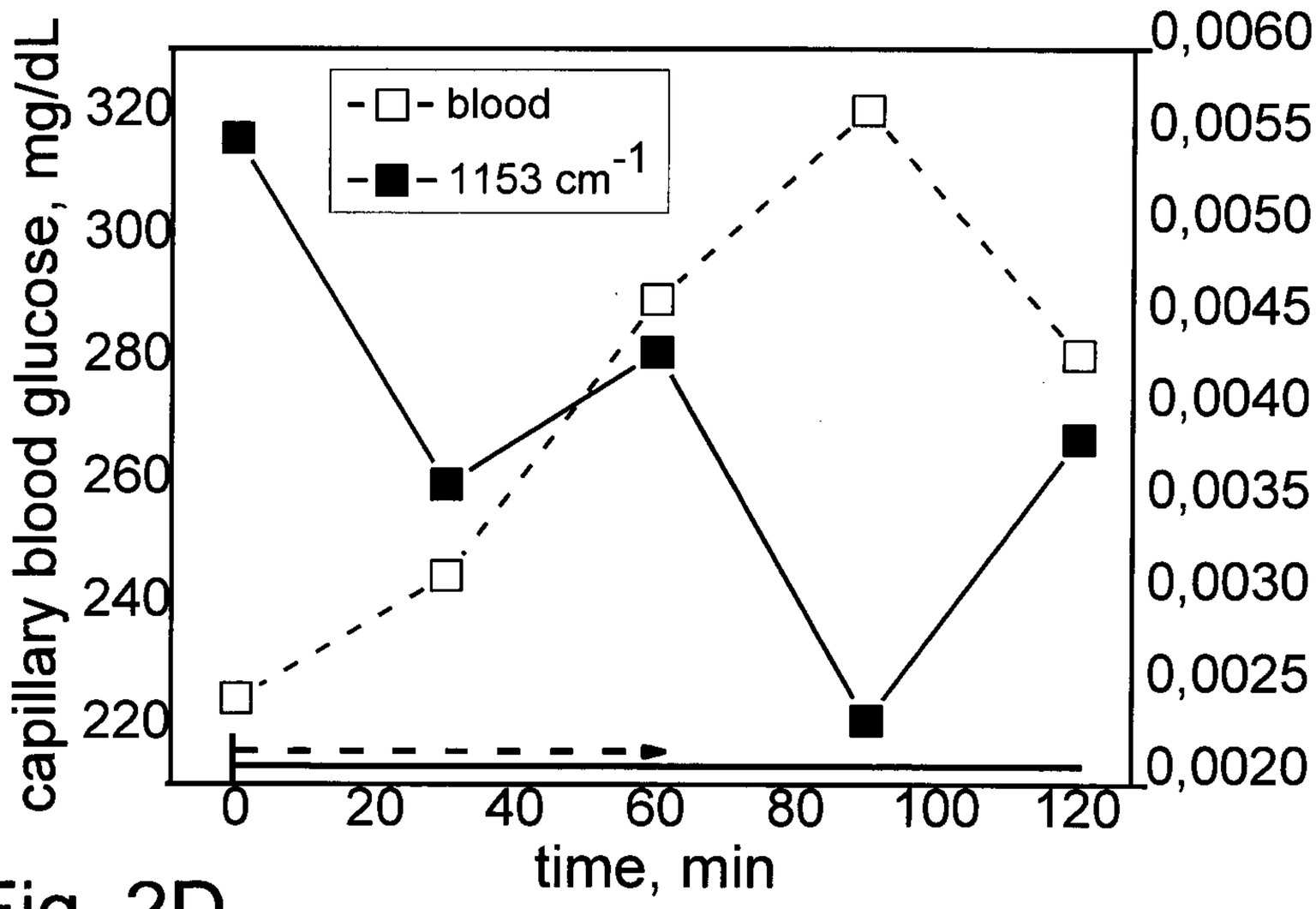
capillary blood glucose, mg/dL
320
300
280
260
240
220
blood
1153 cm-1
0,0060
0,0055
0,0050
0,0045
0,0040
0,0035
0,0030
0,0025
0,0020
ATR-FTIR, a.u.
0
20
40
60
80
100
120
time, min

Fig. 2D

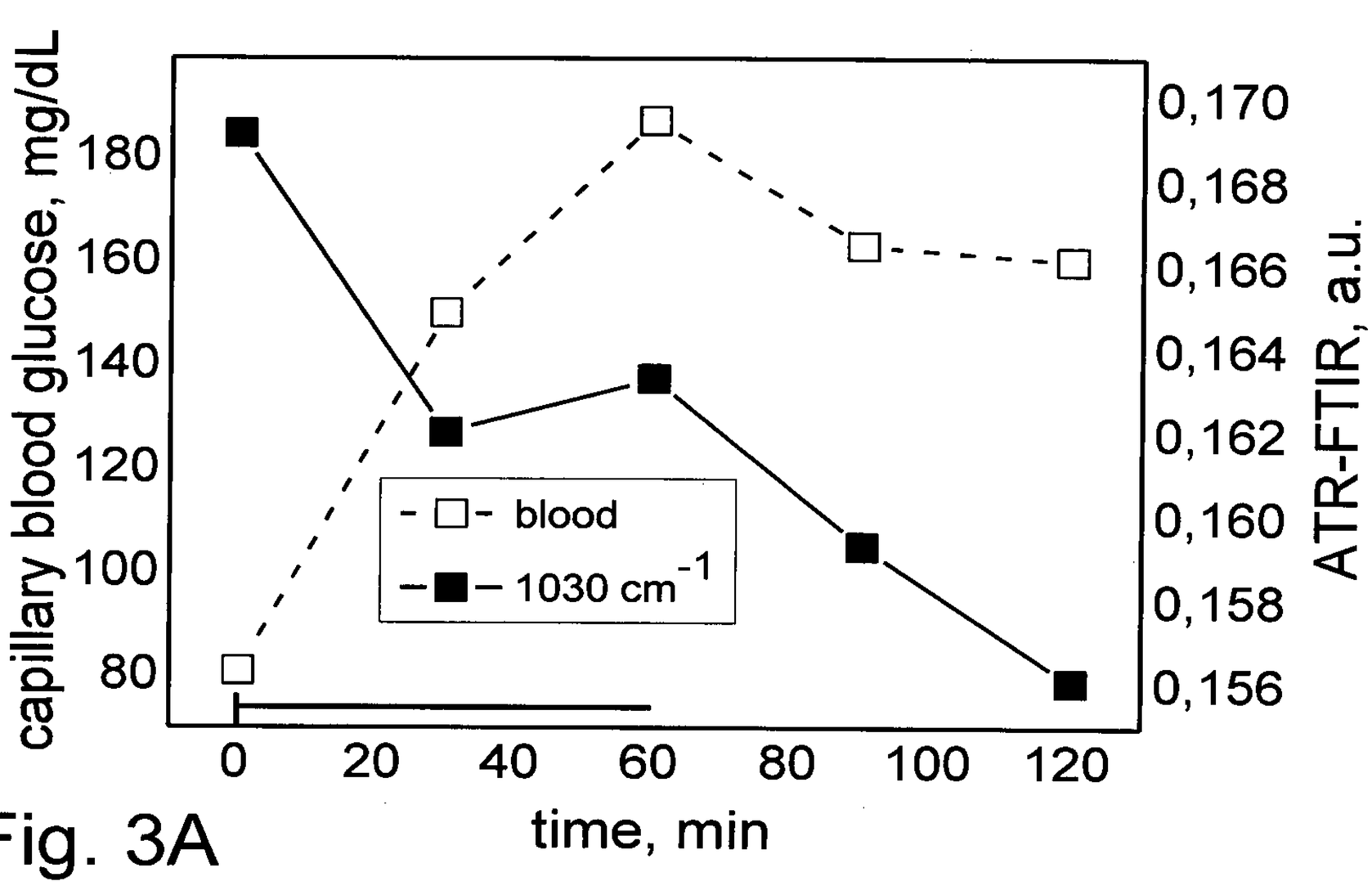
capillary blood glucose, mg/dL
180
160
140
120
100
80
0,170
0,168
0,166
0,164
0,162
0,160
0,158
0,156
ATR-FTIR, a.u.
blood
1030 cm-1
0
20
40
60
80
100
120
time, min

Fig. 3A

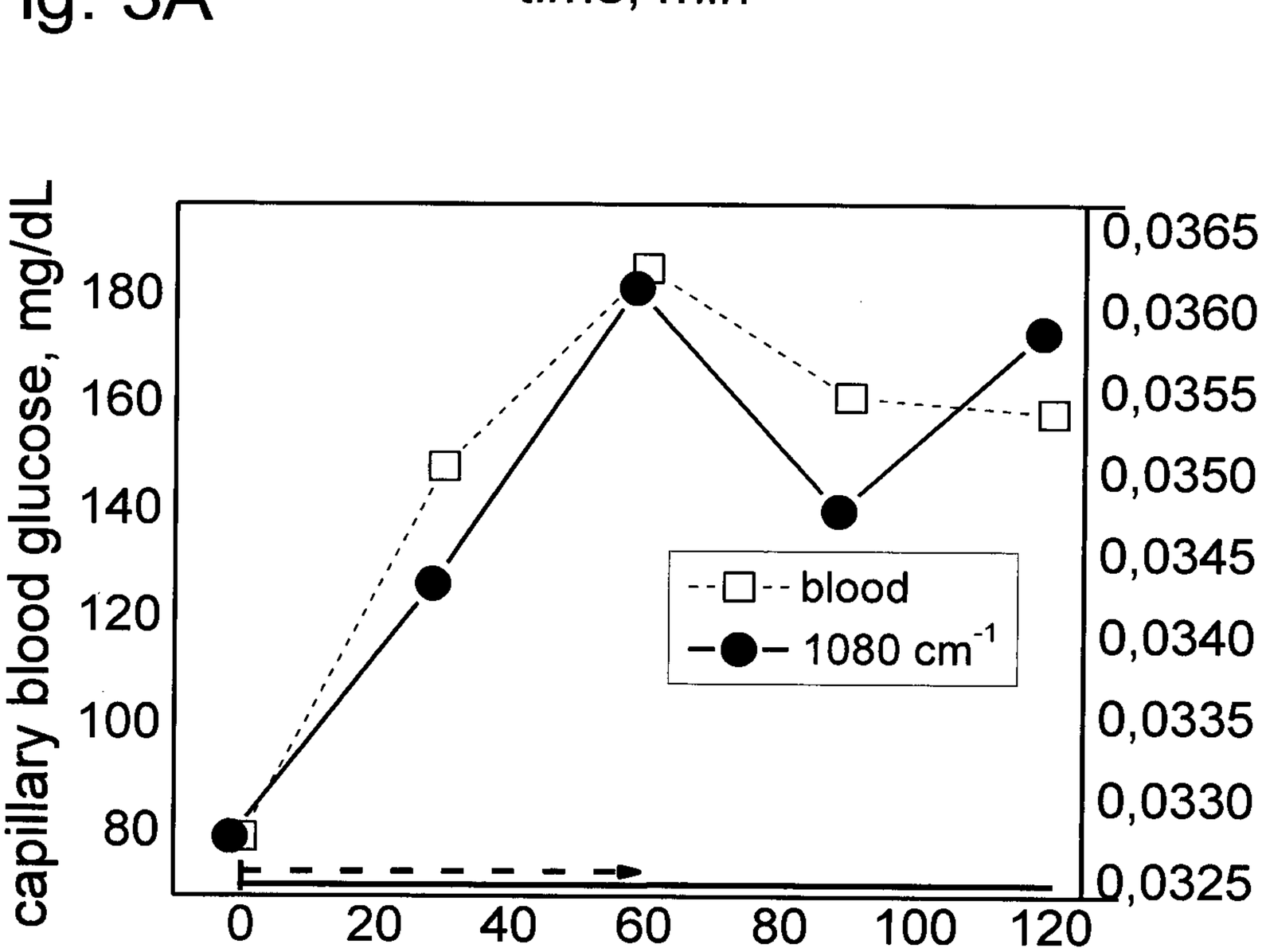
capillary blood glucose, mg/dL
180
160
140
120
100
80
0,0365
0,0360
0,0355
0,0350
0,0345
0,0340
0,0335
0,0330
0,0325
ATR-FTIR, a.u.
blood
1080 cm-1
0
20
40
60
80
100
120
time, min

Fig. 3B

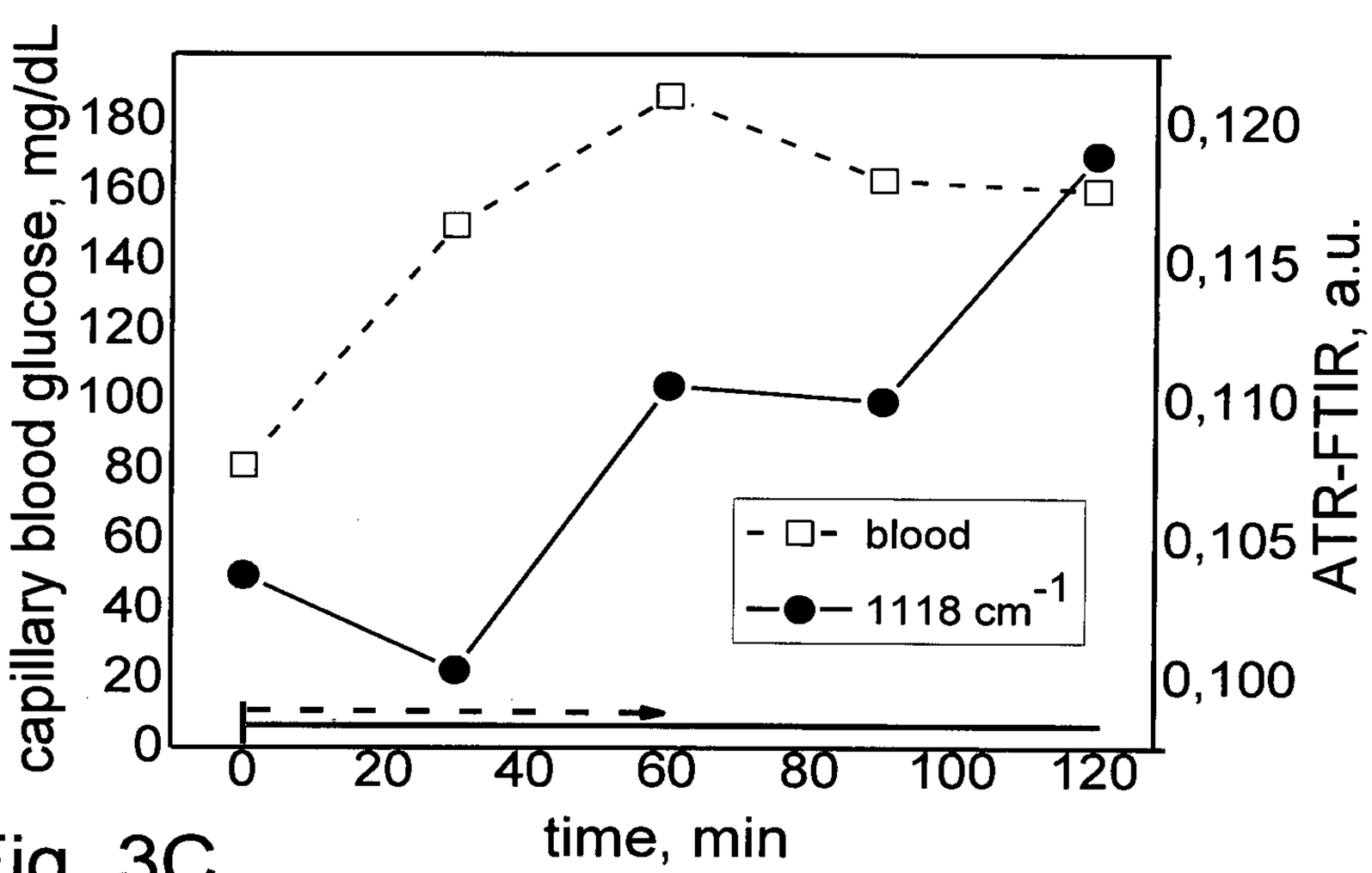
capillary blood glucose, mg/dL
180
160
140
120
100
80
60
40
20
0
0,120
0,115
0,110
0,105
0,100
ATR-FTIR, a.u.
blood
1118 cm-1
0
20
40
60
80
100
120
time, min

Fig. 3C

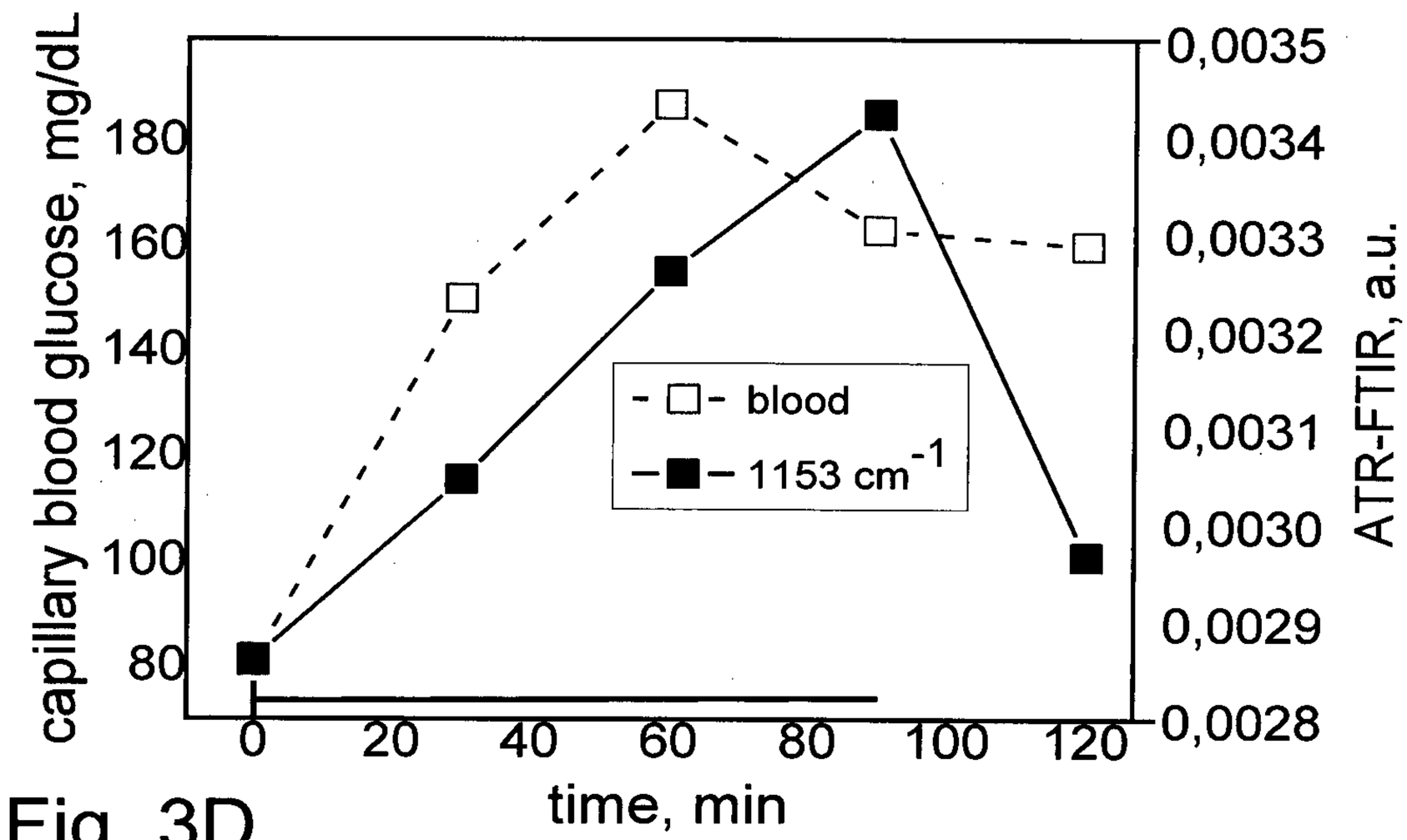
capillary blood glucose, mg/dL
180
160
140
120
100
80
0,0035
0,0034
0,0033
0,0032
0,0031
0,0030
0,0029
0,0028
ATR-FTIR, a.u.
blood
1153 cm-1
0
20
40
60
80
100
120
time, min

Fig. 3D

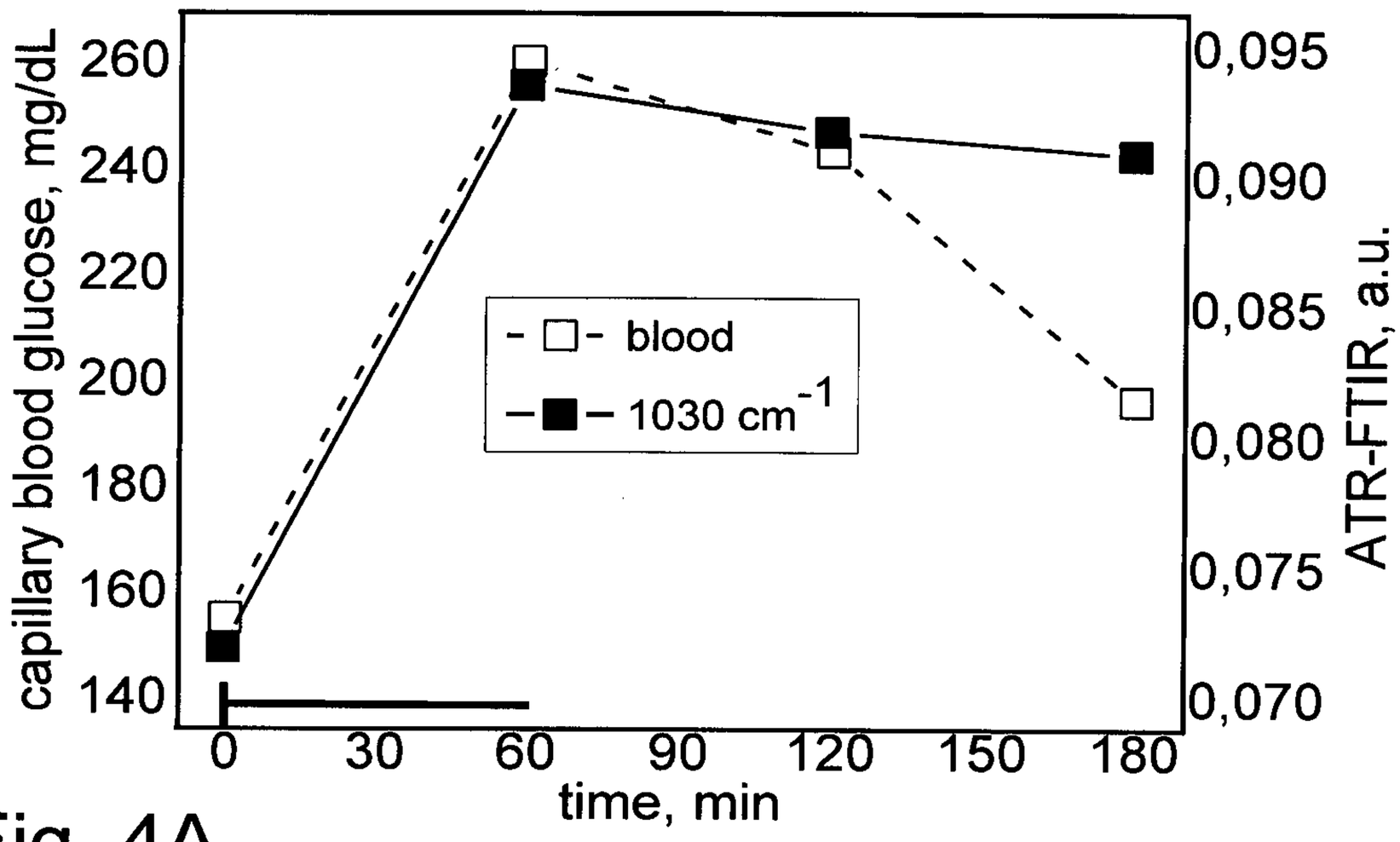
capillary blood glucose, mg/dL
260
240
220
200
180
160
140
0
30
60
90
120
150
180
time, min
0,095
0,090
0,085
0,080
0,075
0,070
ATR-FTIR, a.u.
blood
1030 cm-1

Fig. 4A

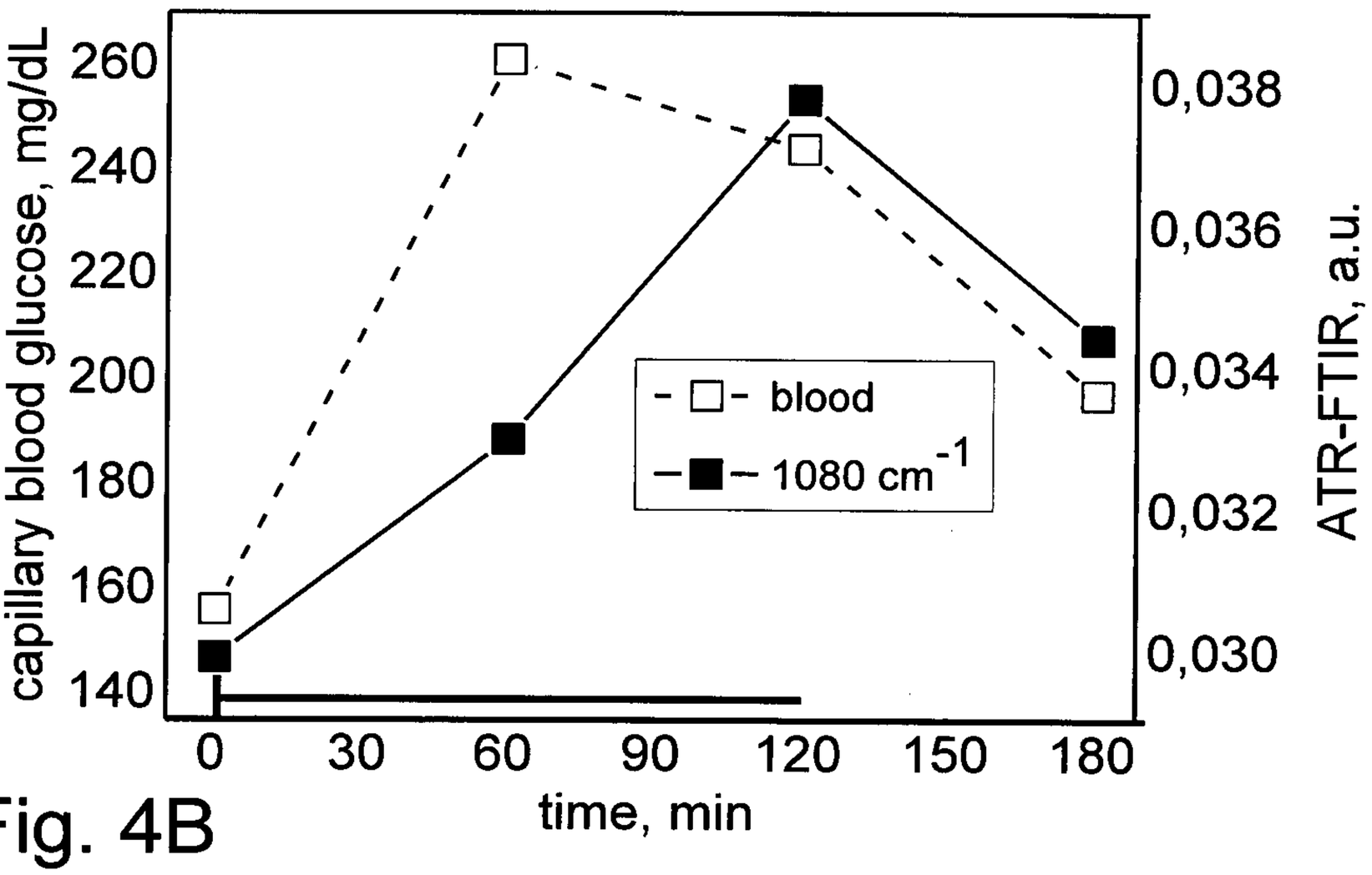
capillary blood glucose, mg/dL
260
240
220
200
180
160
140
0
30
60
90
120
150
180
time, min
0,038
0,036
0,034
0,032
0,030
ATR-FTIR, a.u.
blood
1080 cm-1

Fig. 4B

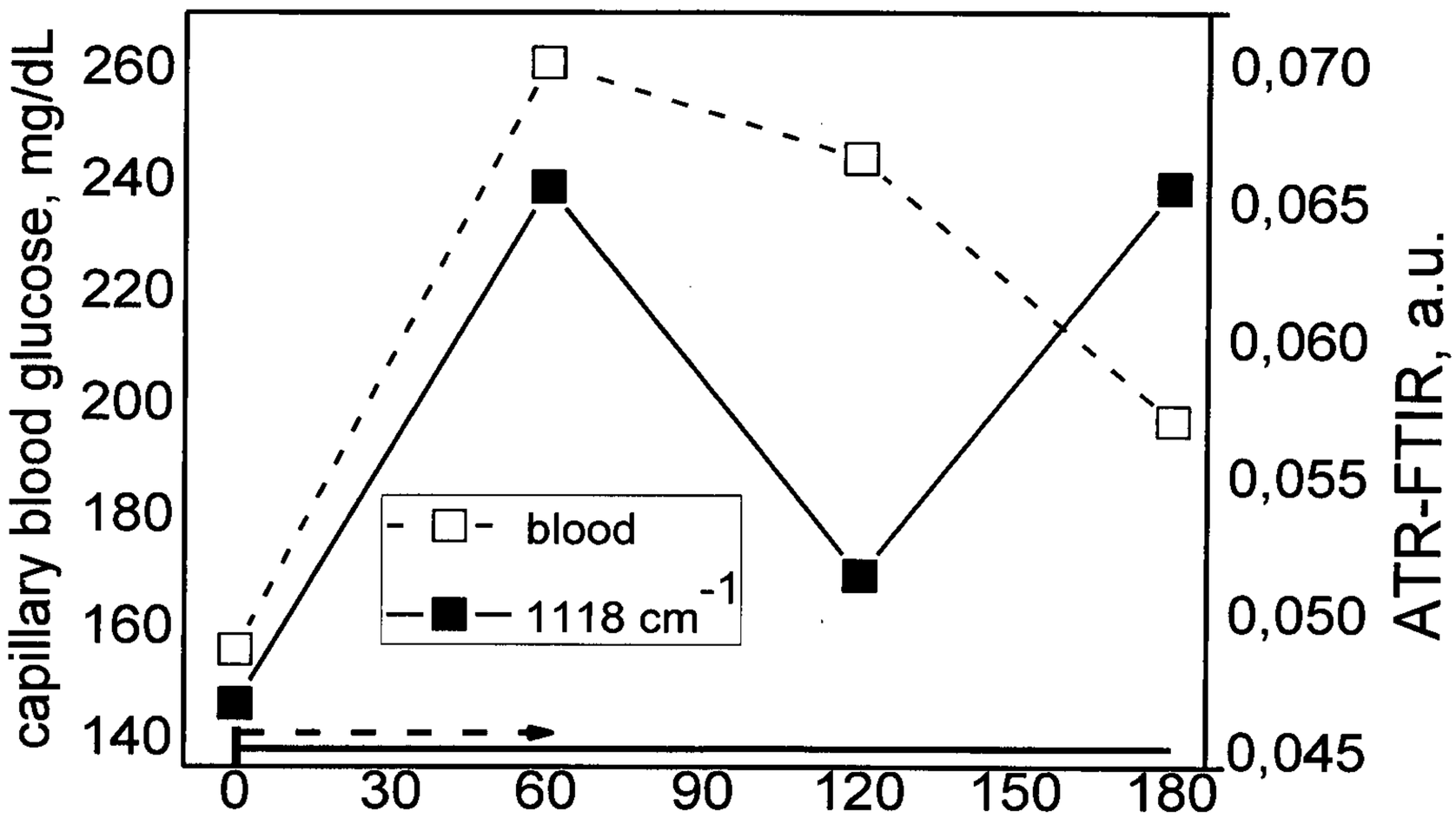
capillary blood glucose, mg/dL
260
240
220
200
180
160
140
0
30
60
90
120
150
180
time, min
0,070
0,065
0,060
0,055
0,050
0,045
ATR-FTIR, a.u.
blood
1118 cm-1

Fig.4C

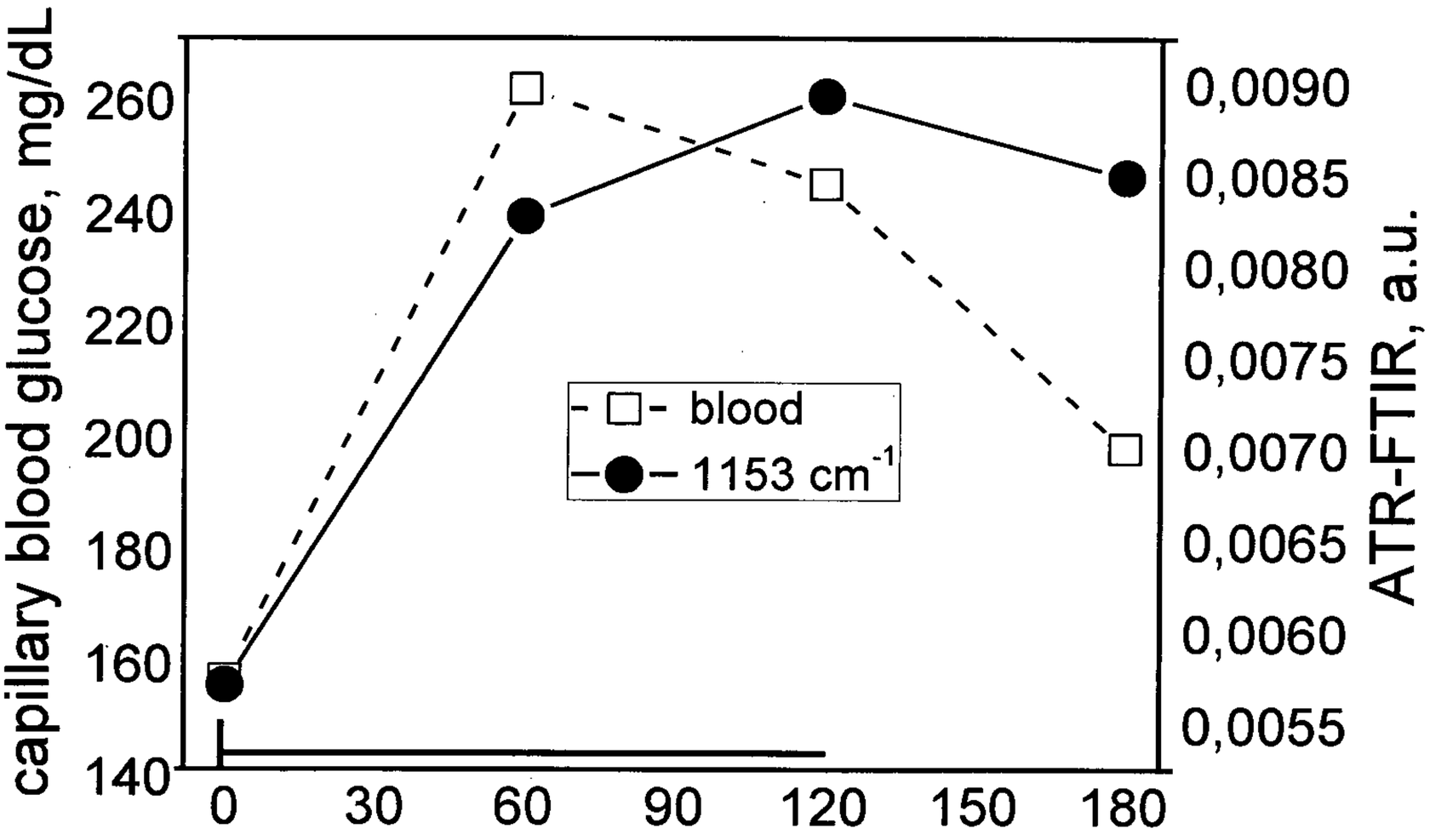
capillary blood glucose, mg/dL
260
240
220
200
180
160
140
0
30
60
90
120
150
180
time, min
0,0090
0,0085
0,0080
0,0075
0,0070
0,0065
0,0060
0,0055
ATR-FTIR, a.u.
blood
1153 cm-1

Fig. 4D

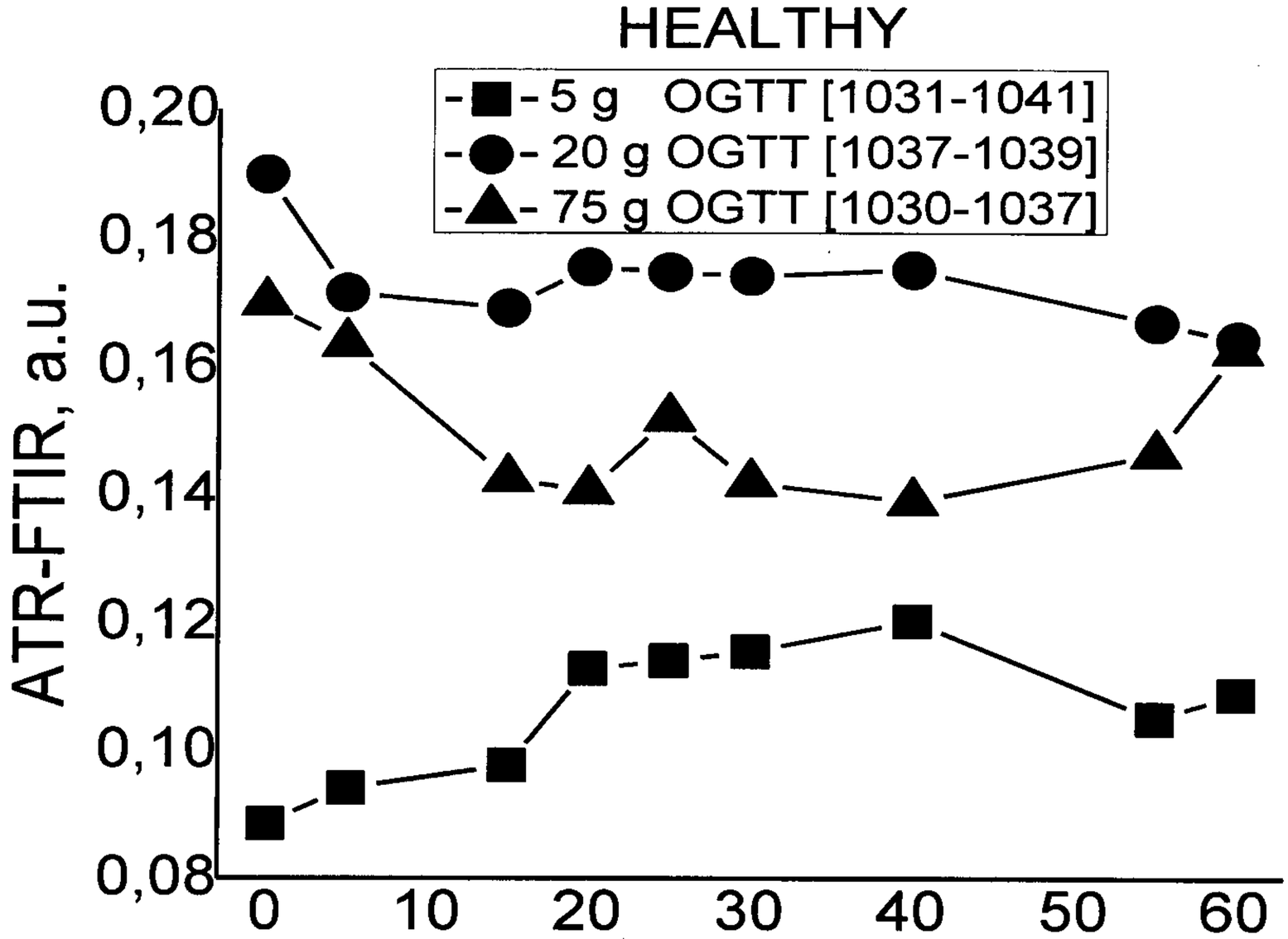
HEALTHY
5 g OGTT [1031-1041]
20 g OGTT [1037-1039]
75 g OGTT [1030-1037]
ATR-FTIR, a.u.
0,20
0,18
0,16
0,14
0,12
0,10
0,08
0
10
20
30
40
50
60
time, min

Fig. 5A

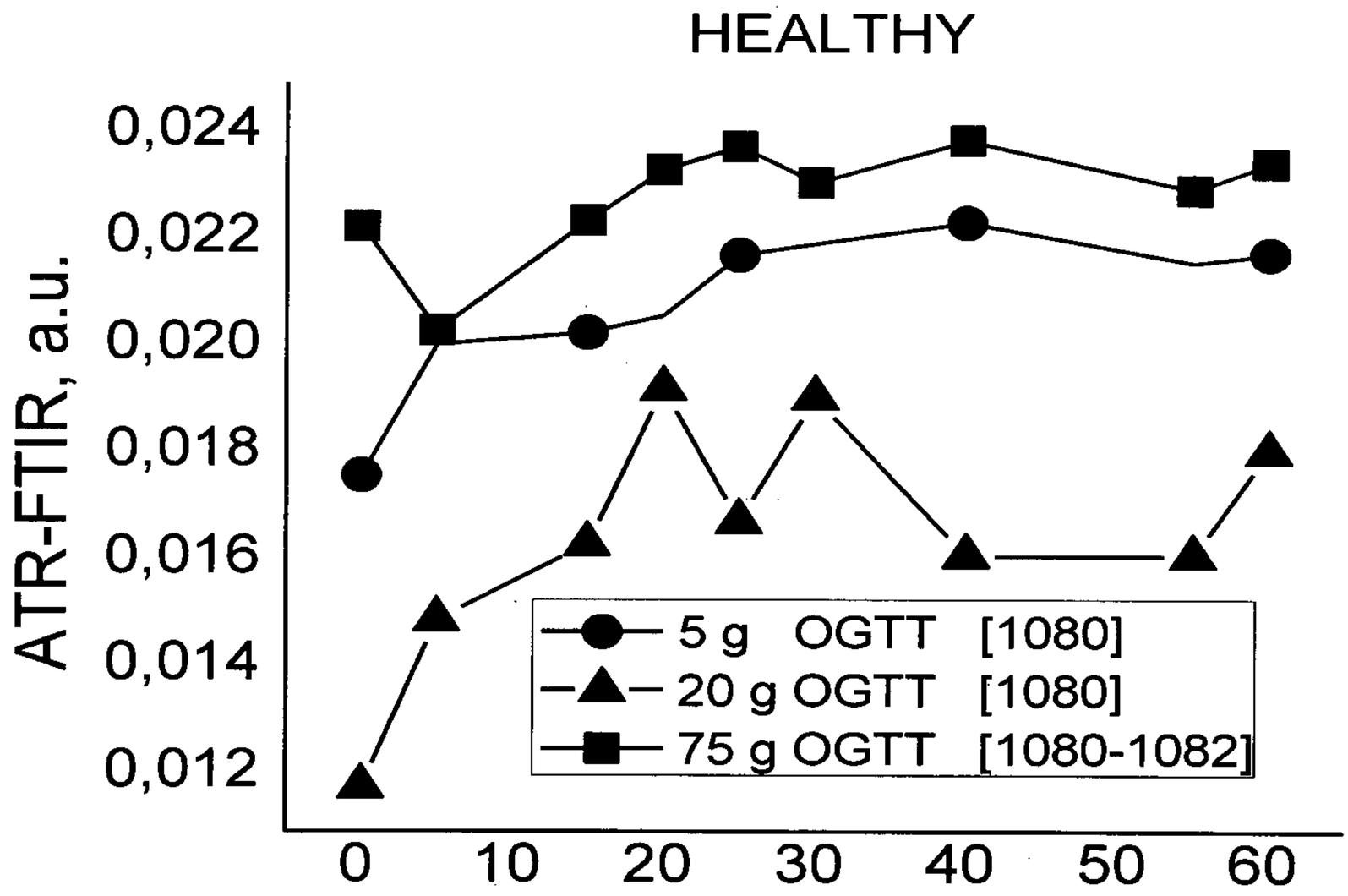
HEALTHY
ATR-FTIR, a.u.
0,024
0,022
0,020
0,018
0,016
0,014
0,012
5 g OGTT [1080]
20 g OGTT [1080]
75 g OGTT [1080-1082]
0
10
20
30
40
50
60
time, min

Fig. 5B

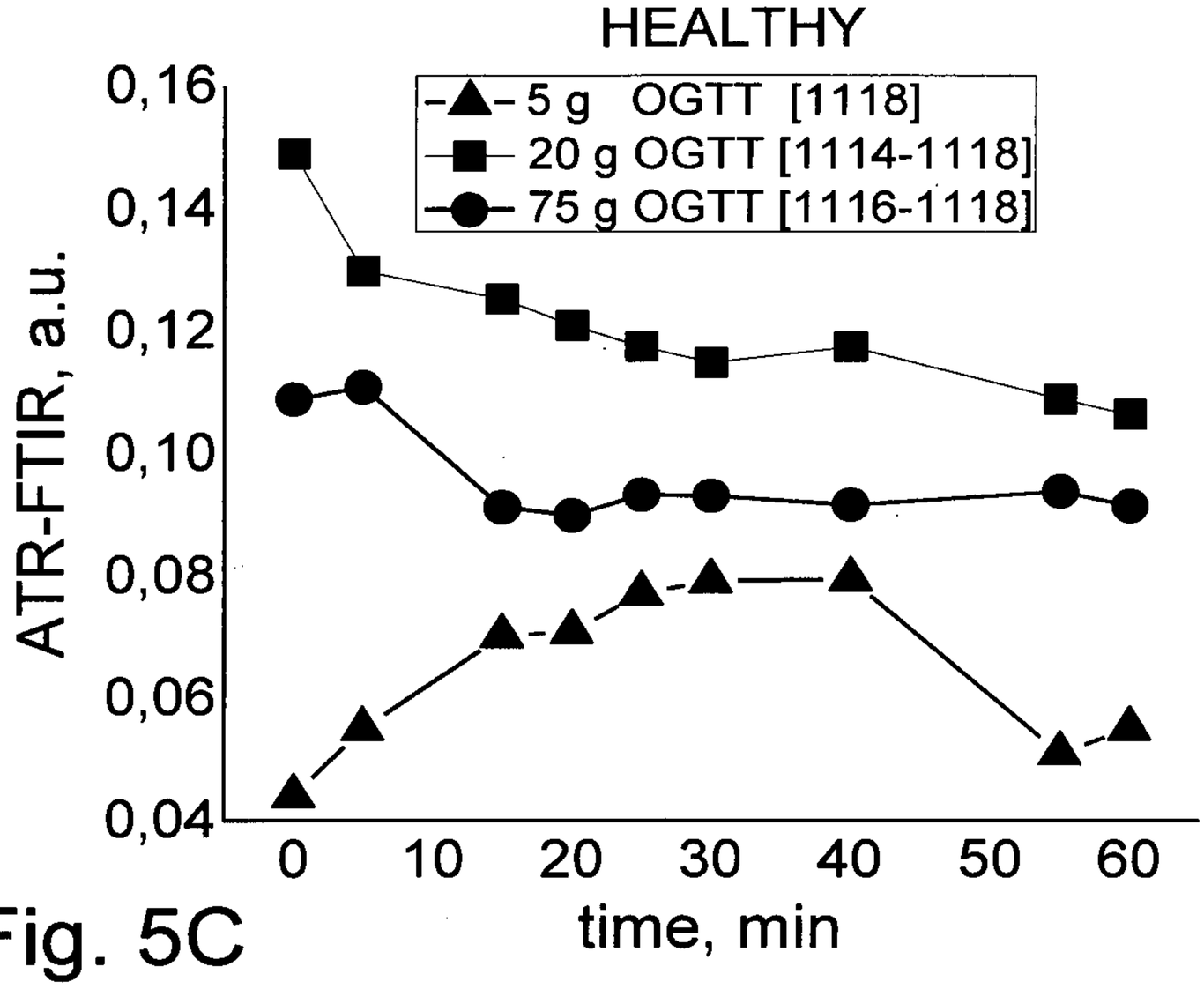
HEALTHY
5 g OGTT [1118]
20 g OGTT [1114-1118]
75 g OGTT [1116-1118]
ATR-FTIR, a.u.
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0
10
20
30
40
50
60
time, min

Fig. 5C

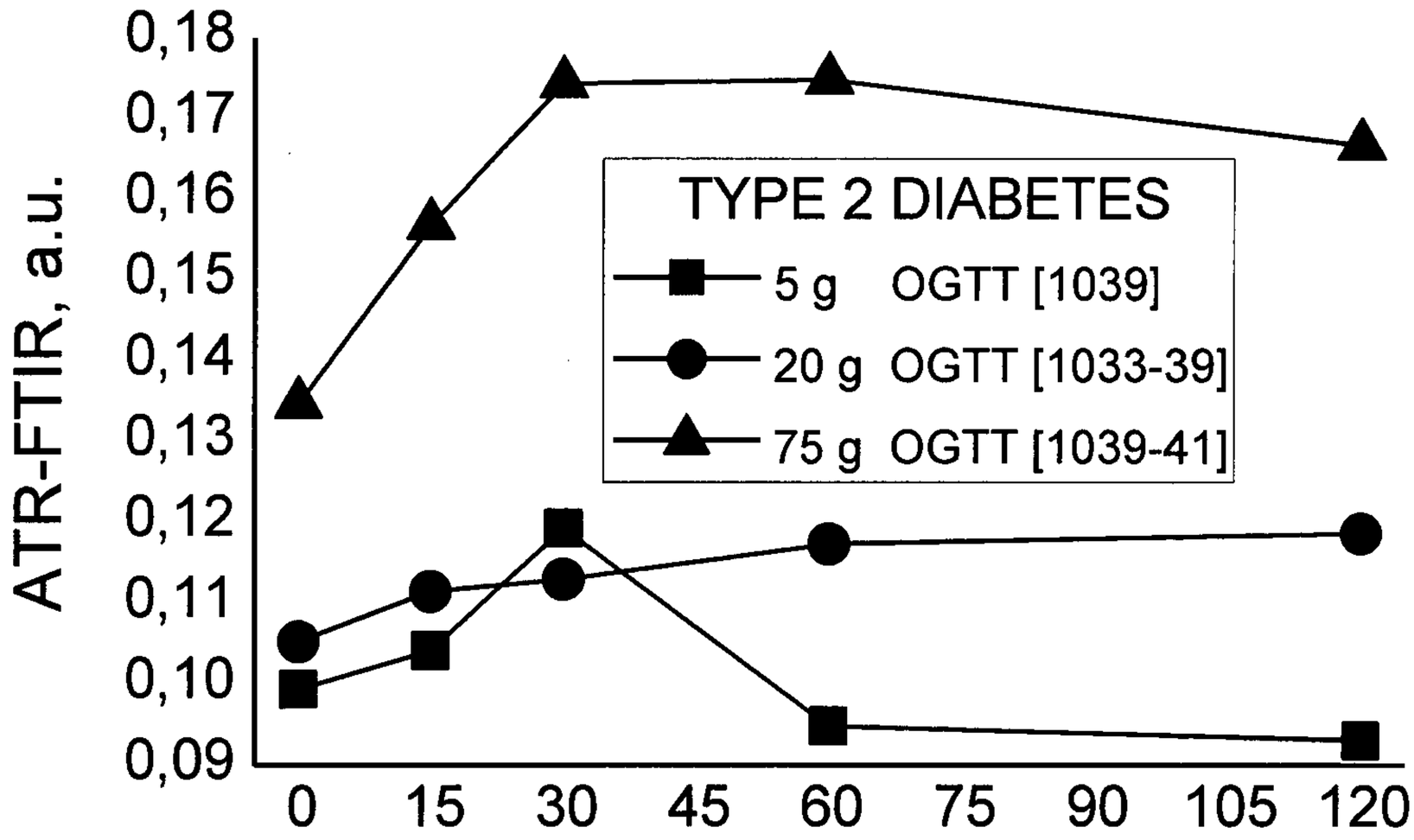
TYPE 2 DIABETES
5 g OGTT [1039]
20 g OGTT [1033-39]
75 g OGTT [1039-41]
ATR-FTIR, a.u.
0,18
0,17
0,16
0,15
0,14
0,13
0,12
0,11
0,10
0,09
0
15
30
45
60
75
90
105
120
time, min

Fig. 6A

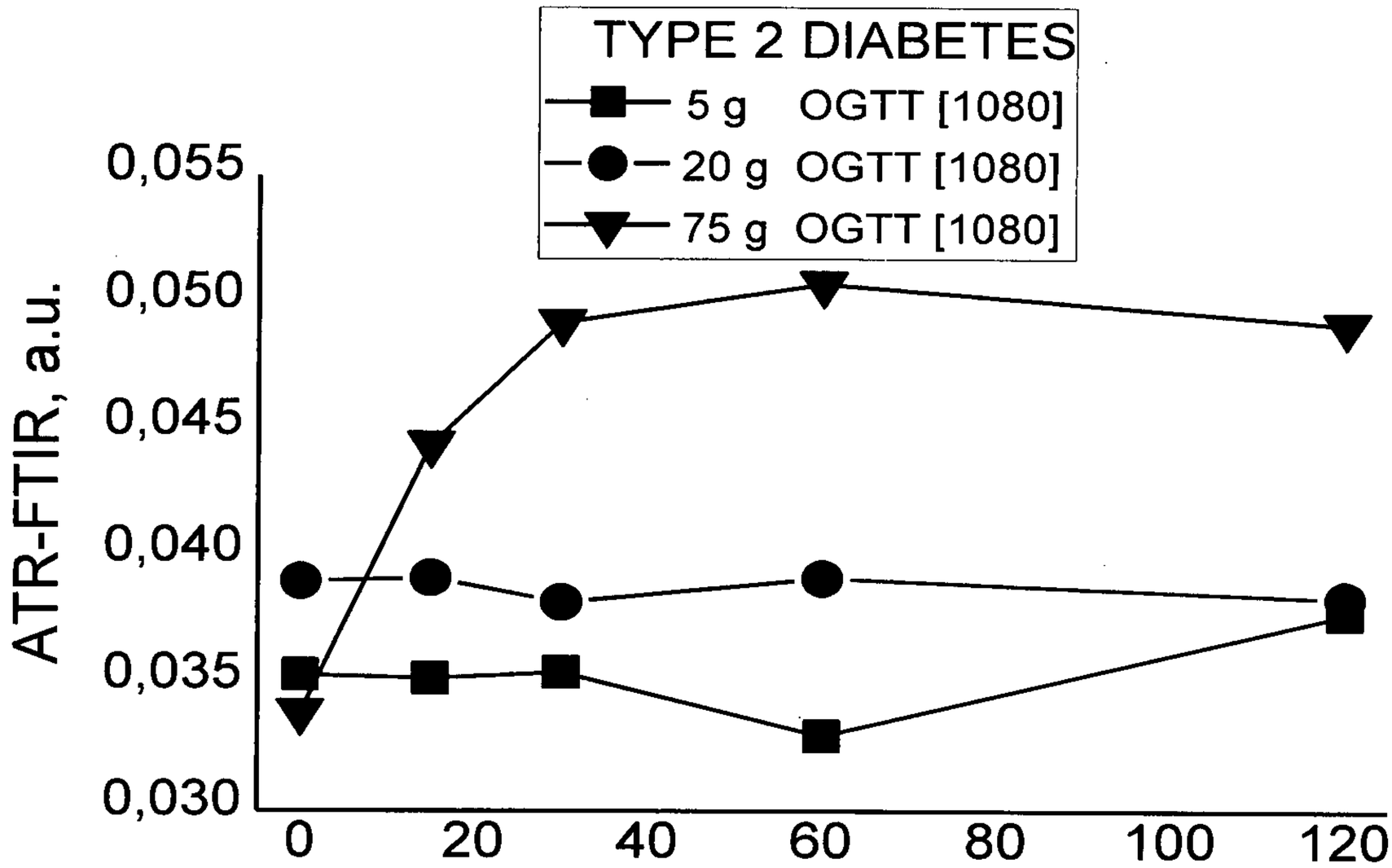
TYPE 2 DIABETES
5 g OGTT [1080]
20 g OGTT [1080]
75 g OGTT [1080]
ATR-FTIR, a.u.
0,055
0,050
0,045
0,040
0,035
0,030
0
20
40
60
80
100
120
time, min

Fig. 6B

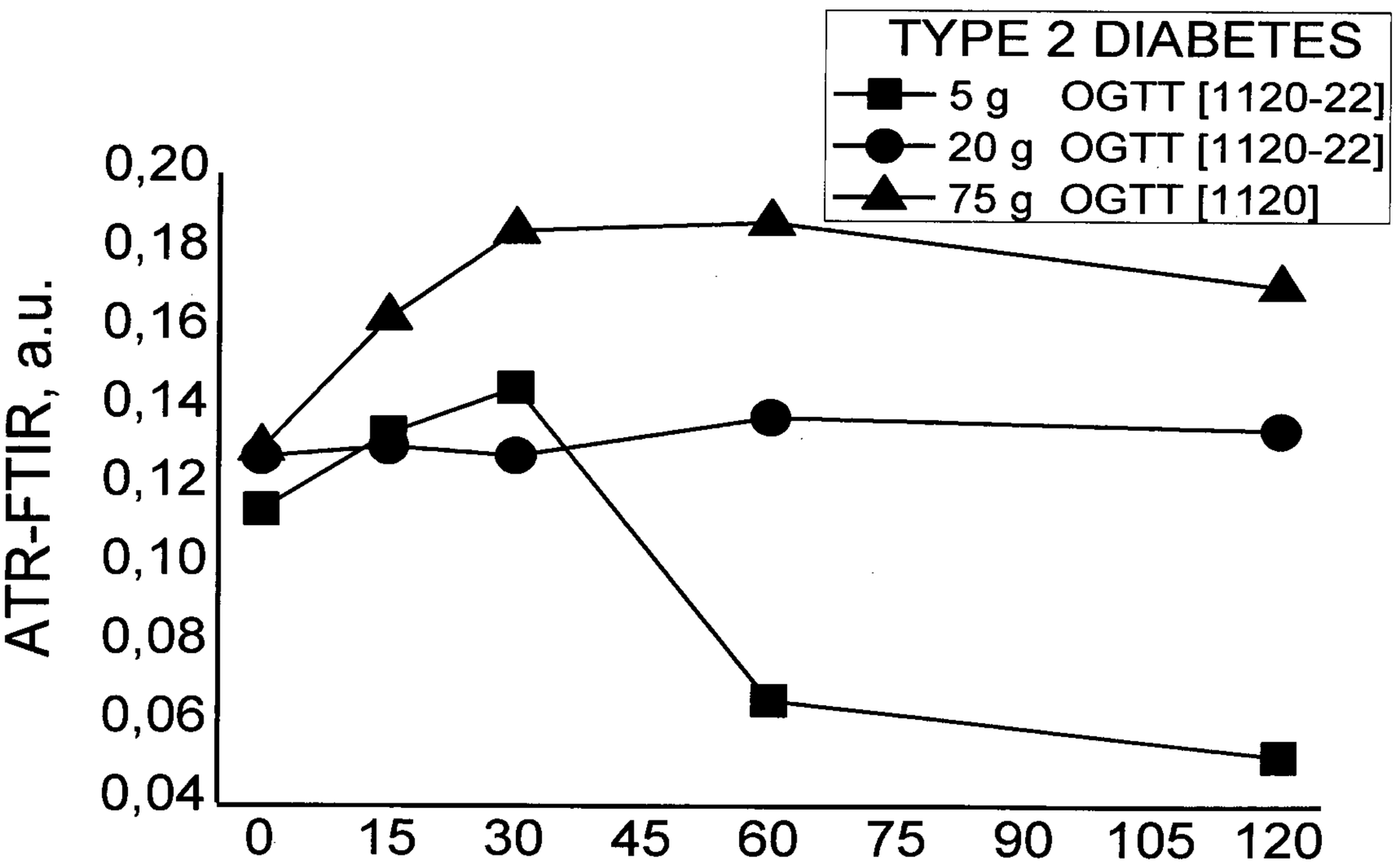
TYPE 2 DIABETES
5 g OGTT [1120-22]
20 g OGTT [1120-22]
75 g OGTT [1120]
ATR-FTIR, a.u.
0,20
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0
15
30
45
60
75
90
105
120
time, min

Fig. 6C

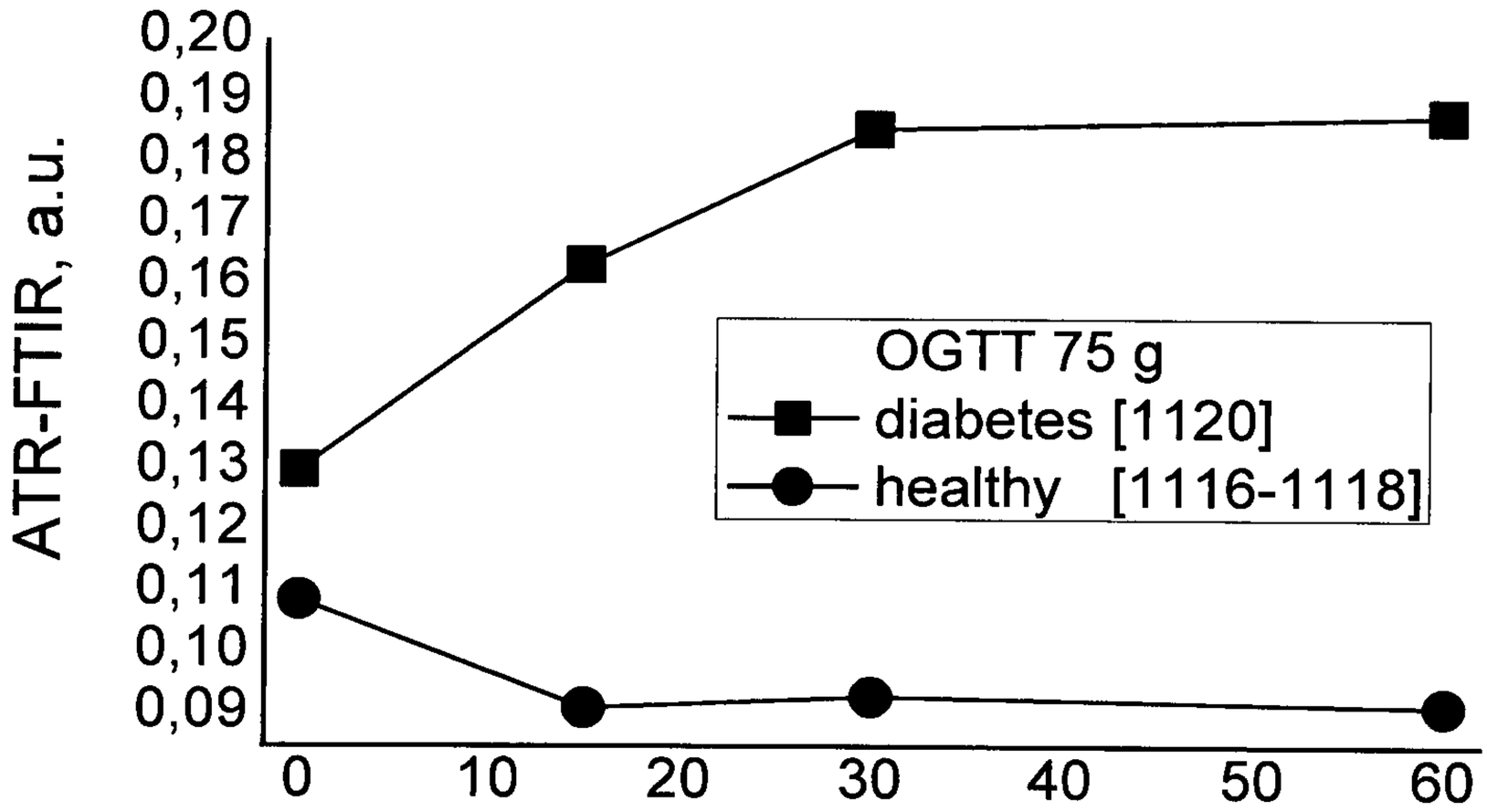
ATR-FTIR, a.u.
0,20
0,19
0,18
0,17
0,16
0,15
0,14
0,13
0,12
0,11
0,10
0,09
0
10
20
30
40
50
60
time, min
OGTT 75 g
diabetes [1120]
healthy [1116-1118]

Fig. 7A

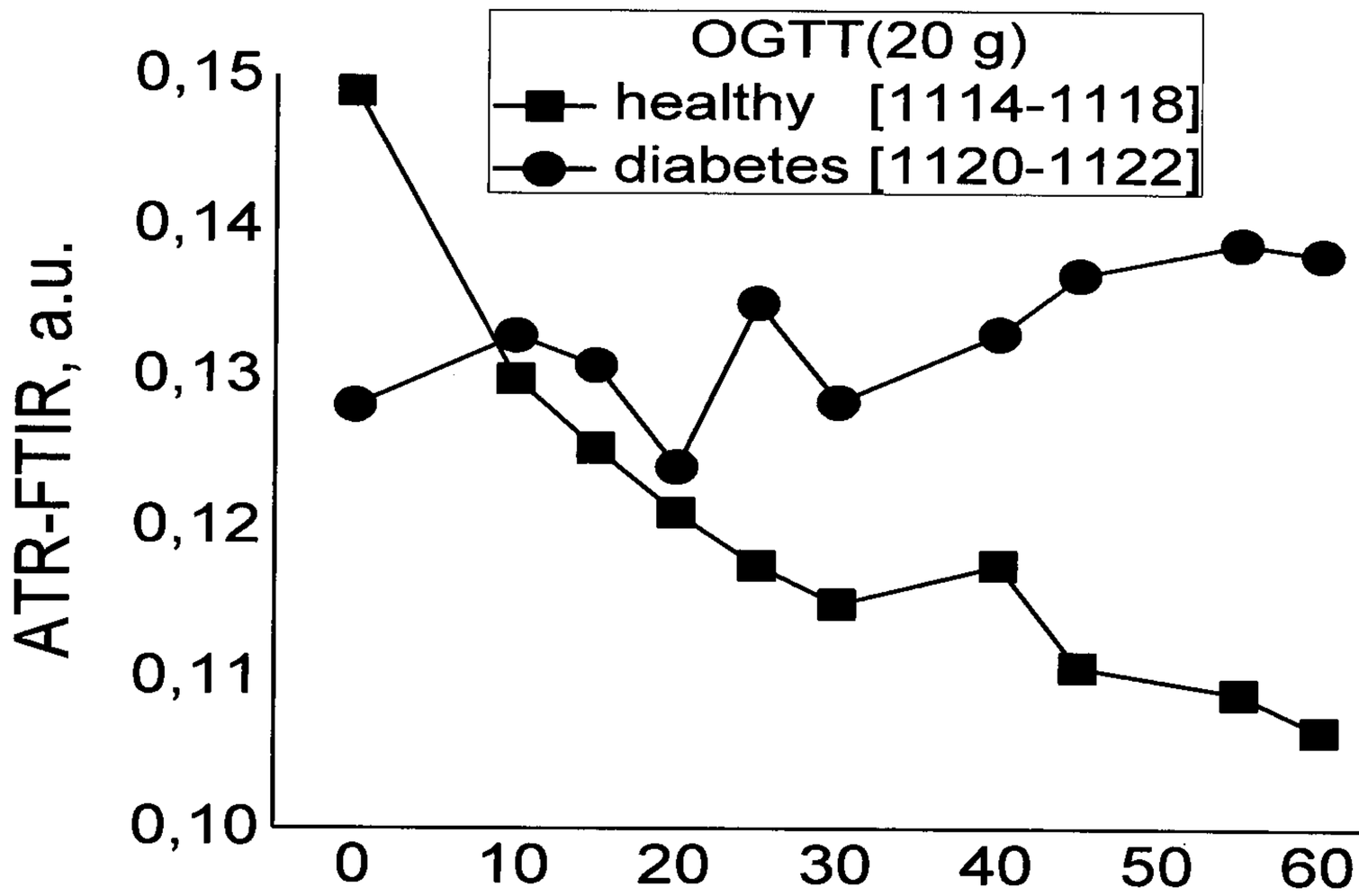
ATR-FTIR, a.u.
0,15
0,14
0,13
0,12
0,11
0,10
0
10
20
30
40
50
60
time, min
OGTT(20 g)
healthy [1114-1118]
diabetes [1120-1122]

Fig. 7B

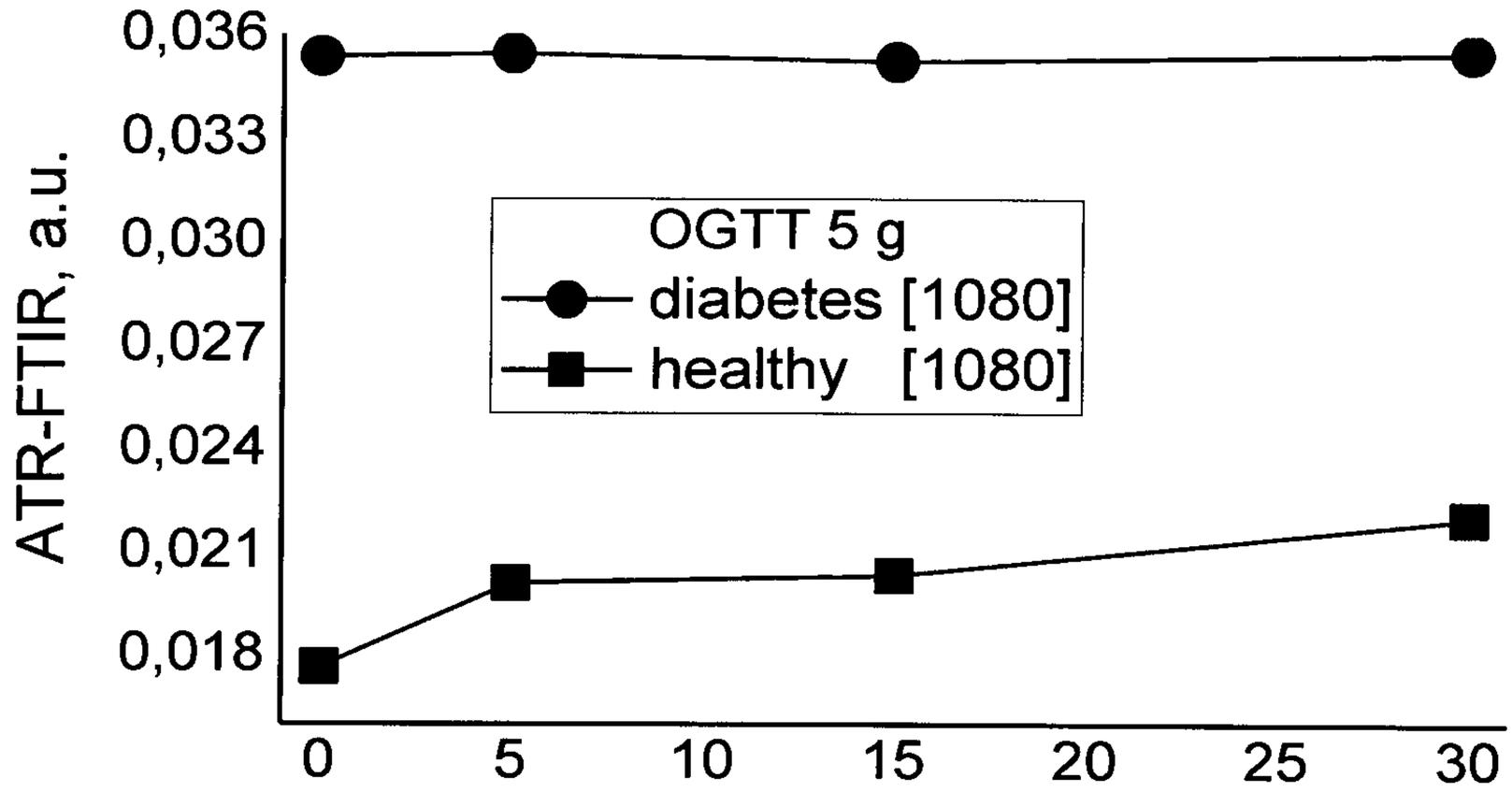
0,036
0,033
0,030
0,027
0,024
0,021
0,018
ATR-FTIR, a.u.
OGTT 5 g
diabetes [1080]
healthy [1080]
0
5
10
15
20
25
30
time, min

Fig. 7C


TYPE 2 DIABETES
1039-1041
1080
1122
1153
0,20
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
ATR-FTIR, a.u.
0
30
60
90
120
150
180
210
240
time, min

Fig. 8A

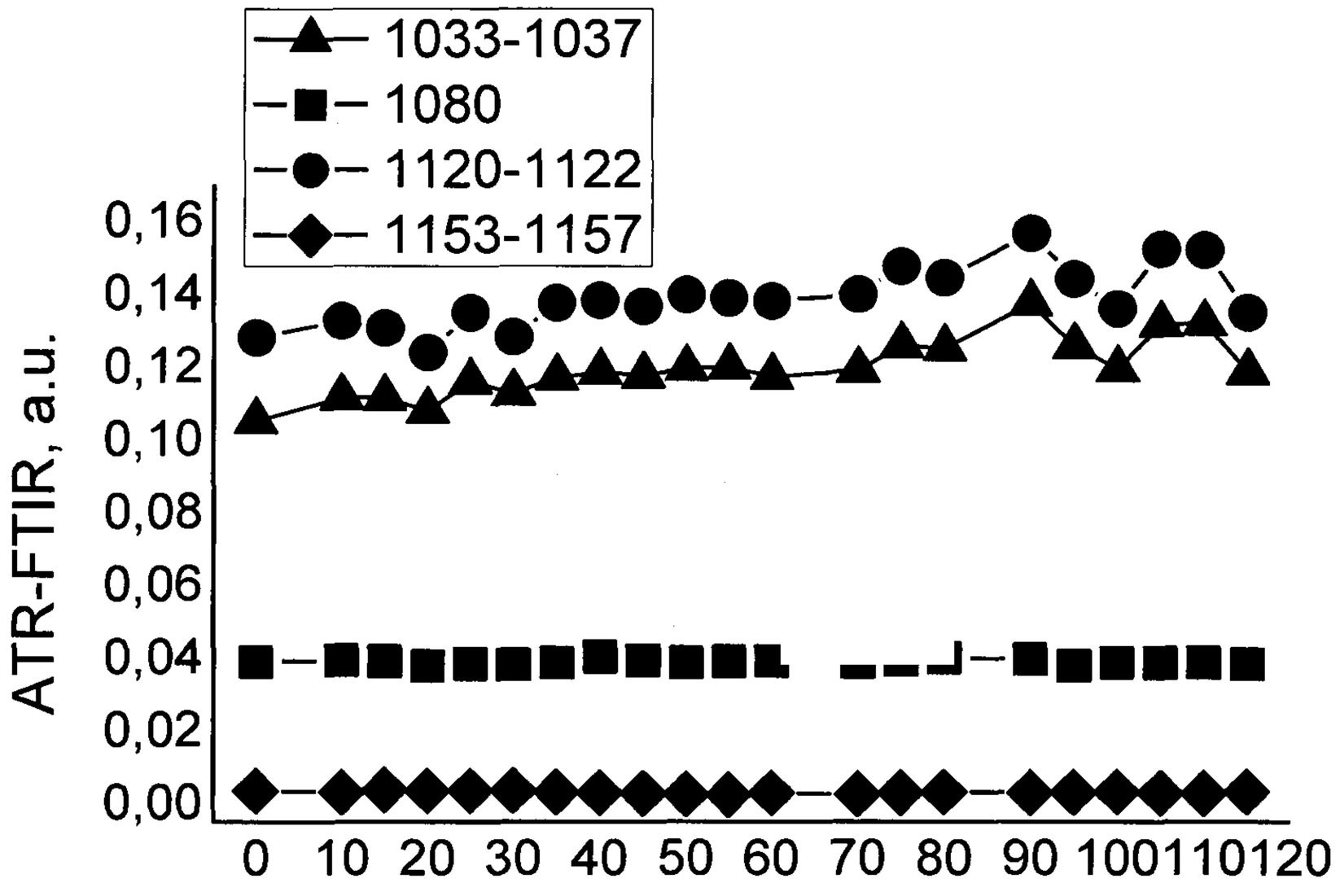
1033-1037
1080
1120-1122
1153-1157
ATR-FTIR, a.u.
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
0 10 20 30 40 50 60 70 80 90 100110120
time, min

Fig. 8B

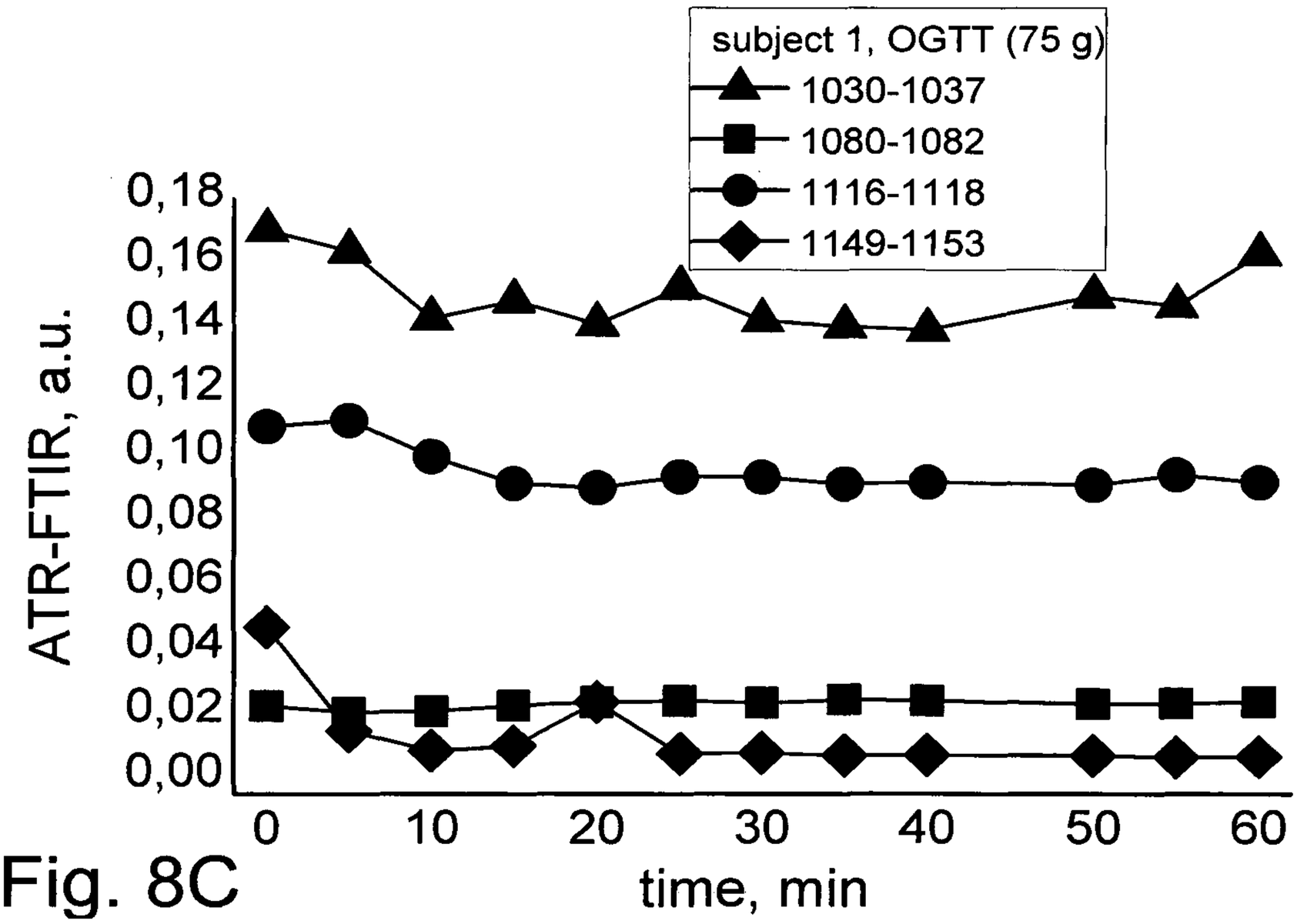
subject 1, OGTT (75 g)
1030-1037
1080-1082
1116-1118
1149-1153
ATR-FTIR, a.u.
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
0 10 20 30 40 50 60
time, min

Fig. 8C

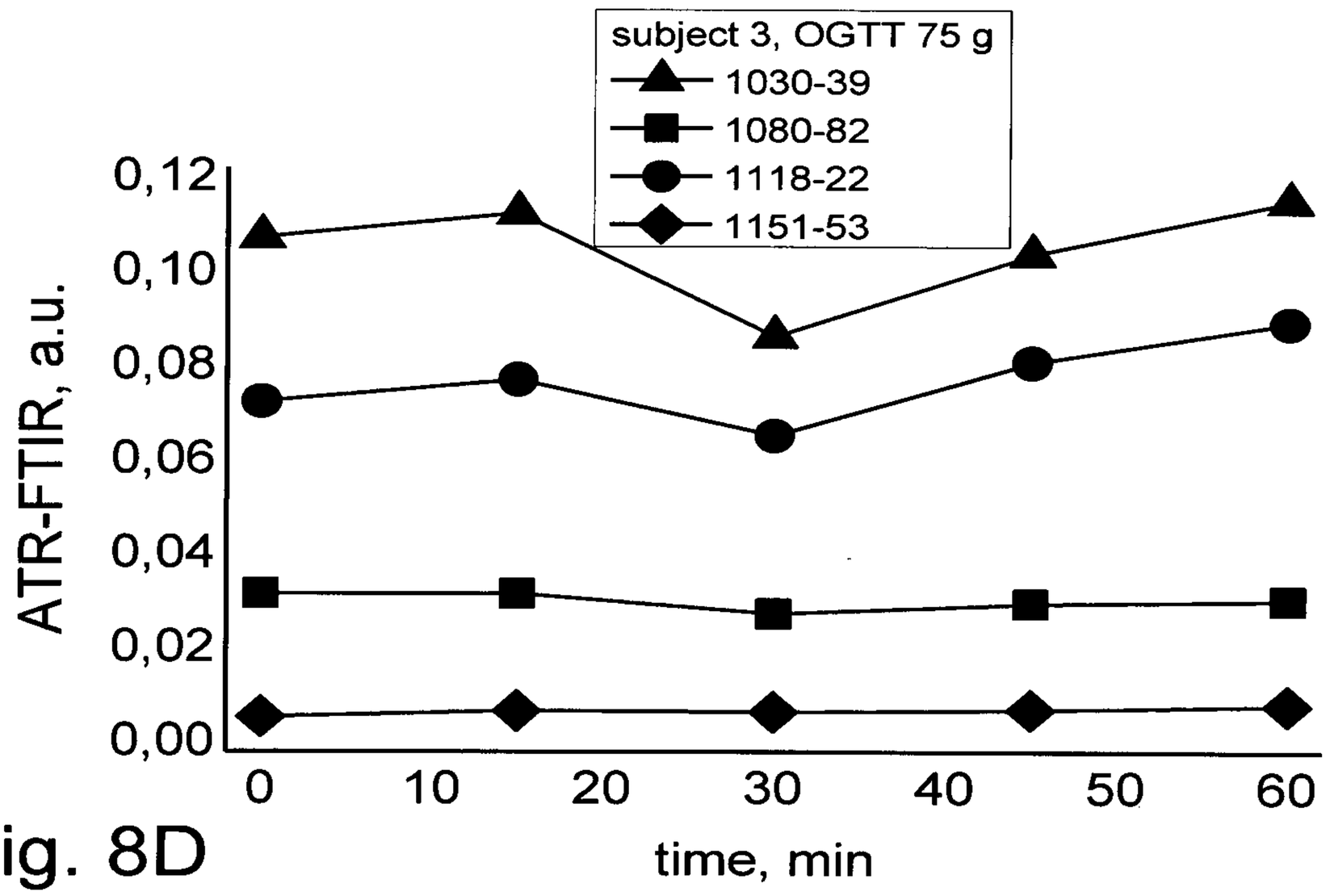
subject 3, OGTT 75 g
1030-39
1080-82
1118-22
1151-53
ATR-FTIR, a.u.
0,12
0,10
0,08
0,06
0,04
0,02
0,00
0
10
20
30
40
50
60
time, min

Fig. 8D

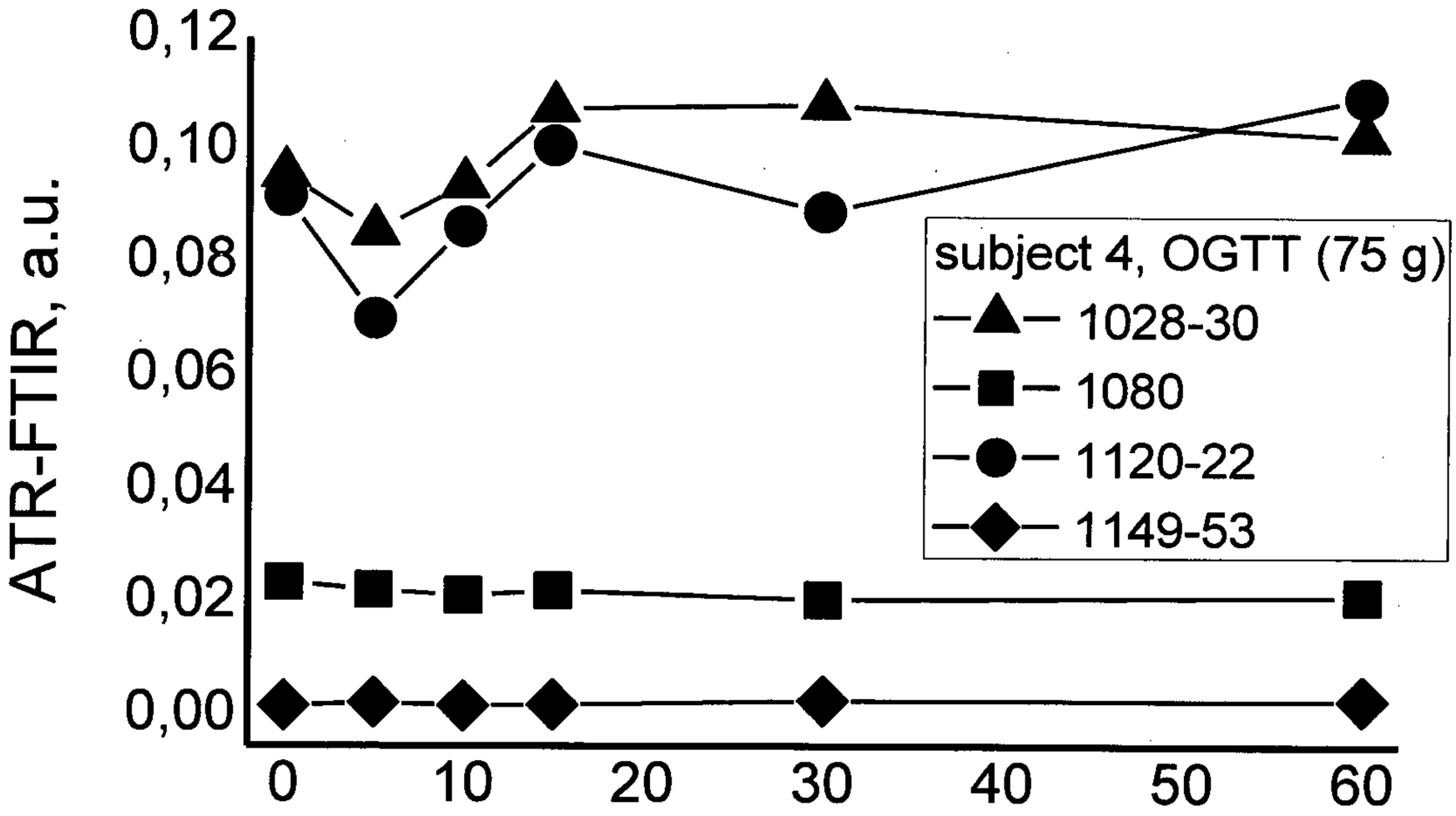
subject 4, OGTT (75 g)
1028-30
1080
1120-22
1149-53
ATR-FTIR, a.u.
0,12
0,10
0,08
0,06
0,04
0,02
0,00
0
10
20
30
40
50
60
time, min

Fig. 8E

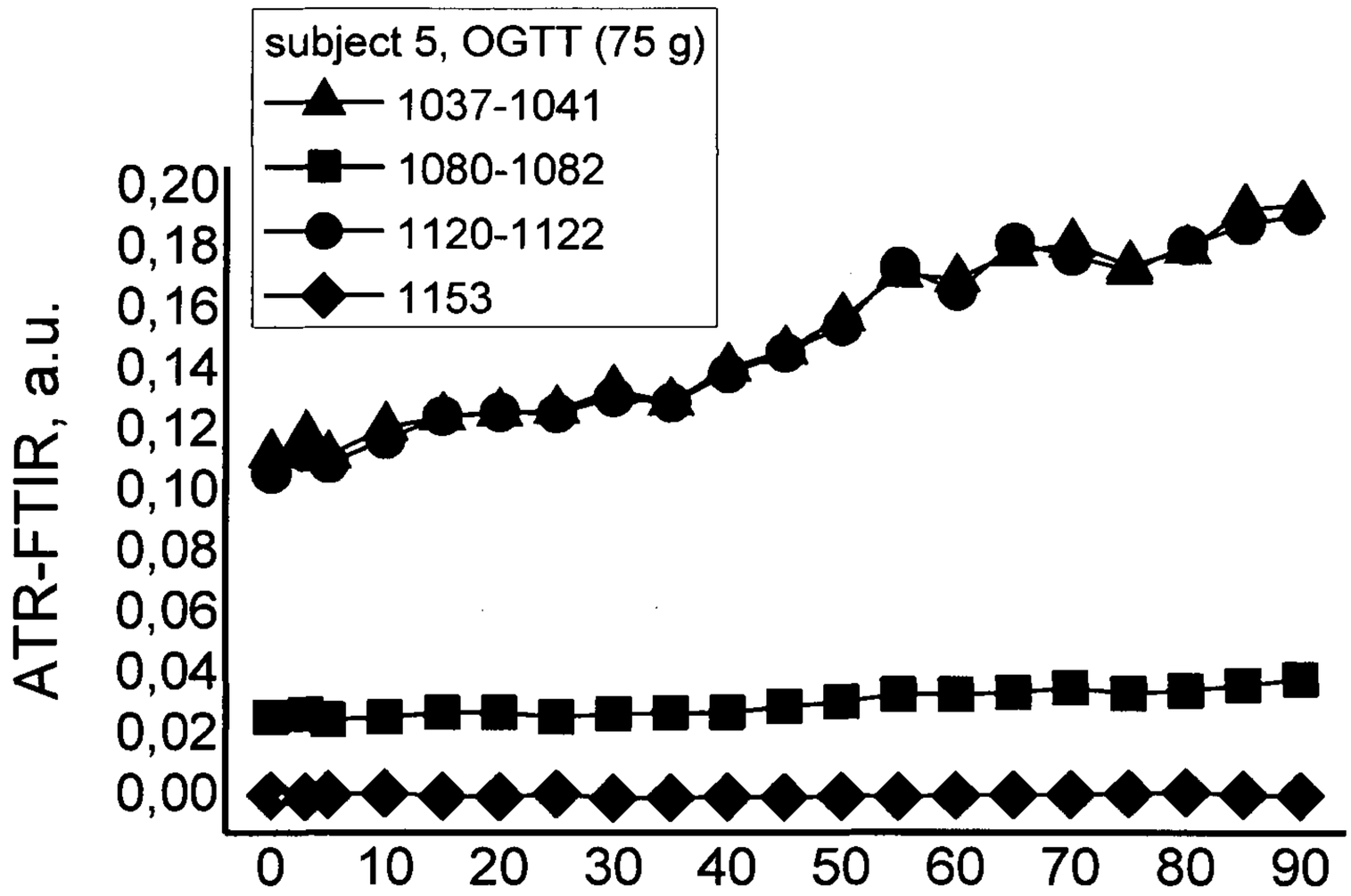
subject 5, OGTT (75 g)
1037-1041
1080-1082
1120-1122
1153
ATR-FTIR, a.u.
0,20
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
0
10
20
30
40
50
60
70
80
90
time, min

Fig. 8F

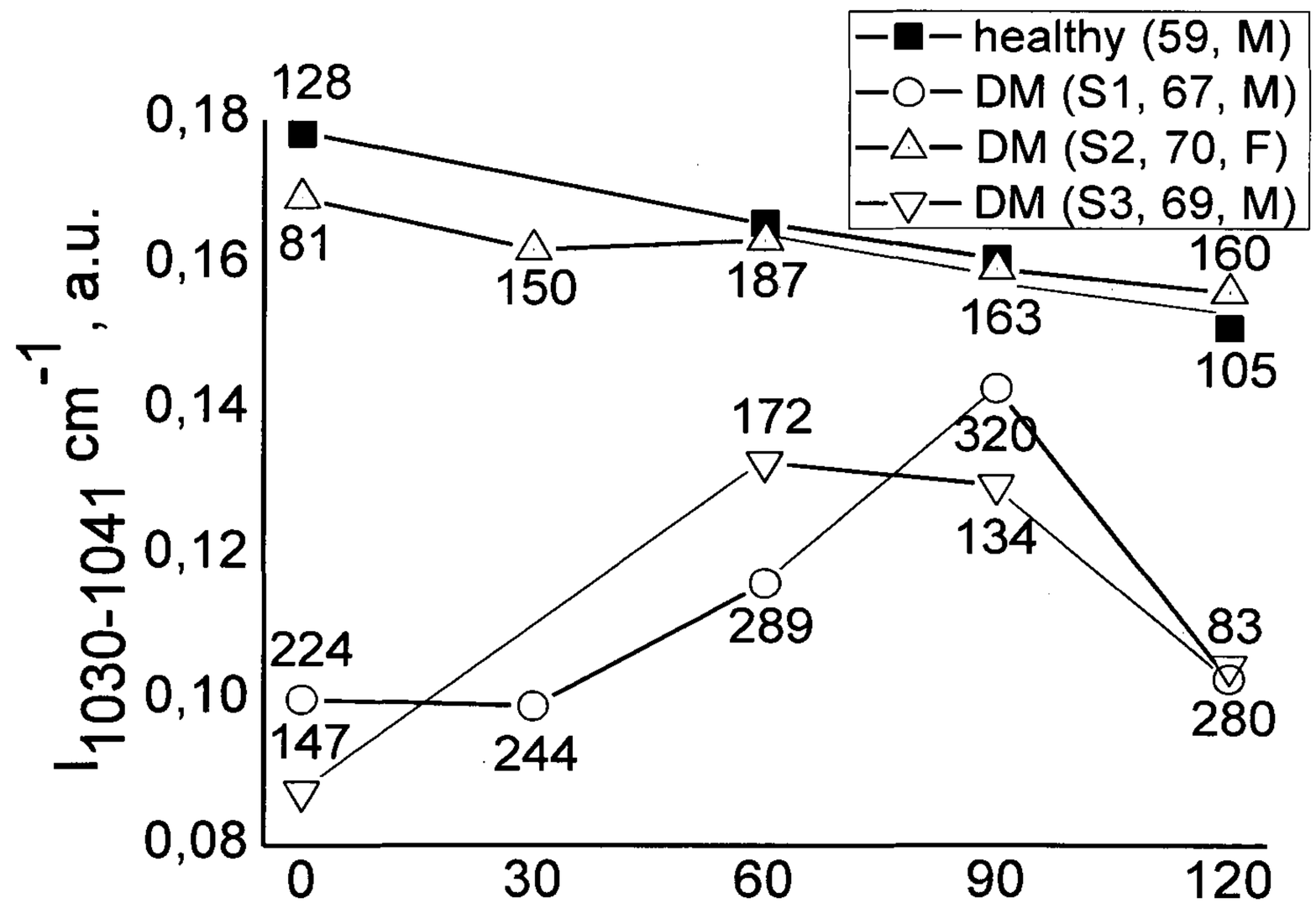
healthy (59, M)
DM (S1, 67, M)
DM (S2, 70, F)
DM (S3, 69, M)
I1030-1041 cm-1, a.u.
0,18
0,16
0,14
0,12
0,10
0,08
128
81
150
187
163
160
105
172
320
134
83
280
224
147
244
289
0
30
60
90
120
time, min

Fig. 9A

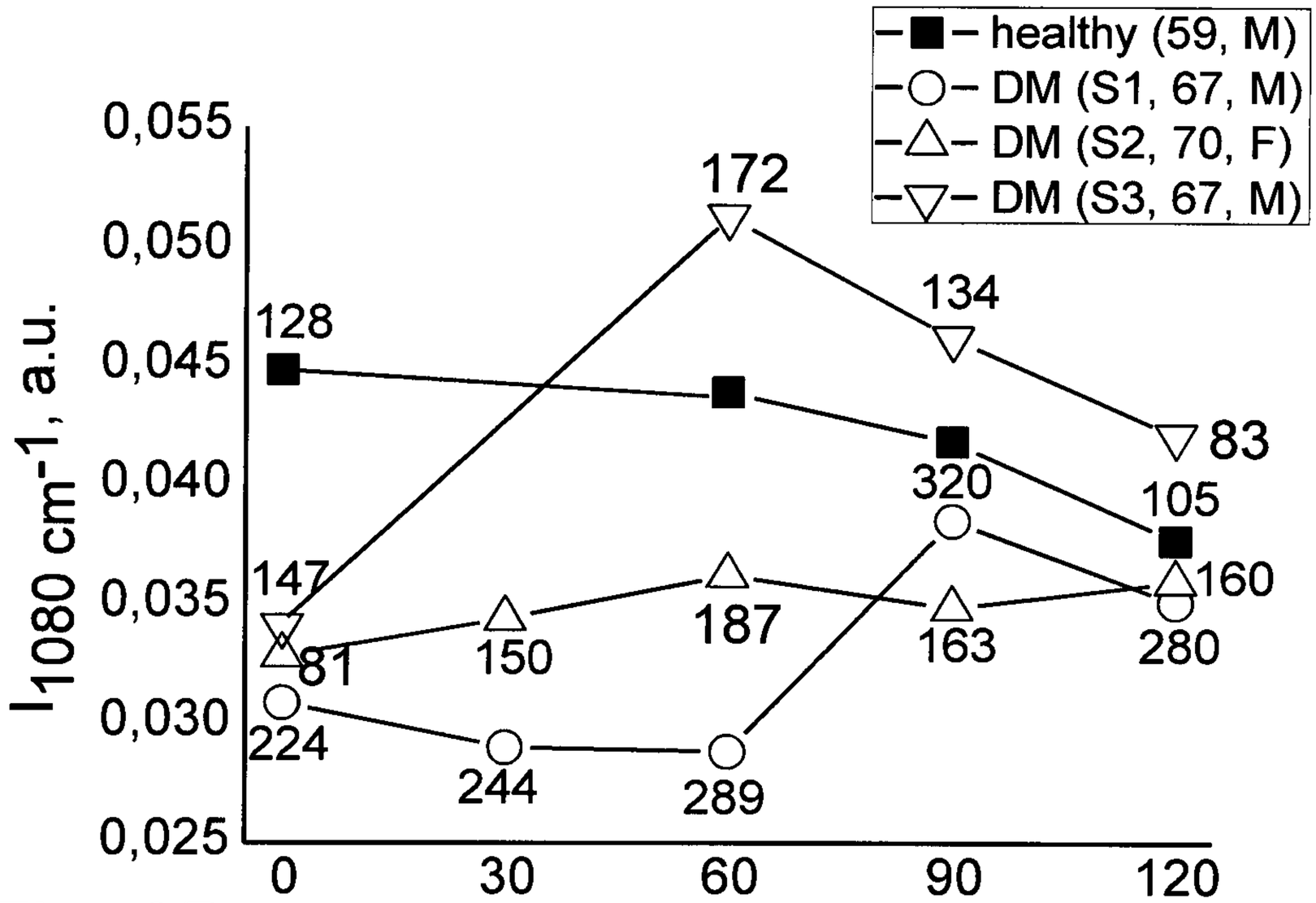
healthy (59, M)
DM (S1, 67, M)
DM (S2, 70, F)
DM (S3, 67, M)
I_{1080} cm^{-1}, a.u.
0,055
0,050
0,045
0,040
0,035
0,030
0,025
0
30
60
90
120
time, min
128
172
134
83
320
105
147
81
150
187
163
160
280
224
244
289

Fig. 9B

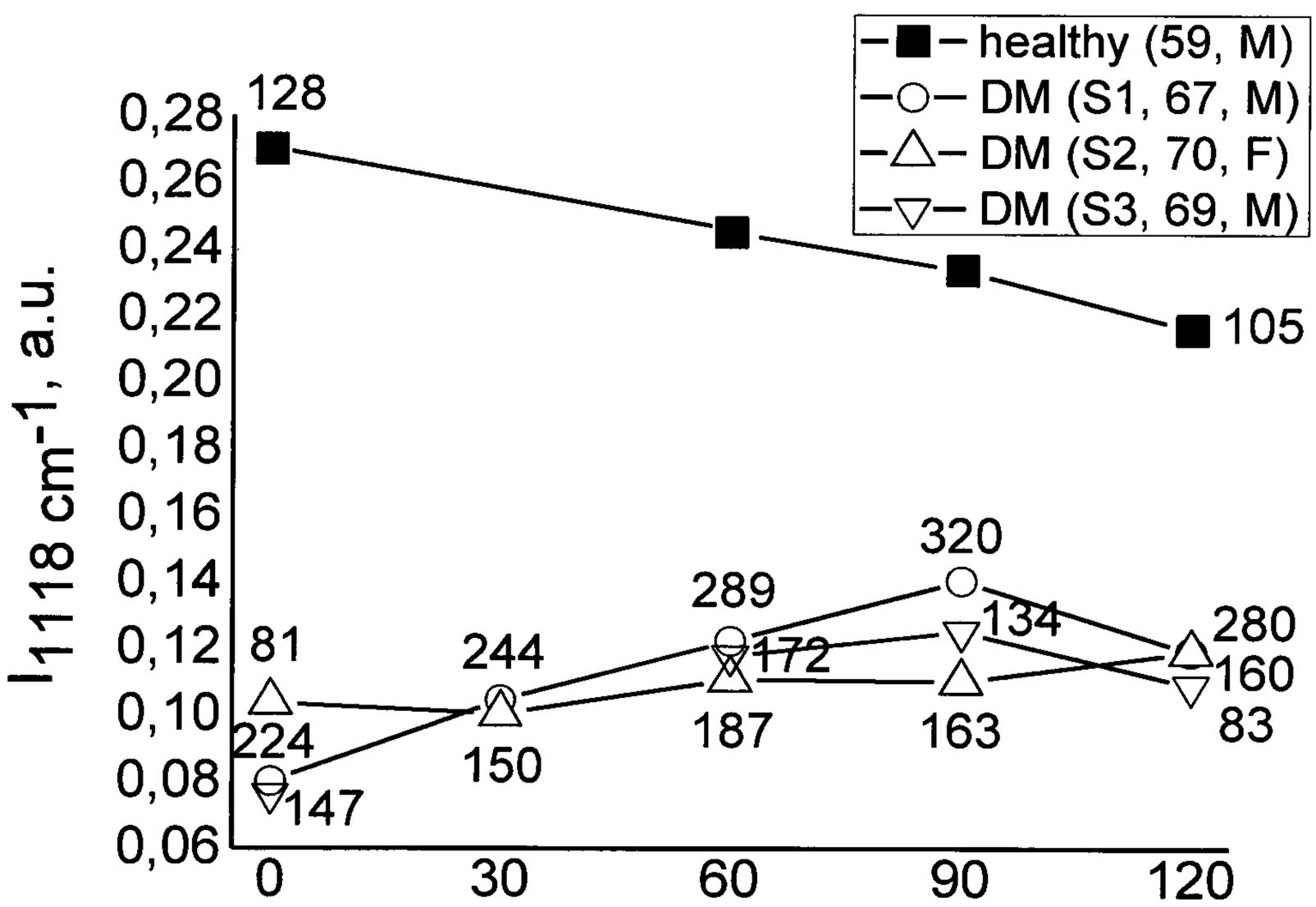
healthy (59, M)
DM (S1, 67, M)
DM (S2, 70, F)
DM (S3, 69, M)
I_{1118} cm^{-1}, a.u.
0,28
0,26
0,24
0,22
0,20
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0
30
60
90
120
time, min
128
105
320
289
134
172
280
160
83
81
244
224
150
187
163
147

Fig. 9C

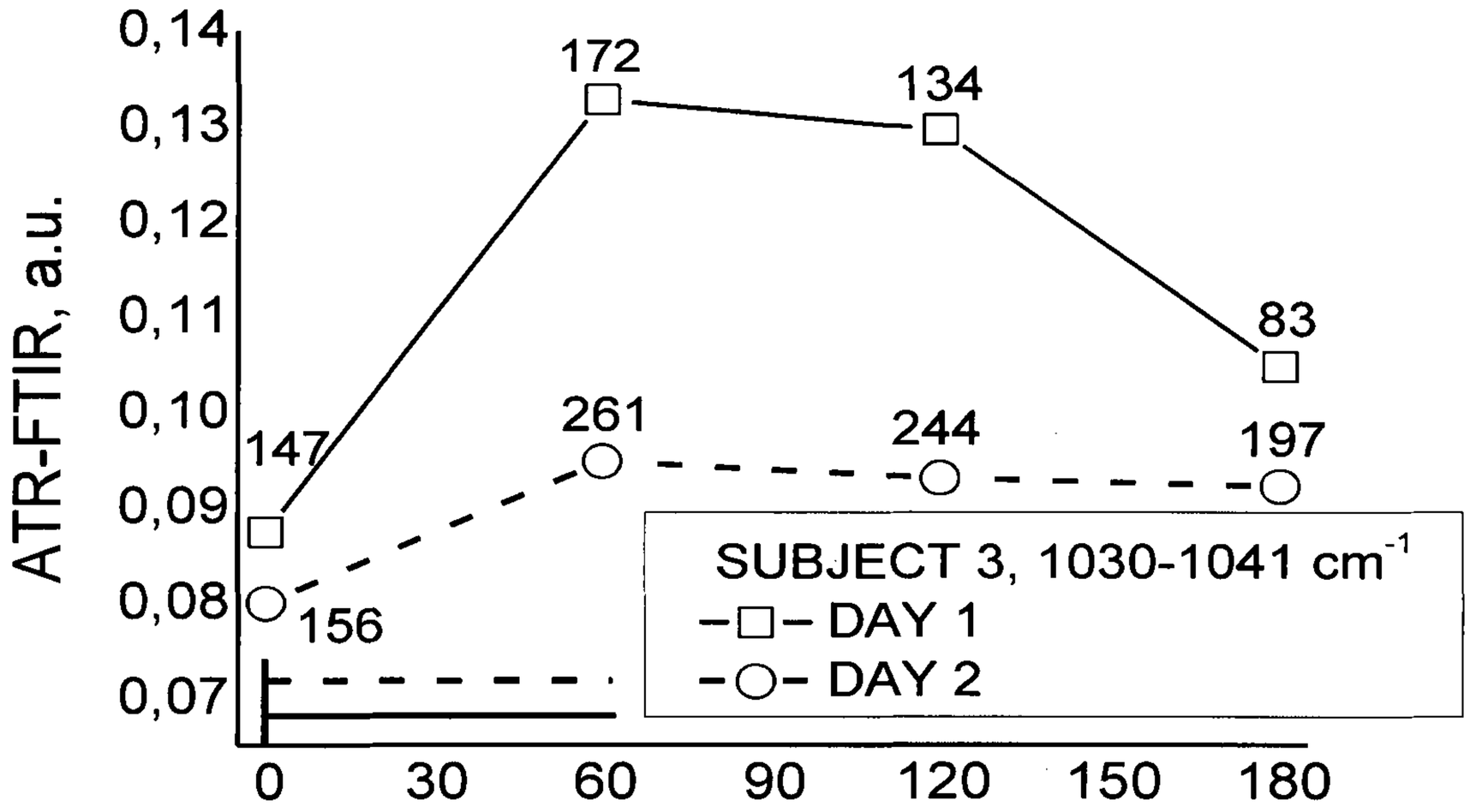

Fig. 10A

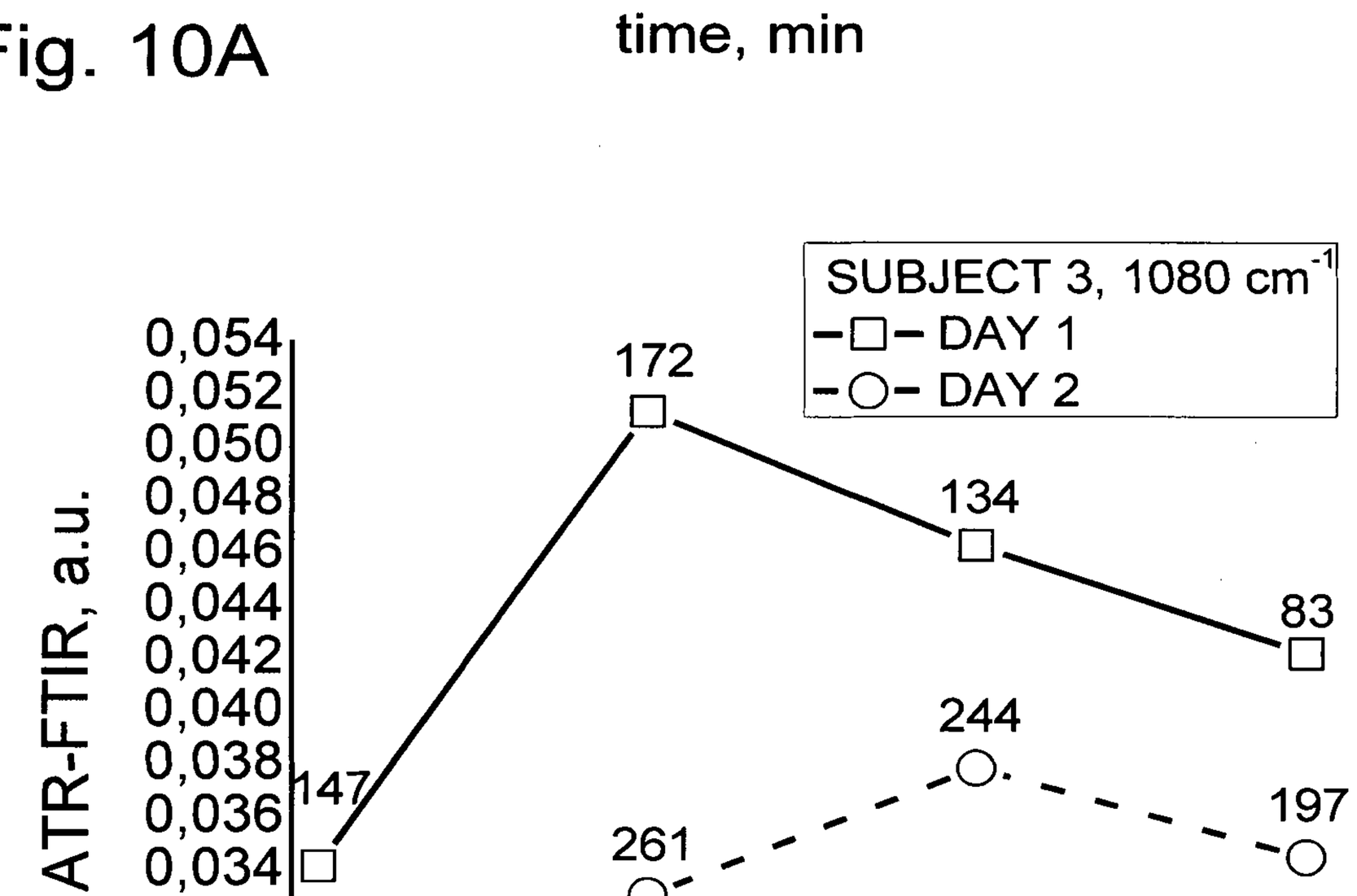

Fig. 10B

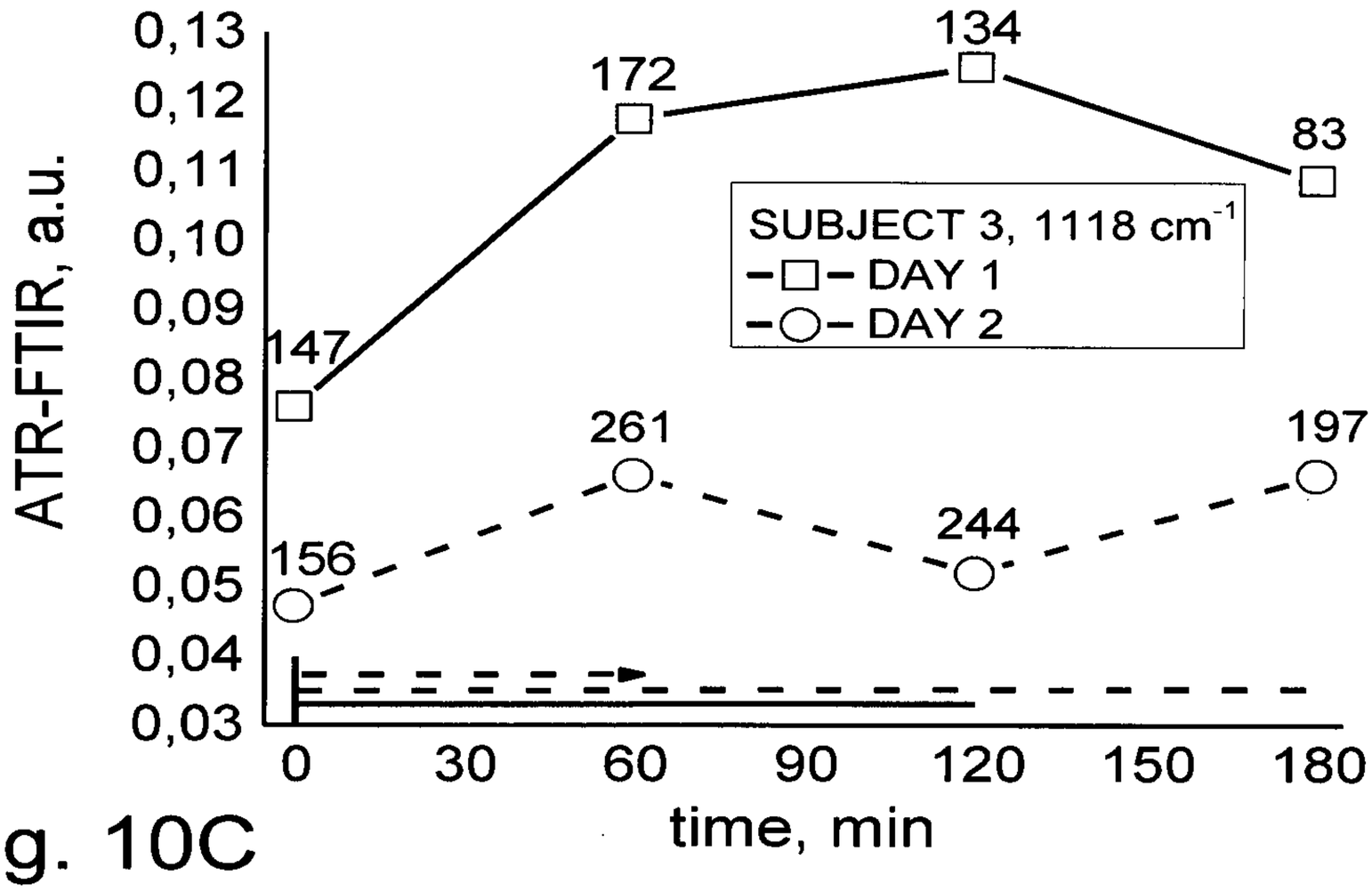

Fig. 10C

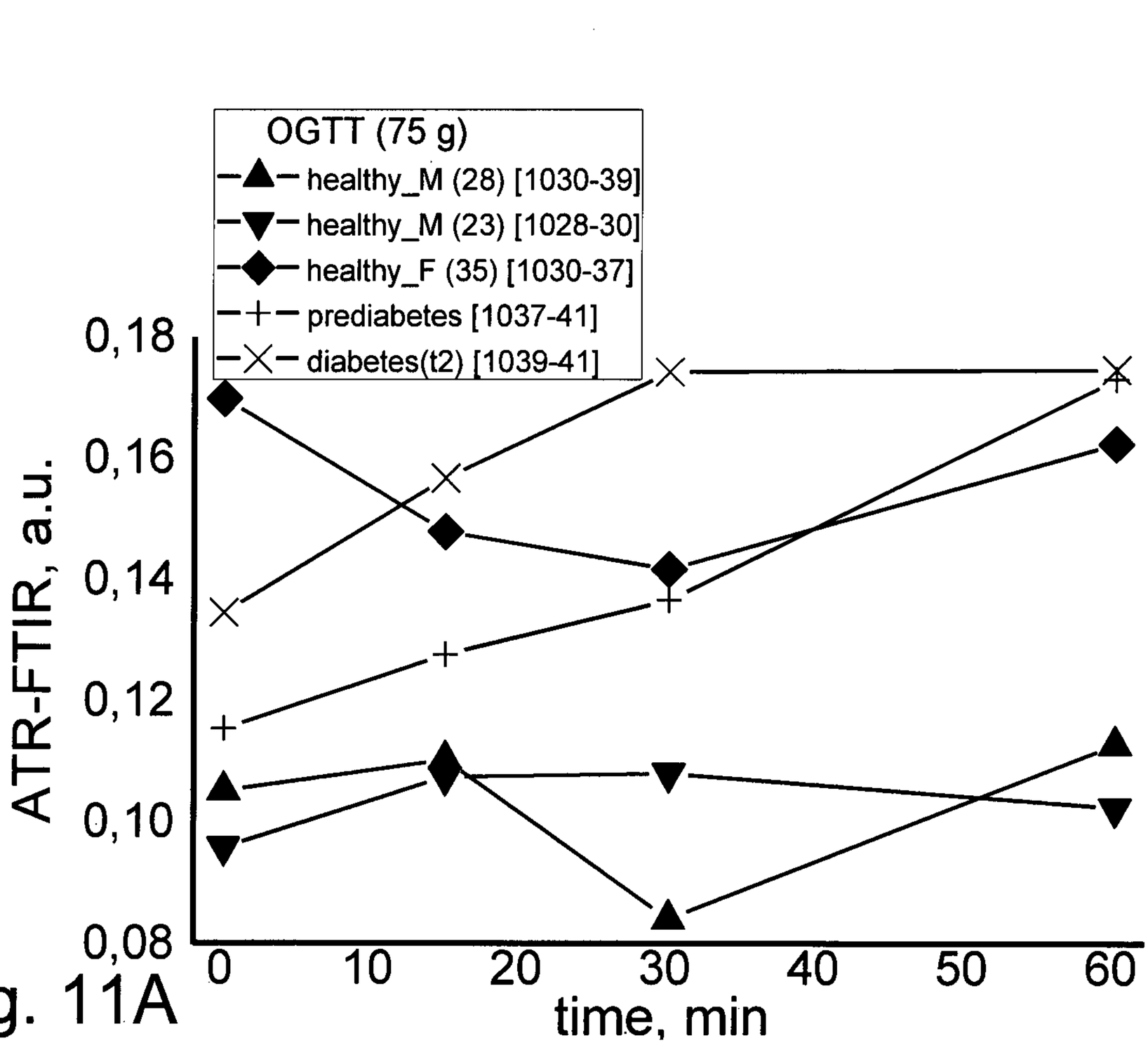

Fig. 11A

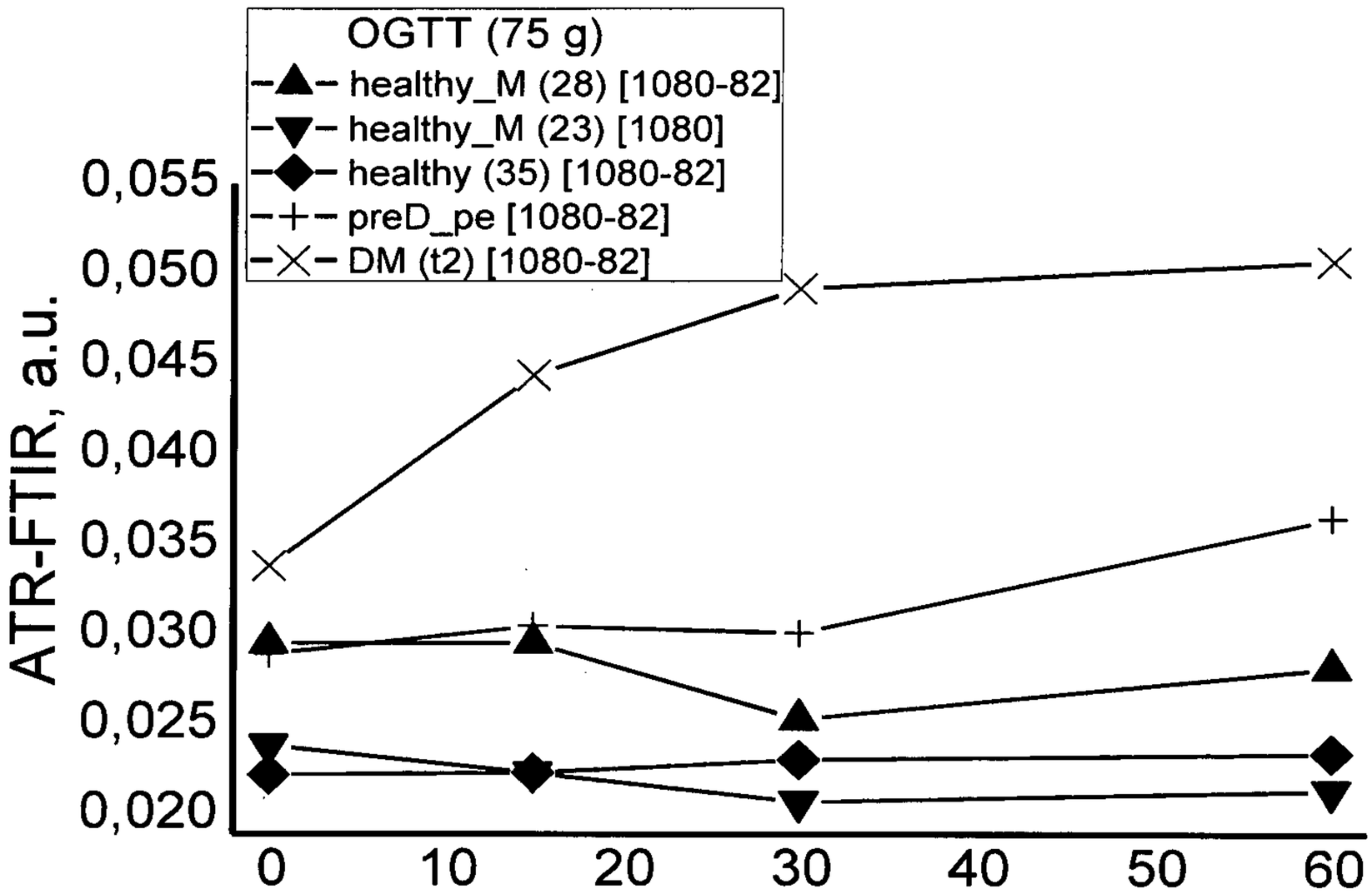

Fig. 11B

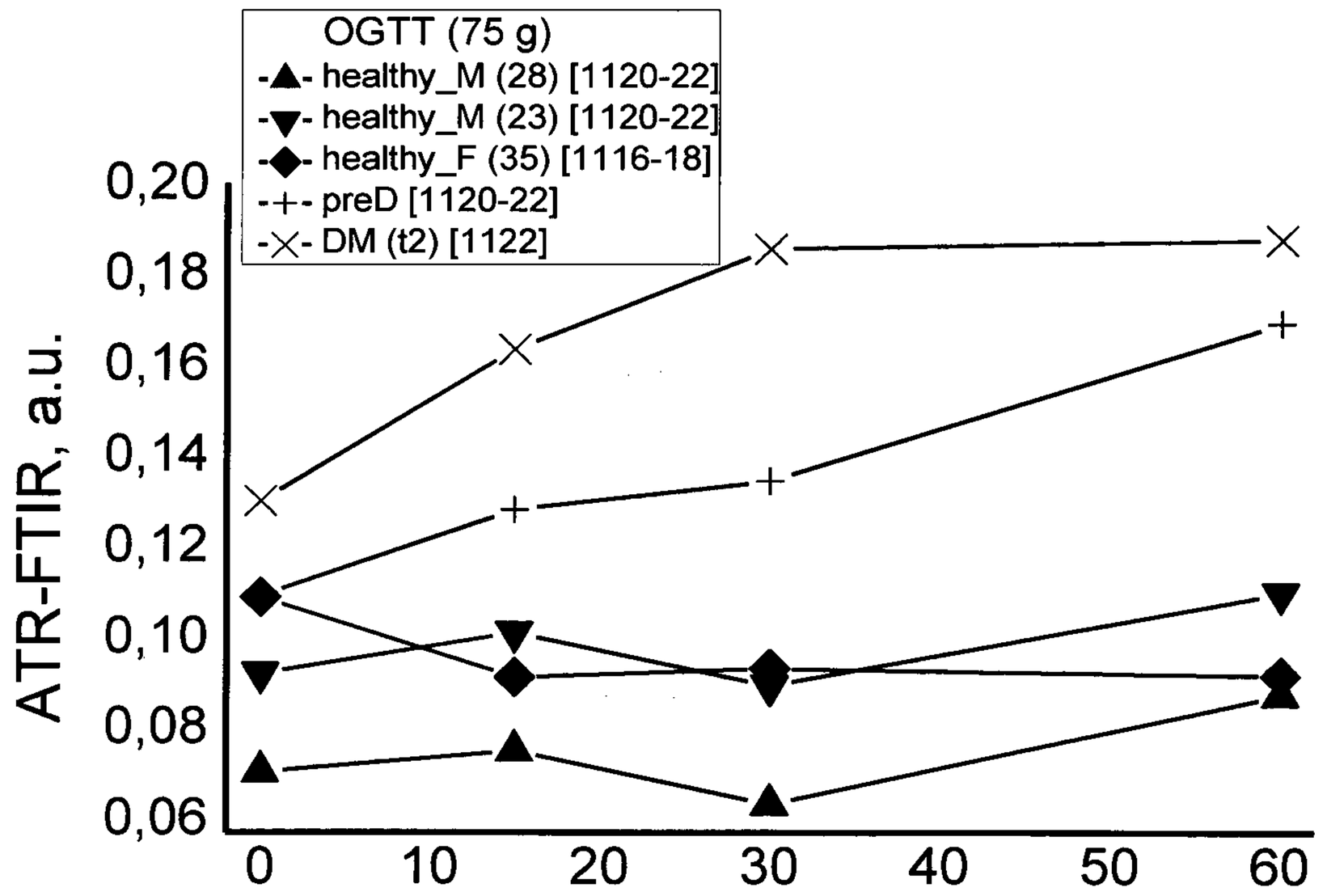

Fig. 11C

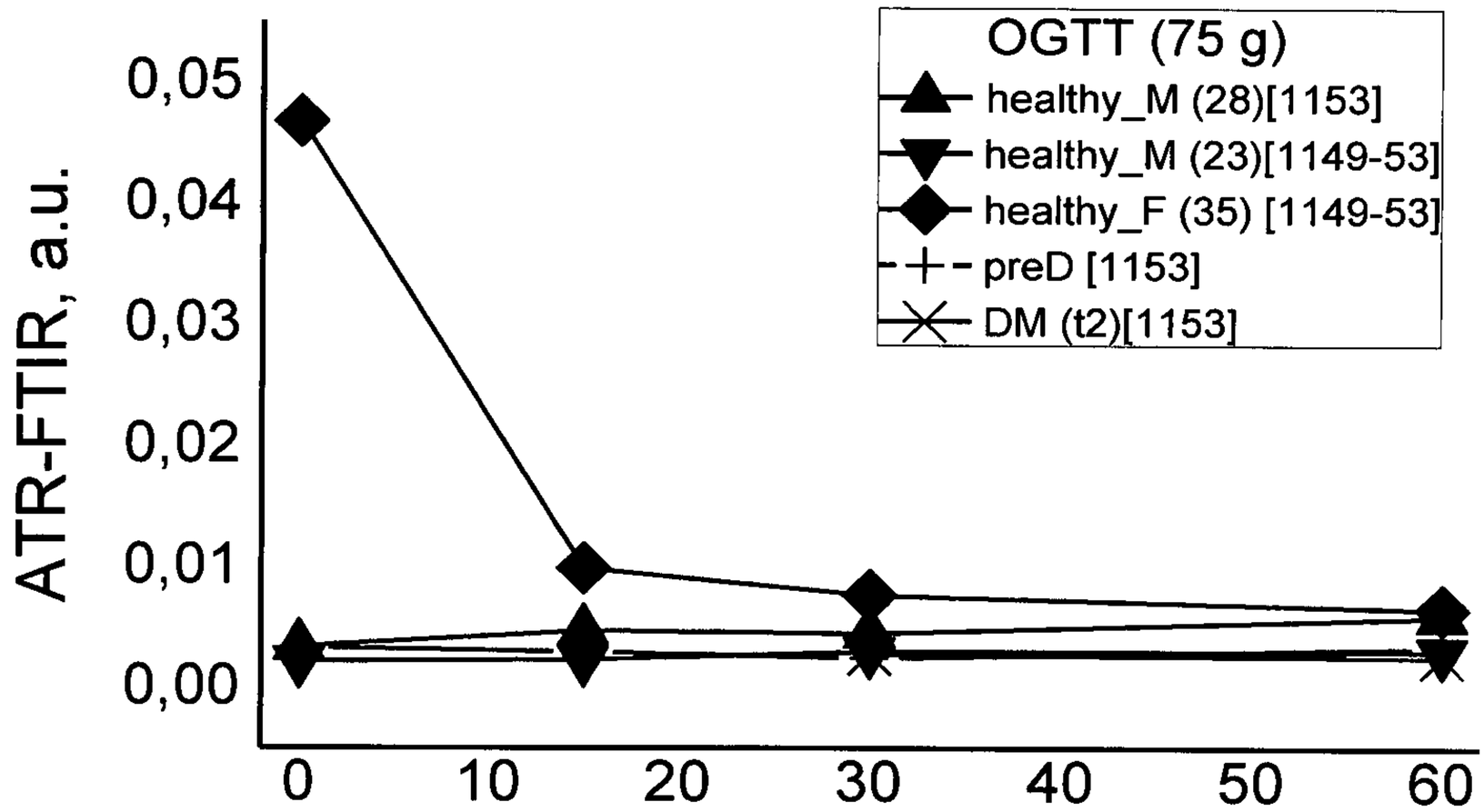
OGTT (75 g)
healthy_M (28)[1153]
healthy_M (23)[1149-53]
healthy_F (35) [1149-53]
preD [1153]
DM (t2)[1153]
ATR-FTIR, a.u.
0,05
0,04
0,03
0,02
0,01
0,00
0
10
20
30
40
50
60
time, min

Fig. 11D