Interstitial measurements of glucose, glycerol and lactate in adolescents with decompensated type 1 diabetes

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Abstract

OBJECTIVE: The study was undertaken to assess the interstitial, adipose tissue concentrations of glucose, lactate and glycerol in teenagers with diabetes type 1 who suffered from the disease for a minimum of 5 years, in whom it was impossible to reach a satisfactory level of metabolic control of the disease.

METHODS: Using microdialysis technique interstitial concentrations of glucose lactate and glycerol was measured in adipose tissue during 24–48 hours. Nineteen teenagers with poorly controlled type 1 diabetes (HbA1c 8.9 ± 2.85%) were compared with six adolescent control subjects.

RESULTS: A statistically significant differences in concentration values of interstitial glucose between the investigated and control groups were found (10.4 vs. 4.26 mmol/l p=0.001). The values of interstitial concentrations of lactates did not significantly differ in the two groups (2.96 vs. 2.54 mmol/l NS). The average daily glycerol concentrations in the investigated group were statistically significantly lower than those in the control group (258.26 vs. 397.88 μmol/l, p=0.019). No such difference was detected in average night concentrations of glycerol (157.78 vs. 361.4 μmol/l, NS).

CONCLUSIONS: Authors conclude that microdialysis is the only one minimal invasive method for investigating adipose tissue metabolism *in vivo* and provides a novel opportunity for glucose and lipids metabolism monitoring in adolescents with diabetes type 1. In our observations interstitial glycerol concentrations, measured in abdominal subcutaneous adipose tissue as an index of lipolysis, were not significantly influenced by hyperglycemia in diabetic adolescents.

ORIGINAL ARTICLE

INTRODUCTION

Many diagnostic and therapeutic decisions in medical practice are based on measuring blood concentrations of endogenous molecules. However, most biochemical and pharmacological processes take place in the tissues. Assessing tissue chemistry should theoretically provide more accurate data, as this can now be achieved relatively cheaply and minimally invasively with microdialysis. Microdialysis has been shown to offer information about substances directly at the site of action while being well tolerable and safe (Müller 2002).

Principles of microdialysis

An appropriate probe is the most important part of the diagnostic kit. Microdialysis is based on the sampling of interstitial fluid. In general, the central part of the apparatus is a semipermeable membrane, which allows an exchange between interstitial and perfusion fluids. The membrane is constantly rinsed with a fluid similar to the physiological solution by means of special micropump. The pump is propelled by batteries; its syringe contains about 2.5 ml of fluid (Ringer solution). Usually, the fluid is perfused at the speed of 0.3 µl/min, which allows it to work for five days.

A microdialysis probe is inserted into interstitial fluid. Intramuscular, subcutaneous or intracerebral probes are also available. At the tip of the probe perfused interstitial fluid is transferred to microprobes that serve as temporary reservoirs. Small molecules can freely pass through the membrane by means of perfusion. The fluid gathered in microprobes is then analyzed.

Changes in the fluid composition reflect those in interstitial compartment (Arner 1997). Microprobes are exchanged regularly, usually every hour and placed inside the analyzer. Then, the results (of biochemical changes occurring in the interstitial fluid) are presented on the monitor.

An extremely important aspect of the technique described is its simplicity and safety in use. It can be widely used in children, elderly people, unconscious patients lying on intensive care units (Ungerstedt 1991; Hildingsson *et al.* 2000; Hildingsson *et al.* 1996). A probe insertion does not require special skills and may be performed by nurses or auxiliary medical staff only after a short training. The operation is as painful as the insertion of an ordinary intravenous line. The exemption are intracerebral probes that must be handled by qualified staff only in sterile environment, such as operating theatre.

Microdialysis has been used to obtain long term (more than 24 hours) profiles of endogenous or exogenous substances in different tissues. It can be a valuable tool in patients with minimal blood supply, such as neonates.

Monitoring of substance concentrations

There are hundreds, if not thousands of substances possible to be studied by means of microdialysis. They are divided into several categories:

1. metabolites of energetic pathways
2. neurotransmitters
3. chemical markers of tissue damage (destruction)
4. various substances of exogenous origin (drugs) (Herkner *et al.* 2002; Müller 2000).

For the clinician, if the information obtained by means of microdialysis could reflect the present state of cellules or, ideally, if it would make it possible to anticipate and prevent some irreversible changes, that would make the method a powerful diagnostic tool. However, in spite of its many advantages it should be underlined that in practice only few substances can be measured by means of this method. The main obstacle is the price and availability of chemical reagents required for the procedure. Moreover, the pores of semi-permeable membrane do not allow to pass molecules larger than 50 kD which makes the measurement of larger molecules impossible.

The state-of-the-art knowledge gives priority to measurements of substances that reflect energetic phenomena (changes) in tissues, such as episodes of ischaemia. These can be, for example, indices of a degree of organ damage. The first studies used a microdialysis technique analyzing concentrations of neurotransmitters in experimental rats' brains. The first studies in humans investigated glucose concentrations in fat tissue in 1997.

The most commonly measured metabolites are: glucose, lactate, pyruvate and glutamate.

Glucose measurements

Measurements of glucose, lactate and pyruvate allow to investigate the metabolism of glucose in brain and peripheral tissue. Glucose concentration is in strict relation to blood supply and cellular metabolism. Pyruvate and lactate levels reflect the character and degree of energetic changes, the lactate/pyruvate ratio is a well-known index of tissue ischaemia.

Glycerole measurements

An interpretation of glycerol concentration varies according to the place of measurement. In subcutaneous fat tissue glycerol level reflects the degree of lipolysis. Because the latter is controlled by noradrenalin excretion from nerve fibers it may be interpreted as a direct index of the present sympathetic activity (Marcus *et al.* 1989; Arner & Bolinder 1991; Kamel *et al.* 1999; Lafontan & Arner 1996). An increase of glycerol level inside the central nervous system points out to a desintegration of cellular membranes. A probable explanation of this phenomenon may be that ischaemia (i.e. the insufficient supply of oxygen and glucose) causes disturbances of proton pump, calcium influx and phos-

pholipase activation which triggers membrane destruction (Hillered, Persson 1999; Eblad *et al.* 1996).

The aim of the study was to assess the interstitial, adipose tissue concentrations of glucose, lactate and glycerol in teenagers with diabetes type 1 who suffered from the disease for a minimum of 5 years, in whom it was impossible to reach a satisfactory level of metabolic control of the disease.

MATERIALS AND METHODS

The study was conducted in a cohort of 19 patients, 13 to 19 years of age (X=16.89±1.53), whose diabetes type 1 was diagnosed when they were approximately 10.7±3.14 years of age. The investigated group comprised 5 boys and 14 girls. The control group consisted of 6 healthy teenagers aged 18 years of age (17.9±0.9), including 1 boy and 5 girls. The BMI of the investigated and control groups differed significantly [22.7 (17.6–32.0) vs 22.2 (18.7–25.4)].

All the patients in the investigated group were in the 4^{th} (1 person) or 5^{th} (18 persons) phase of puberty. 6 patients were diagnosed with a comorbid disease, and two patients suffered from autoimmunological thyroidits, treated with a substitution treatment with a good metabolic control. In the investigated group 13 patients had a family history of diabetes type 1 or 2. All the patients in the investigated group were given an intensive insulin treatment. The time of treating was approximately 5.6 (0.6–11.4) years. The requirement for insulin amounted to roughly 0.8 (0.34–1.4) U/kg of body mass per day. 5 patients used personal insulin pumps. All the probands were the patients of the Clinic of Pediatrics, Endocrinology, Diabetology, Metabolic Diseases and Cardiology of the Developmental Age at the Pomeranian Medical University of Szczecin, Poland and the Specialist Diabetic Clinic of the Developmental Age for Children and Young People of the SPSK Hospital No 1 in Szczecin.

All the patients were subjected to 24-hour blood pressure measurements by means of a round-the-clock automatic recording method (ABPM). Additionally, the proportion of glycosylated hemoglobin (HbA_1c) was measured and some selected parameters of lipid metabolism (cholesterol, triglycerides, HDL-C, LDL-C), as well as the amount of albumins excreted in daily urine were determined.

The percentage of glycosylated hemoglobin (HbA_1c) was measured as the proportion of total concentration of hemoglobin in the investigated sample by means of immunoturbidimetric and colorimetric method (total Hb) on Immulite apparatus, using Tina-grant test manufactured by Roche. Cholesterol concentration was determined by means of enzymatic-colorimetric method with cholesterol esterase and oxidase (CHOD-PAP). The concentration of triglycerides was determined by means of enzymatic-colorimetric method with glycerophosphate oxidase (GPO-PAP). The concentration of HDL cholesterol was measured using a direct improved immunological method and the concentration of LDL cholesterol was assessed by means of a direct method with catalase. Microalbuminuria was measured by means of immunturbidimetric method on the apparatus manufactured by Olympus.

The round-the-clock assessment of arterial blood pressure was made with Holter's method using an apparatus manufactured by Mobil-O-Graph. According to the guidelines set by the Task Force on Blood Pressure Control in Children, the correct values of systolic and diastolic pressure were assumed not to exceed 95 percentile for the age and sex for the measurements made during the day and the values were lowered by 10% for the measurements made during the night.

From the analysis of the round-the-clock assessment of arterial blood pressure the following values were calculated:

- the average daily systolic and diastolic pressure
- the average daytime and night systolic and diastolic pressure
- the maximum daytime and night systolic and diastolic pressure
- the lowering of daytime and night systolic and diastolic pressure.

Subcutaneous microdialysis was used to determine concentrations of glucose, lactate and glycerol. A CMA 60 catheter (length 30 mm, CMA Microdialysis, Solna, Sweden) was inserted in the umbilical subcutaneous fat in every subject. The catheter had a dialyzing membrane with a molecular cutoff of 20 kDa and was continuously perfused with a Ringer's solution (Perfusion fluid, CMA Microdialysis, Solna, Sweden) at a flow rate of 0.3 μL/min.

The perfusate was administered using the catheter with a CMA 106 pump. Samples were collected every 60 min during the 24 to 48 hours and analyses were performed bedside at the CMA 600 (CMA Microdialysis, Solna, Sweden). No complications with the technique occurred. The study was approved by the local Ethical Review Board.

Statistical methods

The statistical analysis was conducted using Statistica 8.0 software. The goodness of fit of the variables compared with normal distribution was checked using Shapiro-Wilk, Lilliefors and Kołmogarow-Smirnoff tests. In order to compare independent tests, non-parametric tests were used – U-Mann-Whitney and Wald-Wolfowitz's tests. In order to compare dependent tests, Wilcoxon's test and a sign test were used. The differences in average values $p<0.05$ were assumed to have a statistical significance.

Tab. 1. Biochemical parameters in the study group.

Value	Study group		
	N	Mean	SD
Microalbuminuria [mg/day]	19	14.9 (0.4–92.0)	22.34
Total cholesterol concentration [mg/dl]	19	197.2 (120.0–303.0)	60.21
TG [mg/dl]	18	160.2 (46.0–626.0)	173.42
HDL cholesterol [mg/dl]	16	54.5 (39.0–86.0)	13.41
LDL cholesterol [mg/dl]	17	107.5 (59.0–216.0)	44.72
HbA1c-mean from the last 5 years [%]	19	8.6 (6.6–12.8)	1.78
HbA1c actual [%]	19	8.9 (5.0–13.8)	2.85
Diurnal creatinine clearance [ml/min/sqm]	13	118.3 (48.9–261.0)	58.75
Nocturnal creatinine clearance [ml/min/sqm]	13	85.5 (21.0–241.9)	55.66

Tab. 2. Blood pressure measurements in the study group.

Value	Study group		
	N	Mean	SD
Mean diurnal systolic blood pressure [mm Hg]	19	114.5 (93.0–135.0)	12.29
Mean nocturnal systolic blood pressure [mm Hg]	19	70.8 (50.0–94.0)	12.93
Mean diurnal diastolic blood pressure [mm Hg]	19	96.9 (51.0–137.0)	19.43
Mean nocturnal diastolic blood pressure [mm Hg]	19	56.9 (0.0–87.0)	16.37

RESULTS

The paper compared interstitial concentrations of glucose, glycerol and lactate obtained using microdialysis method in a group of patients suffering from diabetes type 1 and in healthy people. The average concentration of glucose in venous blood plasma in the investigated subjects amounted to 10.1 mmol/L.

The percentage values of glycosylated hemoglobin, plasma lipoid fractions, creatinine clearance and microalbuminuria were determined in all the subjects. The results of biochemical tests are presented in Table 1.

All the patients were subjected to a round-the-clock assessment of arterial blood pressure using the Holter's method. The obtained values are shown in Table 2.

The microdialysis filtrate of the investigated and control groups yielded concentrations of glucose, lactate and glycerol. Daily changes in the twenty-four hour concentrations of the above mentioned metabolites were analyzed during the study. The results are presented in Table 3 and Figures 1–12.

A statistically significant differences in concentration values of interstitial glucose between the investigated and control groups were found. The values of interstitial concentrations of lactates did not significantly differ in the two groups. The average twenty-four hour and daily glycerol concentrations in the investigated group were statistically significantly lower than those in the control group. No such difference was detected in average night concentrations of glycerol.

A relationship between the average values of the night and daily diastolic pressure and the average concentrations of glycerol in the interstitial fluid was found. The equivalent regression equations can be seen in Figures 13–14.

DISCUSSION

Many reports have been recently published in the literature on the usefulness of microdialysis in monitoring many substances metabolically active (Müller 2000; Kamel *et al.* 1999; Eblad *et al.* 1996; Kennergren *et al.* 1999). Special attention was paid to patients with diabetes types both 1 and 2 (Bolinder *et al.* 1993; Jungheim *et al.* 2001; Bolinder *et al.* 1997; Jansson *et al.* 1993; Blaak *et al.* 1999). The method used in these investigations proved to be useful in determining concentrations of glucose, lactate and glycerol (and therefore also useful in continuous monitoring of glycaemia). Our own earlier published papers also focused on the issue of the usefulness of the method in monitoring interstitial blood-glucose levels in states of physiological hypoglycaemia in healthy young volunteers who remained fasting for approximately 24 hr (Alken *et al.* 2008).

Because of exceptionally wavering course of diabetes in children and young people scientists for years have been trying to find non-invasive methods which could assess both a patient's metabolism control and the efficacy of treatment. The basic advantages of microdialysis lie in the fact that it only requires a single puncture into the subcutaneous fat tissue during the introduction of a catheter, while at the same time it makes it possible to measure bloodlessly several times per day concentrations of active substances using very small amounts of microdialysate. Consequently, the method turned out to be particularly useful in the youngest patients. Holzinger *et al.* 2006, described how the method can be used to monitor glycaemia in a patient with neonatal diabetes (Holzinger *et al.* 2006). Also in 2006, German scholars (Baumeister *et al.*) described how microdialysis can be used to non-invasive monitoring of glycaemia in a 6-month old newborn with neonatal diabetes (Baumeister *et al.* 2001). Friedrich *et al.* 2001, used the technique for constant monitoring of glycaemia in newborns and infants with a low birth weight

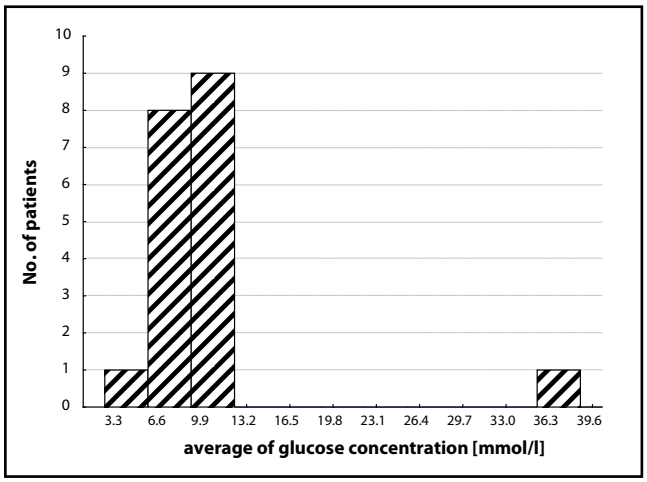

Fig.1. Mean concentration of glucose in the interstitial tissue in the study group.

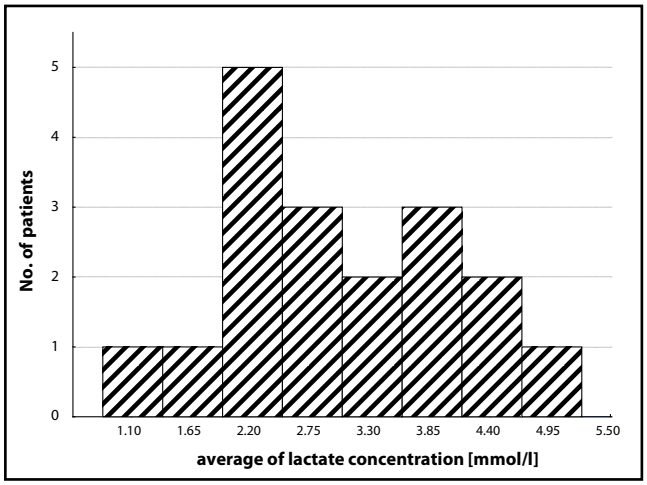

Fig.2. Mean concentration of lactate in the interstitial tissue in the study group.

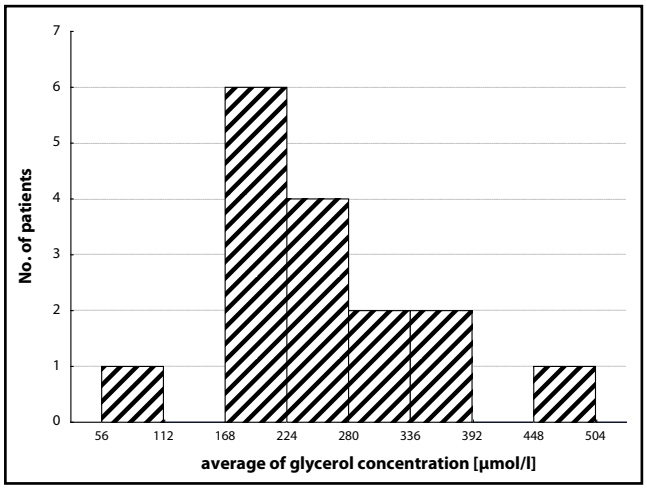

Fig.3. Mean concentration of glycerol in the interstitial tissue in the study group.

Tab. 3. Mean interstitial concentrations of glucose, lactate and glycerol in both groups.

Value	Study group			Control group			*p*-value
	N	mean	SD	N	mean	SD	
Glucose mean daily conc. [mmol/l]	19	10.40	7.1	6	4.26	0.6	0.0012
Glucose mean diurnal conc. [mmol/l]	19	10.08	5.7	6	4.23	0.6	0.0012
Glucose mean nocturnal conc. [mmol/l]	19	10.68	9.9	6	4.36	0.7	0.0012
Lactate mean daily conc. [mmol/l]	18	2.97	1.0	5	2.54	0.8	NS
Lactate mean diurnal conc. [mmol/l]	18	3.06	1.1	5	2.48	0.8	NS
Lactate mean nocturnal conc. [mmol/l]	18	2.72	1.2	5	3.15	1.4	NS
Glycerol mean daily conc. [µmol/l]	16	257.19	89.7	3	384.58	49.0	0.0336
Glycerol mean diurnal conc. [µmol/l]	16	258.26	86.1	3	397.88	33.8	0.0189
Glycerol mean nocturnal conc. [µmol/l]	16	257.78	108.1	3	361.40	138.8	NS

(Friedrich *et al.* 2001). The authors also safely used the method in children with very low birth weight (lower than 1 000 g) hospitalized at intensive care units (Horal *et al.* 1995). In 2007, Vlasselaers *et al.* used microdialysis to continuous monitoring of glucose levels in children hospitalized at intensive care units (Vlasselaers *et al.* 2007). They stressed the fact that based on their experience the method is useful in detecting episodes of both hypoglycaemia and hyperglycaemia. Close monitoring of glucose levels significantly lowers the number of organ complications and reduces mortality rate (Holzinger *et al.* 2006; Baumeister *et al.* 2001; Friedrich *et al.* 2001; Horal *et al.* 1995; Vlasselaers *et al.* 2007). A very interesting report was published in 2004 by Ahlson *et al.* about a treatment of extreme hyperglycemia monitored with subcutaneous and intracerebral microdialysis. The technique was used in an 11-yr-old boy with new-onset diabetes who had a blood glucose concentration of 100 mmol/l (1 800 mg/dl) and serum osmolality of 448 mOsm/kg. The authors concluded, that microdialysis can be used to monitor the brain/subcutaneous glucose ratio in patients with extreme hyperglycemia (Ahlson *et al.* 2004). A definite majority of authors describe microdialysis as a minimally invasive and safe method. This observation was also confirmed in our patients. Microdialysis catheters were inserted without complications in 26 investigated subjects (in 19 in the investigated group and in 6 controls).

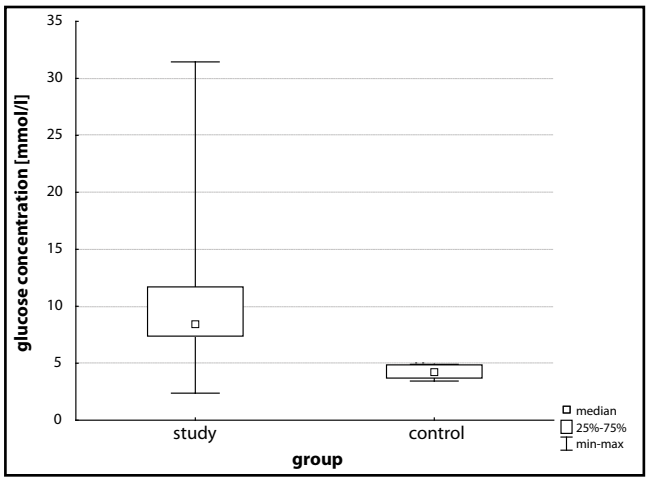

Fig.4. Difference in diurnal glucose concentrations in both groups.

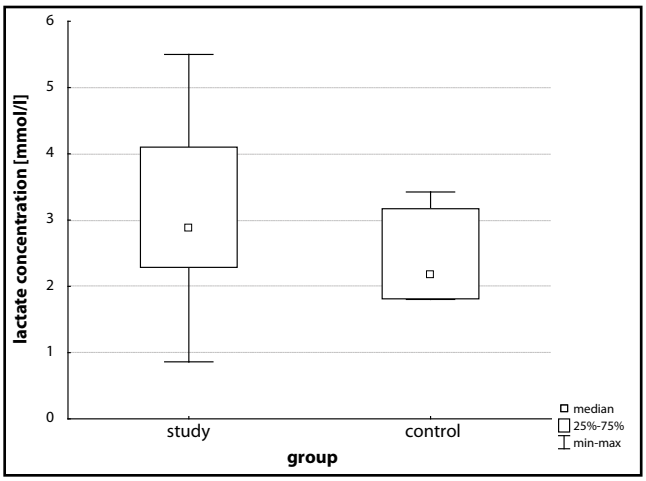

Fig.7. Difference in diurnal lactate concentrations in both groups.

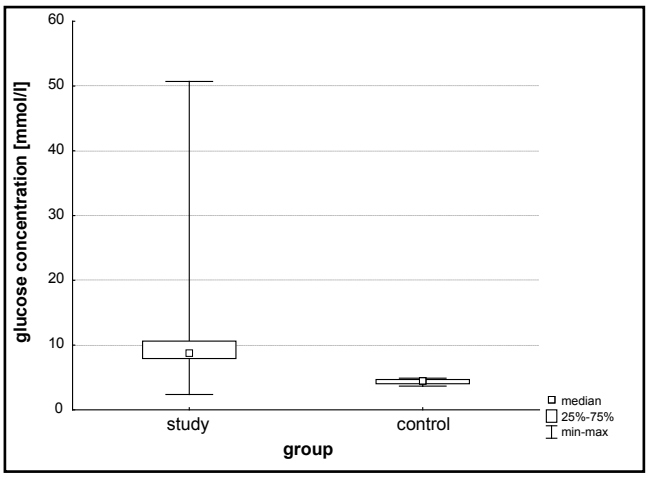

Fig.5. Difference in nocturnal glucose concentrations in both groups.

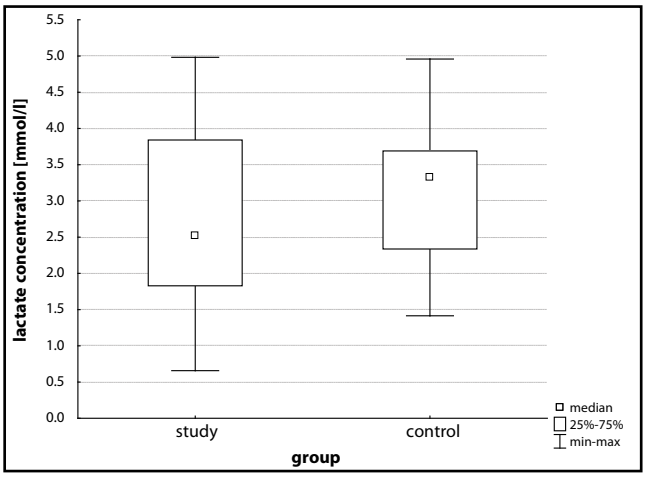

Fig.8. Difference in nocturnal lactate concentrations in both groups.

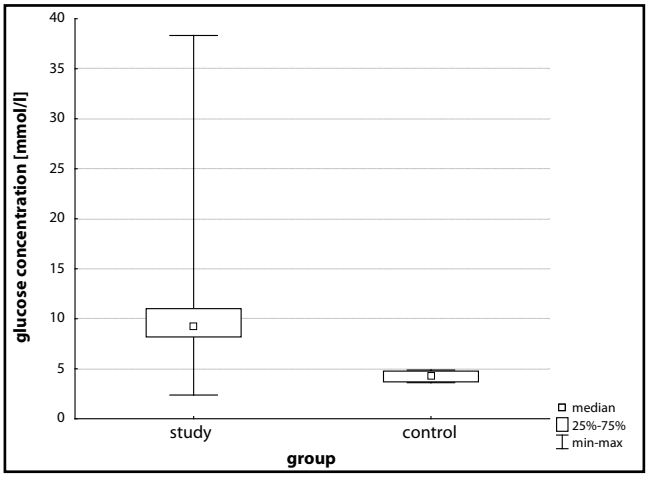

Fig.6. Difference in mean glucose concentrations in both groups.

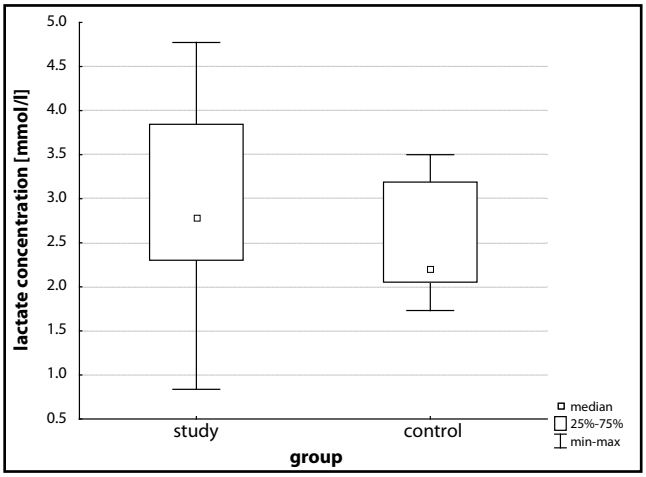

Fig.9. Difference in mean lactate concentrations in both groups.

Metabolites were measured, bedside, every 60 minutes, for 24–36 hrs.

It should also be mentioned that Radman *et al.* described in 2008, a rare case of complications connected with microdialysis method in a 31 year-old patient with diabetes type 1 in whom the hollow fiber dialysis chamber was disconnected from the nylon tube. The hollow fiber was localized using a radiograph of the abdominal wall, and was later successfully removed by a plastic surgeon (Radman *et al.* 2008). However, it seems

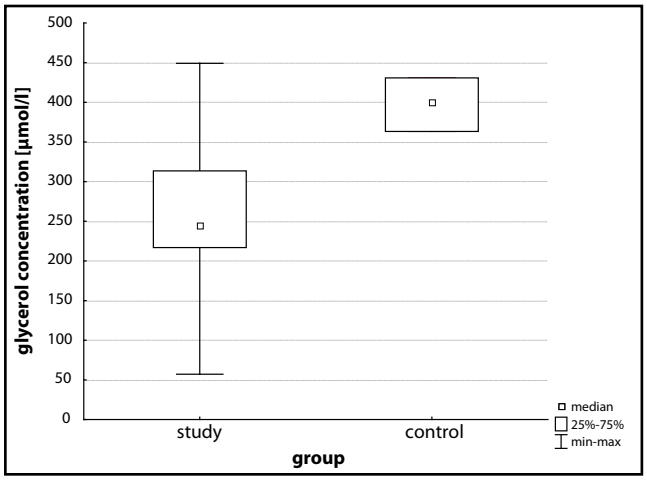

Fig.10. Difference in diurnal glycerol concentrations in both groups.

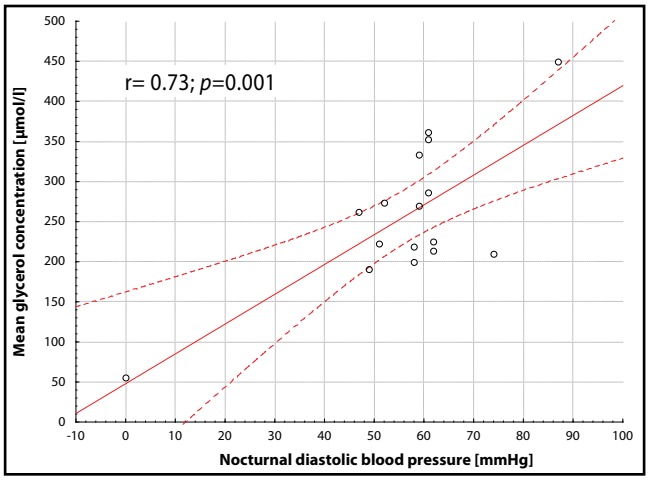

Fig.13. Correlation between mean glycerol concentration and nocturnal diastolic blood pressure.

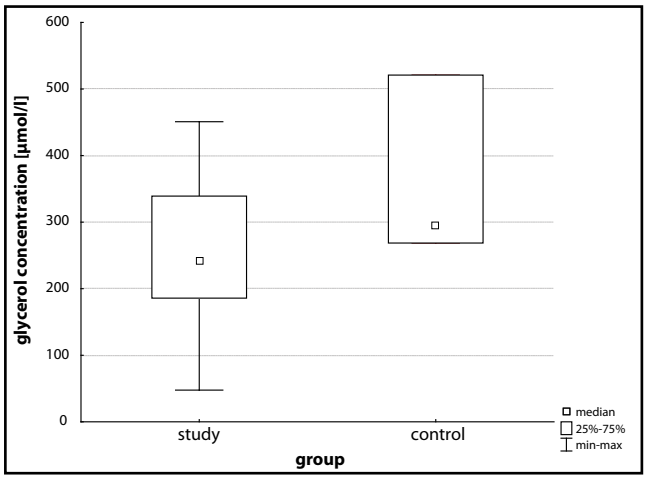

Fig.11. Difference in nocturnal glycerol concentrations in both groups.

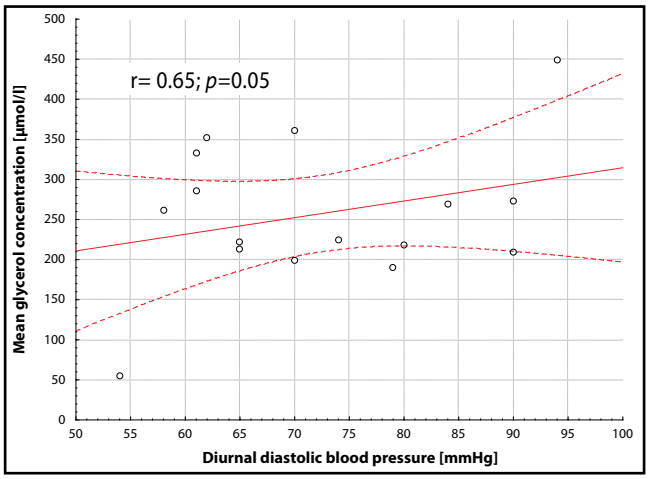

Fig. 14. Correlation between mean glycerol concentration and diurnal diastolic blood pressure.

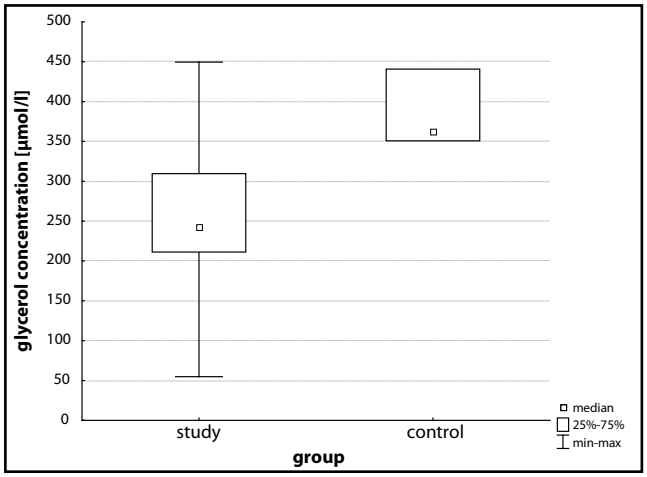

Fig.12. Difference in mean glycerol concentrations in both groups.

that was the only case of complications connected with using the method, which proved to be particularly safe in inserting catheters in subcutaneous fat tissue.

Fat tissue is an organ which plays a very important role in maintaining a correct homeostasia of glucose. Both glucose utilization and lipolysis taking place in fat tissue are controlled by insulin. In the investigated group the authors confirmed significantly different tissue concentrations of glucose and glycerol between the investigated subjects and the controls. Similar observations were made by Ciechanowska *et al.* , who used microdialysis to monitor concentrations of glucose, glycerol and lactate in 31 adult patients with acute complications of diabetes (diabetic ketoacidosis and hyperglycemic hyperosmolar state). The concentrations of interstitial glycerol in the group of patients described by Ciechanowska *et al.* were very similar to those recorded in our group of patients (267 ± 41 µmol/l vs 257.2 ± 89 µmol/l) (Ciechanowska *et al.* 2008). The analysis of glycerol could be used for studies of lipolysis and the antilipolytic effect of insulin. All the patients in our group were administered insulin; 5 patients were given constant insulin infusion to subcutaneous tissue using a personal insulin pump, 14 were on intensive insulin therapy, most of whom used analogues of long activity insulin. Heptulla *et al.* 2003, described observations opposite to those made in our group of patients

and concerning concentrations of glycerol in teenagers with diabetes type 1 (Heptulla *et al.* 2003). We analyzed metabolic changes in a group of 19 patients with average glycosylated hemoglobin of 8.9 ± 2.85%, whereas Heptulla *et al.* investigated 10 young patients with average glycosylated hemoglobin of 10.2 ± 0.2%. Both studies were controlled by determining glycerol concentrations in a control group of 6 healthy teenagers. In Heptulla's study, incorrect higher than in the control group, concentrations of glycerol were recorded. This could be an evidence of incorrect lipolysis in patients with uncontrolled diabetes type 1. In our study, we did not find the differences in mean nocturnal glycerol concentrations between study and control group. The daily and diurnal glycerol concentrations were slightly higher in control group. Similar to our observations were made by Merwe at al, in seven lean patients with type 1 diabetes (Merwe *et al.* 1994). In that paper interstitial glycerol concentrations, measured in abdominal subcutaneous adipose tissue as an index of lipolysis, were not significantly influenced by hyperglycemia in diabetic adolescents.

It is worth pointing out that so far only a several reports have been published on using microdialysis in a group of teenage patients with bad metabolic control of diabetes type 1. Our own study conducted on nearly twice as large group as described previously is probably going to be the one of the few published reports on the issue.

In the investigated group, no statistically significant differences in interstitial concentrations of lactate were found between the investigated and control groups, which is in agreement with most reports on the problem available in the literature (Heptulla *et al.* 2003; Merwe *et al.* 1994). The authors also tried to assess the influence of changes in arterial blood pressure on the concentrations of determined metabolites. A statistically significant relationship was found between night values of increased systolic pressure and average concentration of glycerol. Increased blood pressure is associated with higher concentrations of glycerol. Changes in perfusion probably affect changes in the rate of interstitial lipolysis. Further research on the issue is to be continued.

On the basis of the present investigations the following conclusions can be drawn:

CONCLUSIONS

Microdialysis is the only one minimal invasive method for investigating adipose tissue metabolism *in vivo* and provides a novel opportunity for glucose and lipids metabolism monitoring in adolescents with diabetes type 1.

In our observations interstitial glycerol concentrations, measured in abdominal subcutaneous adipose tissue as an index of lipolysis, were not significantly influenced by hyperglycemia in diabetic adolescents.

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