Eur J Med Res (2005) 10: 419-425

Subcutaneous Microdialysis for Children – Safe Biochemical Tissue Monitoring Based on a Minimal Traumatizing No Touch Insertion Technique

A. Hack[1], V. Busch[1], K. Gempel[2], F. A. M. Baumeister[1]

[1]Children´s Hospital of the Technical University Munich, Munich, Germany
[2]Institute for Clinical Chemistry, Molecular Diagnostics, and Mitochondrial Genetics, Academic Hospital Schwabing, Munich, Germany

Abstract
Background: Microdialysis (MD) enables analysis of extracellular metabolites without performing blood tests. Changes in the concentration of various metabolites can be monitored frequently on almost every type of human tissue. Microdialysis of subcutaneous tissue (sc MD) is of particular significance in the case of pediatric patients because diurnal profiles can be generated without repeated blood sampling.

There are only a few scientific articles that describe the application of sc MD on neonates, infants, or children. So far, side effects have not been investigated comprehensively.

This prospective study scrutinizes side effects of sc MD in pediatric patients, focusing on a Minimal Traumatizing Insertion Technique of the MD catheter.
Patients and Methods: 42 pediatric patients within four age categories participated in the study which involved bedside monitoring using sc MD, including 5 extremely low birth weight (ELBW) infants with a body weight <1000g. A total of 48 sc MD catheters were inserted. Selection criteria were risk of hypoglycaemia (n = 29), elevated lactate levels (n = 16), or aminoacidopathies (n = 3). Duration of sc MD ranged from 1 to 16 days. We used a Minimal Traumatizing Insertion Technique to safely insert the MD catheter into the subcutaneous tissue, characterized by blunt dissection of the tissue and by the use of a plastic cannula guidance to avoid desterilisation of the catheter. Complications and side effects related to sc MD were documented in standardized forms.
Results: The MD probe was easily placed even in the scanty adipose tissue of ELBW infants. During insertion of sc MD catheters accidental venous puncture occurred to 8%, and minor bleeding to 27%. Even with local anaesthesia insertion was painful for 40%. During the course of sc MD complications were rare: disturbance of perfusion flow 4%, catheter dislocation 4%, local bleeding 4%. No signs of systemic or local infection were observed, there were no cases of local incompatibility. All catheters were withdrawn completely without leaving a scar.

Repeated measurements allowed the generation of diurnal metabolic profiles. In some cases (respiratory chain complex I deficiency, PDH-deficiency) significant therapeutical effects on the patients´ metabolism were demonstrated.

Conclusions: The present study proves long-term sc MD to be suitable and safe for biochemical tissue monitoring. Using our insertion technique, it can be applied to children of all ages without causing discomfort or severe side effects. As it permits frequent sampling it allows evaluating and optimizing therapy and means a substantial progress for pediatric observation.

Key words: microdialysis, tissue monitoring, children, minimal traumatizing, side effects

Abbreviations: sc MD, subcutaneous Microdialysis; ELBW, extremely low birth weight; PDH, pyruvate dehydrogenase

Introduction

Microdialysis is a sampling method that enables repeated analysis of extracellular metabolites without performing blood tests. Clinical applications of MD became possible in recent years with the introduction of commercially available MD catheters, CE certified for application in human tissue. The microdialysis probe mimics a capillary blood vessel by perfusing a thin dialysis tube implanted into the tissue of interest, making it possible to measure frequently the concentration of small molecules in the surrounding interstitial fluid space [2, 15, 16, 19]. Microdialysis can be used to tightly monitor changes in the concentration of various metabolites in almost every type of human tissue, most commonly used in brain tissue.

Microdialysis of the subcutaneous tissue can be of particular significance in the case of pediatric patients since it reduces diagnostic blood loss and pain caused by repeated blood sampling on infants and children. At the same time it allows intensive monitoring of tissue metabolism by continuous sampling at very short intervals (e.g. every 15min) over long periods of time (days-weeks). Dialysate samples can easily be obtained even while the patient is sleeping.

There are only a few scientific articles that describe the application of sc MD on neonates, infants or older children for monitoring or diagnostic purposes. So far sc MD has been used for glucose monitoring in children with hypoglycaemia [1, 3, 13, 14, 17, 18], for a metabolic monitoring (lactate, glycerol) after surgery [11, 12], and to monitor glycerol (lipolytic index) for

HIV-infected children with lipohypertrophy [4], for obese adolescent girls [7], and for adolescents with poorly controlled type 1 diabetes [10]. To our knowledge side effects of sc MD have rarely been comprehensively analyzed for pediatric patients or adults.

In this study sc MD was applied to pediatric patients with mitochondrial diseases, lactic acidosis, aminoacidopathies or hypoglycemia, ketogenic patients and neonates with various complications. In addition to bedside monitoring of glucose, lactate, pyruvate and glycerol we measured carnitines and amino acids in the dialysates. We prospectively investigated side effects, technical difficulties and discomfort related to sc MD for the biochemical monitoring of our patients, focusing on a Minimal Traumatizing No Touch Insertion Technique of the MD catheter.

Patients and Methods

The studies were approved by the local ethics committee, and written consent was obtained from the parents. The microdialysis device used (CMA/Microdialysis, Solna, Sweden) is CE certified for the clinical application on human brain and subcutaneous tissue.

Patients

42 pediatric patients participated in the studies which involved continuous bedside monitoring using sc MD. A total of 48 sc MD catheters were inserted. Patients were divided into 4 categories: (1) 21 neonates and preterm infants with a median weight of 2.5kg (0.6 to 4.0kg) and a median gestational age of 38.3weeks (24.4 to 42.8weeks), (2) 7 infants up to 1 year of age with a median age of 4.8month (1.5 to 12month), (3) 13 children up to 6 years with a median age of 2.8years (1.1 to 6.5y) and (4) 7 children older than 6 years with a median age of 11.8years (7.5 to 15.33y). Five patients in the first age category were ELBW infants with a body weight of 0.6 to 0.9kg.

As shown in Table 1, selection criteria for study participants were proven or suspected mitochondrial disease with lactate acidosis (n = 12, lactate and pyruvate monitoring), risk for hypoglycaemia (n = 20, glucose monitoring), metabolic monitoring of ketogenic diet (n = 9, glucose and lactate monitoring), aminoacidopathies (n = 3, amino acid monitoring), and diverse severe neonatal complications (n = 4, lactate and glucose monitoring).

Microdialysis

The principle of the microdialysis technique used has previously been described in detail [2, 15, 16, 19].

We used a CMA 70 microdialysis catheter (CMA / Microdialysis, Solna, Sweden) with a dialysis membrane length of 10mm (diameter 0.6mm) for neonates and of 20mm for older children. The molecular exclusion size of the polyamide membrane was 20kD, and the relative recovery of the MD system used was tested previously to be about 90% in vivo during long-term MD and about 100% in vitro [3]. The high relative recovery in vitro and in vivo is a prerequisite for clinical investigations if approximation of "true" values is mandatory [15].

The microdialysis catheter was inserted under sterile conditions upon transdermal local anaesthesia (EMLA, Wedel, Germany). EMLA was performed for at least 1 hour to ensure local anaesthesia and vasoconstriction to minimize bleeding. The subcutaneous adipose tissue of the lateral thigh (neonates, infants) or the forearm (children) was chosen as insertion site. MD catheters were inserted subcutaneously tight under the corium. To ensure minimal traumatic insertion of the catheters we used a normal intravenous canula (Vasofix Braunüle, 18 G; Braun Melsungen, Melsungen, Germany) as guidance. After perforating the skin the needle was withdrawn slightly and the tissue was punctured blunt by the surrounding plastic canula. Then the needle was removed completely and the tip of the MD catheter (10mm or 20mm) was inserted through the plastic cannula into the subcutaneous tissue under sterile conditions. Finally, the plastic cannula was extracted and remained on the shaft of the MD catheter outside the skin. After implantation, the catheter was fixed on the skin with adhesive tape strips and a transparent sterile plastic film. Steps of minimal traumatizing insertion of MD catheter are shown in Fig. 1a-d.

The catheter was continuously perfused with a sterile isotonic solution (NaCl 0.9% Braun Melsungen, Melsungen, Germany). The low flow rate of 0.3µL/min was ensured by a battery-driven pump (CMA 106 microdialysis pump, CMA/Microdialysis AB, Solna, Sweden) using disposable precision syringes. The dialysate samples were collected in microvials (CMA/ Microdialysis AB, Solna, Sweden) in a vial holder fixed at the end of the catheter outlet tube (Fig. 1e, f).

An enzymatic analysis was performed on the dialysate samples using the CMA 600 Microdialysis

Table 1. Selection criteria for test subjects undergoing monitoring with subcutaneous Microdialysis.

criteria for sc MD	**number**	**parameters of interest**
mitochondrial disease, lactic acidosis	12	lactate, pyruvate
risk for hypoglycaemia (SGA, neonatal diabetes mellitus, neonatal hyperinsulinism, Type 1 diabetes mellitus)	20	glucose
aminoacidopathies (phenylketonuria, maple syrup disease, glutaraciduria)	3	amino acids
monitoring of ketogenic diet (risk for hypoglycemia)	9	glucose, lactate, carnitines
neonatal complications (asphyxia, necrotic enterocolitis, pneumothorax)	4	lactate, glucose

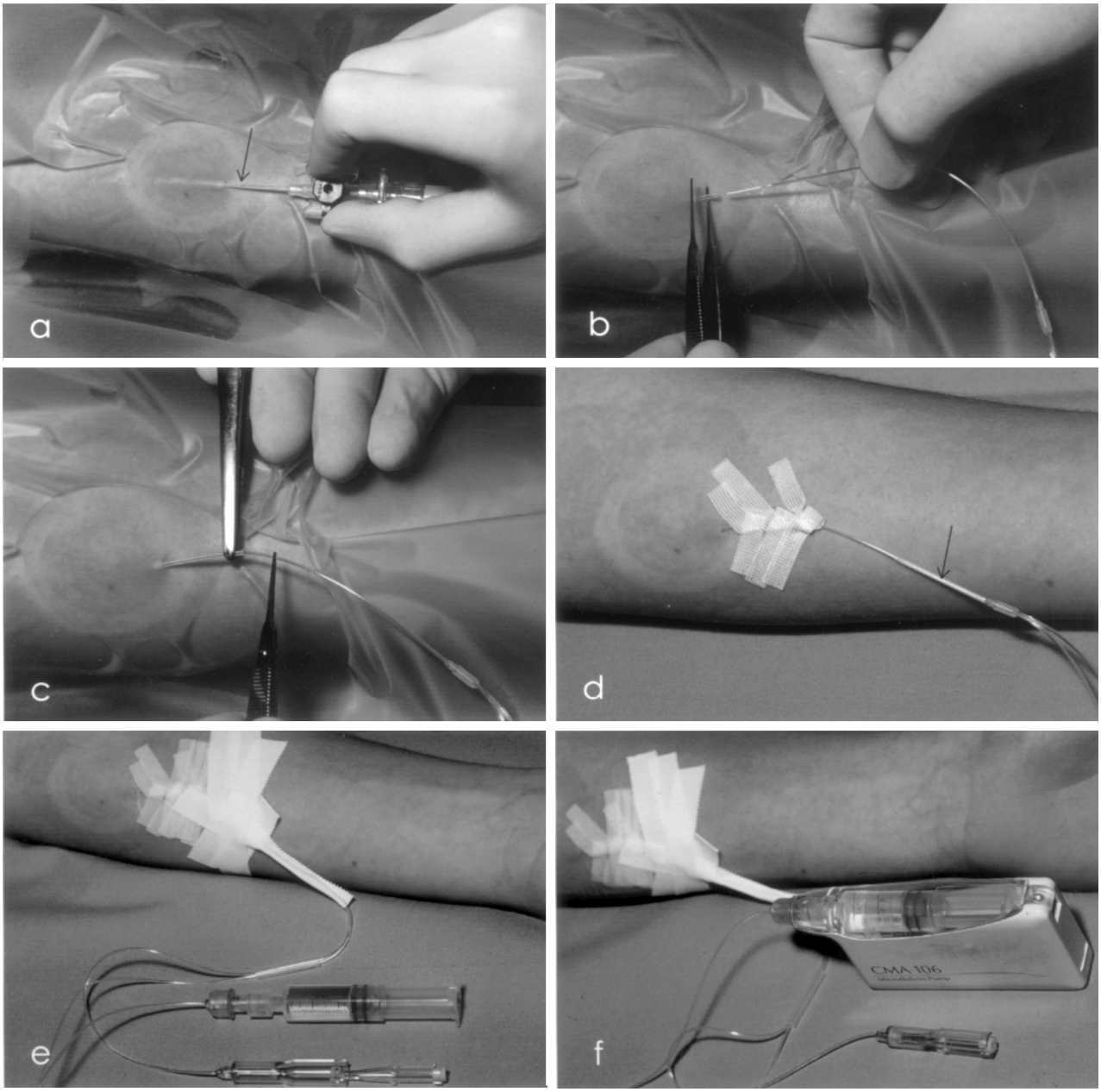

Fig 1a-f. Stepwise insertion of MD catheter with Minimal Traumatizing No Touch Technique (adolescent, forearm). Preparation: Plastic tube of a venous canula is cut 20 mm from the tip (arrow in Fig a points at dissection site), CMA 106 syringe is filled with 2.5ml NaCl 0.9% and MD system is perfused 0.3 µl/min. Preparation and draping of insertion site is performed under sterile conditions.
a. The skin is perforated with the prepared intravenous canula as for venous puncture. The needle is withdrawn slightly and the plastic tube is placed under the corium by blunt dissection of the subcutaneous tissue.
b. To avoid desterilization, the distal part of the plastic tube is gripped with a forceps and the needle together with the proximal part of the venous canula is removed. The semipermeable tip of the MD catheter (10mm, white colour) is inserted via the plastic tube into the subcutaneous tissue without touching the skin.
c. The plastic tube is pulled back along the MD catheter using two forceps and remains around the shaft of the catheter above the skin (arrow in Fig d points at remaining plastic tube).
d. The catheter is fixed on the skin with adhesive tape strips.
e. Implantation site is covered with a transparent sterile plastic film. CMA 106 syringe is connected to the inlet tube, vial holder is at the outlet tube of the MD catheter. Microvial is outside the vial holder.
f. Syringe is placed in the lightweight CMA 106 Microdialysis Pump and microvial is put in the vial holder. The whole system is now portable and can be fixed on the skin.

Analyser (CMA/Microdialysis AB, Solna, Sweden). Free carnitines and acylcarnitines were quantified by tandem mass spectrometry using a serum method with slight modifications [9]. Amino acids were analyzed using ion exchange high performance liquid chromatography.

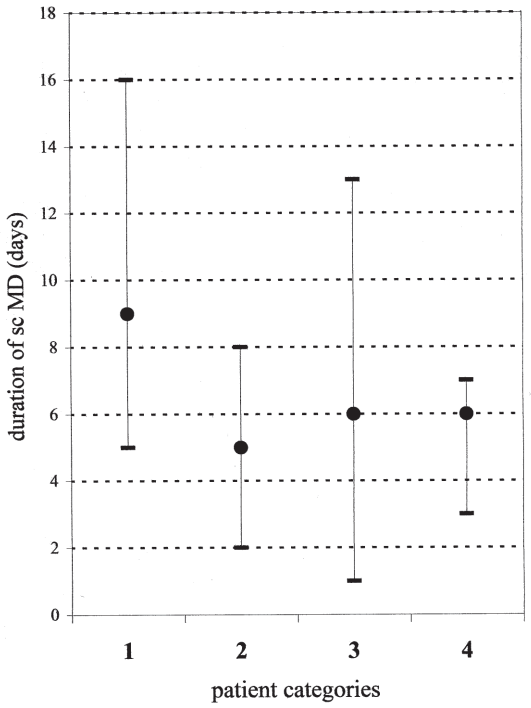

Fig. 2. Duration of subcutaneous microdialysis in 4 different age categories. Median (●) and range (-) is given for neonates and preterm infants (n = 21, category 1), infants up to 1 year of age (n = 7, category 2), children aged 1-6.5years (n = 13, category 3), and children above 6 years (n = 7, category 4).

Documentation Form

Complications and side effects caused by insertion of MD catheters or occurring during observation were documented prospectively in a standardized format. All 48 applications of sc MD were fully documented and evaluated.

Statistics

Statistical calculations were performed using the SPSS software package version 11.5. Statistical significances were tested using the Kruskal-Wallis-Test or the Mann-Whitney-Test.

Results

48 subcutaneous microdialysis catheters were inserted into pediatric patients of various age categories. Microdialysis duration ranged from 1 to 16 days (median 7 days, Fig. 2). For the 5 ELBW infants sc MD lasted 9 to 16 days.

By choosing the lateral thigh or forearm as insertion site, the microdialysis probe could be safe and easily placed even in ELBW infants with scanty abdominal adipose tissue.

Upon insertion of MD catheters, an accidental venous puncture occurred to 8% (4/48) and minor bleeding (1-3 drops of blood) to 27% (13/48). Bleeding upon insertion was more frequent in the case of younger children (23 to 33% in age categories 1 to 3 versus 0% in category 4). Despite locally performed anaesthesia (EMLA) insertion was painful for 40% (19/48). Pain reaction, as measured by facial expression and defence reaction, can be compared to the insertion of a venous canula. Two patients showed a local reaction due to EMLA.

During the course of sc MD complications were rare. Microdialysis was terminated in two patients after 4 and 12 days because perfusion flow was disturbed. In two other patients it was interrupted after 5 and 9 days due to dislocation of the MD catheter. Local bleeding around the insertion site occurred in the case of another two patients, but MD could be continued. Two patients of age category 2 died due to the advancement of their underlying illnesses, and MD was aborted after 2 and 4 days.

There were no signs of local or systemic infection. No case of local incompatibility was observed. Eight infants received antibiotic treatment during the whole duration of sc MD due to their underlying illness. All MD catheters were removed completely and left no scars. (Table 2)

The catheters were well tolerated and did not substantially impede movements. By changing the collecting tube at the end of the perfusion line dialysate samples could easily be obtained even while patients were sleeping.

Repeated measurements under standardized conditions allowed the generation of diurnal metabolic profiles. With monitoring of sc MD significant effects of a therapy on lactate levels could be demonstrated eg. in

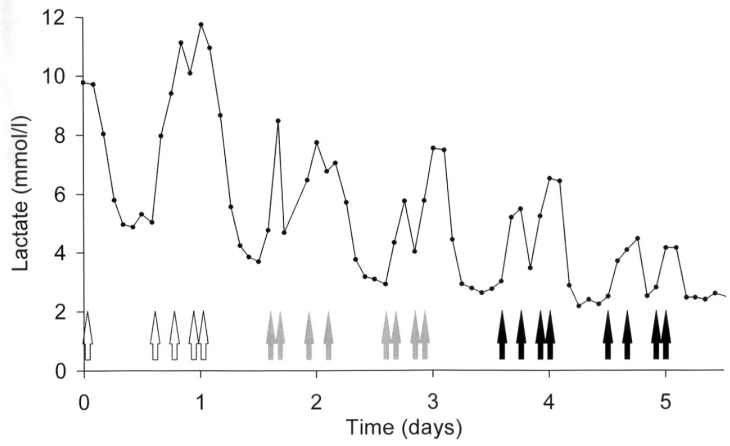

Fig. 3. Lactate monitoring on a 6 year-old girl with respiratory chain complex I deficiency treated with ketogenic diet. Monitoring with sc MD demonstrates diurnal fluctuations of lactate levels in association with feeding. Lactate concentrations were low before feeding (arrows) and rise postprandial. The fat content of ketogenic diet was increased every two days from 70% (white arrows) to 80% (grey arrows) and finally to 90% of caloric intake (black arrows). With increasing fat content, lactate values decreased significantly both before feeding ($p = 10^{-5}$) and postprandial ($p = 2 \times 10^{-5}$, Kruskal-Wallis-Test)

Table 2. Complications, side effects and malfunctions associated with subcutaneous microdialysis (n = 48 applications)

time of occurrence	side effects	frequency (%)
insertion	accidental venous puncture	8
	minor bleeding	27
	pain reaction	40
course	local infection	0
	systemic infection	0
	local bleeding	4
	local incompatibility	0
	catheter dislocation	4
	flow disturbance	4
	breaking of catheter tip*	0
	scarring	0

*determined by loupe inspection after catheter withdrawal

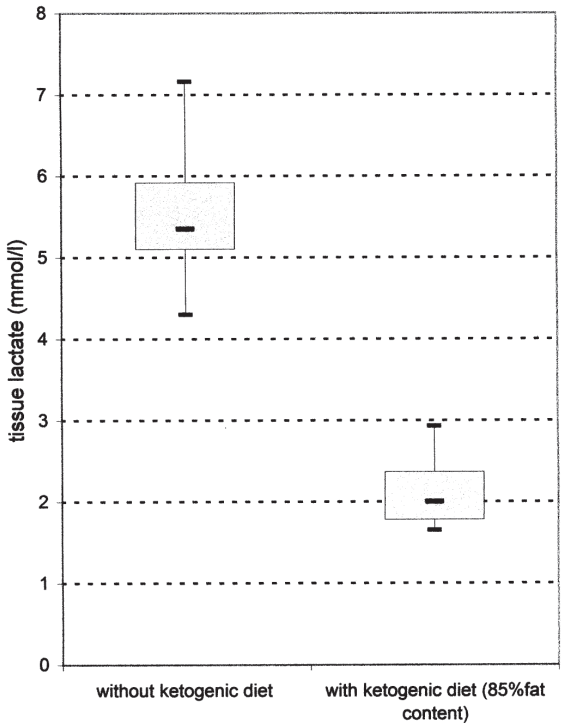

Fig. 4. Treatment with ketogenic diet reduced subcutaneous extracellular lactate levels in an infant with PDH deficiency. Using continuous subcutaneous microdialysis, tissue lactate was monitored at the age of 3 months before treatment, and again at the age of 5 months while the patient was being treated with ketogenic diet (LCT-diet, 85% fat of caloric intake).The differences between tissue lactate values without and with ketogenic diet are significant ($P = 3 \times 10^{-9}$, Mann-Whitney Test). Boxplots are based on 23 and 25 values, respectively.

patients with respiratory chain complex I deficiency and PDH deficiency (Fig. 3, 4).

Discussion

The present study demonstrates that microdialysis of subcutaneous adipose tissue is a safe and well-tolerated method for the biochemical tissue monitoring on pediatric patients.

Insertion of the microdialysis catheter into subcutaneous tissue in accordance with the described Minimal-Invasive-No-Touch-Technique is easy and can be compared with the implementation of a venous canula. A No-Touch Technique has already been described by neurosurgeons as a useful method to decrease cerebrospinal fluid shunt infections. Minimal handling of shunt components, not touching the skin and keeping shunt instruments separate from skin dissection instruments resulted in a threefold decrease in shunt infection rate [8].

The short dialysis membrane used in our studies (10mm for neonates) and the chosen site for insertion of the MD catheter (lateral thigh, forearm) enabled the safe application for children of all ages, especially these with scanty adipose tissue as ELBW infants.

Except for two recent studies by our group [3, 17], sc MD in pediatric patients has always been performed in the abdominal region, and the dialysis membranes had a length of 30mm [1, 4, 7, 10-14, 18]. Especially in the case of neonates there is a higher risk of perforation into the abdominal cavity. Therefore the lower weight limit in all these studies was stipulated at 1000g to ensure the safe location of the catheter in consideration of the small amount of abdominal subcutaneous adipose tissue and the length of the probe [13].

During our study, the insertion of catheters and the execution of sc MD were performed safely and without great difficulty, even for 5 ELBW infants with scanty subcutaneous adipose tissue. However, minor complications associated with insertion of the MD catheter (bleeding, pain reaction) occurred frequently among younger children.

There are only a few reports that describe the application of sc MD for pediatric patients, and to our knowledge comprehensive investigations of complications and side effects on both adult and pediatric patients monitored with sc MD are rare.

Subcutaneous microdialysis has previously been performed up to 4 days on neonates after birth [13] and after surgery [11, 12]. No side effects have been noted, especially no inflammation around the probe [13], no bleeding, infection or local skin irritation [11]. On another 12 newborn infants sc MD of the abdominal adipose tissue was easy to perform and produced no side effects [18]. In previous studies by our group side effects of sc MD have been scrutinized upon observation of 13 neonates/infants and even two ELBW infants (0.8 and 0.9kg) with scanty subcutaneous adipose tissue [3]. The length of the membrane (10mm) was shorter than in the other studies (30mm), and the insertion site was changed to the lateral thigh. These 13 subjects were also included in our present evaluation. Several papers did not mention side effects of sc MD on children [1,4,7,10,14,17]. (Table 3)

To our knowledge for adult patients only one case of malfunction due to MD catheter obstruction was recorded [5]. No other side effects have yet been reported in the literature, specifically no local or generalized infections, catheter dislocations, or bleeding complications [6].

Table 3. Summary of previous applications of subcutaneous microdialysis for pediatric patients.

corresponding author, year	subjects	weight range [kg]	parameters at interest				duration [days]	anaes-thesia	side effects examined
			glucose	lactate	glycerol	others			
Horal, 1995	7 neonates	1.0-3.2	X	X			1.5 - 4	n.s.†	
Hildingsson, 1996	14 neonates	1.9-4.1	X	X	X		1-4,5	general	X*
Kamel, 1999	6 children	n.s.†	X		X		1	n.s.†	
Enoksson, 2000	17 adolescents	n.s.†			X		n.s.†	local‡	
Hildingsson, 2000	13 neonates, 12 children	1.9-4.8, 5.0-20.0	X	X			1	general	
Baumeister, 2001	13 neonates§	0.8-7.0	X	X		urea	4-16	local‡	X▯
Rolinski, 2001	5 neonates§	1.2-2.8	X	X		amino acids	8-16	local‡	
Stenninger, 2001	12 neonates	3.4-5.3	X	X	X		1	none	
Beregszaszi, 2003	20 children	n.s.†			X		n.s.†	local‡	
Heptulla, 2003	16 adolescents	62.7**			X		1	local‡	
Ahlsson, 2004	1 child	32.0	X				3	general	
present study, 2005	21 neonates, 7 infants, 20 children	0.6-49.5	X	X		pyruvate, amino acids, carnitines	1-16	local‡	X

* inflammation (0/14), bleeding(0/14), infection(0/14), local skin irritation (0/14)
† not specified
‡ EMLA
§ subjects also included in the present study
▯ accidental venous puncture (4/13), catheter accidental withdrawn (2/13), disturbance of perfusion flow (1/13), minor bleeding around catheter (1/13); local/systemic infection (0/13), skin irritation (0/13), scarring (0/13)
** mean weight

We conclude that long-term subcutaneous microdialysis is a suitable and safe approach for a continuous biochemical tissue monitoring especially in pediatrics. Using our insertion technique, it can be applied to children of all ages without causing discomfort or severe side effects. As it permits frequent sampling it allows evaluating and optimizing therapy and in addition it is a substantial progress for pediatric research.

References

1. Ahlsson F, Gedeborg R, Hesselager G, Tuvemo T, Enblad P. (2004) Treatment of extreme hyperglycemia monitored with intracerebral Microdialysis. Pediatr Crit Care Med 5:89-92
2. Arner P, Bolinder J. (1991) Microdialysis of adipose tissue. J Intern Med 230:381-386
3. Baumeister FAM, Rolinski B, Busch R, Emmrich P. (2001) Glucose Monitoring With Long-Term Subcutaneous Microdialysis in Neonates. Pediatrics 108:1187-1192
4. Beregszaszi M, Jaquet D, Levine M, et al. (2003) Severe insulin resistance contrasting with mild anthropometric changes in the adipose tissue of HIV-infected children with lipohypertrophy. Int J Obes Relat Metab Disord 27:25-30
5. Bolinder J, Ungerstedt U, Arner P. (1993) Long-term continuous glucose monitoring with microdialysis in ambulatory insulin-dependent diabetic patients. Lancet 342:1080-1085
6. Damian MS, Sauter W, Reichmann H. (2001) Subcutaneous microdialysis in mitochondrial cytopathy. Muscle Nerve 24:648-653
7. Enoksson S, Talbot M, Rife F, Tamborlane WV, Sherwin RS, Caprio S. (2000) Impaired in vivo stimulation of lipolysis in adipose tissue by selective beta2-adrenergic agonist in obese adolescent girls. Diabetes 49:2149-2153
8. Faillace WJ. (1995) A no-touch technique protocol to diminish cerebrospinal fluid shunt infection. Surg Neurol 43:344-350
9. Gempel K, Kiechl S, Hofmann S, et al. (2002) Screening for carnitine palmitoyltransferase II deficiency by tandem mass spectrometry. J Inherit Metab Dis 25:17-27
10. Heptulla RA, Stewart A, Enocksson S, et al. (2003) In situ evidence that peripheral insulin resistance in adolescents with poorly controlled type 1 diabetes is associated with impaired suppression of lipolysis: a microdialysis study. Pediatr Res 53:830-835
11. Hildingsson U, Selldén H, Ungerstedt U, Marcus C. (1996) Microdialysis for metabolic monitoring in neonates after surgery. Acta Paediatr 85:589-594
12. Hildingsson U, Lönnqvist P-A, Selldén H, Eksborg S, Ungerstedt U, Marcus C. (2000) Age-dependent variations in white adipose tissue glycerol and lactate production after surgery measured by microdialysis in neonates and children. Paediatr Anaesth 10:283-289

13. Horal M, Ungerstedt U, Persson B, Westgren M, Marcus C. (1995) Metabolic adaptation in IUGR neonates determined with microdialysis - A pilot study. Early Hum Dev 42:1-14
14. Kamel A, Norgren S, Persson B, Marcus C. (1999) Insulin induced hypoglycaemia: comparison of glucose and glycerol concentrations in plasma and microdialysate from subcutaneous adipose tissue. Arch Dis Child 80:42-45
15. Landolt H, Langemann H. (1996) Cerebral microdialysis as a diagnostic tool in acute brain injury. Eur J Anaesthesiol 13:269-278
16. Nakahara D, Ozaki N, Nagatsu T. (1993) In vivo microdialysis of neurotransmitters and their metabolites. In: Parvez SH, Naoi M, Nagatsu T, Parvez S (eds) Methods in neurotransmitter and neuropeptide research. Paris,France: Elsevier Science Publishers; 219-248
17. Rolinski B, Baumeister FAM, Roscher AA. Determination of amino acid tissue concentrations by microdialysis: method evaluation and relation to plasma values. Amino Acids 2001; 21:129-138
18. Stenninger E, Eriksson E, Stigfur A, Schollin J, Aman J. Monitoring of early postnatal glucose homeostasis and cerebral function in newborn infants of diabetic mothers. A pilot study. Early Hum Dev. 2001;62:23-32
19. Ungerstedt U. (1991) Microdialysis- principles and applications for studies in animal and man. J Intern Med 230:365-373

Received: April 27, 2005 / Accepted: September 2, 2005

Address for correspondence:
Priv. Doz. Dr. F.A.M. Baumeister
Pediatric Neurology
Children´s Hospital of the Technical University Munich
Kinderklinik München Schwabing
Kölner Platz 1
D-80804 Munich, Germany
Tel.: +49-89-3068-3352, -2277
Fax.: +49-89-3068-3887
e-mail: FAM.Baumeister@lrz.uni-muenchen.de