Differences in muscle metabolism in patients with type I diabetes – influence of gender and nephropathy studied by ^{31}P MR spectroscopy

Petr Sedivy[1,2], Monika Dezortova[1], Miloslav Drobny[1], Zuzana Vlasakova[3], Vit Herynek[1], Milan Hajek[1]

[1)] MR-Unit, Department of Diagnostic and Interventional Radiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

[2)] 1st Medical Faculty of Charles University, Prague, Czech Republic

[3)] Center of Diabetology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Corresponding Author:

Ing. Milan Hájek, DrSc.

MR-Unit

Department of Diagnostic and Interventional Radiology

Institute for Clinical and Experimental Medicine

Vídeňská 1958/9, 140 21 Prague 4, Czech Republic

e-mail: miha@medicon.cz

Phone: +420-236055245

Short title: Dynamic 31P MR spectroscopy in DM1 patients

Summary

Introduction

Type I diabetes mellitus (DM1) is a complex disease with adverse effects on organs and tissues despite compensation by insulin treatment. The goal of our study was to study how kidney diseases change ^{31}P MR parameters of muscle metabolism in DM1 patients with respect to gender.

Materials and Methods

59 DM1 patients (19m/14f without and 13m/5f with nephropathy) and 26 (14m/12f) healthy volunteers were examined using ^{31}P magnetic resonance spectroscopy at 3T tomograph at rest, and during and after a calf muscle exercise. The exercise consisted of a six-minute plantar flexion using a pedal ergometer followed by a six-minute recovery.

Results

It is reflected by reduced relative β-ATP and increased Pi and phosphodiester signals to phosphocreatine (PCr) at rest and prolongation of the PCr recovery time after the exercise.

Measurement on healthy volunteers indicated differences between males and females in pH at the rest and after the exercise only. These differences between patients groups were not significant.

Conclusions

We have proven that nephropathy affects the metabolism in diabetic patients and our results confirm significant difference between patients with and without nephropathy. Gender differences in pH were observed only between male and female healthy volunteers.

Keywords

Magnetic Resonance Spectroscopy, Diabetes Mellitus Type 1, Energy Metabolism

Introduction

Phosphorous magnetic resonance spectroscopy (^{31}P MRS) is a noninvasive method allowing for in vivo investigation of energy metabolism in muscles based on detecting ^{31}P signals originated from phosphocreatine (PCr), inorganic phosphate (Pi), adenosine triphosphate (ATP), phosphodiesters (PDE), phosphomonoesters (PME) and nicotinamide adenine dinucleotide (NADH) (Valkovič et al. 2017, Argov et al. 2000). In addition, intramyocellular (IMCL) pH can be determined from chemical shift differences between PCr and Pi (Moon and Richards 1973). During dynamic ^{31}P MRS, physical exercise and measurement of ^{31}P MR spectra from muscle are combined (Sedivy *et al.* 2015, Kemp *et al*. 2015, Kemp and Radda 1994).

During a physical exercise, the muscle uses energy from ATP which is immediately resynthesized from PCr through creatine-kinase reaction. PCr drop is stopped by two mechanisms that produce ATP during exercise. The first mechanism is anaerobic glycolysis that provides ATP during the exercise activity. It generates protons and causes a decrease in muscle pH (Kemp *et al.* 2001, Robergs *et al.* 2004). The second process is oxidative phosphorylation which continues after the exercise. During exercise and recovery period changes of PCr, Pi and pH are monitored and other important parameters as mitochondrial capacity could be calculated (Q_{max}) (Kemp and Radda 1994). Mitochondrial capacity expresses the maximal possible extent of mitochondrial aerobic metabolism. A high mitochondrial capacity calculated from ^{31}P MRS is associated with a good function of mitochondria and correlates well with oxidative capacity of mitochondria isolated from muscle biopsies (Lanza *et al.* 2011).

Patients with type I diabetes mellitus (DM1) suffer from the lack of insulin due to the autoimmune destruction of the insulin-producing beta cells in the pancreas. Insulin regulates the uptake of glucose into muscle cells via the GLUT-4 transporter. Insulin also promotes

glycogen, lipid and protein synthesis in muscle cells, while suppressing lipolysis and gluconeogenesis from muscle amino acids; therefore, it is closely related to energy metabolism (Wilcox 2005).

Even in the case of insulin substitution therapy, glycemia in DM1 patients often oscillates between hyper- and hypoglycemic levels. Poorly managed DM1 may lead eventually to cardiovascular diseases, diabetic neuropathy, retinopathy, nephropathy or a diabetic foot syndrome (Brownlee 2001). A number of DM1 patients also exhibit insulin resistance (Bergman *et al.* 2012, Cree-Green *et al.* 2015) and higher deposition of IMCL fat (Perseghin *et al.* 2003) similarly as in DM2 patients owing to impaired mitochondrial metabolism (Szendroedi *et al.* 2008, Petersen *et al.* 2004).

Changes in mitochondrial metabolism in DM1 were proven by in vitro studies, which revealed changes in mitochondrial gene expression (Antonetti *et al.* 1995, Karakelides *et al.* 2007) with a reduction in ATP production. The change of mitochondrial function in diabetes mellitus is explained by glucose toxicity (Rossetti *et al.* 1995, Rabol *et al.* 2009), lipotoxicity (Perseghin *et al.* 2003), the effect of chronic hyperinsulinemia (Karakelides *et al.* 2007) or by reduced nutrient delivery due to limited insulin action and glucose transport (Yki-Jarvinen et al. 1990) or as a result of a reduced muscle blood flow and oxygen supply attributable to microangiopathy (Cree-Green *et al.* 2015).

Only a few dynamic *in vivo* studies were performed on type I diabetes mellitus (Cree-Green *et al.* 2015, Crowther *et al.* 2003, Item *et al.* 2011). A significantly decreased mitochondrial capacity was found in DM1 patients (males only) compared to controls (Crowther *et al.* 2003). Mean mitochondrial capacity in female patients did not change in comparison with female controls (Item *et al.* 2011) but a negative correlation between the value of glycosylated hemoglobin and the individual mitochondrial capacity was noted. Although specific differences were described separately for males and females (Crowther *et al.* 2003, Item *et al.*

2011), these studies did not compare males and females directly and provided somewhat contradictory results.

One of the most serious complications of diabetes mellitus is nephropathy. It is characterized as insufficient kidney function; it means that concentrations of several ions, creatine, urea, etc. are increasing in blood. Also metabolism of amino acids is negatively affected and patients suffer from the erythropoietin and vitamin D deficiency. In this condition the skeletal muscles are atrophied (Fahal 2014) and energy metabolism is impaired (Táborský et al. 1993, Kemp et al. 1995). Nephropathy patients have bigger drop of PCr and pH during exercise (Kemp et al. 1995) and lower PCr/Pi ratio at rest (Táborský et al. 1993).

The aim of our study was to answer the following two questions:

1) Can we see gender specific differences in the rest and dynamic ^{31}P parameters in our DM1 patients and controls?

2) How nephropathy in DM1 patients changes the rest and dynamic ^{31}P parameters?

Methods

Subjects

Overall 59 diabetic patients (32m/19f) were recruited for the study from our Department of diabetology according to their clinical examinations and laboratory results. In addition 12 healthy females (Cf) and 14 healthy males (Cm) participated in the study as control groups. Based on questionnaire none of them was an active sportsman and all had predominantly sedentary jobs.

The subjects were divided into subgroups according the gender (male - m; female - f) and clinical diagnosis (controls - C; patients without nephropathy - DM1; patients with nephropathy - DM1N), see Table 1. Subjects with a low workload during the exercise (drop of PCr lower than 15 %) were excluded (6m/2f patients) from the study. Diabetic patients were

treated by insulin substitution therapy; patients with nephropathy suffered from kidney failure and were on a waiting list for kidney transplantation. The age, BMI, glycosylated hemoglobin (HbA1c) and creatinine from blood samples (results from clinical reports) of all subgroups are listed in Table 1.

In addition, ten healthy volunteers were examined to assess the quality measurement of the ^{31}P MR spectroscopy of the calf muscle at rest: five of them were examined three times in independent sessions and five volunteers once with three measurements. Long-term reproducibility of the dynamic protocol for the assessment of mitochondrial capacity was tested on two healthy subjects (25 and 65 years old): 3 and 5 dynamic ^{31}P MRS examinations were performed in three and five subsequent weeks in the same time of the day.

All subjects were informed about the examination protocol and they signed their consent with the study. The study was approved by the local ethics committee. All subjects also filled out a questionnaire about their physical condition, sport activities and living habits.

MR examination

MR examinations were performed using a whole-body 3T MR system TRIO (Siemens, Erlangen, Germany) with a dual-channel ^{1}H/^{31}P surface coil (Rapid Biomedical, Rimpar, Germany). All subjects were examined in a supine position with the coil fixed under the musculus gastrocnemius. The positioning of the muscle over the coil was verified using a localizer sequence. ^{31}P MR spectra at rest were acquired by a non-localized acquisition sequence FID with the following parameters: acquisition delay TE* = 0.4 ms, TR = 15 s, number of acquisitions NA = 16, vector size of 1024. Magnetic field homogeneity was optimized by the localized shimming of the water signal.

Dynamic ^{31}P MR spectra were obtained by the FID sequence with the following parameters: TE* = 0.4 ms, TR = 2 s, NA = 1, vector size of 1024; number of measurements = 420. Our

standard exercise examination protocol was divided into three parts: a two-minute rest period, a six-minute exercise period and a six-minute recovery period. The exercise was performed with a home-built ergometer by the plantar flexion twice per repetition time (2 s) with a power below 60% of maximal power that had been measured by dynamometer, for more details see (Sedivy *et al.* 2015). Acoustic synchronization was used to navigate the subjects during the exercise period of the experiment.

Spectra evaluation

Spectra were analyzed by the AMARES time domain fitting routine (drawing upon prior knowledge) (Vanhamme *et al.* 1997) in the jMRUI 5.0 software package. Lorentzian line shapes were used for the fitting of singlets of PCr, Pi, PDE (glycerol-3-phosphorylcholine and glycerol-3-phosphorylethanolamine), PME (phosphorylcholine and phosphorylethanolamine) and NADH signals. The ATP peaks were fitted as two doublets (α-ATP, and γ-ATP) and a triplet (β-ATP). Integral intensities were related to total integral of the whole spectra.

The relative chemical shift of Pi and PCr (δ in ppm) was used to calculate the intracellular pH according to the Henderson-Hasselbalch equation (Moon and Richards 1973):

$$pH = 6.75+\log [(\delta P_i - 3.27)/(5.63 - \delta P_i)] \quad (1)$$

The PCr changes during the recovery period were fitted by a mono-exponential function to evaluate the PCr recovery rate:

$$[PCr](t) = [PCr]_{e\_ex}+\Delta[PCr](1 - e^{-t/\tau_{PCr}}) \quad (2)$$

where t is time, $[PCr]_{e\_ex}$ is the PCr amount at the end of the exercise, $\Delta[PCr]$ is the difference in the PCr amount at rest and at the end of the exercise, and τ_{PCr} is the time constant of the PCr recovery rate.

The initial PCr recovery rate (V_{iPCr}) roughly representing the ATP turnover at the end of the exercise was calculated as follows:

$$V_{iPCr} = [PCr]/\tau_{PCr} \quad (3)$$

Mitochondrial capacity Q_{max} was calculated according to the model of Michaelis & Menten, taking into account adenosine diphosphate at the end of the exercise activity ($[ADP]_{e\_ex}$, V_{iPCr}, and the Michaelis-Menten constant (K_m), which was assumed to be 30 μM (Kemp 1994):

$$Q_{max}\_ADP = V_{iPCr}\,(1+K_m/[ADP]_{e\_ex}) \quad (4)$$

where $[ADP]_{e\_ex}$ was calculated according to the method described by Kemp (Kemp *et al.* 1993), assuming constant total creatine concentration throughout all measurements and 15% of total creatine not being phosphorylated in the resting state (Boska 1994)

$$[ADP]_{e\_ex} = [Cr][ATP]/[PCr][H^+]\,K_{CK} \quad (5)$$

where $[H^+]$ is the concentration of proton ions and K_{CK} is the equilibrium constant.

Absolute concentrations of PCr necessary for evaluation were calculated from PCr/β-ATP ratios assuming constant ATP concentration of 8.2 mM in the muscle tissue (Kemp *et al.* 2007, Taylor *et al.* 1986).

Statistical evaluation

For the comparison of individual parameters of the patient and control groups, statistics was done using the Prism6 software. According to the Shapiro-Wilk normality tests, parametric or non-parametric multiple comparison (ANOVA Sidak's multiple comparisons and/or Kruskal-Wallis) was undertaken (the probability level of $p<0.05$ was considered as statistically significant). As some data did not follow normal distribution, Spearman's correlation analysis was conducted (the probability level of $p<0.005$ was considered as statistically significant).

Results

An initial test on healthy volunteers revealed that the reproducibility of metabolic ratios measurements at rest are: β-ATP/P_{tot} - 3%, Pi/P_{tot} - 6%, PDE/P_{tot} - 8%, PCr/Pi - 8%, pH - 0.2%. The reproducibility of Q_{max} and τ_{PCr} was found 10%, and 25%, resp.

Groups of patients and controls did not significantly differ in terms of the mean age and BMI independently of gender (see Table 1). As expected, the creatinine was significantly higher in patient groups with nephropathy from the others. Similarly, significantly higher values of HbA1c were found in DM1 and DM1N groups compared to healthy controls.

The results of ^{31}P MR spectroscopy at rest and exercise are summarized in Tables 2 and 3. Signal intensities of PCr, Pi, β-ATP, and PDE related to the total spectrum integral (P_{tot}) and pH were evaluated at rest. Significantly decreased β-ATP/P_{tot} and increased Pi/P_{tot} and PDE/P_{tot} ratios were observed in nephropatic DM1 patients to controls; β-ATP/P_{tot} was also able to distinguish groups of patients with and without nephropathy. When dividing groups according to gender, only the β-ATP/P_{tot} ratio was significantly different between male groups of nephropatic patients and controls. In addition, we found significantly higher pH in male control group compared to female healthy controls. These gender effects were not observed in patients groups.

The six-minute exercise was sufficient to create equilibrium between the consumption and creation of PCr in most of the subjects, and the six-minute recovery period was also sufficient to fully restore the PCr signal intensity to the original values in both controls and patients (see Figure 1). We found prolongation of τ_{PCr} in patients in the order of C < DM1 < DM1N. However, statistical significance was seen only between the control and nephropathy groups. In addition, a significantly lower pH was found in DM1 patients compared to controls. When dividing groups according to gender only trends in dynamic parameters were observed due to

a high variance in groups of patients. Measurement on healthy volunteers revealed differences between males and females only in pH after the exercise, similarly as at rest. The differences in pH between Cm and DM1m were observed, see Table 3.

Correlation analysis

The length of the disease correlated with the patients' age ($r = 0.46$; $p = 0.0001$, Spearman's r coefficients and corresponding p values). An increasing BMI correlated with the age ($r = 0.338$; $p = 0.003$) and was coincidental with the length of the disease ($r = 0.46$; $p = 0.0006$). From rest ^{31}P MRS parameters β-ATP/P_{tot} positively correlated with PDE/P_{tot} ($r = 0.475$; $p = 0.0001$) and β-ATP/P_{tot} negatively correlated with age ($r = -0.425$; $p = 0.0001$). From dynamic ^{31}P MRS parameters pH after exercise negatively correlated with HbA1c ($r = -0.376$; $p = 0.001$).

Discussion

^{31}P MR spectroscopy at rest and under exercise is an interesting tool for investigating energy metabolism in muscles but it has some limitations. A different physical effort of each subject may influence the results. A low drop of the PCr signal (ΔPCr) may indicate an insufficient depletion of PCr and also brings an additional error into the calculation. Therefore, only subjects with $\Delta PCr > 15\%$ were included in the study. A higher workload helps determine correct mitochondrial capacity but may provoke anaerobic metabolism and acidosis which can inhibit oxidative phosphorylation while also affecting the results (Robergs *et al.* 2004). This was probably reflected by prolonged time of PCr recovery τ_{PCr} in subjects with a lower pH after the exercise (significant negative correlation between these two parameters was observed too). Moreover, acidosis may be another marker related to the DM1 disease and probably depends on sex. We found a significant difference between the pH values of male and female

controls. It is in agreement with the findings described for quadriceps muscles (Schunk et al. 1999). Crowther (Crowther *et al.* 2003) described decreased pH values in DM1 males; on the other hand, Item (Item *et al.* 2011) found no change in the DM1 females. Our findings are similar but without statistical significance.

Although one would expect a substantial impairment of the energy metabolism in relation to diabetes, we did not find any significant differences in metabolite ratios measured at rest between controls and diabetic patients without nephropathy (DM1m, DM1f, DM1). The DM1N group (all patients with nephropathy) had higher Pi/P_{tot} and PDE/P_{tot} while having lower β-ATP/P_{tot} in comparison to healthy controls. In addition, DM1N group was distinguished from DM1 group in β-ATP/P_{tot}. However, when we differentiated patients according to sex, the only β-ATP/P_{tot} was significantly lower in only males (Cm vs. DM1mN). In females' groups a similar trend in β-ATP/P_{tot} was visible. Thus we can assume that the β-ATP/P_{tot} ratio can be the best marker to separate patients with and without nephropathy.

Decreased ATP and increased Pi can be generally associated with kidney failure and uremia (Nishida *et al.* 1991, Taborsky *et al.* 1993). Similar metabolite changes were also described in mitochondrial disorders (Mattei *et al.* 2004) and indicate a reducing phosphorylation potential. It is in agreement with negative correlation between age and β-ATP/P_{tot}. Positive correlation between age and PDE/P_{tot} can be considered consistent with an increasing BMI (Valkovic *et al.* 2016). In addition, the pH decrease after the exercise and an increase of Pi/P_{tot} negatively influence τ_{PCr}.

Similar gender changes as at rest were observed in pH after the exercise between male and female controls. This difference was not observed in respect of the patients. Pooled data of the males and females without severe nephropathy (DM1) as well as the DM1m group only had a decreased pH after the exercise compared with the control group, which is probably related to

the increased demand for PCr supply reflected also by a higher (but not statistically significant) drop of the PCr signal.

We should mention that an increased variation of obtained experimental data can also be explained by the variability of a patient's physical condition. Although all subjects filled in a questionnaire concerning their physical conditions, there was difficult to find out an objective parameter which could help with the explanation of the data variability. Thus, only qualitative trends have to be discussed. It is the case of e.g. longer recovery time τ_{PCr} in all DM1 groups compared to controls which reflects a decreased mitochondrial capacity. From this qualitative point of view, our findings of V_{iPCr} and Q_{max} in the DM1 females are not consistent with the finding outlined in Item (Item *et al.* 2011), neither did we find any significant correlation of glycosylated hemoglobin reported in that paper.

Lower V_{iPCr} and mitochondrial capacity Q_{max} in the case of the DM1 male patients compared to DM1 females without kidney failure suggests that male metabolism is influenced by DM1 more than female metabolism. However, the fact that we did not observe a similar difference in patients with severe nephropathy remains unexplained; we may speculate that severe metabolic impairment smoothed moderate gender differences.

It is known that females have a lower efficiency in the effective use of ATP for muscle contraction (Mattei *et al.* 1999). We hypothesize that the lower efficiency is related to a different proportion of muscle fibers between males and females which could also cause a different (smaller) effect of DM1 on the muscle metabolism in females.

Gender differences may also reflect DM1-related changes in levels of several important hormones and factors affecting skeletal muscle atrophy, growth, and regeneration (Krause *et al.* 2011) and hypothetically may differ in males and females.

Conclusion

We have proven that nephropathy further negatively affects the energy metabolism in diabetic patients. It is reflected by reduced relative β-ATP and increased Pi and PDE signals to P_{tot} at rest and prolongation of the PCr recovery time after the exercise. Gender specific changes can be seen in healthy subjects in pH values both at rest and after the exercise. We have not confirmed any significant gender differences in the DM1 patients by ^{31}P MR spectroscopy in our groups of patients.

Acknowledgements

The study was supported by the project Grant Agency of Charles University (project No. 315) and MHCZ-DRO 00023001IKEM.

References

ANTONETTI DA, REYNET C, KAHN CR: Increased expression of mitochondrial-encoded genes in skeletal muscle of humans with diabetes mellitus. *J Clin Invest* **95**:1383-1388, 1995.

ARGOV Z, LÖFBERG M, ARNOLD DL: Insights into muscle diseases gained by phosphorus magnetic resonance spectroscopy. *Muscle Nerve* **23**: 1316-34, 2000.

BERGMAN BC, HOWARD D, SCHAUER IE, MAAHS DM, SNELL-BERGEON JK, ECKEL RH, PERREAULT L, REWERS M: Features of hepatic and skeletal muscle insulin resistance unique to type 1 diabetes. *J Clin Endocrinol Metab* **97**: 1663-1672, 2012.

BOSKA M: ATP production rates as a function of force level in the human gastrocnemius/soleus using 31P MRS. *Magn Reson Med* **32**: 1-10, 1994.

BROWNLEE M: Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**: 813-820, 2001.

CREE-GREEN M, NEWCOMER BR, BROWN MS, BAUMGARTNER AD, BERGMAN B, DREW B, REGENSTEINER JG, PYLE L, REUSCH JE, NADEAU KJ: Delayed skeletal muscle mitochondrial ADP recovery in youth with type 1 diabetes relates to muscle insulin resistance. *Diabetes* **64**: 383-392, 2015.

CROWTHER GJ, MILSTEIN JM, JUBRIAS SA, KUSHMERICK MJ, GRONKA RK, CONLEY KE: Altered energetic properties in skeletal muscle of men with well-controlled insulin-dependent (type 1) diabetes. *Am J Physiol Endocrinol Metab* **284**: E655-662, 2003.

DEVRIES DA, MARSH GD, RODGER NW, THOMPSON RT: Metabolic response of forearm muscle to graded exercise in type II diabetes mellitus: effect of endurance training. *Can J Appl Physiol* **21**:120-33, 1996.

FAHAL IH: Uraemic sarcopenia: aetiology and implications. *Nephrol Dial Transplant* **29**: 1655-65, 2014.

ITEM F, HEINZER-SCHWEIZER S, WYSS M, FONTANA P, LEHMANN R, HENNING A, WEBER M, BOESIGER P, BOUTELLIER U, TOIGO M: Mitochondrial capacity is affected by glycemic status in young untrained women with type 1 diabetes but is not impaired relative to healthy untrained women. *Am J Physiol Regul Integr Comp Physiol* **301**: R60-66, 2011.

KARAKELIDES H, ASMANN YW, BIGELOW ML, SHORT KR, DHATARIYA K, COENEN-SCHIMKE J, KAHL J, MUKHOPADHYAY D, NAIR KS: Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. *Diabete*s **56**: 2683-2689, 2007.

KEMP GJ: Interactions of mitochondrial ATP synthesis and the creatine kinase equilibrium in skeletal muscle. *J Theor Biol* **170**: 239-46, 1994.

KEMP GJ, AHMAD RE, NICOLAY K, PROMPERS JJ: Quantification of skeletal muscle mitochondrial function by 31P magnetic resonance spectroscopy techniques: a quantitative review. *Acta Physio* **213**: 107-144, 2015.

KEMP GJ, MEYERSPEER M, MOSER E: Absolute quantification of phosphorus metabolite concentrations in human muscle in vivo by 31P MRS: a quantitative review. *NMR Biomed* **20**: 555-565, 2007.

KEMP GJ, RADDA GK: Quantitative interpretation of bioenergetic data from 31P and 1H magnetic resonance spectroscopic studies of skeletal muscle: an analytical review. *Magn Reson Q* **10**: 43-63, 1994.

KEMP GJ, ROUSSEL M, BENDAHAN D, LE FUR Y, COZZONE PJ: Interrelations of ATP synthesis and proton handling in ischaemically exercising human forearm muscle studied by 31P magnetic resonance spectroscopy. *J Physiol* **535**: 901-928, 2001.

KEMP GJ, TAYLOR DJ, RADDA GK: Control of phosphocreatine resynthesis during recovery from exercise in human skeletal muscle. *NMR Biomed* **6**:66-72, 1993.

KEMP GJ, THOMPSON CH, TAYLOR DJ, RADDA GK: ATP production and mechanical work in exercising skeletal muscle: a theoretical analysis applied to 31P magnetic resonance spectroscopic studies of dialyzed uremic patients. *Magn Reson Med* **33**: 601-9, 1995.

KRAUSE MP, RIDDELL MC, HAWKE TJ: Effects of type 1 diabetes mellitus on skeletal muscle: clinical observations and physiological mechanisms. *Pediatric Diabetes* **12**: 345-664, 2011.

LANZA IR, BHAGRA S, NAIR KS, PORT JD: Measurement of human skeletal muscle oxidative capacity by 31P-MR spectroscopy: a cross-validation with in vitro measurements. *J Magn Reson Imaging* **34**: 1143-1150, 2011.

MATTEI JP, BENDAHAN D, COZZONE P: P-31 magnetic resonance spectroscopy. A tool for diagnostic purposes and pathophysiological insights in muscle diseases. *Reumatismo* **56**: 9-14, 2004.

MATTEI JP, BENDAHAN D, ROUSSEL M, LEFUR Y, COZZONE PJ: Gender modulates the energy cost of muscle contraction in untrained healthy subjects. A 31P magnetic resonance spectroscopy analysis. *FEBS Lett* **450**:173-177, 1999.

MOON RB, RICHARDS JH: Determination of intracellular pH by 31P magnetic resonance. *J Biol Chem* **248**: 7276-7578, 1973.

NISHIDA A, KUBO K, NIHEI H: Impaired muscle energy metabolism in uremia as monitored by 31P-NMR. *Nihon Jinzo Gakkai Shi* **33**: 65-73, 1991.

PERSEGHIN G, LATTUADA G, DANNA M, SERENI LP, MAFFI P, DE COBELLI F, BATTEZZATI A, SECCHI A, DEL MASCHIO A, LUZI L: Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab* **285**: E1174-1181, 2003.

PETERSEN KF, DUFOUR S, BEFROY D, GARCIA R, SHULMAN GI: Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* **350**: 664-671, 2004.

RABOL R, HOJBERG PM, ALMDAL T, BOUSHEL R, HAUGAARD SB, MADSBAD S, DELA F: Effect of hyperglycemia on mitochondrial respiration in type 2 diabetes. *J Clin Endocrinol Metab* **94**: 1372-1378, 2009.

ROBERGS RA, GHIASVAND F, PARKER D: Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* **287**: R502-516, 2004.

ROSSETTI L: Glucose Toxicity - the Implications of Hyperglycemia in the Pathophysiology of Diabetes-Mellitus. *Clinical and Investigative Medicine* **18**: 255-260, 1995.

SCHUNK K, PITTON M, DÜBER C, KERSJES W, SCHADMAND-FISCHER S, THELEN M. Dynamic phosphorus-31 magnetic resonance spectroscopy of the quadriceps muscle: effects of age and sex on spectroscopic results. *Invest Radiol.* **34**:116-125, 1999.

SEDIVY P, KIPFELSBERGER MC, DEZORTOVA M, KRSSAK M, DROBNY M, CHMELIK M, RYDLO J, TRATTNIG S, HAJEK M, VALKOVIC L: Dynamic 31P MR spectroscopy of plantar flexion: influence of ergometer design, magnetic field strength (3 and 7 T), and RF-coil design. *Med Phys* **42**: 1678-1689, 2015.

SZENDROEDI J, RODEN M: Mitochondrial fitness and insulin sensitivity in humans. *Diabetologia* **51**: 2155-2167, 2008.

TABORSKY P, SOTORNIK I, KASLIKOVA J, SCHUCK O, HAJEK M, HORSKA A: 31P magnetic resonance spectroscopy investigation of skeletal muscle metabolism in uraemic patients. *Nephron* **65**: 222-226, 1993.

TAYLOR DJ, STYLES P, MATTHEWS PM, ARNOLD DA, GADIAN DG, BORE P, RADDA GK: Energetics of human muscle: exercise-induced ATP depletion. *Magn Reson Med* **3**: 44-54, 1986.

VALKOVIČ L, CHMELÍK M, KRŠŠÁK M: In-vivo 31P-MRS of skeletal muscle and liver: A way for non-invasive assessment of their metabolism. *Anal Biochem* **529**: 193-215, 2017.

VALKOVIC L, CHMELIK M, UKROPCOVA B, HECKMANN T, BOGNER W, FROLLO I, TSCHAN H, KREBS M, BACHL N, UKROPEC J, TRATTNIG S, AND KRSSAK M: Skeletal muscle alkaline Pi pool is decreased in overweight-to-obese sedentary subjects and relates to mitochondrial capacity and phosphodiester content. *Scientific reports* **6**: 20087, 2016.

VANHAMME L, VAN DEN BOOGAART A, VAN HUFFEL S: Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* **129**: 35-43, 1997.

WILCOX G. INSULIN AND INSULIN RESISTANCE: *Clin Biochem Rev* **26**: 19-39, 2005.

YKI-JARVINEN H, SAHLIN K, REN JM, KOIVISTO VA: Localization of rate-limiting defect for glucose disposal in skeletal muscle of insulin-resistant type I diabetic patients. *Diabetes* **39**:157-167, 1990.

Tables

Table 1. Age, body mass index (BMI), disease length, and selected biochemical data (glycosylated hemoglobin - HbA1c, and creatinine evaluated from blood tests) of the evaluated groups of patients and controls. Mean values ± standard deviations are listed.

Subject group	Age (years)	BMI (kg/m^2)	Length of the disease (years)	HbA1c (%)	Creatinine (µmol/L)
Cm (14)	32±9	26±4	0	3.1±0.1	84±8
DM1m (19)	35±11	24±4	12±8	7.2±1.8	81±13
DM1mN (13)	44±9	27±4	28±6	7.1±1.5	491±360
Cf (12)	40±10	25±4	0	3.7±0.3	70±7
DM1f (14)	38±13	27±4	17±13	7.5±1.5	64±11
DM1fN (5)	33±7	24±4	22±5	7.4±1.9	503±225
C (26)	36±10	25±4	0	3.4±0.4	77±11
DM1 (33)	36±12	25±4	18±10	7.3±1.7	74±15
DM1N (18)	41±9	26±4	26±6	7.2±1.6	494±321

Cm – control males, Cf - control females, C - control males and females, DM1m - diabetic males, DM1f - diabetic females, DM1 - diabetic males and females, DM1mN - diabetic males with nephropathy, DM1fN – diabetic females with nephropathy, DM1N - diabetic males and females with nephropathy; significant differences ($p<0.05$) are labeled

Table 2. Metabolic concentrations of phosphocreatine (PCr), inorganic phosphate (Pi), adenosine triphosphate (signal of the second phosphate, β-ATP), and phosphodiesters (PDE) related to total integral of the phosphorous spectra (P_{tot}), and pH measured at rest evaluated for the patients' groups and controls. Mean values ± standard deviations are listed.

Subject group	**PCr/P_{tot}**	**Pi/P_{tot}**	**β-ATP/P_{tot}**	**PDE/P_{tot}**	**pH**
Cm	0.49±0.02	0.06±0.02	0.101±0.009	0.050±0.017	7.047±0.032
DM1m	0.50±0.03	0.07±0.02	0.101±0.015	0.051±0.019	7.032±0.022
DM1mN	0.50±0.02	0.08±0.01	0.083±0.004	0.069±0.021	7.032±0.018
Cf	0.50±0.02	0.06±0.02	0.093±0.007	0.053±0.020	7.019±0.026
DM1f	0.49±0.04	0.08±0.03	0.089±0.011	0.056±0.015	7.019±0.019
DM1fN	0.50±0.03	0.08±0.01	0.083±0.004	0.058±0.023	7.040±0.020
C	0.497±0.0214	0.063±0.016	0.097±0.009	0.052±0.018	7.03±0.030
DM1	0.494±0.0334	0.071±0.022	0.096±0.015	0.053±0.017	7.03±0.022
DM1N	0.497±0.0248	0.078±0.015	0.085±0.009	0.066±0.022	7.03±0.019

Cm - control males, Cf - control females, C - control males and females, DM1m - diabetic males, DM1f - diabetic females, DM1 - diabetic males and females, DM1mN - diabetic males with nephropathy, DM1fN – diabetic females with nephropathy, DM1N - diabetic males and females with nephropathy; significant differences ($p<0.05$) are labeled

Table 3. Dynamic parameters – recovery time of phosphocreatine after exercise (τ_{PCr}), drop of phosphocreatine during exercise (ΔPCr), speed of PCr replenishment (V_{iPCr}), mitochondrial capacity (Q_{max}), and pH after exercise of the patients' groups and controls. Mean values ± standard deviations are listed.

Subject group	**τ_{PCr} (s)**	**ΔPCr (%)**	**V_{iPCr} (mmol/s)**	**Q_{max} (mmol/s)**	**pH after exercise**
Cm	53±24	36±18	0.32±0.12	0.58±0.21	7.002±0.169
DM1m	60±29	42±18	0.25±0.09	0.50±0.17	6.876±0.197
DM1mN	87±70	45±21	0.30±0.14	0.51±0.19	6.895±0.219
Cf	42±11	38±11	0.42±0.18	0.71±0.25	6.945±0.155
DM1f	61±23	45±16	0.36±0.12	0.63±0.16	6.826±0.134
DM1fN	80±56	41±17	0.31±0.15	0.52±0.24	6.970±0.174
C	48±19	36±14	0.36±0.16	0.64±0.23	6.976±0.162
DM1	60 ±26	43±17	0.30±0.11	0.55±0.17	6.855±0.173
DM1N	85±65	44±20	0.30±0.14	0.51±0.20	6.916±0.206

Cm – control males, Cf - control females, C - control males and females, DM1m - diabetic males, DM1f - diabetic females, DM1 - diabetic males and females, DM1mN - diabetic males with nephropathy, DM1fN – diabetic females with nephropathy, DM1N - diabetic males and females with nephropathy; significant differences ($p<0.05$) are labeled

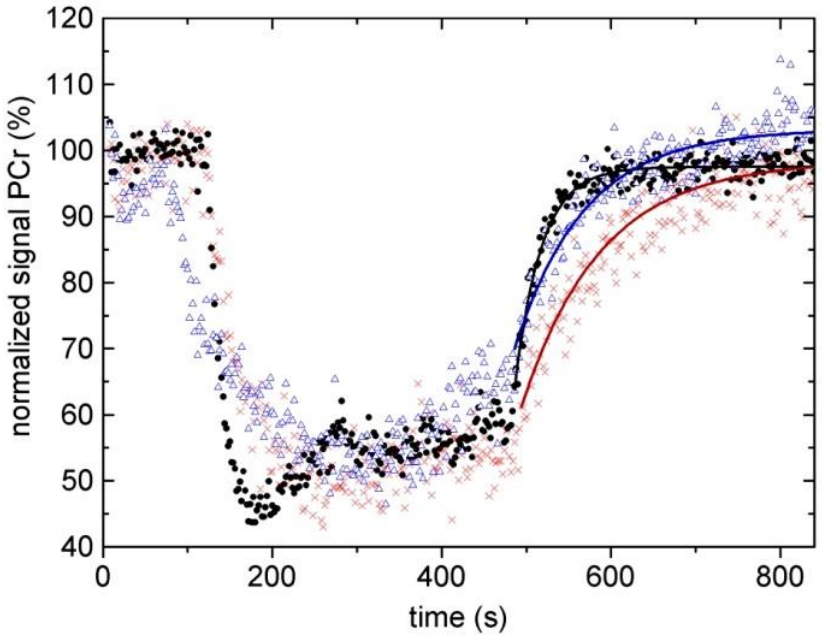

Fig. 1. Typical changes in phosphocreatine (PCr) during the rest – exercise – recovery periods during the examination of a female control (black), DM1 patient without (blue) and with (red) nephropathy.