

# Advanced skeletal muscle imaging in S-Adenosylhomocysteine Hydrolase Deficiency: A case series

**Ivana Kuhtic**

KBC Zagreb: Klinicki Bolnicki Centar Zagreb

**Ivo Baric**

KBC Zagreb: Klinicki Bolnicki Centar Zagreb

**Danijela Petkovic Ramadza**

KBC Zagreb: Klinicki Bolnicki Centar Zagreb

**Mislav Cavka**

KBC Zagreb: Klinicki Bolnicki Centar Zagreb

**Maja Prutki** (✉ [maja.prutki@gmail.com](mailto:maja.prutki@gmail.com))

KBC Zagreb: Klinicki Bolnicki Centar Zagreb <https://orcid.org/0000-0002-2409-801X>

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## Case report

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# Abstract

## Background

S-Adenosylhomocysteine hydrolase (SAHH) deficiency is a rare inherited multisystemic disease with muscle involvement associated with increased activity of creatine kinase being one of the most prominent and poorly understood feature. Therefore, skeletal muscles were analyzed by magnetic resonance imaging (MRI) and MR spectroscopy (MRS) in three brothers with SAHH deficiency in a different age group.

## Case presentation

At the time of this study, the brothers were at age of 13, 11, and 8 years, respectively. They presented with similar symptoms in early infancy. Motor developmental delay began in the first months of life. Myopathy was more pronounced in the lower extremities and proximal skeletal muscle groups. MRI revealed lipid infiltration, and MRS curve showed an elevated muscle lipid fraction (higher peak of lipid) increased with age and in proximal skeletal muscle in lower extremities. The data was consistent with muscle biopsy findings in two of them, and third patient had no specific pathological changes in examined muscle tissue.

## Conclusions

These findings open the possibility of insight into the extent of muscle involvement, monitoring the course of SAHH deficiency and response to therapy with an accessible and non-invasive method of MRI and MRS.

## Background

S-Adenosylhomocysteine hydrolase (SAHH) (EC 3.3.1.1) is the enzyme that catalyzes the hydrolysis of S-adenosylhomocysteine (AdoHcy) to adenosine and homocysteine [1]. In SAHH deficiency (OMIM 613752) pathogenesis is only partially elucidated, but considering the critical role of methylation in various cellular processes, it is assumed that elevation of AdoHcy, a potent inhibitor of transmethylation reactions, plays a significant part in causing clinical abnormalities [2, 3].

SAHH deficiency has been reported in about 15 patients [4–14] It is a multisystemic, clinically variable, and autosomal recessive inherited metabolic disease characterized most frequently by psychomotor delay, myopathy and liver dysfunction. Hypermethioninemia and elevated creatine kinase (CK) are frequently observed. Disease onset is typically in infancy but may occur already *in utero* or only in adult age [10]. In infancy, the clinical presentation typically consists of developmental delay and hypotonia due to myopathy, and more variably with cerebral hypomyelination, coagulation abnormalities and hepatopathy. Microcephaly, behavioral deviations and strabismus are frequent. *In utero* presentation is characterized by fetal hydrops and congenital brain anomalies (pontine and cerebellar hypoplasia,

hypoplastic corpus callosum) followed after birth by synthetic liver failure, respiratory insufficiency due to severe muscle weakness and death in early infancy. Specific biochemical abnormalities are markedly increased plasma AdoHcy and S-adenosylmethionine (AdoMet) in combination with normal or near normal total homocysteine (tHcy) and hypermethioninemia which is sometimes missing, particularly in early infancy. Although the disease is usually severe with poor developmental outcomes, the phenotype can be mild (later onset, mild weakness and mild developmental delay) or even asymptomatic. Hepatocellular carcinoma was reported in a single patient [10]. Reports on muscle biopsy are rare. Numerous myelin figures were detected in muscle by electron microscopy, and the authors described the muscle histology as indicative of slowly progressive myopathy [3–6].

MR spectroscopy (MRS) is a non-invasive method of metabolic imaging with magnetic resonance imaging (MRI) which detects a quantified signal of water, lipids and other metabolites in the tissue, possibly representing the metabolism of interest. With water-suppression scheme, a fraction of lipids can be analyzed with more accuracy. In muscle, the intracellular and extracellular lipid fractions can be analyzed separately. Intracellular lipids (IMCL) are located near mitochondria while extracellular (EMCL) is in adipocytes between muscle cells [15, 16]. Figure 1 shows the normal curve of metabolite fractions in skeletal muscle [17].

Molecular identification of specific metabolic markers is potentially useful for characterization of musculoskeletal abnormalities to help guide treatment decisions and follow-up [18–20]. A few studies in the literature have explored the role of MRS lipid content in musculoskeletal imaging for muscle diseases [15, 16, 21, 22]. Furthermore, lipid infiltration has been associated with disease progression, age, and clinical functional tests in Duchenne muscular dystrophy (DMD) [23]. Using single-voxel  $^1\text{H}$ -MRS, a measure dependent on lipid infiltration, shows a higher value in DMD compared to controls, indicating increased muscle damage and inflammation/oedema, which can be effective in monitoring disease progression [24–26].

In SAHH deficiency, one of the dominant symptoms is myopathy. Therefore, in this case series, MRI and MRS findings of skeletal muscles in lower extremities and proximal muscle groups in upper extremity three brothers with the proven SAHH deficiency were analyzed.

The results were partially presented as a poster at the Annual Symposium of the Society for the Study of Inborn Errors of Metabolism 2015 in Lyon, France [27].

## Materials And Methods

All procedures performed in this case series involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

The study was based on analysis of MRI and MRS of the skeletal muscles of the lower and proximal muscle groups of upper extremities in three siblings with SAHH deficiency.

MRI and MRS were performed on the same day in all three brothers at the age of 13, 11 and 8 years, respectively. MRI and MRS were acquired on a 3T scanner (Siemens Healthcare, Erlangen, Germany) using a four-element 'body matrix' receiver coil and a circularly polarized (CP) body transmit coil.

On MRI, T2, T2 with fat suppression (Slice: 4 mm, Dist: 5.2 mm, TR: 3600 ms, TE: 92 ms) and T1 (Slice: 5 mm, Dist: 6.5 mm, TR: 500 ms, TE: 15 ms) sequences were made in axial, coronal and sagittal planes. Axial T2 weighted anatomic images (TR/TE 3000 ms/30 ms, FOV 20cm, slice thickness 7mm, acquisition time 4 minutes) were collected to provide a guide for spectroscopy voxel localization within vastus lateralis muscle, soleus muscle and biceps brachii muscle. Prior to data collection, field homogeneity was optimized using linear, manual shimming. The voxel was positioned in the muscles with attention to avoid blood vessels, subcutaneous and other fat, and the femur bone. For each voxel, a single voxel Point-Resolved Spectroscopy Sequence (PRESS) (TR 2 s; TE 135 ms, voxel size 1×1×1 cm (1 cm<sup>3</sup>), 128 averages, acquisition time 4 min 20s) spectrum was acquired with a 4-pulse CHES water-suppression scheme, followed by two acquisitions without water suppression (16 averages, scan time 40 sec), one collected with 'body matrix' receive and the other with the CP-transmit coil used as received.

Fatty infiltration of the lower extremities and proximal muscle groups of upper extremities was graded using semi-quantitative and quantitative methods. The semi-quantitative method using MR images (based on the morphological findings) entailed consensus scoring by two radiologists blinded to patient data to minimize bias, resulting in the agreement of both readers. Semi-quantitative method was performed at the largest cross-sectional area of each muscle, we used the scale described by Kim et al. [28], as follows: grade 0, homogeneous muscle signal intensity without fatty infiltration; grade 1 (minimal), predominantly homogeneous muscle signal intensity with minimal scattered fatty infiltration (often seen in soleus muscle); grade 2 (mild), mild fatty infiltration with additional patchy areas of intramuscular high T1 signal intensity involving less than 30% of muscle bulk; grade 3 (moderate), moderate fatty infiltration involving 30–60% of muscle bulk, and preserved differentiation between muscle and subcutaneous fat; and grade 4 (severe), severe fatty infiltration involving more than 60% of muscle bulk with loss of demarcation between muscle and subcutaneous fat.

Quantitative measures of muscle fatty infiltration were obtained by determining the amount of intramuscular adipose tissue on MRS in the vastus lateralis muscle, soleus muscle and biceps brachii muscle. The results were compared between the brothers.

## Case Presentation And Results

The patients were the children of healthy, unrelated parents, born at term by normal delivery after a normal pregnancy. The definite diagnosis was made by sequence analysis of the *AHCY* gene which revealed two mutations: the maternally derived c.336G>A (p.W112X) and the paternally derived c.428A>G (p.Y143C).

All three brothers had similar symptoms: psychomotor delay, myopathy, mild hepatopathy with disturbed coagulation, behavioral problems, and cognitive impairment. Myopathy was the most prominent symptom, and unamenable to treatment. At the time of skeletal muscle MRI and MRS, all patients had hypotonia and muscle weakness, more prominent in the proximal muscle groups especially in the lower extremities, fatigability and obesity due to low physical activity, but all walked unassisted, and were able to perform everyday chores.

### **CASE 1 [3]**

Index patient had delayed psychomotor development since birth. He presented at the age of eight months with severe developmental delay, hypotonia, more in lower than upper extremities, convergent strabismus and microcephaly. Diagnostic work-up showed increased activities of CK and aminotransferases, low albumin and prolonged prothrombin time (the later as signs of impaired liver synthetic function). Electromyography showed myopathic potentials. Histopathological examination of skeletal muscle revealed variability in fiber size with few necrotizing fibers undergoing phagocytosis and some basophilic regenerating fibers, histochemistry demonstrated no specific pathological changes, whereas electron microscopy showed numerous myelin figures of different sizes and shapes in muscle fibers and extracellularly and numerous enlarged and abnormally shaped mitochondria within some fibers. In the liver tissue there were signs of mildly active chronic hepatitis. Brain MRI revealed white matter atrophy and impaired myelination. The diagnosis of SAHH deficiency was confirmed at the age of 12.8 months by measuring low SAHH activity in red blood cells, fibroblasts and liver, and confirming two pathogenic mutations in the *AHCY* gene. Methionine-restricted diet and supplementation of phosphatidylcholine and creatine were started at the age of 13 months. Treatment resulted in marked decrease of biochemical biomarkers of this disorder (AdoMet, AdoHcy and methionine), and gradual, but constant, clinical improvement. Patient became more alert, communicative, and muscle strength improved. He started to walk unassisted at age 19 months. Control brain MRI, after seven months of treatment, showed near normal myelination for age [4]. Elevated CK and aminotransferases remained despite the treatment. Second muscle biopsy was obtained at 12.5 years and showed fiber variability, endomysial oedema, with some fatty infiltration and inflammation. Histochemistry and immunohistochemistry demonstrated no significant changes, and electron microscopy showed normal-sized and shaped mitochondria with swollen cristae and subsarcolemmal myelin figures [3]. At the time of skeletal muscle MRI and MRS patient was 13 years old.

### **CASE 2 [5]**

Patient was hypotonic since birth. At first clinical evaluation at 15 days of life he had reduced spontaneous movements, generalized hypotonia and absent tendon reflexes. As his older brother, he had elevated CK and aminotransferases, and delayed myelination and frontotemporal atrophy on brain MRI. In contrast to his brother, patient had neither manifesting liver disease nor coagulation disturbance. The diagnosis of SAHH deficiency had been clearly established at the age of 3.4 months (elevated AdoMet and AdoHcy, very reduced activity of SAHH in red blood cells, and confirmation of two biallelic family

mutations in the *ACHY* gene) At the time of diagnosis, the boy had hypotonia, convergent strabismus, and developmental delay, although less severe than his older brother at corresponding age. At that time, treatment was started (low methionine diet with phosphatidylcholine and creatine supplementation), which resulted in improved strength, alertness and spontaneous movements. In subsequent period strabismus disappeared, tendon reflexes, although weak, could be elicited and muscle hypotonia was less evident. CK and aminotransferases remained permanently elevated. Patient was able to sit unsupported at 10 months, and to stand and walk with support at 13 months. Control brain MRI performed seven months after the treatment initiation showed almost normal myelination for age. The biopsy of the right deltoid muscle was done at age 13.5 months and histological examination revealed fairly normal muscle fibers except for slightly increased variation in fiber size. Immunohistochemically, expressions of dystrophin, merosin and alpha-sarcoglycan were normal. Electron microscopy revealed a small number of myelin figures with different sizes and shapes, and focal myofibrillar degeneration in the subsarcolemmal regions of an occasional muscle fiber [5]. Long term follow-up showed that patient 2 had the best outcome considering muscle strength and endurance (he was able to play football with his peers), but cognitive abilities as well. He was 11 years old at the time of MRI and MRS

### **CASE 3 [4]**

Third patient, who harboured the same pathogenic mutations of the *ACHY* gene, exhibited clear signs of myopathy since birth: sluggishness, shallow breathing, floppiness, diminished spontaneous activity and absent tendon reflexes. Comparison to his brothers regarding presentation of their inherited disease was somewhat complicated, as this patient experienced mild perinatal hypoxia (Apgar score 8/9) which might have contributed to the clinical symptoms. He also had elevated AdoMet, AdoHcy, and CK. Low methionine diet and oral phosphatidylcholine supplementation were started at age 18 days, and creatine was added a month later. On treatment patient gradually gained strength, became more alert with better contact and spontaneous activity. Patient had permanently elevated CK and delayed milestones (unsupported walking at 19 months of age). A muscle sample was taken during orthopedic procedure of the hip at the age of 4.4 years and histopathology, immunohistochemistry, and electron microscopy showed normal findings [4]. This patient was 8 years old at the time of MRI and MRS.

## **Results**

MRI revealed that the most affected was the posterior group of proximal skeletal muscles of lower extremities, followed by the distal muscle groups of lower extremities, and the muscle groups of the proximal part of the upper extremities. MRI showed an abnormal fatty infiltration of skeletal muscle in the proximal parts of the lower extremities, especially in the posterior muscle group (vastus lateralis and adductor magnus muscle). On the other hand, the gracilis and adductor longus and brevis muscle were spared. The most pronounced changes were found in the oldest brother (Patient 1), and the least in the youngest brother (Patient 3) (Table 1, Fig. 2).

In the skeletal muscles of the distal part of the lower extremities, the most pronounced pathology was detected in the soleus and peroneus muscle. The most prominent changes were also found in the oldest brother (Patient 1), while in the middle brother only minor changes were present (Patient 2). The changes in the youngest brother were moderate (Table 1, Fig. 3). There was significant oedema in the soleus muscle without signs of pseudohypertrophy (muscle diameter is in the referral interval for age).

In the area of the proximal part of the upper extremities, MRI showed fatty infiltration of the biceps brachii muscle in the oldest brother (Patient 1). In the two younger brothers, the finding was normal (Table 1).

Table 1  
Fatty infiltration of the muscles on MRI using the semi-quantitative method [24]

<b>Skeletal muscle</b>			
	<b>Proximal muscle groups of lower extremities</b>	<b>Distal muscle groups of lower extremities</b>	<b>Proximal muscle groups of upper extremities</b>
<b>Patient 1</b>	4	4	2
<b>Patient 2</b>	3	1	0
<b>Patient 3</b>	2	3	0

MRS with voxel placed within vastus lateralis muscle of the proximal part of the lower extremity showed a high peak of EMCL ( $\text{CH}_2$  and  $\text{CH}_3$ ) lipids (Table 2). The highest peak of EMCL lipids was detected in the oldest brother (Patient 1), slightly lower in the youngest brother (Patient 3), and the lowest in the middle-aged brother (Patient 2). No increase in lipid IMCL ( $\text{CH}_2$  and  $\text{CH}_3$ ) was detected in either sibling (Fig. 4).

The elevated peak of EMCL ( $\text{CH}_2$ ) was detected in the soleus muscle in the distal part of the lower extremity of the oldest brother (Patient 1), slightly lower in the youngest brother (Patient 3), and again the smallest peak in the middle-aged brother (Patient 2). EMCL ( $\text{CH}_3$ ) was detected only in the youngest sibling (Patient 3). No increase in IMCL ( $\text{CH}_2$  and  $\text{CH}_3$ ) was detected in either sibling (Fig. 5).

MRS within the biceps brachii muscle in the proximal part of the upper extremity showed a slightly elevated peak of EMCL ( $\text{CH}_2$ ) in the oldest brother (Patient 1). The findings of the MRS in the two younger brothers was normal (Fig. 6).

Table 2  
Lipid amplitude in MRS in vastus lateralis muscle and soleus muscle

Skeletal muscle						
Ppm	Vastus lateralis muscle			Soleus muscle		
	Patient 1	Patient 2	Patient 3	Patient 1	Patient 2	Patient 3
0.9 IMCL (CH <sub>3</sub> ) – lipids						
1.1 EMCL (CH <sub>3</sub> ) – lipids	<b>67.5</b>	11.8	28.3	0	0	26.7
1.3 IMCL (CH <sub>2</sub> ) – lipids						
1.5 EMCL (CH <sub>2</sub> ) – lipids	<b>250.5</b>	<b>54.7</b>	<b>132.8</b>	<b>191.9</b>	<b>43.5</b>	<b>50.7</b>
2.2 METHYLENE GROUP OF ESTER BINDING	<b>70</b>	11.5	23	<b>43.2</b>	9.9	12.2
* IMCL – intracellular lipids; EMCL – extracellular lipids						

## Discussion And Conclusions

SAHH deficiency is a rare multisystemic disease caused by disorder of methionine cycle. Clinical presentation is variable, but myopathy is a constant feature [29, 30].

Muscle histology changes in our patients were unspecific, and in one patient even absent, albeit that patient had clear clinical and biochemical signs of muscle involvement. Possible explanation for absence of significant pathological changes in the muscle of youngest brother is, that sample was taken during orthopedic procedure and not from clinically most severely affected muscles (as it is done during muscle biopsy for diagnostic purposes).

It is still unclear how SAHH deficiency affects muscle. Choline depletion may lead to muscle disease [31], but the persistent elevation of CK despite phosphatidylcholine (and creatine) supplementation suggests that lack of these compounds is not the sole cause of adverse muscle effects in the present patients. Partial improvement following the decrease of AdoHcy during the methionine-restricted diet is consistent with this hypothesis [5]. As SAHH deficiency is at least partially amenable to the treatment, an important prerequisite for the patient outcome is an early recognition.

MRI findings in this study are in accordance with myopathy as a characteristic finding in SAHH deficiency. MR imaging finding showed an abnormal fatty infiltration, oedema and atrophy of skeletal muscle in the extremities, predominantly in proximal part of the lower extremities. The changes were most extensive in the index patient, what corresponds with the clinical observation, as that patient had more severe myopathy, and was diagnosed and treated at later age than his brothers. Presented findings

are consistent with previously described skeletal muscle changes characteristic for muscular dystrophies such as Duchenne muscular dystrophy [23, 24, 28, 32–36].

The MRS is available, a non-invasive method which can measure the peak of metabolites and lipids that suggest the fatty infiltration in the observed skeletal muscle. MRS showed that the content of lipids in the target muscles was most pronounced in the eldest brother (Patient 1). This may reflect the later diagnosis and treatment of the index patient, or the natural possibly progressive course of the disease.

In patient 2, MRS showed a slightly lower proportion of increased lipids in muscles among the siblings but also more severe muscle oedema. Clinically, patient 2 had less severe myopathy, attained developmental milestones earlier and had less marked histological changes in muscle than patient 1, manifested neither liver disease nor clotting disturbance at the time of diagnosis, and besides better muscle strength, displayed better cognitive outcome during long-term follow-up. Although these lines of evidence may suggest that patient 2 is less affected than his older brother, this conclusion is equivocal for several reasons: treatment in patient 2 started at earlier onset of disease; there were age differences (and corresponding body protein synthesis rates) at which metabolite measurements were made, and there were differences in dietary methionine intakes in the period just before the diagnoses were made (patient 2 was breast-fed; patient 1 was taking mixed infant food) [4, 5]. In addition, the first patient has been exposed to higher amounts of methionine and consequently higher amounts of AdoHcy, which probably led to significantly impaired transmethylation reactions in the body, and thus in the muscles. On the other hand, the fact that does not support this thesis is more pronounced changes in the youngest brother in whom treatment was started earlier, and it would be expected that he has less changes than patient 2.

The morphological finding of the target muscles was almost equal in the patient 2 and 3 with more pronounced fatty infiltration of the distal lower extremity in the youngest brother (patient 3). Overall, the most pronounced fatty infiltration was in posterior muscle groups of the proximal lower extremity, followed by the groups of the distal lower extremity, and only in the older brother in the proximal muscle groups of upper extremities.

To the best of our knowledge, this is the first study describing comparative MRI and MRS findings in patients with AHcy deficiency. The Stender et al mentioned that MRI of the legs showed muscular atrophy in these patients [10]. The results showed that the progression of fatty infiltration goes towards the distal, primarily in the lower extremities. For understanding and monitoring muscle involvement, the primary focus was on magnetic resonance imaging (MRI) and spectroscopy (MRS) which showed a significant pathological finding consistent with biopsy of the skeletal muscle.

These findings open the possibility of insight into the extent of muscle involvement, detection, monitoring the course of SAHH deficiency and response to currently available and future therapies with an accessible and non-invasive method of MRI and MRS. MRI and MRS is also a possible excellent substitute for the invasive biopsy method since coagulopathy is also present in these patients.

# Abbreviations

AdoHcy - S-Adenosylhomocysteine

AdoMet - S-adenosylmethionine

CK - Creatine kinase

DMD - Duchenne Muscular Dystrophy

EMCL – extracellular lipids

IMCL – intracellular lipids

MD - Muscular Dystrophy

MRI - magnetic resonance imaging

MRS - magnetic resonance imaging and spectroscopy

SAHH S-adenosylhomocystein hydrolase

# Declarations

**Ethics approval and consent to participate:** All procedures performed in this case series involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All data were viewed retrospectively and no personal data was contained in this study.

**Consent for publication:** Informed consent was obtained from the parents for all patient studies.

**Availability of data and materials:** The study was based on institutional data that included insight into MR spectroscopy images of the skeletal muscle. The clinical data are contained in the previous studies as follow:

Patient 1 - Barić I, Fumić K, Glenn B, et al. S-Adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. *Proc Natl Acad Sci USA*. 2004;101:4234–4239. doi:10.1073/pnas.0400658101

Patient 2 - Barić I, Cuk M, Fumić K, et al. S-Adenosylhomocysteine hydrolase deficiency: a second patient, the younger brother of the index patient, and outcomes during therapy. *J Inherit Metab Dis*. 2005; 28(6):885-902. doi:10.1007/s10545-005-0192-9

Patient 3 - Čuk M, Lovrić M, Fumić K, Mudd SH, Vugrek O, Sarnavka V, et al. The fourth S-adenosylhomocysteine hydrolase deficient patient: Further evidence of congenital miopathy. *Clin Chem*

Lab Med 2007;A43.

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests:** The authors declare that they have no competing interests

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**Authors' contributions:** All authors contributed to the study conception and design. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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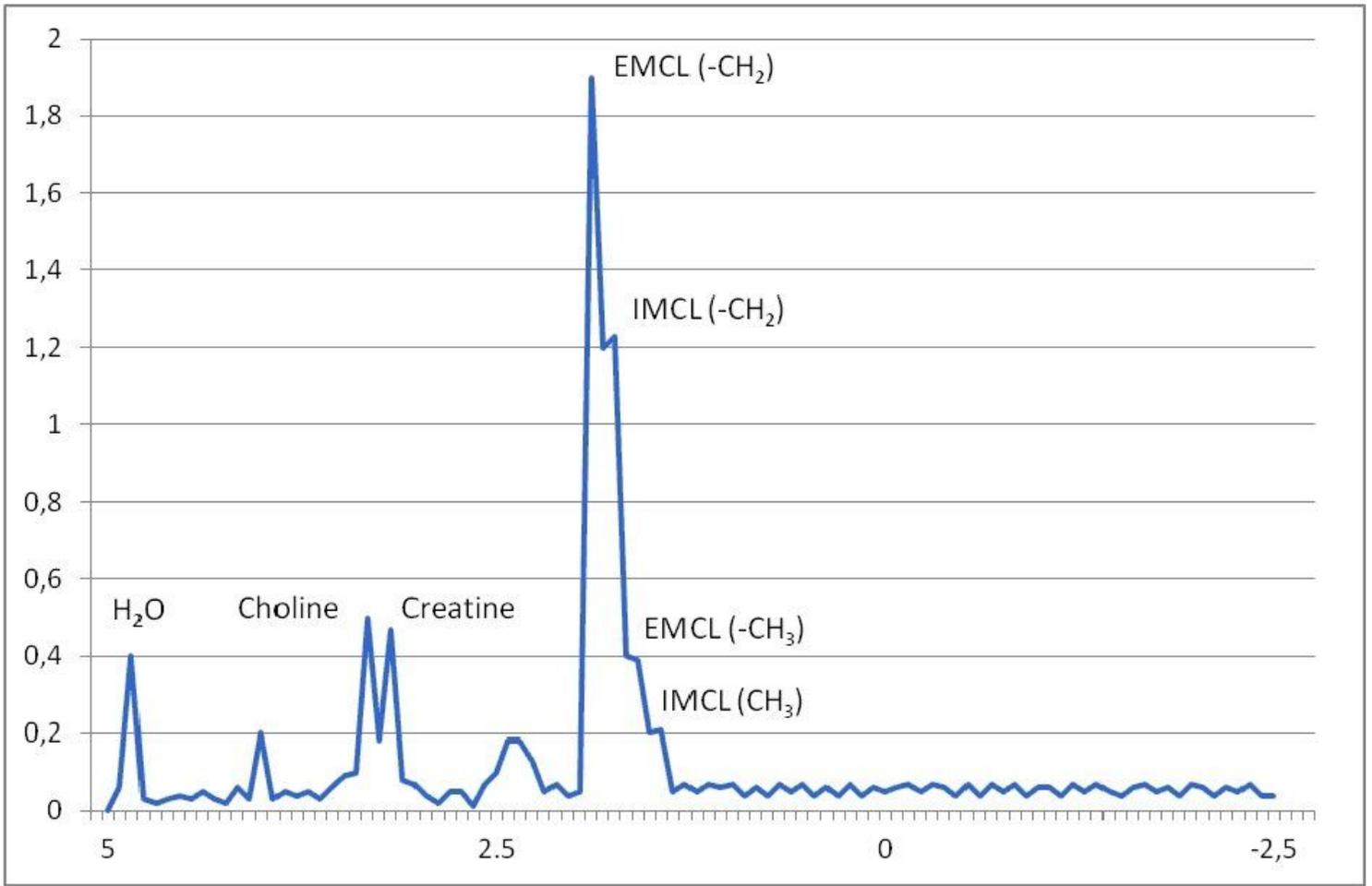
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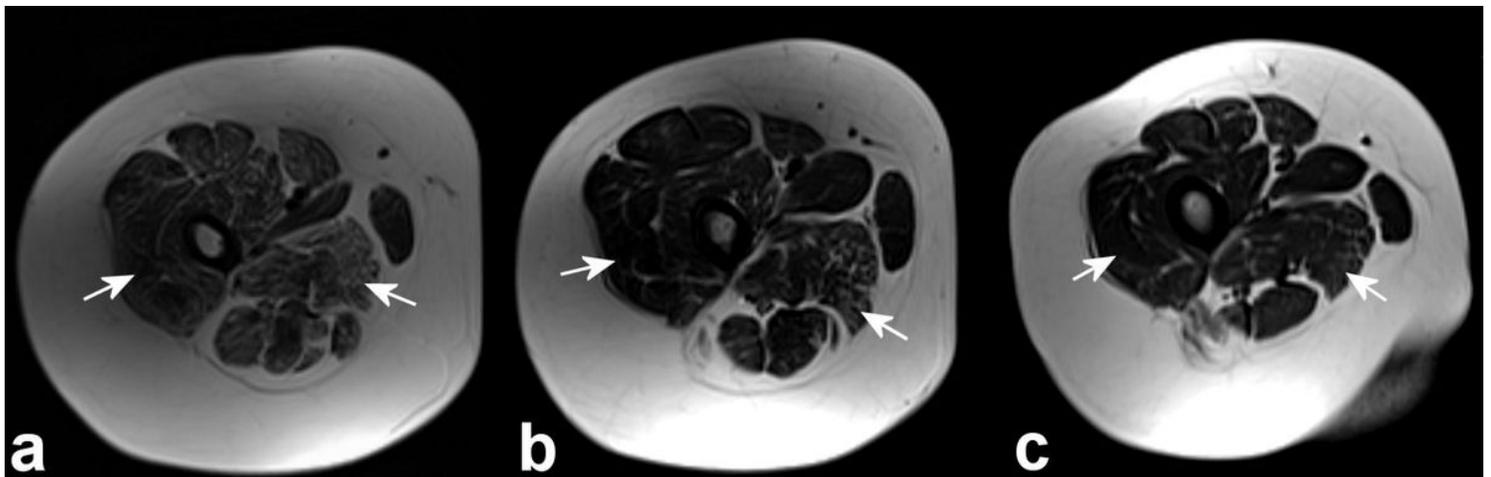
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## Figures



**Figure 1**

Schematic representation of the MRS curve demonstrates different molecular resonance for a variety of metabolites in the normal skeletal muscle. The spectral dispersion in muscles: IMCL (-CH<sub>3</sub>), intramyocellular lipid methyl protons at 0.9 ppm; EMCL (-CH<sub>3</sub>), extramyocellular lipid methyl protons at 1.1 ppm; IMCL (-CH<sub>2</sub>), intramyocellular lipid methylene protons at 1.3 ppm; EMCL (-CH<sub>2</sub>), extramyocellular lipid methylene protons at 1.5 ppm



**Figure 2**

MRI (axial T2-weighted images) of proximal muscles groups of lower extremities showing the dominant fatty infiltration in the most affected muscles (vastus lateralis muscle and adductor magnus muscle) in Patient 1 (a), Patient 2 (b) and Patient 3 (c)

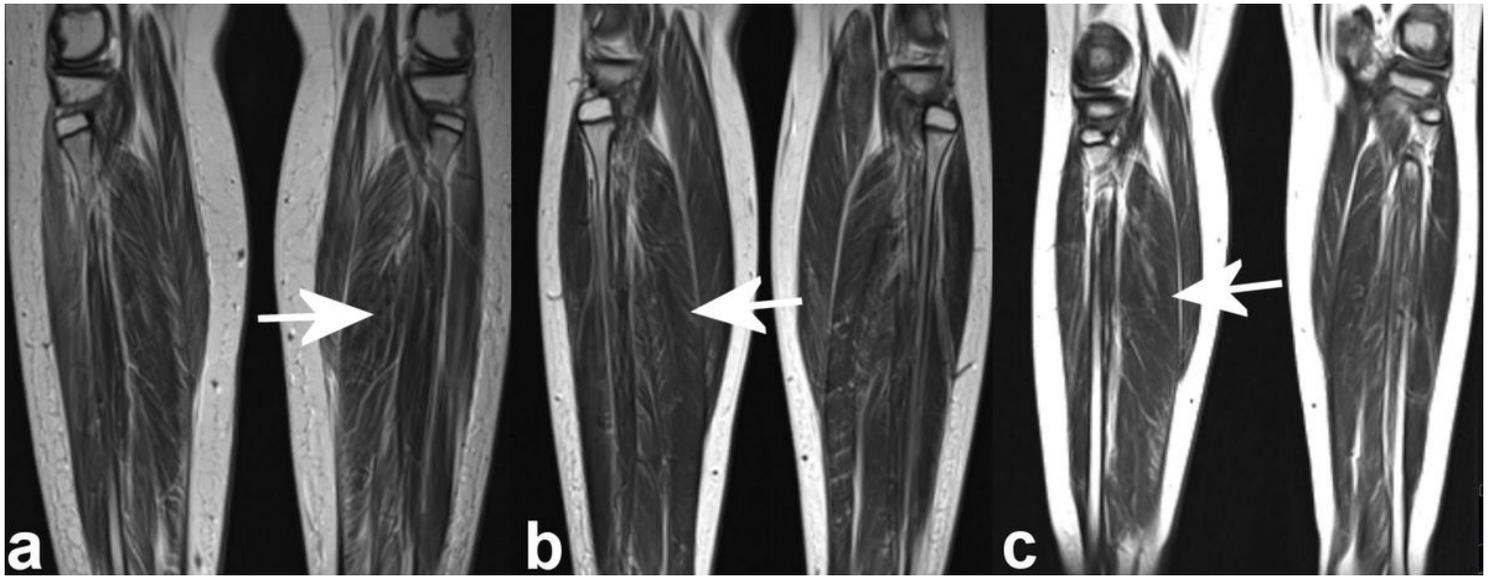


Figure 3

MRI of distal muscles groups of lower extremities (coronal T2-weighted images) showing fatty infiltration in the most affected muscle (soleus muscle) in Patient 1 (a), Patient 2 (b) and Patient 3 (c)

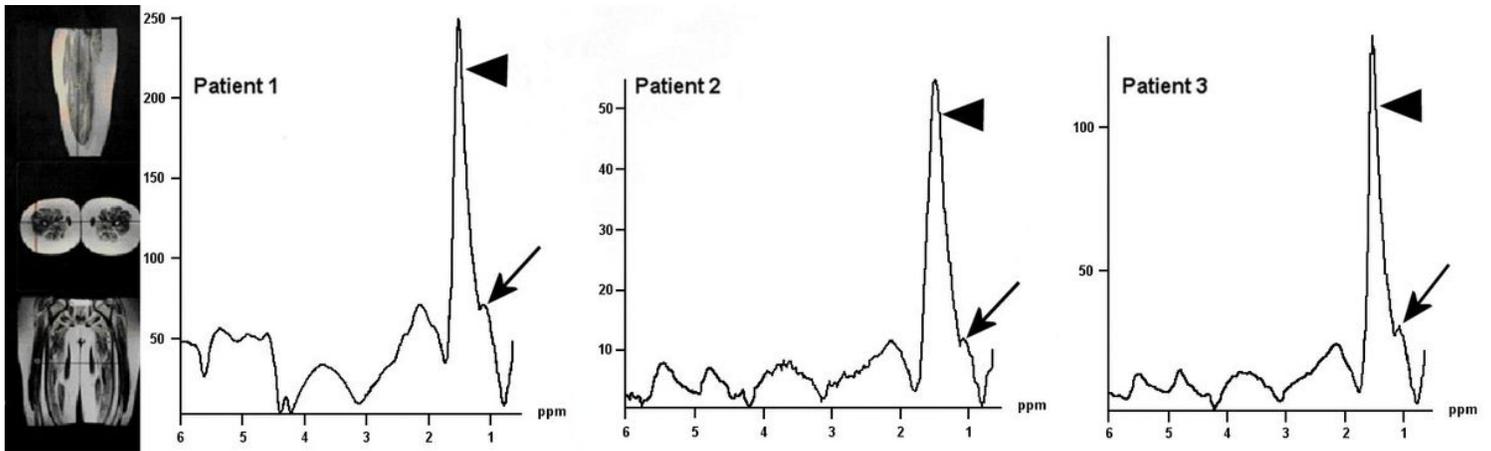


Figure 4

MRS obtained in three patients within vastus lateralis muscle showing the highest fraction of EMCL (CH<sub>2</sub>) (arrowhead) and EMCL (CH<sub>3</sub>) (arrow) in the oldest patient (Patient 1) and lowest in middle-aged patient (Patient 3)

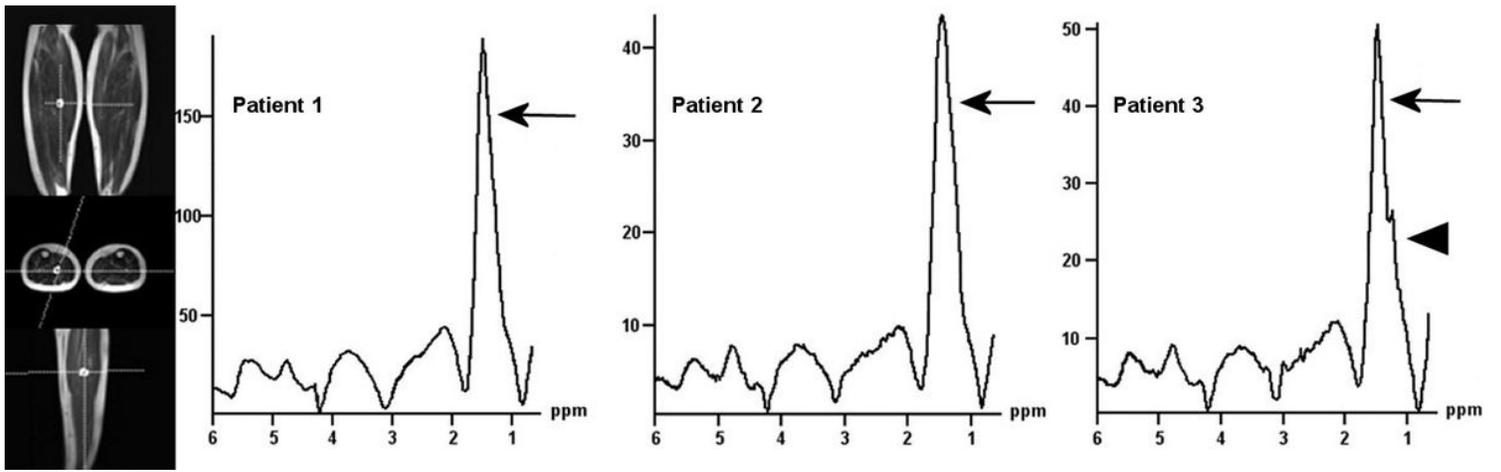
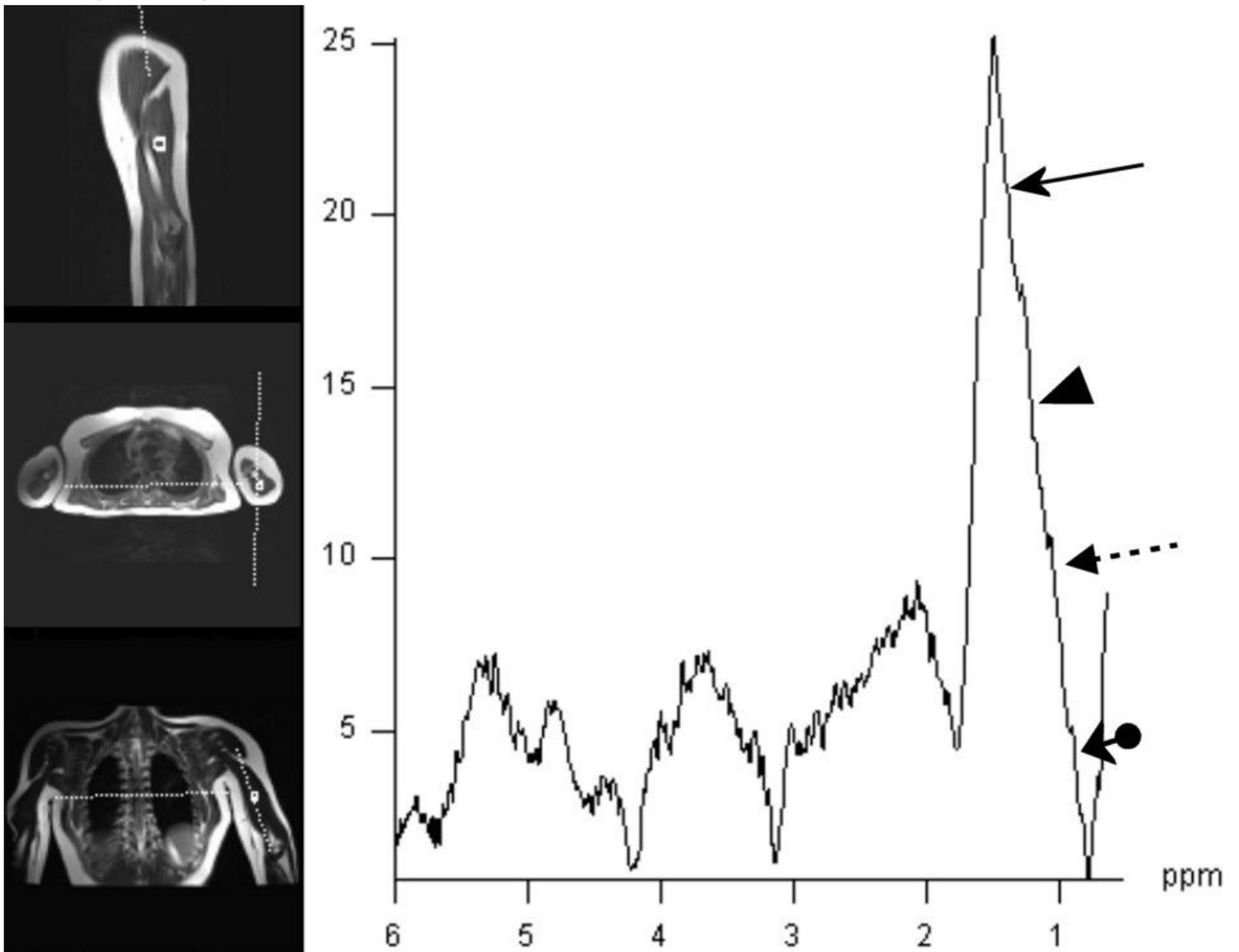


Figure 5

MRS obtained in three patients within soleus muscle demonstrating the highest peak of EMCL (CH2) (arrow) in the oldest patient (Patient 1), while EMCL (CH3) (arrowhead) is present only in the youngest patient (Patient 3)



## Figure 6

MRS obtained in the youngest patient (Patient 3) within biceps brachii muscle in showing normal curve of lipids fractions: EMCL (CH<sub>2</sub>) at 1.5 ppm (arrow), EMCL (CH<sub>3</sub>) at 1.1 ppm (dashed arrow), IMCL (CH<sub>2</sub>) 18 at 1.3 ppm (arrowhead) and IMCL (CH<sub>3</sub>) at 0.9 ppm (arrowhead - ball).