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# Acetaminophen treatment in children and adults with spinal muscular atrophy: a lower tolerance and higher risk of hepatotoxicity

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ABSTRACT

Acute liver failure has been reported sporadically in patients with spinal muscular atrophy (SMA) and other neuromuscular disorders with low skeletal muscle mass receiving recommended dosages of acetaminophen. It is suggested that low skeletal muscle mass may add to the risk of toxicity. We aimed to describe the pharmacokinetics and safety of acetaminophen in patients with SMA. We analyzed acetaminophen metabolites and liver biomarkers in plasma from SMA patients and healthy controls (HC) every hour for six or eight hours on day 1 and day 3 of treatment with therapeutic doses of acetaminophen. Twelve patients with SMA (six adults and six children) and 11 HC participated in the study. Adult patients with SMA had significantly lower clearance of acetaminophen compared to HC (14.1 L/h vs. 21.5 L/h). Formation clearance of acetaminophen metabolites, glucuronide, sulfate, and oxidative metabolites were two-fold lower in the patients compared to HC. The liver transaminases and microRNAs increased nine-fold in one adult SMA patient after two days of treatment. The other patients and HC did not develop abnormal liver biomarkers. In this study, patients with SMA had lower clearance and slower metabolism of acetaminophen, and one patient developed liver involvement. We recommend giving 15 mg/kg/dose to SMA adults (with a maximum of 4000 mg/day) and monitoring standard liver biomarkers 48 h after first-time treatment of acetaminophen.



## 1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the survival motor neuron 1 (*SMN1*) gene on chromosome 5q12 [1]. Bi-allele deficiency of *SMN1* causes degeneration of the anterior horn cells of the spinal cord, resulting in progressive muscular weakness and atrophy. Based on the age of onset and disease severity, SMA is divided into subtypes from I to IV [1]. The natural history of untreated SMA type I and type II (SMA II) renders patients wheelchair dependent and unable to walk at any time [1]. Current treatment options consist of three types of gene-modifying or gene therapy treatments for the most severe forms

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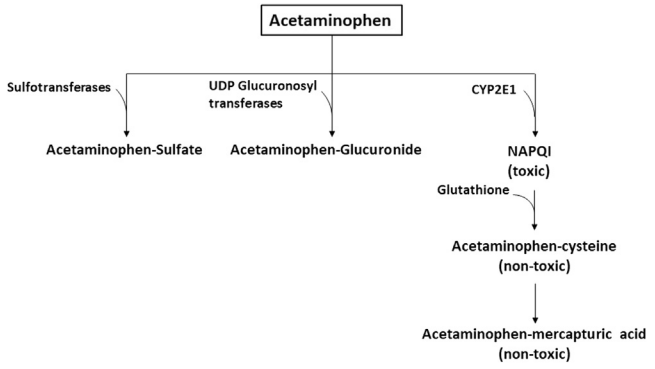


**Fig. 1.** Acetaminophen metabolism. Abbreviations: NAPQI= N-acetyl-p-benzo-quinone imine, UDP= uridine diphosphate, CYP= cytochromes.

(SMA I and SMA II) [2]. These treatments slow disease progression and improve motor function [2]. Independently of treatment, comorbidities such as malnutrition, low bone mineral density, and scoliosis are common in this patient population [3–5]. Many patients need scoliosis surgery during childhood or adolescence to prevent lung function deterioration and improvement of sitting position [6]. As a part of postoperative mild-to-moderate pain treatment and antipyretic treatment, patients with SMA often receive acetaminophen for several days at same dose levels as healthy subjects without considering the patient's lower muscle mass. Thus, several individual case reports involving children and adults with SMA, limb-girdle muscular dystrophy, Duchenne muscular dystrophy (DMD) and congenital muscular dystrophy with low skeletal muscle mass have reported hepatotoxic side effects of acetaminophen administered in therapeutic doses after surgery, infections, or critical illness [7–12]. In line with this, we have experienced one adult patient with SMA II that developed fatal acute liver failure following abdominal surgery, suspected to be caused by acetaminophen toxicity (unpublished data). Furthermore, a DMD boy in our clinic recently developed acute liver failure after intake of therapeutic doses of acetaminophen during hospitalization (unpublished data).

The majority of acetaminophen is conjugated to sulfate and glucuronide to form nontoxic metabolites (Fig. 1). A small portion undergoes CYP-mediated metabolism, forming the reactive and potentially toxic metabolite N-acetyl-p-benzo-quinone imine (NAPQI) (Fig. 1). NAPQI is conjugated by glutathione (GSH) to the nontoxic oxidative metabolites cysteine and mercapturic acid [13]. In toxic doses, the usual metabolic pathways are overloaded, and acetaminophen is shunted to the oxidative pathway, leading to the depletion of GSH stores. Hepatic cellular injury and necrosis occur as NAPQI accumulates [13].

Glutathione is a tripeptide consisting of glutamate, cysteine, and glycine. However, GSH synthesis depends on glutamine from the skeletal muscle to form glutamate [14]. Thus, patients with SMA may have a lower concentration of GSH compared to healthy due to their altered body composition, i.e., low skeletal muscle mass [15,16]. Furthermore, some children and adults with SMA are malnourished and are at increased risk of becoming critically ill. Several studies have shown that there may be a correlation between malnutrition, fasting, critical illness, and GSH deficiency [17–20]. This may increase the risk of acetaminophen-induced hepatotoxicity in the patients, even when treated with therapeutic doses [19,21,22]

Traditionally, alanine aminotransferase (ALT) is analyzed as a marker of acute liver toxicity. However, sometimes the ALT is within the normal range even though the hepatic function is impaired [23,24]. This warrants the use of more specific markers for liver injury. Studies have found that the liver specific microRNAs 122 and 192 (miRNA 122, miRNA 192) increase earlier and at lower acetaminophen doses than ALT [25–28]. Thus, the liver specific miRNAs may have the potential of being diagnostic biomarkers of drug-induced liver injury and it would be interesting to investigate these liver-specific miRNAs during acetaminophen treatment in SMA patients. Furthermore, it would be beneficial to have a biomarker in clinical settings that can be used for risk stratification of the patients and prediction of which patients are at increased susceptibility to acetaminophen toxicity in therapeutic doses. miRNA 122 is for now one of the most promising prognostic biomarkers for prediction of development of later liver injury in patients with normal ALT [29]. Two other studies have investigated the difference in miRNA 122 in patients with acetaminophen-induced liver injury or acute liver injury compared to 76 and 135 healthy participants, respectively. The studies found a significant increase in miRNA 122 levels among patients with ongoing liver injury compared to the healthy controls [25,27,29,30]. Thus, the biomarker could have a great potential in patient groups we suspect to have a lower tolerance for acetaminophen, such as patients with SMA and other patients with low skeletal muscle mass.

No prior prospective investigation of the safety of acetaminophen treatment in patients with SMA or other neuromuscular disorders with low skeletal muscle mass has been published. We aimed to investigate the safety, pharmacokinetics, and pharmacogenetic polymorphisms of acetaminophen in children and adults with SMA.

## 2. Methods

### 2.1. Study population

This was a non-randomized, open-label, single-site clinical trial investigating the pharmacokinetics and safety of therapeutic doses of acetaminophen in children and adults with SMA II from the age of 6 to 45 years. Patients were eligible for inclusion if genetically verified. The study conduct was between February 2019 and April 2021 at the Copenhagen Neuromuscular Center, Rigshospitalet. We recruited healthy adults from official recruitment sites for healthy controls and Facebook.com. Exclusion criteria were intake of medications (that may interfere with the results and that may affect gastric emptying), failure to obtain consent, or the participant being considered unsuitable by the treating physician. The Danish National Center of Ethics advises against using children as healthy controls in studies where they do not receive direct benefits of the study. Therefore, due to ethical considerations, we did not include age-matched healthy children.

### 2.2. Study visits

The participants were treated with an oral liquid formulation of acetaminophen in therapeutic doses, 15 mg/kg/dose every six hours, with a maximum of 1 gram x 4 per day, for three consecutive days (Fig. 2). The pharmacy of the Capital Region prepared the investigational product in doses corresponding to the participants' weight in the study. One peripheral venous catheter was inserted in the dorsal vein of the hand or in the cubital vein of the arm to draw blood samples. The participants were randomly divided into two plasma sampling schemes to minimize blood volume (groups A and B), each consisting of six or seven sampling times at each study visit (Fig. 2). Acetaminophen parent compound and its metabolite concentrations were measured every hour for six or eight hours after the initial dosing on study days 1 and 3 (Fig. 2). The participants were at home on day 2 without blood sampling. Liver plasma biomarkers (ALT,

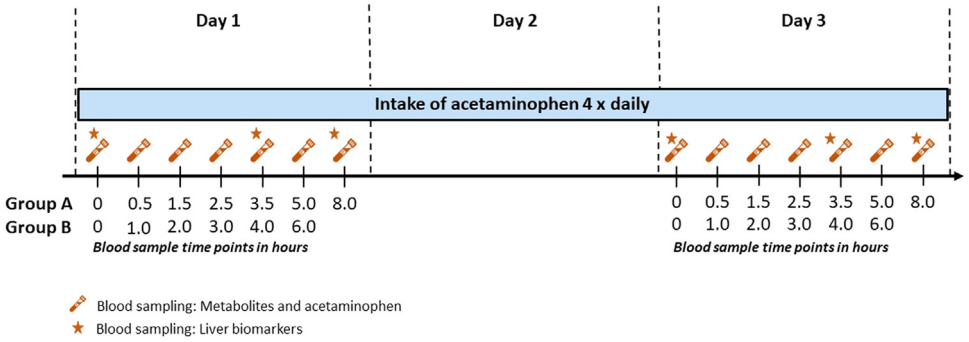


**Fig. 2.** Study flow. The patients were treated with acetaminophen from day 1 to day 3. Blood samples measuring acetaminophen, metabolites, and liver biomarkers were taken on day 1 and day 3.

aspartate aminotransferase (AST), alkaline phosphatase, INR, lactate dehydrogenase (LDH), bilirubin, miRNA 122, and miRNA 192), kidney plasma biomarkers (creatinine, potassium, sodium, and urea) and creatinine kinase (CK) were collected at three different time points during study days 1 and 2 (Fig. 2). The treatment of acetaminophen was stopped if the ALT and/or AST were elevated compared to the baseline sample during the study days judged by the investigator on a patient-by-patient basis or if any other adverse events occurred. All participants completed a journal during the study period to report and monitor compliance and side effects.

### 2.3. Data collection

Data collection included weight, sex, diagnosis, co-medications, body composition, genetic data, and plasma samples. The liver and kidney biomarkers were analyzed at the Department of Clinical Biochemistry. Body composition and the amount of muscle mass were estimated by use of dual-energy x-ray absorptiometry scans (DXA scans). Results from the DXA scans were obtained from the medical file; the scan was taken before or after the study visits as a part of the clinical follow-up. Fat-free mass in kilograms (kg) was used to calculate the fat-free mass index (FFMI), a measurement of the amount of muscle mass. DXA scans were not available for the healthy controls and two of the patients. We assumed the FFMI in the healthy controls to be within the normal range based on literature and a previous study where we DXA scanned healthy controls [31]. The FFMI in the two patients without DXA scan were assumed to be the median value of the DXA results from the other patients in the study, as they did not differ from the other patients.

### 2.4. Analytical methods for acetaminophen metabolites

Venous blood was transferred to cooled tubes with ethylenediaminetetraacetic acid (0.33 M, 10 $\mu$l ml$^{-1}$) and centrifuged at 1200 g for 10 min. Plasma was distributed to Eppendorf tubes, immediately frozen on dry ice, and stored at −80 °C until analysis. Acetaminophen and its metabolites were from Toronto Research Company and the respective internal standards for all compounds. They were analyzed on an Acquity-UPLC I-Class LC-system with a BEH C18 column coupled to a Waters Xevo TQ-XS MS/MS system (all Waters, Milford, MA, USA). Eluents were water (A) and acetonitrile (B), containing 0.1% formic acid with a gradient from 95 % to 80 % A for the first minute, followed by a gradient going to 40 % A within the next minute. The compounds were detected in electrospray positive mode, exact settings can be seen in the supplemental material A. Samples, calibrators, and controls were prepared by mixing plasma with methanol containing the internal standards (1:3, volume) in an Agilent Captiva ND Lipids 96-well filter plate, vortexed, filtered and then the addition of 1 vol waters to the filtrate. Calibrators and controls were prepared in plasma from healthy controls.

### 2.5. Pharmacokinetic model

Population pharmacokinetic parameters estimates were obtained using a nonlinear mixed effects model, implemented in NONMEM 7.4, interfaced with PsN 4.4.0 and Pirana 2.9.9. We assumed the bioavailability of oral acetaminophen to be 100 %, and the pathways illustrated in Fig. 1 account for all elimination of acetaminophen and its metabolites. The structure of the model involves single compartments of acetaminophen and its metabolites in plasma. Since acetaminophen-cysteine and acetaminophen-mercapturic acid were derived from CYP-mediated oxidation, we summed them together into one compartment called oxidative metabolites. The estimated pharmacokinetic parameters in the model were total clearance of acetaminophen, formation clearances of each metabolite, unmetabolized clearance of acetaminophen, the volume of distribution of acetaminophen and its metabolites, renal clearances of each metabolite, and half-life time of acetaminophen and each metabolite. Unmetabolized clearance of acetaminophen was the estimated acetaminophen that the three metabolic pathways could not explain. All the concentrations of acetaminophen and its metabolites were converted from mass to molecular weight to be expressed in acetaminophen equivalents. Our model selection criteria included objective function value changes, estimate results, goodness of fit, and visual predictive check. The bootstrap resampling method was applied to assess the final model's stability and quantify uncertainty in parameter estimates. Bootstrap datasets ($n = 500$) were randomly sampled in PsN with replacement from the original dataset [32]. More details about the pharmacokinetic model process can be provided upon request and will be published in a separate paper.

### 2.6. Analytical methods for miRNA 122 and miRNA 192

MiRNA was isolated from 300 µl plasma according to the manufacturer's instructions (Nuleospin miRNA Plasma, Macheray-Nagel, Düren, Germany) from each of three-time points: before acetaminophen was administered on day 1, six or eight hours after acetaminophen was administered on day 1 and 3. cDNA was made from the miRNA according to the manufacturer's instructions (TaqMan Advanced miRNA cDNA Synthesis Kit, ThermoFisher Scientific, Waltham, MA). qPCR was performed using TaqMan Advanced assay mix and TaqMan Advanced Assay miRNA probes for miR-122–5p (477,855_mir) and miR-192–5p (478,262_mir) as well as internal controls let-7a-5p (478,575_mir), miR-26a-5p (477,995_mir) and miR-221–5p (478,778_mir). The assay mix and all miRNA probes were from (ThermoFisher Scientific), and the

PCR reactions and PCR program were set up according to the manufacturer's instructions and performed on a CFX96 real-time PCR system (Bio-Rad, Hercules, CA). Relative expression was calculated by normalizing the geometric mean of the relative expression of the internal control miRNAs [33].

### 2.7. Pharmacogenetics

DNA for investigation of pharmacogenetics was available in our biobank for the SMA patients. The prevalence of pharmacogenetics variants in healthy controls was assumed to be similar to the North-western European population, which had already been studied in a large sample of 4294 individuals, and thus not investigated in the healthy controls in this study [34]. The investigated single-nucleotide polymorphisms (SNPs) were selected after a review of the literature, researching the reported SNPs with possible effects on acetaminophen metabolism [8,28,35–37]. We selected 16 different SNPs. A comprehensive list of the SNPS can be found in the supplemental material (Supplemental material B). Sequences surrounding the selected SNPs were PCR amplified using AmpliSeq single pool custom design (Thermofisher) and resulting PCR products were subsequently sequences and a GeneStudio S5 (Thermofisher) using standard protocols. Data were analyzed through IonReporter (Thermofisher). Technical details are available upon reasonable request. The pharmacogenetic profiles in the SMA patients were compared to the prevalence of homozygous carriers of the alternative alleles in the specific SNPs found in the North-western European population of 4294 individuals [34].

### 2.8. Outcome measures

The primary outcome measure was the clearance of total acetaminophen, acetaminophen-glucuronide, acetaminophen-sulfate, acetaminophen-oxidative metabolites, and unmetabolized acetaminophen in patients with SMA. The co-primary outcome was the volume of distribution of acetaminophen in patients with SMA in comparison to healthy controls.

Secondary outcome measures were liver biomarkers (ALT, LDH, bilirubin, miRNA 122, miRNA 192) and eGFR in patients with SMA during acetaminophen treatment, pharmacokinetic parameters, and pharmacogenetic screening of SNPs in acetaminophen-metabolizing enzymes in SMA patients.

### 2.9. Statistical methods

R version 4.2.0 with R studio version 2022.02.2 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform the statistical analyses. Continuous data are presented as a median with range. We compared the data by using the Wilcoxon test and the Kruskal-Wallis test. A p-value $< 0.05$ was considered statistically significant. The two-sample binomial test, Boschloo's test, was used to compare the percentages of homozygous carriers of the alternative alleles in the investigated SNPs between the patient group and the North-western European population.

### 2.10. Approval and registration

The study was approved by the ethics committee of Copenhagen, Denmark (H-18,032,928), the Danish medicine agency (EudraCT-number 2018–002,295–40), and the Data Protection Agency (VD-2019–65). All participants and their parents, if appropriate, were informed about the study both orally and in writing and provided their written consent to participate. The study was registered at the ClinicalTrials.gov ID (NCT03648658) titled "Paracetamol Study in Patients with Low Muscle Mass".

## 3. Results

### 3.1. Population characteristics

Six adults and six children with SMA II were included in the study, and the results were compared to 11 healthy adults. The demographics of the participants are shown in Table 1. We matched the adult patients with healthy controls based on age. The body weight was significantly lower in the adults with SMA compared to healthy controls. All participants completed the three study days. Two children with SMA were treated with nusinersen, a disease-modifying treatment for SMA. No medications with a known inducing or inhibitory influence of acetaminophen were co-administered. All SMA patients had low skeletal muscle mass with an FFMI below the 10th percentile. The median baseline CK levels with range of the SMA adults, SMA children and healthy controls were 25.5 U/l ([12]–139), 94.0 U/l (57–521), 122.0 U/l (49–275), respectively (normal range: 55–365 U/l in children up to 15 years and 30–200 U/l in adults).

### 3.2. Primary outcome measures: clearance of acetaminophen and its metabolites and volume of distribution

The pharmacokinetic parameters are compared in the three groups in Table 2. The total clearance of acetaminophen (actual total clearance) was significantly lower in the adult patients compared to the healthy controls. The clearance was also lower in the children but did not reach significance (Table 2). Formation of the nontoxic metabolites sulfate and glucuronide was significantly lower in the patient group compared to the healthy (see the formation rate of glucuronide and sulfate in Table 2 and Fig. 3). Furthermore, the formation of the nontoxic oxidative metabolites mercapturic acid and cysteine downstream from NAPQI were significantly lower in the patients (see formation rate of oxidative metabolites in Table 2 and Fig. 3). The volume of distribution of acetaminophen and its metabolites was significantly lower in the patients compared to healthy controls (see V in Table 2). The fraction of unmetabolized acetaminophen that could not be explained by the measured metabolites was high in all but higher in the SMA group (see metabolite fractions in Table 2). There was no difference in the acetaminophen metabolism in the two SMA patients who received nusinersen, compared to the other SMA patients (Table 1).

### 3.3. Secondary outcome measures: safety and pharmacogenetics

#### 3.3.1. Safety

One adult with SMA developed increased ALT and AST after two days of acetaminophen intake (Fig. 4). ALT increased from 7 U/L to 98 U/L (normal range: 10–70 U/L), and AST increased from 21 U/L to 147 U/L (normal range: 15–45 U/L). Furthermore, the miRNA 122 and miRNA 192 were elevated 3-fold and 25-fold, respectively, from the baseline when measured six hours after the first dose of acetaminophen on day 1 (Fig. 4). The patient was clinically unaffected and did not differ clinically from the other patients with SMA. The ALT normalized to 11 U/L when measured eight days after completion of the acetaminophen treatment. The patient did not receive any other medications or had any hepatic history explaining the sudden elevation of liver biomarkers besides the treatment of acetaminophen. The patient's oxidative clearance, glucuronidation, and sulphation clearance were like the average in the adult patient group (see Table 1). The liver biomarkers, both liver transaminases, and miRNAs, did not change markedly in the rest of the participants, and there was no difference between the patients and healthy controls (Fig. 5).

**Table 1**
Demographics and individual pharmacokinetic parameters

| | Gender | Age (years) | Weight (kg) | APAP dose (mg) | Actual total clearance (L/h) | Formation rate of glucuronide (L/h) | Formation rate of sulfate (L/h) | Formation rate of oxidative metabolites (L/h) | Elimination rate of glucuronide (L/h) | Elimination rate of sulfate (L/h) | Elimination rate of oxidative Metabolites (L/h) | Unmetabolized clearance APAP (L/h) | Volume of Distribution APAP (L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Children** | | | | | | | | | | | | | |
| 1 | F | 16 | 27 | 405 | 14.14 | 3.84 | 3.39 | 0.10 | 2.79 | 10.00 | 1.54 | 6.80 | 49.82 |
| 2¤ | M | 6 | 31 | 465 | 20.34 | 3.97 | 4.85 | 0.11 | 3.09 | 11.09 | 2.64 | 11.42 | 38.94 |
| 3 | M | 10 | 22 | 330 | 19.94 | 3.37 | 3.48 | 0.09 | 2.39 | 8.58 | 1.45 | 13.01 | 44.05 |
| 4 | F | 11 | 57 | 855 | 26.6 | 6.58 | 8.40 | 0.17 | 4.88 | 17.52 | 2.69 | 11.46 | 67.69 |
| 5 | M | 8 | 26 | 390 | 9.07 | 1.95 | 4.30 | 0.10 | 2.71 | 9.72 | 1.75 | 2.72 | 32.76 |
| 6¤ | F | 6 | 22 | 330 | 13.57 | 2.72 | 5.17 | 0.09 | 2.39 | 8.58 | 1.30 | 5.59 | 35.74 |
| **Adults** | | | | | | | | | | | | | |
| 1 | M | 31 | 39 | 585 | 20.69 | 4.00 | 5.71 | 0.13 | 3.67 | 13.18 | 4.51 | 10.85 | 54.28 |
| 2 | F | 37 | 25 | 375 | 10.53 | 2.02 | 3.11 | 0.09 | 2.63 | 9.44 | 1.45 | 5.31 | 32.09 |
| 3* | M | 28 | 35 | 525 | 15.04 | 3.96 | 4.50 | 0.12 | 3.39 | 12.15 | 2.67 | 6.45 | 55.28 |
| 4 | M | 18 | 30 | 450 | 15.90 | 3.76 | 4.90 | 0.11 | 3.02 | 10.82 | 2.14 | 7.14 | 39.62 |
| 5 | M | 21 | 50 | 750 | 13.22 | 4.31 | 6.76 | 0.16 | 4.42 | 15.88 | 2.96 | 1.99 | 51.76 |
| 6 | F | 24 | 31 | 465 | 11.97 | 3.37 | 2.51 | 0.11 | 3.09 | 11.09 | 2.37 | 5.99 | 56.47 |
| **HC** | | | | | | | | | | | | | |
| 1 | F | 23 | 78 | 1000 | 37.68 | 13.85 | 8.96 | 0.22 | 6.17 | 22.16 | 2.93 | 14.64 | 48.39 |
| 2 | M | 31 | 80 | 1000 | 21.51 | 4.17 | 8.12 | 0.23 | 6.29 | 22.59 | 3.91 | 8.99 | 82.73 |
| 3 | F | 20 | 51 | 750 | 21.10 | 4.90 | 6.66 | 0.16 | 4.49 | 16.12 | 3.56 | 9.34 | 68.44 |
| 4 | M | 25 | 83 | 1000 | 17.58 | 4.95 | 9.13 | 0.23 | 6.47 | 23.22 | 4.95 | 3.27 | 90.59 |
| 5 | M | 23 | 76 | 1000 | 30.20 | 9.01 | 11.40 | 0.22 | 6.06 | 21.74 | 2.43 | 9.58 | 30.91 |
| 6 | M | 22 | 92 | 1000 | 24.97 | 7.77 | 12.52 | 0.25 | 6.99 | 25.08 | 3.82 | 4.44 | 96.50 |
| 7 | F | 36 | 53 | 795 | 19.15 | 5.10 | 8.39 | 0.17 | 4.62 | 16.59 | 2.64 | 5.51 | 62.10 |
| 8 | M | 26 | 76 | 1000 | 24.17 | 8.23 | 11.42 | 0.22 | 6.06 | 21.74 | 4.57 | 4.30 | 81.65 |
| 9 | M | 24 | 84 | 1000 | 17.83 | 4.17 | 12.24 | 0.23 | 6.53 | 23.43 | 2.89 | 1.19 | 69.46 |
| 10 | F | 27 | 103 | 1000 | 26.90 | 11.30 | 10.78 | 0.27 | 7.61 | 27.30 | 3.86 | 4.56 | 68.97 |
| 11 | F | 26 | 72 | 1000 | 19.90 | 7.25 | 9.50 | 0.21 | 5.81 | 20.87 | 3.96 | 2.95 | 79.20 |

* Patient with increased liver biomarkers, ¤Patients who were treated with nusinersen during the project period. Abbreviations: APAP= acetaminophen, HC= healthy controls, *F*= female, *M*= male.

**Table 2**
Pharmacokinetic parameters in adults and children with SMA and healthy controls

| Pharmacokinetics | | | |
|---|---|---|---|
| | HC | SMAa | SMAc |
| **Actual total clearance (L/h)** | 21.51 | 14.13** | 17.05 |
| **Formation rate of glucuronide (L/h)** | 7.25 | 3.87*** | 3.61** |
| **Elimination rate of glucuronide (L/h)** | 6.18 | 3.24** | 2.75** |
| **Formation rate of sulfate (L/h)** | 9.50 | 4.70*** | 4.58** |
| **Elimination rate of sulfate (L/h)** | 22.16 | 11.62** | 9.86** |
| **Formation rate of oxidative metabolites (L/h)** | 0.22 | 0.12** | 0.10** |
| **Elimination rate of oxidative metabolites (L/h)** | 3.82 | 2.52 | 1.65** |
| **Unmetabolized acetaminophen clearance (L/h)** | 4.56 | 6.22 | 9.11 |
| **V acetaminophen (L)** | 69.46 | 53.02* | 41.50* |
| **Acetaminophen $t_{1/2}$ (h)** | 2.34 | 2.33 | 1.79 |
| **Glucuronide $t_{1/2}$ (h)** | 1.68 | 1.74 | 1.80 |
| **Sulfate $t_{1/2}$ (h)** | 0.47 | 0.49 | 0.50 |
| **Oxidative metabolites $t_{1/2}$ (h)** | 2.40 | 2.45 | 3.29 |
| **Metabolite fractions** | | | |
| **Unmetabolized acetaminophen** | 26.8 % | 44.1 % | 46.6 % |
| **Glucuronidation** | 30.0 % | 24.9 % | 24.9 % |
| **Sulfation** | 42.2 % | 30.2 % | 28.4 % |
| **Oxidation** | 0.96 % | 0.81 % | 0.67 % |

The values are given as medians. P-values are calculated by comparing healthy controls with the adults, and healthy controls with the children,.

* $=p<0.05$,.

** $=p<0.01$,.

*** $=p<0.001$. The formation rates and elimination rates are clearances, the clearance of acetaminophen formed to metabolites and clearance of acetaminophen metabolites excreted in the urine. The clearance is equal to the rate of acetaminophen removed from plasma (mg/min) divided by the concentration of acetaminophen in the plasma (mg/mL). The unit of the formation and elimination rates is L/h. Abbreviations: HC= healthy controls, SMAa= spinal muscular atrophy adults, SMAc= spinal muscular atrophy children, oxidative metabolites= cysteine and mercapturic acid that are downstream metabolites of NAPQI, $V$ = volume of distribution, t ½ = half-life.

*3.3.2. Pharmacogenetics*

We found that 75 % of the patients with SMA (9/12) were homozygous carriers of the alternative allele in the UGT1A-3 gene important for the glucuronide pathway, including the SNPs rs10929303 (C/C), rs1042640 (C/C) and rs8330 (C/C). These SNPs are associated with decreased glucuronidation activity [35–37]. The frequencies of homozygous carriers of the alternative alleles in the North-western European population are 62 %, 64 % and 60 %, respectively [34]. There was no significant difference in the percentages of SNPs in the SMA patients compared to the North-western European population (p-values= 0.46, 0.55, 0.37). Furthermore, 67 % of the SMA patients were homozygous carriers of the alternative allele of the gene CYP1A2, important in the oxidation pathway with SNP rs762551 (A/A), associated with increased CYP1A2 activity and thus increased activation of the CYP-mediated pathway [35,36]. The frequency of homozygous carriers in the North-western European population is 51 % [34]. The frequency in the SMA patients was not significantly different compared to the North-western European population (p-value= 0.36). The SMA patients were wildtype for SNPs in CYP2E1 gene, one of the most important enzymes in bioactivation of the CYP-mediated pathway, forming NAPQI [36].

## 4. Discussion

In this interventional study, investigating the treatment of acetaminophen in patients with SMA, we found that 1) the clearance of acetaminophen was lower in patients with SMA compared to healthy controls, 2) miRNA 122 and miRNA 192 increased on day 1 in one adult patient and 3) the same adult patient had elevated ALT and AST after only two days of acetaminophen treatment. Thus, we advise clinicians to be more aware when treating SMA patients with acetaminophen for a longer period (> 48 h).

Both formation and elimination clearance of the different metabolites in all three metabolic pathways were lower in the patient group compared to the healthy adults. With a lower total clearance, the patient's elimination of the drug from the body is prolonged. Prolonged treatment of acetaminophen may therefore lead to the accumulation of acetaminophen and more of the drug being metabolized in the oxidative pathway. Thus, monitoring of the standard liver biomarkers such as ALT, gamma glutamyl-transferase (GGT), and LDH during treatment with acetaminophen in SMA patients could be an important safety measurement, especially the first time a patient is treated. Unexpectedly, one out of 12 patients had elevated ALT and AST after only two days of acetaminophen intake. Other case reports have described liver toxicity at least 72 h after the initial dose of acetaminophen in patients with SMA and other neuromuscular disorders with low skeletal muscle mass [7–11]. Furthermore, in our study, the miRNAs 122 and 192 were markedly elevated after the first dose of acetaminophen in the adult patient that developed increased liver transaminases. This supports miRNA 122 and 192 as early predictive biomarkers for liver injury [26,27]. Thus ideally, measuring these miRNAs together with the standard liver biomarkers (ALT, AST, GGT, and LDH) could facilitate the detection of early liver toxicity. However, the miRNAs have not yet been implemented in the clinic and are not ready as screening biomarkers. Therefore, standard liver biomarkers are still crucial in assessing possible liver injury.

The oxidative pathway is the most important pathway regarding acetaminophen's toxic effects and safety. We measured mercapturic acid and cysteine; the nontoxic metabolites produced when NAPQI is conjugated with GSH. As expected, the clearance of these two conjugated metabolites was lower in the patient group. This may be explained by low skeletal muscle mass and therefore suspected lower concentrations of GSH [16]. Unfortunately, most of the measured GSH were under the lower limit of detection in our study (LOQI < 5 ng/ml). Thus, we were not able to investigate an association between plasma-GSH and pharmacokinetics in the participants. Other studies of patients with anorexia and critically ill patients in intensive care units with severe catabolism have

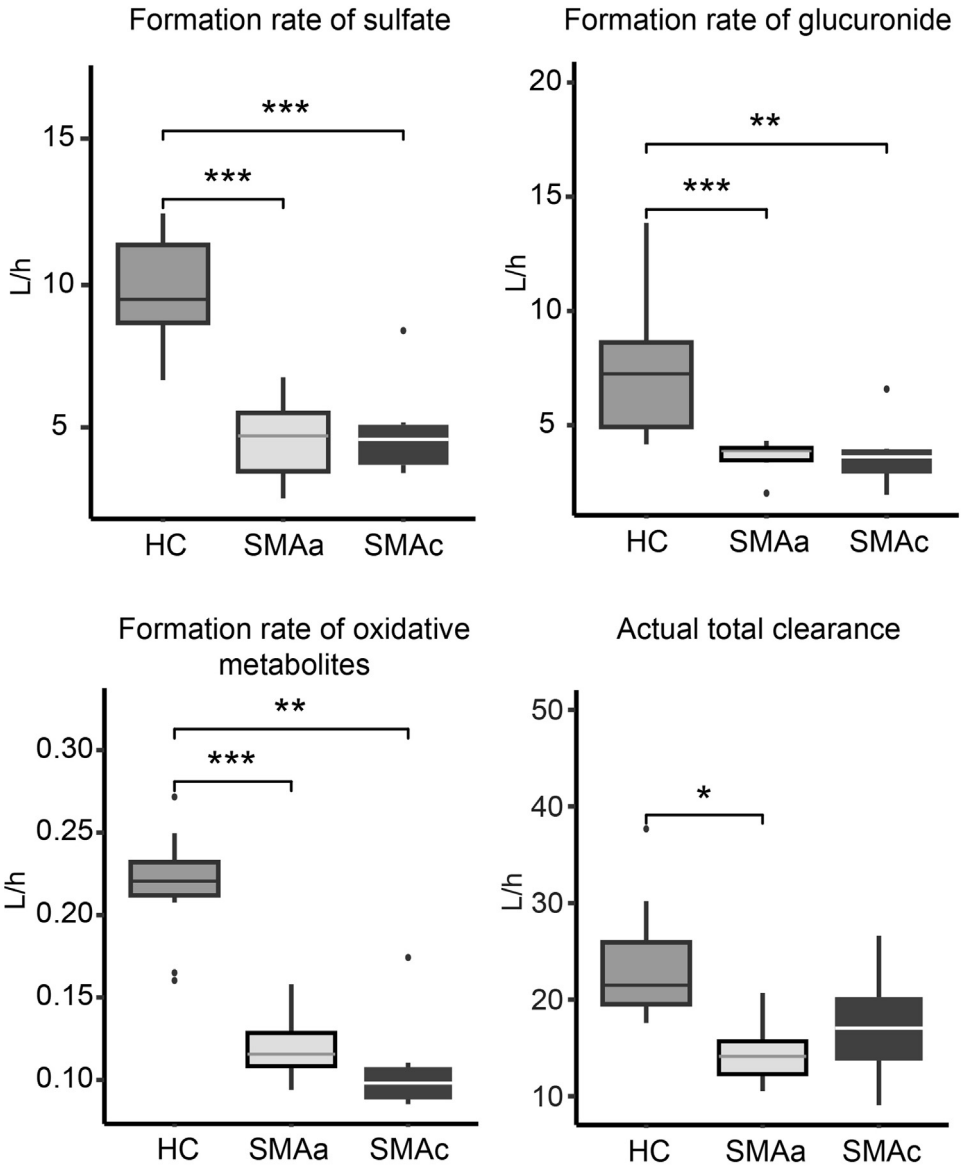


**Fig. 3.** Boxplots of formation rates of glucuronide, sulfate, oxidative metabolites, and total clearance in the SMA patients and the healthy controls. Abbreviations: HC = healthy controls, SMAa = spinal muscular atrophy adults, SMAc= spinal muscular atrophy children. $^{*}p < 0.05$ versus healthy controls, $^{**}p < 0.01$ versus healthy controls, $^{***}p < 0.001$ versus healthy controls.

shown a lower concentration of GSH compared to healthy [18,38]. We suspect that SMA patients resemble these groups. Furthermore, the patients in our study had a high percentage of unmetabolized acetaminophen. This may be due to the suggested slow turnover in the CYP pathway, with less ability to detoxify acetaminophen. This is supported by the finding of a lower formation rate of the nontoxic oxidative metabolites of NAPQI in the patient group compared to the healthy controls. Unfortunately, we could not measure NAPQI as it is extremely reactive [39]. In addition, the adult patients had a lower clearance of acetaminophen compared to the pediatric patients. This may be explained by the adult patients having less skeletal muscle mass than the pediatric patients. Although adult patients were taller than the pediatric patients, they had comparable weights. Furthermore, as the disease progresses, it is expected that adults will have less muscle mass than pediatric patients.

The patients were homozygous for common polymorphisms previously implicated in acetaminophen metabolism, associated with decreased glucuronidation and increased CYP-activity. These findings may contribute further to an increased risk of liver toxicity in SMA patients. The patient in our study that developed increased liver biomarkers during treatment was one of the nine patients homozygous for the SNPs. If the patients have a reduced/slower metabolism of the main pathway, the glucuronide pathway, there will be more acetaminophen available for CYP-mediated metabolism, increasing the risk of toxicity. The percentages of the SNPs in the SMA patients were not significantly higher than in the North-western European population. We therefore do not think that testing pharmacogenetic variants in SMA patients will add significant value in future clinical practice. Pharmacogenetic testing will require both economic resources and time. Thus, the pharmacogenetics are probably adding to the risk of increased susceptibility to acetaminophen toxicity but cannot be interpreted alone. There should be a greater awareness of the unfortunate combination of low skeletal muscle mass, pharmacogenetic variants and treatment of acetaminophen in SMA patients.

As mentioned in the introduction, an adult SMA patient with a weight of 30 kg died of acute liver failure on the suspicion of acetaminophen toxicity. The patient received acetaminophen postoperatively for pain management, 417 mg x 4 daily for three

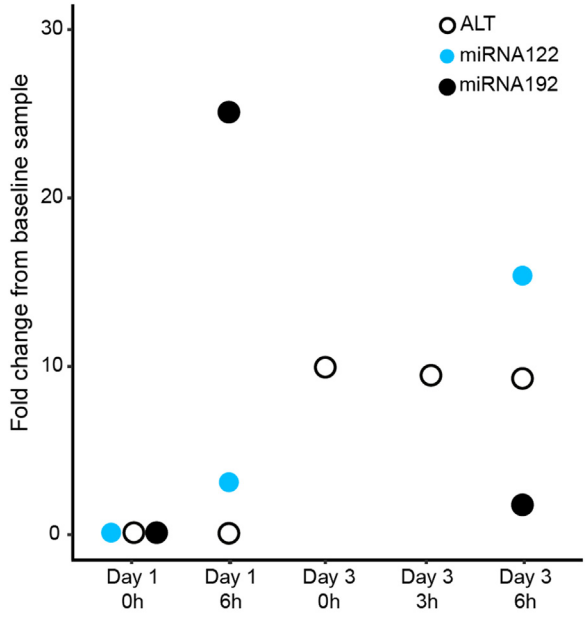


**Fig. 4.** Fold change of ALT, miRNA 122, and miRNA 192 from baseline sample in one adult SMA patient. Abbreviations: ALT= alanine aminotransferase, miRNA 122 and 192 = microRNA 122 and 192.

consecutive days. The ALT level before acetaminophen treatment was 12 U/L and increased to 3980 U/L after three days of analgesic treatment. AST and LDH increased from 26 U/L to 6660 U/L and 154 U/L to 14300 U/L (normal range of LDH: 105–205 U/l). Serum acetaminophen was measured at 0.04 mmol/l (> 1 mmol/l is toxic) on the third day of acetaminophen treatment. A combination of acetaminophen treatment together with postoperative hypotension with hypoperfusion probably explains the development of acute liver failure in this patient. No postmortem autopsy was made. Furthermore, a 14-year-old boy with DMD weighing 50 kg received acetaminophen 1000 mg x 4 daily during hospitalization due to respiratory failure in our clinic. He developed acute liver toxicity after four days of acetaminophen treatment. Unfortunately, he was given the adult dose instead of the pediatric dose of 15 mg/kg/dose, which corresponds to 750 mg per dose. The ALT and LDH levels were 2730 U/L and 2170 U/L on day four. Serum acetaminophen at 0.28 mmol/l was well below the upper reference value. However, the pediatric hepatologists suspected acute acetaminophen toxicity. We measured the concentration of acetaminophen and metabolites in the DMD boy, where we found an acetaminophen concentration of 2975 ng/ml in the DMD boy compared to 909 ng/ml in the healthy controls in this study. The liver transaminases decreased after withdrawal of acetaminophen. This DMD case highlights that susceptibility to acetaminophen toxicity may not be limited to SMA patients, but also to those with other neuromuscular disorders and low skeletal muscle mass, as seen in other case reports [8–11]. The question is whether the toxic limit of serum acetaminophen of 1.0 mmol/L is lower in patients with low skeletal muscle mass.

An additional risk factor to acetaminophen susceptibility may be the presence of liver impairment prior to treatment of acetaminophen. We conducted a metabolic study where we found that 4/8 (50 %) of the children with SMA II in the study had liver fibrosis and/or steatosis [40]. Other studies have also found

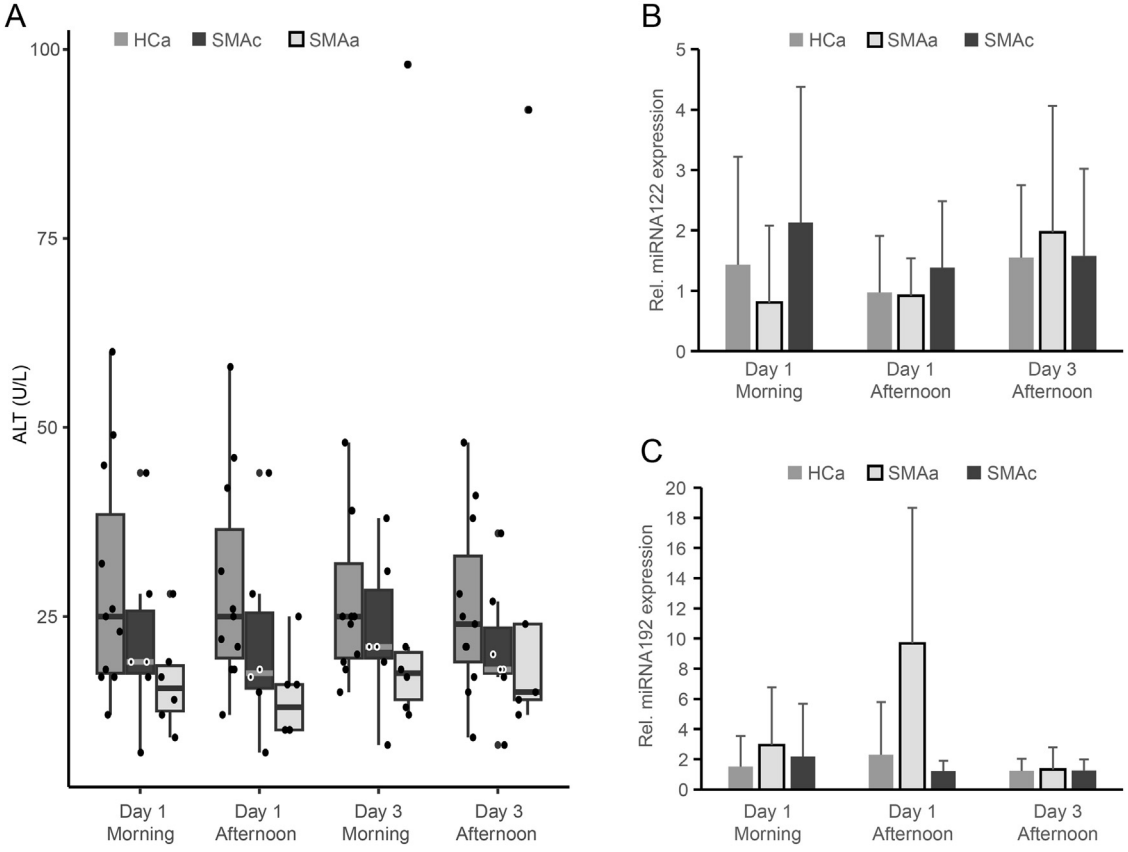


**Fig. 5.** (A) Boxplot of measured plasma-ALT during the study days in the three groups, (B) barplot of measured plasma miRNA 122 during the study days in the three groups, and (C) barplot of measured plasma miRNA 192 during the study days in the three groups. Boxplots and bar plots of plasma-ALT and plasma miRNA 122 and 192 measured on study days 1 and 2 show no difference between the three groups. Abbreviations: ALT= alanine aminotransferase, HCa= healthy controls, Rel= relative, SMAa= spinal muscular atrophy adults, SMAc= spinal muscular atrophy children.

a proportion of SMA patients (in particular those with the severe phenotypes) to have non-alcoholic fatty liver disease (NAFLD) and altered fatty acid metabolism [23,41]. Some studies with a small number of included children and adults with NAFLD and non-alcoholic steatohepatitis (NASH) have found altered metabolism of acetaminophen ([42]–45). However, related to this, there are no published studies about acetaminophen metabolism deficiency in fatty acid oxidation disorders. Hence, more research is warranted to investigate how these defects impact acetaminophen metabolism.

We treated the participants in our study with the recommended doses of acetaminophen in our country and recommended by the Food and Drug Administration, which is 15 mg/kg/dose and a maximum of 4000 mg/day. It is important to note that some other countries suggest lower doses. For instance, China recommends a maximum of 600 mg/dose and no more than 2000 mg/day. However, most SMA patients in our study received doses below 600 mg/dose, while the patient with elevated ALT and AST received only 525 mg/dose and 2100 mg/day. We are confident that the participants in our study did receive a dosage that was within the acceptable range.

We did not define a specific change in ALT and AST as a criterion for withdrawal of the acetaminophen treatment during the study days. The evaluation was subjective, as it is in a clinical setting, which could be problematic. In future studies, we recommend using a defined ALT change to determine whether acetaminophen treatment can continue. However, except for one subject, all participants had ALT and AST levels within the normal range during the study days. Therefore, it is unlikely that there was any damage to the liver parenchyma in the subjects with normal ALT and AST. Furthermore, the patient with elevated ALT and AST had received the last dose of acetaminophen before the increase was detected. Thus, all participants completed the trial and the treatment regimen. The participants felt fine without any clinical symptoms of acetaminophen toxicity.

We did not measure urine metabolites and had a small sample size which are limitations of this study. The fraction of unmetabolized acetaminophen was much higher than in other studies in all participants, including healthy controls [13,41,42]. This is probably due to the pharmacokinetic model being developed with measured plasma metabolites and not urine metabolites. Ideally, we should have had both plasma and urine samples to build the pharmacokinetic model. Since SMA is a rare disease, the number of eligible participants was limited. Future studies investigating other patient groups with low skeletal muscle mass treated with acetaminophen are needed.

In conclusion, patients with SMA have several risk factors that may increase their susceptibility to acetaminophen-induced hepatotoxicity, such as lower clearance of acetaminophen, low skeletal muscle mass, and common pharmacogenetic polymorphisms. We recommend monitoring standard liver biomarkers of ALT, LDH, and GGT in SMA patients 48 h after first-time treatment of acetaminophen or if the patient is metabolically deranged, malnourished, or critically ill. If the liver biomarkers increase during treatment, a lower dose or longer dosing intervals should be considered in SMA patients. Lastly, we recommend that adult patients with SMA are treated with a pediatric dose of acetaminophen calculated after weight (15 mg/kg/dose), with a maximum of 4000 mg/day.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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