Received: 17 April 2019 | Accepted: 25 July 2019

DOI: 10.1111/bcpt.13304

ORIGINAL ARTICLE

BCPT Basic & Clinical Pharmacology & Toxicology

Acetaminophen toxicity induces mitochondrial complex I inhibition in human liver tissue

Karoline Maise Chrøis[1] | **Steen Larsen**[1,2] | **Julie Steen Pedersen**[3] | **Marte Opseth Rygg**[3] | **Astrid Elisabeth Bruun Boilsen**[4] | **Flemming Bendtsen**[3] | **Flemming Dela**[1,5]

[1]Xlab, Centre for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

[2]Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland

[3]Gastrounit, Medical Division, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark

[4]Gastrounit, Surgical Division, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark

[5]Department of Geriatrics, Bispebjerg University Hospital, Copenhagen, Denmark

Correspondence
Flemming Dela, MD, Xlab, Center for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, Denmark.
Email: fdela@sund.ku.dk

Abstract

Acetaminophen (APAP) is used worldwide and is regarded as safe in therapeutic concentrations but can cause acute liver failure in higher doses. High doses of APAP have been shown to inhibit complex I and II mitochondrial respiratory capacity in mouse hepatocytes, but human studies are lacking. Here, we studied mitochondrial respiratory capacity in human hepatic tissue ex vivo with increasing doses of APAP. Hepatic biopsies were obtained from 12 obese patients who underwent a Roux-en-Y gastric bypass (RYGB) or a sleeve gastrectomy surgery. Mitochondrial respiration was measured by high-resolution respirometry. Therapeutic concentrations (≤0.13 mmol/L) of APAP did not inhibit state 3 complex I-linked respiration. APAP concentrations of ≥2.0 mmol/L in the medium significantly reduced hepatic mitochondrial respiration in a dose-dependent manner. Complex II-linked mitochondrial respiration was not inhibited by APAP. We conclude that the mitochondrial respiratory capacity is affected by a hepato-toxic effect of APAP, which involved complex I, but not complex II.

KEYWORDS

Acetaminophen (APAP), drug-induced liver injury, hepatotoxicity, mitochondrial respiratory capacity, overdose

1 | INTRODUCTION AND BACKGROUND

Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) is widely used due to its analgesic and antipyretic effects.[1] At therapeutic doses, the drug is harmless, and after absorption, it is mainly metabolized in the liver and the metabolites subsequently excreted in the urine, but it can cause severe hepatic toxicity at higher doses leading to acute liver failure.[2] Davidson & Eastham were the first to report that APAP caused hepatotoxicity in high doses. They analysed liver tissue from two patients with an APAP overdose at autopsy with findings of fulminant hepatic necrosis.[3] It has been hypothesized that mitochondria play an important role in APAP-induced liver injury.[4,5] The majority of studies have been performed in rodent models, and studies on human hepatic tissues include only one study [6] in which mitochondrial function in human hepatocytes was evaluated following APAP exposure. The measures included estimation of the mitochondrial membrane potential, but no respirometric measurement was reported.[6] Burcham & Harman [7] exposed isolated permeabilized mouse hepatocytes to a high concentration (5 mM) of APAP which resulted in an inhibition of the mitochondrial respiratory capacity through complex I and II, accompanied by a decrease in cytosolic ATP levels and the ATP/ADP ratio in the cytosolic and mitochondrial compartment measured at 30°C using a Clark-type oxygen probe.

APAP is absorbed from the gastrointestinal tract and is metabolized in the liver and kidney by cytochrome P-450 (CYP) enzymes to form N-acetyl-p-benzoquinone imine

(NAPQI), which depletes glutathione (GSH) levels, induces protein adduct formation and elicits oxidative stress in the mitochondria and hepatotoxicity at high doses.[1,8] These findings were confirmed by McGill et al[9] in a human model cell line, HepaRG. N-acetylcysteine (NAC) can be used as treatment for APAP intoxication and prevents further hepatic injury by increasing the concentration of reduced GSH. However, the efficacy of NAC is highly dependent on the time of intake of APAP to administration of NAC, and unintentional APAP overdosing is typically discovered after NAPQI formation with presence of liver damage.[10] Saito et al found in mice that GSH was more effective than NAC in preventing liver injury caused by APAP overdose.[11] They observed that reduced liver injury correlated with recovery of ATP, mitochondrial GSH concentrations and increased substrate supply through TCA cycle. Elucidation of the exact mechanisms of APAP-induced hepatotoxicity in humans can contribute to improve the development of treatment strategies for hepatotoxicity and decrease morbidity and mortality due to APAP overdosing. To this end, in the present study, we investigated the effect of increasing doses of APAP on the mitochondrial respiratory capacity in human hepatic tissue.

2 | MATERIALS AND METHODS

2.1 | Participants

Hepatic tissue samples were obtained per-operatively from 12 obese patients (two men and 10 women) who underwent Roux-en-Y gastric bypass (RYGB) or a sleeve gastrectomy surgery. One patient was in daily treatment with APAP. The average age and body mass index (BMI) was 44 ± 3 years and 40 ± 2 kg/m^2. The concomitant medications for all participants are listed in Table 1. All patients were Caucasian of race and fulfilled the criteria for bariatric surgery according to the national guidelines.

The study was performed in accordance with the Helsinki declaration and approved by the Ethical Committee of Copenhagen (ref. no. H-16030784). All participants were informed about the experimental procedures, and potential risks before written consent were obtained from all participants. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[12]

2.2 | Experimental protocol

Height, weight, body mass index (BMI), waist-hip ratio, blood pressure, concomitant medication and smoking status were recorded and measured before the patients underwent surgery. A hepatic biopsy was sampled during the laparoscopic bariatric procedure by the surgeon. The biopsy was obtained from the right liver lobe (margo inferior) immediately after trocar placement but before the actual bariatric procedure. Immediately after extraction, the biopsy was placed in ice-cold relaxing buffer BIOPS (CaK_2EGTA 2.77 mmol/L, K_2EGTA 7.23 mmol/L, Na_2ATP 5.77 mmol/L, $MgCl_2.6H_2O$ 6.56 mmol/L, taurine 20 mmol/L, Na_2phosphocreatine

TABLE 1 Participant characteristics and number of patients taking medications (n = 12). Data are mean ± SEM

Sex (m/f) (n)	2/10
Age (years)	44 ± 3
Weight (kg)	117 ± 7
BMI (kg/m^2)	40 ± 1
Type 2 diabetes (n)	3
Hypertension (n)	3
Dyslipidaemia (n)	3
Haemoglobin (mmol/mol)	8.5 ± 0.2
Fasting glucose (mmol/L)	6.2 ± 0.3
Fasting insulin (pmol/L)	154 ± 25
HbA1c (mmol/mol)	37 ± 2
INR[a]	1.0 ± 0.02
ALAT[a] (U/L)	30.6 ± 4.3
ASAT (U/L)	23.5 ± 2.0
GGT (U/L)	31.1 ± 5.1
Albumin (g/L)	37.6 ± 1.2
Bilirubin (μmol/L)	7.3 ± 0.5
NAFLD Activity Score[b] (n)	
0-2	0
3-4	7
5	5
Fibrosis Score (n)	
0—None	1
1—Perisinusoidal or periportal	4
2—Perisinusoidal and periportal	6
3—Bridging fibrosis	1
4—Cirrhosis	0
Medication	
Paracetamol (daily) (n)	1
Glucose lowering (n)	3
ACE inhibitor (n)	3
Statin (n)	3
Aspirin (n)	2

Abbreviation: ALAT, alanine transaminase; ASAT, aspartate transaminase; BMI, body mass index; GGT, gamma-glutamyltransferase; HbA1c, glycated haemoglobin A1c; INR, international normalized ratio; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

[a]Missing data for 1 patient.

[b]NAFLD Activity Score: 0-2: non-NASH/simple steatosis; 3-4: borderline NASH; ≥5: definite NASH.

15 mmol/L, imidazole 20 mmol/L, dithiothreitol 0.5 mmol/L and 4-morpholineethanesulfonic acid 50 mmol/L at pH 7.1). Fasting blood samples were collected from the cubital vein in the morning at the day of surgery.

2.3 | Mitochondrial respiration protocol

After the biopsy was dissected free of blood and connective tissue, it was washed in ice-cold respiration media MiR05 (EGTA 0.5 mmol/L, $MgCl_2.6H_2O$ 3.0 mmol/L, K-lactobionate 60 mmol/L, taurine 20 mmol/L, KH_2PO_4 10 mmol/L, HEPES 20 mmol/L, sucrose 110 mmol/L and BSA 1 g/L at pH 7.1) for 2×10 minutes. Finally, the tissue was weighed and transferred to the respiration chamber where mitochondrial oxidative phosphorylation capacity was measured by high-resolution respirometry (HRR) (Oxygraph-2 k; Oroboros, Innsbruck, Austria). The evaluation of APAP toxicity on mitochondrial respiration capacity was done in duplicate in 37°C with hyperoxygenation to prevent potential oxygen limitations to respiration, as previously described.[13] For assessment of possible complex I inhibition by APAP, the following protocol was used: malate (2 mmol/L) and glutamate (10 mmol/L) to determine leak respiration (state 2), adenosine diphosphate (ADP, 1 mmol/L) and magnesium chloride (MgCl, 1 mmol/L) to measure maximal complex I respiration (state 3, complex I). Subsequently, APAP tablets were pulverized and dissolved in H_2O, and titrated in the respiration chamber (0.025, 0.10, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00 and 10.00 mmol/L), and rotenone added thereafter (0.5 µmol/L) to inhibit complex I-linked respiration. Then, succinate (10 mmol/L) was added to assess complex II-linked respiration (GMS, complex II). Cytochrome *C* (10 µmol/L) was added to test for integrity of the outer mitochondrial membrane. The protocol was ended by addition of antimycin A (5 µmol/L) to measure non-mitochondrial respiration. The respiratory protocol was performed in MiR05. Data were recorded using DatLab software version 7 (Oroboros, Innsbruck, Austria).

2.4 | Statistics

To evaluate mitochondrial complex I-linked respiration with increasing doses of APAP, a one-way ANOVA with repeated measures was performed followed by the Student-Newman-Keuls multiple comparison procedure. If the equal variance test failed, data were log-transformed and re-analysed. To evaluate the effect of APAP on mitochondrial complex II-linked respiration, a two-tailed Student *t* test was performed.

All statistical analyses were performed using software SigmaPlot 13.0 (Systat Software, San Jose, CA, USA). Data are expressed as mean ± SEM *P* value of 0.05 or less was considered statistically significant.

3 | RESULTS

Participant characteristics are presented in Table 1. Study participants were morbidly obese with a mean BMI of 40 kg/m^2. A subset of the study participants held an obesity-related comorbidity diagnosis at the time of enrolment (3 with type 2 diabetes, 3 with hypertension, 3 with dyslipidaemia). Mean fasting glucose was marginally elevated to 6.2 mmol/L, but mean Hba1c was normal (37 mmol/mol). Even though five patients were diagnosed with definite NASH based on liver histopathology and the majority of study participants had mild (grade 1, n = 4) or moderate (grade 2, n = 6) liver fibrosis, all liver function tests (ALT, AST, GGT, bilirubin INR, and albumin) were within normal range.

Therapeutic concentrations of APAP (≤0.13 mmol/L, corresponding to maximal plasma concentrations after 1000 mg oral APAP) did not inhibit state 3 complex I respiration (Figure 1). Mitochondrial respiration was reduced dose-dependently with increasing concentrations of APAP above 2.0 mmol/L (Figure 1) compared with the initial state 3 complex I-linked respiration. The highest concentration of APAP decreased state 3 complex I-linked respiration by 45%. Complex II respiration (GMS) was not affected by APAP (Figure 2). The respiratory data from the single patient who was treated regularly with APAP were not different from the data of the other patients (data not shown).

4 | DISCUSSION

The present study demonstrates that high non-therapeutic doses of APAP induce inhibition of mitochondrial respiration

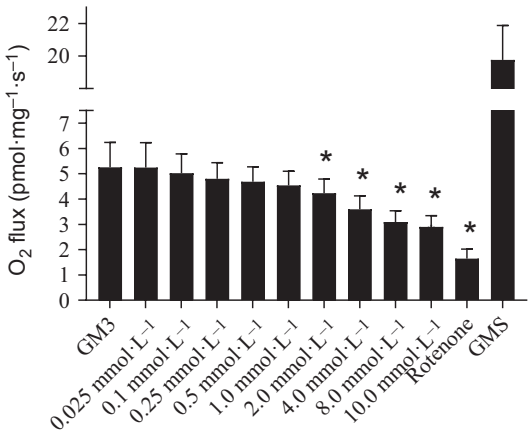

FIGURE 1 Mitochondria state 3 respiration with increasing doses of APAP (0.025, 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 10.0 mmol/L). GM3: state 3 respiration with complex I-linked substrates, glutamate and malate; rotenone: inhibition of complex I respiration; GMS: state 3 respiration with complex II-linked (succinate) substrate. N = 12. Data are mean ± SEM. * different from complex I respiration without APAP ($P \leq .05$)

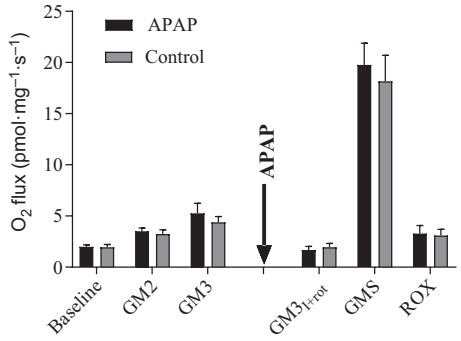

FIGURE 2 Mitochondrial respiration capacity with (black bars) and without (grey bars) APAP. Baseline: buffer and tissue with no substrates for respiration. GM2: sate 2_{LEAK} respiration, complex I.; GM3: state 3 respiration with complex I-linked substrates, glutamate and malate; rotenone inhibition of complex I respiration; GMS: state 3 respiration with complex II-linked (succinate) substrate. ROX: residual respiration after further inhibition of complex III with antimycin A. n = 12. APAP in increasing doses were added to respiration chamber after GM3. Detailed respiratory data are shown in Figure 1. Data are mean ± SEM

through complex I in human liver tissue. Addition of APAP decreased state 3 respiration sustained by complex I-linked substrates, but complex II-linked respiration (GMS) was not affected by APAP. It is not clear precisely where in complex I APAP exerts its inhibitory effect, but it seems likely that APAP induces hepatic toxicity at complex I. The development of APAP-induced mitochondrial dysfunction has previously been reported by others via biomarkers of mitochondrial damage in human plasma.[14] In human primary hepatocytes from either donor livers or liver resections, Xie *et al*[6] concluded that APAP exposure to the cells resulted in a loss of mitochondrial membrane potential (by fluorescence measures of tetraethylbenzimidazolylcarbocyanine iodide (JC-1)), but no respirometric analyses were reported.

Of relevance to the present study, Burcham and Harman[7] reported that APAP-induced mitochondrial toxicity appears through complex I and II in isolated rat hepatocytes, a finding which is partly in contrast to the present data. McGill et al[14] found a correlation between serum biomarkers for mitochondrial damage (glutamate dehydrogenase and mitochondrial DNA) with severity of liver damage in patients with APAP overdose. Increasing levels of biomarkers for mitochondrial damage and nuclear DNA fragmentation have shown to be critical events in APAP hepatotoxicity in humans.[14] However, serum biomarkers for mitochondrial damage only provide a limited insight in the mechanisms of APAP toxicity.

A study showed that metformin (a drug used in the treatment of type 2 diabetes) seems to protect against APAP toxicity.[15] Mitochondrial respiration was measured in HepaRG cells by the Seahorse technique, but this protocol does not allow for determination of which specific complex is affected. In liver tissue from metformin-treated mice, the authors then measured (by spectrophotometric analysis) a metformin-induced reduction in complex I activity, but also that APAP in fact increased complex I activity, and with the combination of the two (Metformin + APAP) in between. From this, it is difficult to conclude that complex II is affected by APAP and the finding that APAP increased complex I activity may just underline potential species differences. In addition, only high doses of metformin elicit an inhibitory effect on respiration (affecting both complex I and II) and in patients treated with metformin, complex I respiration in skeletal muscle is not decreased.[16]

In support of the present findings of an effect on complex I-sustained respiration is a recent study in mouse liver tissue, in which rotenone (a specific inhibitor of complex I) treatment for three days resulted in a diminished APAP-induced liver injury, as estimated histologically, via enzyme release and measurements of GSH.[17] Further arguments for specific role of complex I are that this is the complex which is considered to be the major ROS-generating site, and ROS has been shown in many studies to be a major player in the APAP-induced hepatotoxicity.

An APAP dose of 1000 mg (orally taken) elicits a maximal APAP concentration of ≤0.13 mmol/L. The present study shows no inhibitory effect of complex I-linked respiration at therapeutic doses of APAP and concentrations below 2.0 mmol/L. Serum concentrations at 1-4 mmol/L have been measured in participants with APAP overdose.[1,18] The concentration of APAP in the mitochondria is unknown, but given the metabolism of APAP in the liver, the peak APAP concentration in liver mitochondria is expected to be higher than the plasma level, APAP concentrations above 1 mmol/L in the mitochondria might for that reason be considered an overdose. The present study observed a decreased mitochondrial state 3 complex I-linked respiration at APAP concentrations at 2.0 mmol/L and above which may be considered an overdose. Whether these findings in mitochondrial respiration are due to a time-dependent decrease is unlikely. The present study was undertaken to examine the effect of APAP inhibition on mitochondrial respiratory capacity. Given APAP is the leading cause for acute liver failure in the US and many other countries[19] and the treatment of NAC is time-dependent, it is important to clarify the exact mechanisms of APAP and to resolve other possible treatment strategies for hepatotoxicity due to APAP overdose. In ischaemia-reperfusion injury, another clinical syndrome that includes mitochondrial dysfunction, it has been shown that pre-ischaemic administration of aminooxyacetate, an inhibitor of the malate-aspartate shuttle, provides some cardioprotection.[20]

Liver tissue for mitochondrial analyses was sampled from obese patients with various degrees of histologically proven non-alcoholic fatty liver disease (NAFLD), some with non-alcoholic steatohepatitis (NASH) and some with fibrosis, but not with cirrhosis.

In patients with liver cirrhosis, half-life of orally administered APAP is doubled, but risk of hepatic injury is generally low if APAP dose is less than 4 g/d.[21-23] Impaired hepatic mitochondrial function is considered to be a co-factor in the development of NASH,[24] and it can be speculated that the histological presence of NAFLD/NASH in our study participants could have increased liver sensitivity to APAP and hence influenced our results. Data from experimental rodent studies have previously shown that NAFLD is associated with an increased risk of liver injury after a single APAP overdose.[25-27] Human data on APAP and NAFLD are sparse and conflicting. Radosevich et al found no increased risk of hepatotoxicity in obese compared with non-obese,[28] while in the study by Nguyen et al,[29] presence of NAFLD was reported to increase susceptibility to acute liver failure following APAP overdose. However, we have previously shown that normal hepatic mitochondrial oxidative phosphorylation capacity was maintained in obese, bariatric patients.[30]

None of our study participants were cirrhotics, and all had normal liver function tests (Table 1). In that light and since therapeutical doses of APAP was not associated with impaired mitochondrial capacity, we believe that the presence of NAFLD only minorily would influence the results. However, further research is needed to elucidate the influence of NAFLD severity on mitochondrial function in the setting of APAP administration and metabolism.

In summary, mitochondrial complex I respiration capacity in human hepatic tissue is inhibited with high non-therapeutic concentrations of APAP but no effect is seen in complex II-linked respiratory capacity. We speculate that the observed decreased mitochondrial complex I respiration may be the primary reason for hepatic toxicity with APAP overdose, but future research is required to determine this.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCE

1. Yoon E, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-induced hepatotoxicity: a comprehensive update. *J Clin Transl Hepatol.* 2016;4(2):131-142.
2. Shah AD, Wood DM, Dargan PI. Understanding lactic acidosis in paracetamol (acetaminophen) poisoning. *Br J Clin Pharmacol.* 2011;71(1):20-28.
3. Davidson DG, Eastham WN. Acute liver necrosis following overdose of paracetamol. *Br Med J.* 1966;2(5512):497-499.
4. Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. *Handb Exp Pharmacol.* 2010;196:369-405.
5. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *J Clin Invest.* 2012;122(4):1574-1583.
6. Xie Y, McGill MR, Dorko K, et al. Mechanisms of acetaminophen-induced cell death in primary human hepatocytes. *Toxicol Appl Pharmacol.* 2014;279(3):266-274.
7. Burcham PC, Harman AW. Acetaminophen toxicity results in site-specific mitochondrial damage in isolated mouse hepatocytes. *J Biol Chem.* 1991;266(8):5049-5054.
8. Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. *Proc Natl Acad Sci U S A.* 1984;81(5):1327-1331.
9. McGill MR, Yan HM, Ramachandran A, Murray GJ, Rollins DE, Jaeschke H. HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. *Hepatology.* 2011;53(3):974-982.
10. Mutsaers A, Green JP, Sivilotti MLA, et al. Changing nomogram risk zone classification with serial testing after acute acetaminophen overdose: a retrospective database analysis. *Clin Toxicol (Phila).* 2019;57:380-386.
11. Saito C, Zwingmann C, Jaeschke H. Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. *Hepatology.* 2010;51(1):246-254.
12. Tveden-Nyborg P, Bergmann TK, Lykkesfeldt J. Basic & clinical pharmacology & toxicology policy for experimental and clinical studies. *Basic Clin Pharmacol Toxicol.* 2018;123(3):233-235.
13. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods Mol Biol.* 2012;810:25-58.
14. McGill MR, Jaeschke H. Mechanistic biomarkers in acetaminophen-induced hepatotoxicity and acute liver failure: from preclinical models to patients. *Expert Opin Drug Metab Toxicol.* 2014;10(7):1005-1017.
15. Du K, Ramachandran A, Weemhoff JL, et al. Metformin protects against acetaminophen hepatotoxicity by attenuation of mitochondrial oxidant stress and dysfunction. *Toxicol Sci.* 2016;154(2):214-226.
16. Larsen S, Rabol R, Hansen CN, Madsbad S, Helge JW, Dela F. Metformin-treated patients with type 2 diabetes have normal mitochondrial complex I respiration. *Diabetologia.* 2012;55(2):443-449.
17. Ge X, Hua H, Wang P, et al. Inhibition of mitochondrial complex I by rotenone protects against acetaminophen-induced liver injury. *Am J Transl Res.* 2019;11(1):188-198.
18. Wang GS, Monte A, Bagdure D, Heard K. Hepatic failure despite early acetylcysteine following large acetaminophen-diphenhydramine overdose. *Pediatrics.* 2011;127(4):e1077-e1080.
19. Larson AM, Polson J, Fontana RJ, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology.* 2005;42(6):1364-1372.
20. Jespersen NR, Yokota T, Støttrup NB, et al. Pre-ischaemic mitochondrial substrate constraint by inhibition of malate-aspartate shuttle preserves mitochondrial function after ischaemia-reperfusion. *J Physiol.* 2017;595(12):3765-3780.
21. Benson GD, Koff RS, Tolman KG. The therapeutic use of acetaminophen in patients with liver disease. *Am J Ther.* 2005;12(2):133-141.
22. Villeneuve JP, Raymond G, Bruneau J, Colpron L, Pomier-Layrargues G. Pharmacokinetics and metabolism of acetaminophen in normal, alcoholic and cirrhotic subjects. *Gastroenterol Clin Biol.* 1983;7(11):898-902.
23. Chandok N, Watt KD. Pain management in the cirrhotic patient: the clinical challenge. *Mayo Clin Proc.* 2010;85(5):451-458.

24. Mansouri A, Gattolliat CH, Asselah T. Mitochondrial dysfunction and signaling in chronic liver diseases. *Gastroenterology*. 2018;155(3):629-647.
25. Kučera O, Roušar T, Staňková P, et al. Susceptibility of rat non-alcoholic fatty liver to the acute toxic effect of acetaminophen. *J Gastroenterol Hepatol*. 2012;27(2):323-330.
26. Kon K, Ikejima K, Okumura K, Arai K, Aoyama T, Watanabe S. Diabetic KK-A(y) mice are highly susceptible to oxidative hepatocellular damage induced by acetaminophen. *Am J Physiol Gastrointest Liver Physiol*. 2010;299(2):G329-G337.
27. Aubert J, Begriche K, Delannoy M, et al. Differences in early acetaminophen hepatotoxicity between obese ob/ob and db/db mice. *J Pharmacol Exp Ther*. 2012;342(3):676-687.
28. Radosevich JJ, Patanwala AE, Erstad BL. Hepatotoxicity in obese versus nonobese patients with acetaminophen poisoning who are treated with intravenous N-acetylcysteine. *Am J Ther*. 2016;23(3):e714-e719.
29. Nguyen GC, Sam J, Thuluvath PJ. Hepatitis C is a predictor of acute liver injury among hospitalizations for acetaminophen overdose in the United States: a nationwide analysis. *Hepatology*. 2008;48(4):1336-1341.
30. Lund MT, Kristensen M, Hansen M, et al. Hepatic mitochondrial oxidative phosphorylation is normal in obese patients with and without type 2 diabetes. *J Physiol*. 2016;594(15):4351-4358.

How to cite this article: Chrøis KM, Larsen S, Pedersen JS, et al. Acetaminophen toxicity induces mitochondrial complex I inhibition in human liver tissue. *Basic Clin Pharmacol Toxicol*. 2020;126:86–91. https://doi.org/10.1111/bcpt.13304