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Masterthesis / Original study

Acetaminophen protein adduct concentrations during therapeutic dosing in patients with class II-III obesity compared to non-obese and overweight patients: a prospective observational gender stratified cohort study

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Abstract

Background: **Obese patients may need higher doses of acetaminophen (APAP) for adequate analgesia, due to increased total clearance and distribution volume. APAP-induced hepatotoxicity is mainly caused through CYP2E1 pathway. Its activity is induced by obesity, potentially endangering the safety profile of APAP. Metabolic-dysfunction associated liver disease (MASLD) is an important associated risk factor for APAP induced-hepatotoxicity.**
Objectives: **This pilot study aimed at observing and analyzing CYP2E1 related protein adducts (APAP-cysteine and APAP-mercapturate) in obese compared to non-obese patients during therapeutic dosing of APAP.**
Study design and setting: **Interim analysis of an ongoing prospective observational gender-stratified cohort PK study, conducted at Ghent University Hospital.**
Methods: **35 obese (BMI>35kg/m²) and 18 non-obese (18,5kg/m²<BMI< 30kg/m²) patients undergoing laparoscopy were included. All patients received intravenously 2g APAP and 1g q6h. Plasma concentrations of protein adducts were measured at predefined timepoints after first and fifth dose.**
Main outcome measures: **CYP2E1 activity was indirectly assessed by measuring APAP protein adducts. Linear mixed model analysis was used to assess correlations between the repeated measurements of protein adducts plasma concentrations and: obesity, age, gender, total body weight, lean body mass and metabolic syndrome. Hepatotoxicity was evaluated by assessing liver function markers and observing the 1.0 µmol/L threshold for APAP protein adducts.**
Results: **No statistically significant interaction was observed between obesity and the measurements for APAP-cysteine or APAP-mercapturate. No significant interaction was noted between metabolic syndrome and these adducts. Significant correlations were found for APAP-Cysteine with sex, total body weight, and lean body mass. Statistically significant differences in bilirubin, prothrombin time (PT), and international normalized ratio (INR) were found in obese patients at 30 hours, though without clinical relevance.**
Conclusions: **Obesity and metabolic syndrome did not have a significant impact on CYP2E1 activity. Liver function markers were significantly different in obese patients, without clinical relevance.**

Keywords: **Acetaminophen, analogs & derivatives, Acetaminophen / pharmacokinetics, Acetaminophen / metabolism, Obesity, morbid/metabolism; Cytochrome P-450 CYP2E1/metabolism.**

Manuscript will be submitted at University Ghent as a thesis to attain the degree of master in specialized medicine.
The study has been conducted in the University Hospital of Ghent and was approved by its ethics committee (Corneel Heymanslaan 10, 9000 Ghent, Belgium. Chairperson: Prof. Dr. R. Peleman. Protocol number BC-07469) Approval was obtained on April 20th 2020. Written informed consent was obtained from all included patients. Data was collected from the 1st of September 2020 until the 31st of March 2024.

Introduction

Acetaminophen/paracetamol/N-acetyl-p-aminophenol (APAP) is widely used as a non-opioid analgesic for weak to moderate pain and as an antipyretic drug. The current maximum recommended therapeutic dosing regimen of APAP in adults constitutes 4g a day and does not take obesity in account. In a peri- and postoperative setting, studies have demonstrated that a starting dose of 2g can be given safely in healthy adults and resulted in beneficial outcomes towards nociception[1-3]. APAP is usually considered as a safe drug, however APAP overdose can lead to massive hepatocellular necrosis and acute liver failure[4,5].

Metabolism

APAP metabolism is age- and dose-dependent. The half-life of APAP in healthy adults ranges between 2.0 to 2.5 hours. In the presence of liver dysfunction, half-life can be prolonged, but overall metabolism is quantitatively the same as in healthy subjects. The peak plasma concentrations of APAP after a therapeutic dose are approximately 20 mg/L up to 30 mg/L and concentrations between 10 to 20 mg/L are considered to be therapeutic.

APAP is predominantly metabolized by the liver and to a lesser extent by the kidney and intestine. A small amount (2 to 5%) of APAP is eliminated unchanged. APAP undergoes extensive phase II conjugation (85%) and ultimately produces glucuronidated (55%) and sulphated (30%) metabolites[6,7].

Approximately 10% undergoes phase I oxidation through microsomal cytochromes P450 system (CYP), mainly through CYP 2E1, but to lesser extent CYP3A4 and CYP1A2. This enzymatic metabolization results in production of NAPQI (N-Acetyl-p-benzoquinone), which is a highly reactive metabolite due to its strong electrophilic character. At therapeutic doses, NAPQI is neutralized by glutathione (GSH) conjugation. GSH is an endogenous antioxidant. APAP-GSH is quickly degraded to APAP-cysteine, which can also undergo acetylation to form APAP-mercapturate. APAP can lead to hepatocellular toxicity when insufficient scavenging of NAPQI occurs (Figure 1)[5,8-12].

Obesity

Worldwide, the prevalence rates of obesity (body mass index [BMI] ≥ 30 kg/m) (Table I) are increasing. This epidemic is also associated with a rising prevalence of metabolic syndrome. Metabolic syndrome comprises a cluster of five conditions that pose risks for developing heart disease, diabetes, stroke, and various other health complications. It is diagnosed when an individual exhibits three or more of the following risk factors: 1) elevated glucose levels, 2) reduced levels of HDL cholesterol, 3)

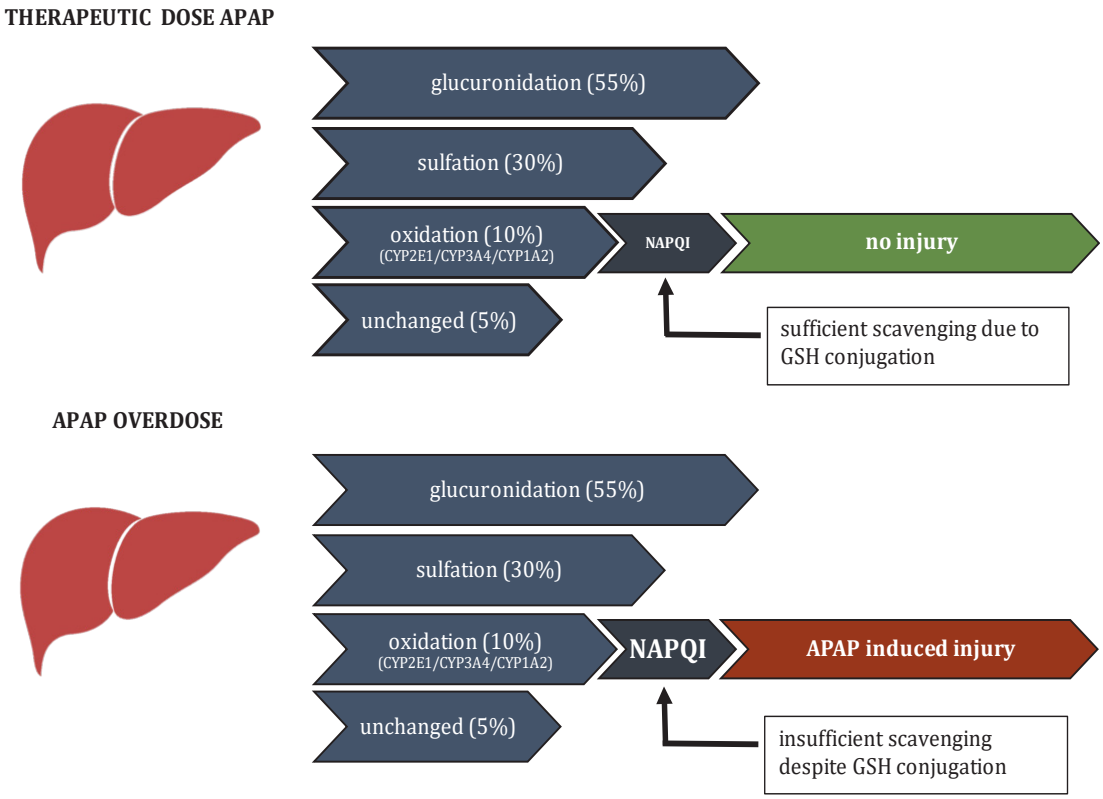

Fig. 1 — Schematic illustration of acetaminophen (APAP) metabolism. When administered in therapeutic doses, NAPQI formation is significantly lower and does not lead to liver injury (upper panel). In overdose, NAPQI formation is significantly higher and does lead to APAP induced liver injury (lower panel).

elevated levels of triglycerides, 4) an increased waist circumference and 5) high blood pressure (Table II)[13-16].

Non-alcoholic fatty liver disease (NAFLD) is another associated health condition in obese patients that might affect APAP metabolism. It refers to the large spectrum of liver lesions in overweight and obese individuals ranging from fatty liver (steatosis) to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. Insulin resistance is a key mechanism contributing to the accumulation of lipids in the liver. It drives fat redistribution from adipose tissue to the liver and enhances de novo lipogenesis within hepatocytes[4,17].

In June 2023, a multi-society consensus was established to revise the nomenclature for NAFLD. The term Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) was introduced to better represent the condition's strong association with cardiometabolic risk factors[18]. The updated diagnostic criteria emphasize the importance of underlying metabolic disorders, such as central obesity, type 2 diabetes, dyslipidemia, and hypertension. This renaming and the corresponding shift in diagnostic focus underscore the need for a broader approach to liver disease that includes comprehensive assessment and management of cardiometabolic health[4,9,13,16-19].

Obesity and acetaminophen

Obesity is recognized to physiologically impact multiple organ systems and as such impact the pharmacodynamics and pharmacokinetics of many drugs, including APAP[20,21].

Abernethy et al. analyzed in 1982 the distinctive metabolism of APAP in obese patients. Morbidly obese patients not only have a much larger distribution volume (Vd), but also have a significant increased total APAP clearance. The increased Vd is partly due to the larger increase in adipose tissue in comparison with the increase in lean body mass. In addition, the Vd is affected by the higher circulating blood volume and cardiac output as well as the reduced proportion of total body water seen in obese patients as compared to the general population[15].

Generally, it has been observed that phase II metabolic pathways are increased in obese individuals, particularly in terms of glucuronide conjugation. However, the influence of obesity on phase I metabolic pathways is more variable. Certain pathways consistently exhibit reduced activity, such as CYP3A4, whereas the CYP2E1 pathway demonstrates significantly increased activity, which is correlated with both higher total body weight and the extent of liver steatosis that gradually occurs in long-standing obesity[4,8,20,22].

Similarly, renal clearance is usually increased in obese patients, due to a higher renal blood flow and glomerular filtration rate; however, with long-standing obesity, renal clearance can be reduced due to obesity-induced chronic kidney disease[20].

Acetaminophen and hepatotoxicity

In most high-income countries hepatotoxicity after unintentional or intentional APAP overdose is the leading cause of acute liver failure. Besides the ingested dose, the risk and severity of hepatotoxicity can be significantly enhanced in the presence of following predisposing factors: malnutrition, chronic alcohol abuse, chronic alcoholic liver disease and hepatitis C virus. An increased risk is also present with comedication with drugs causing cytochromes P450 induction, such as isoniazid, rifampicin and phenobarbital[4,9,17].

Table I. — WHO classification of weight status.

	BMI (kg/m²)	
Underweight	<18.5	
Normal	18.5 – 24.9	
Overweight	25.0 – 29.9	
Obesity	30 – 34.9	Obesity class I
	35.0 – 39.9	Obesity class II
Extreme obesity	≥40	Obesity class III

Table II. — Metabolic syndrome according to NCEP (National Cholesterol Education Program) ATPIII 2005 criteria.

Metabolic syndrome
Blood glucose greater than 5.6 mmol/L (100 mg/dl) or drug treatment for elevated blood glucose
HDL cholesterol < 1.0 mmol/L (40 mg/dl) in men, < 1.3 mmol/L (50 mg/dl) in women or drug treatment for low HDL-cholesterol
Blood triglycerides > 1.7 mmol/L (150 mg/dl) or drug treatment for elevated triglycerides
Waist > 102 cm (men) or > 88 cm (women)
Blood pressure > 130/85 mmHg or drug treatment for hypertension

Overall, an increased CYP2E1 activity seems to play a significant role in the mechanism of APAP-induced liver injury. Increased CYP2E1 might result in higher NAPQI production, making obese patients more vulnerable to hepatotoxic effects of APAP. Despite data confirming an increased CYP2E1 activity in obese individuals, obesity in itself does not seem to be associated with a higher risk of hepatotoxicity. The occurrence of APAP-induced liver injury in an obese individual will depend on a delicate balance between metabolic factors that can be detrimental and factors that can be protective (Table III)[4,9,11,17].

Traditionally, the routine assessment of liver enzymes and APAP concentrations has been critical in managing APAP induced liver injury. There is currently a growing interest in new biomarkers to help risk stratification. Previous research examining APAP-protein adducts in clinical samples has investigated the utility of this biomarker in patients with severe acute liver injury and acute liver failure following APAP overdose. In these studies, a diagnostic threshold of over 1.0 µmol/L for APAP-protein adducts (APAP-Cyst = 254.31 g/mol, APAP-Mercap = 312.34 g/mol was employed to differentiate between cases of APAP-induced acute liver failure and those caused by other factors[23-25].

Objectives of this study

This is a sub-analysis of the main study on pharmacokinetics of therapeutic doses of APAP in obese and non-obese patients after receiving a therapeutic intravenous dosing regimen of a 2g loading dose following 1g per 6 hours. Plasma concentrations of acetaminophen (APAP), acetaminophen-glucuronide (APAP-Gluc), acetaminophen-sulphate (APAP-Sulf), acetaminophen-cysteine (APAP-Cyst) and acetaminophen-mercapturate (APAP-Mercap), were measured.

The aim of this sub-analysis is to specifically investigate the CYP2E1 pathway of APAP in obese and non-obese patients, by analyzing the concentration of its protein adducts: APAP-Cyst and APAP-Mercap. Hepatotoxicity was evaluated by assessing liver function markers as well as observing the suggested threshold of the APAP protein adducts.

Methods

Obese patients (BMI>35kg/m²) undergoing bariatric surgery and non-obese (BMI > 18,5kg/m² < 30kg/m²) patients undergoing abdominal laparoscopic surgery were considered for inclusion in the study (Table IV) and written informed consent was obtained. The study has been conducted in the University Hospital of Ghent and was approved by its ethics committee (C. Heymanslaan 10, 9000 Ghent, Belgium. Chairperson: Prof. Dr. R. Peleman. Protocol number BC-07469) Approval was obtained on April 20th 2020. Written informed consent was obtained from all included patients. Data was collected from the 1st of September 2020 until the 31st of March 2024.

Study design

In this prospective observational single center study, 35 obese patients and 18 non-obese patients were included. All patients received a 2g intravenous dose of APAP (200ml of Fresenius Kabi 10 mg/mL, administered over 15 min using a volumetric pump) after induction of anesthesia. After the loading dose, APAP was scheduled 1g per 6 hours, leading to a cumulative dose of 5g of APAP at the end of study day 1 in both groups.

A total of 16 blood samples were collected for APAP drug assays over two consecutive study days. On study day 1, 11 samples were taken, T1 marks the start of the 2g APAP infusion. On study day 2, 5 samples were taken, T12 marks the start of the fifth administration of APAP (Figure 2).

Liver function markers (PT, INR, total bilirubin, AST, ALT and γ-GT), kidney function markers (creatinine, GFR and urea), metabolic parameters (triglycerides, HDL and fasting glucose) and CRP from serum samples were pre-operatively collected. Thirty hours after the APAP 2g loading dose and four therapeutic 1g doses, blood samples were obtained to measure liver function markers.

Table III. — Metabolic factors influencing NAPQI formation.

Detrimental factors	Protective factors
CYP2E1 induction	Lower APAP gastrointestinal absorption and higher volume of distribution
Low basal levels of glutathione	Lack of CYP2E1 induction or CYP2E1 downregulation
NASH associated mitochondrial dysfunction	Reduced CYP3A4 and CYP1A2 activity
Extent of steatosis and hepatic accumulation of deleterious fatty acids and lipid species	Increased APAP glucuronidation
Presence of lobular inflammation	Exposure and accumulation of protective fatty acids

Table IV. — Eligibility criteria.

Inclusion criteria
1. Adult ≥ 18 ≤ 70 years old
2. Able to comprehend, sign, and date the written informed consent document to participate in the clinical trial
3. Obese scheduled for laparoscopic bariatric surgery or non-obese scheduled for laparoscopic surgery
4. Control group BMI ≥ 18.5 and < 30 kg/m² or Obese group BMI ≥ 35 kg/m²
5. ASA Class I, II or III as assigned by the anesthesiologist
Exclusion criteria
1. Allergy or inability to tolerate "acetaminophen"
2. Documented liver disease or liver enzymes > 3X normal value
3. Kidney disease (eGFR < 30ml/min)
4. Participation in a clinical trial within the past 30 days
5. Chronic alcohol abuse or alcohol intake < 72hrs
6. Gilbert-Meulengracht-syndrome
7. Chronic malnutrition
8. Intake of medication with influence on CYP2E1 or UDP-glucuronosyltransferase
9. Pregnancy

Drug assay

APAP, APAP-metabolites and APAP protein adducts were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

It is important to note that NAPQI is not detectable in vivo due to the numerous reactions it undergoes. These include covalent binding to nucleophilic sites of biomolecules, auto-reduction to APAP and dimerization and/or polymerization. NAPQI formation, due to CYP2E1 activity, is measured indirectly by measuring the stable GSH conjugate and/or its protein adducts such as APAP-Cyst and APAP-Mercap[26,27]. Previous studies have established that at therapeutic dosing, APAP-GSH is present at low, almost undetectable concentrations. Therefore, it was decided not to include APAP-GSH analysis in this study[10,28].

Statistical analysis

The independent student T-test for numerical data and the Chi-square/Fischer's exact test for categorical data were applied to test statistical differences in demographic variables between obese and non-obese patients.

Linear Mixed Model (LMM) analysis was preferred over repeated measures ANOVA because of missing plasma measurements due to failed blood sampling or early discharge from the hospital. Data was hierarchal in structure and followed a 2-level study design. At level 1, we observe patients with variables: age, sex, total body weight and lean body mass. At level 2, we observe groups with variables: obesity and metabolic syndrome. Plasma measurements at subsequent points in time representing peak and trough concentrations (T1, T11, T12, T15, T16) were used for this analysis. LMM was used to analyze correlation between the dependent variables APAP-Cyst and APAP-Mercap and the fixed effects of the measurements, obesity and their interaction. We included each patient as a random intercept with a variance components (VC) covariance structure. Estimated marginal means (EMMeans) were calculated for time of measurements, obesity and their interaction. Multiple comparisons were adjusted using the

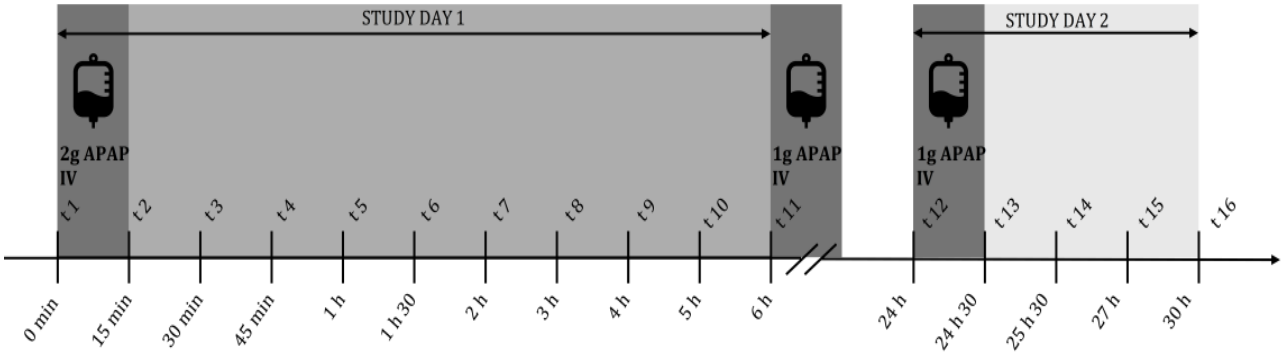

Fig. 2 — Schematic illustration of study design.

Bonferroni method. The same method was used to assess the relationship between the APAP protein adducts and metabolic syndrome. At an individual level, we also assessed the relationship between APAP protein adducts and the fixed effects of age, gender, total body weight and lean body mass.

The Wilcoxon rank test was used to test statistical differences between liver function markers before APAP administration and 30 h after administration.

All statistical analyses were conducted in SPSS (IBM version 29.0.1.0).

A power-analysis was performed using G*Power free software to estimate sample size based on the data published by Van Rongen et al. The sample size was calculated for APAP, APAP-metabolites and protein adducts, using the means and based on the Wilcoxon-Mann-Whitney test (two groups) for a two tailed distribution, a standard alpha error of 0.05, a power of 0.80 and an allocation ratio N2/N1 of 1. Based on this analysis we aimed to enlist a sample size of 70 patients in total (divided in 15 male and female control patients and 20 male and female obese patients).

Results

Patient data

Demographics of patient data are shown in Table V, Figure 3 and Figure 4. A statistically significant difference between the cohorts was found for: age, height, weight, BMI, LBM, waist circumference, Waist to height ratio >0.5 , incidence of metabolic syndrome, ASA score, ALT, CRP and HDL cholesterol.

Observed concentrations of APAP protein adducts

The course of the APAP protein adducts concentrations are shown in Figure 5 and Figure 6. Results of LMM analyses are shown in Table VI, VII, VIII, IX, X and XI. The interaction between measurements and

Table V. — Demographics of obese and non-obese group.

Variable	Obese patients, n = 35	Non-obese patients, n = 18	95% CI	p-value
Gender				0.630
Female [n]	17	10		
Male [n]	18	8		
Age [years]	44.1 (± 13.8)	54.7 (± 11.2)	(-17.7 - -3.5)	0.004
Height [m]	1.73 (± 0.10)	1.70 (± 0.09)	(-0.02 - 0.08)	0.314
Body weight [kg]	126.6 (± 16.9)	75.5 (± 13.3)	(42.5 - 59.6)	<.001
BMI [kg/m²]	42.3 (± 4.9)	25.9 (± 2.9)	14.2 - 18.5	<.001
LBM [a] [kg]	68.4 (± 12.3)	51.4 (± 12.0)	(9.9 - 24.1)	<.001
Waist [cm]	135.6 (± 11.0)	95.8 (± 11.7)	(32.8 - 46.8)	<.001
Waist-to-height ratio > 0.5 [n]	34	17		0.033
Smoking status				1.000
Smoker [n]	6	3		
Non-smoker [n]	29	15		
Metabolic syndrome [b] [n]	20	5		0.060
Increased glucose levels [n]	15	5		
Increased triglycerides levels [n]	13	3		
Reduced levels of HDL cholesterol [n]	18	6		
Increased waist circumference [n]	34	10		
Elevated blood pressure [n]	21	9		
ASA score [n]				0.032
ASA score 1 [n]	1	0		
ASA score 2 [n]	18	16		
ASA score 3 [n]	16	2		
PT [%]	100 (± 13)	100 (± 11)	(-8 - 6)	0.808
INR	0.99 (± 0.08)	0.96 (± 0.07)	(-0.01 - 0.08)	0.149
bilirubin [mg/dL]	0.5 (± 0.3)	0.6 (± 0.3)	(-0.2 - 0.1)	0.651
AST [U/L]	26 (± 14)	24 (± 8)	(-4 - 9)	0.434
ALT [U/L]	39 (± 31)	22 (± 9)	(5 - 28)	0.007
GGT [U/L]	41 (± 26)	28 (± 36)	(-7 - 33)	0.198
Triglycerides [mg/dL]	154 (± 85)	127 (± 82)	(-24 - 76)	0.294
HDL-cholesterol [mg/dL]	45 (± 12)	58 (± 27)	(-24 - -2)	0.02
Glucose [mg/dL]	99 (± 12)	96 (± 19)	(-6 - 14)	0.434
CRP [mg/L]	8.6 (± 3.1)	4.2 (± 0.2)	(1.1. - 8.0)	0.011
Creatinine [mg/dL]	0.90 (± 0.18)	0.89 (± 0.15)	(-0.08 - 0.11)	0.709

Values are expressed as mean (± standard deviation) unless specified otherwise; a calculated according the Janmahastian formula b according to NCEP (National Cholesterol Education Program) ATPIII 2005 criteria.

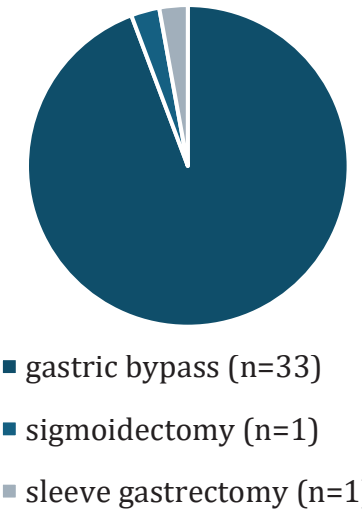

Fig. 3 — Pie chart of types of surgery in obese group.

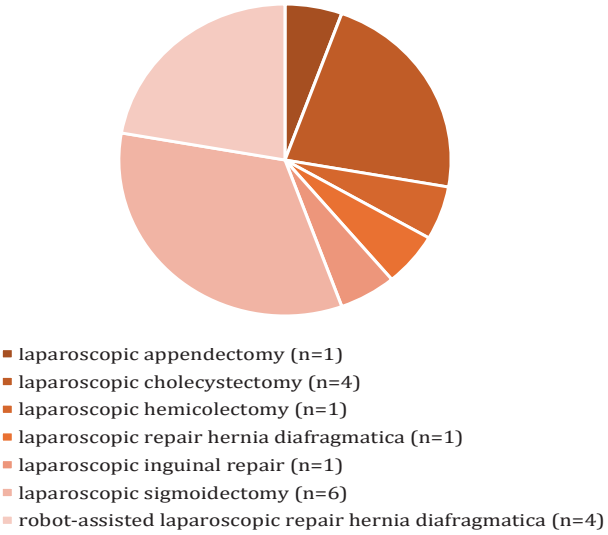

Fig. 4 — Pie chart of types of surgery in non-obese groups.

obesity for APAP-Cyst is not statistically significant (F-value = 0.997, p-value = 0.412). We conclude that the concentration of APAP-Cyst does not vary significantly depending on whether a patient is obese. We note the same result for APAP-Mercap (F-value = 1.027, p-value = 0.396). The analysis for the interaction between the protein adducts and metabolic syndrome also show no statistical significancy (APAP-Cyst: F-value = 1.155, p-value = 0.335; APAP-Mercap: F-value = 0,613, p-value = 0,654). When analyzing the relationship between the concentration of APAP protein adducts and individual factors: age, sex, total body weight and lean body mass. We note that sex (F-value = 3.198, p-value = 0.016), total body weight (F-value = 3.608, p-value = 0.008), and lean body mass (F-value = 2.83, p-value = 0.028) have a significant impact on the concentration of APAP-Cyst. APAP-Mercap did not exhibit any significant interactions with any of the other variables.

Liver function markers

Analysis of the liver function markers at the end of the observation period revealed a statistically significant difference for bilirubin, PT and INR in obese patients. The clinical relevancy of this finding is questionable, since values are within normal range and none of our patients showed clinical signs of hepatotoxicity (Table XII).

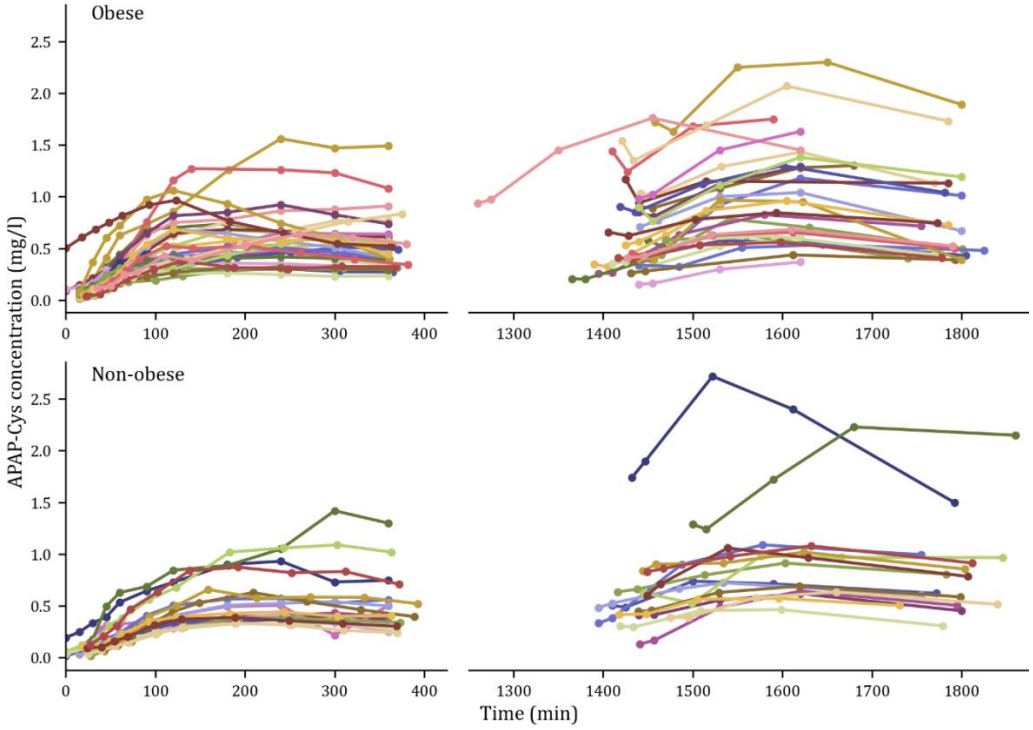

Fig. 5 — Acetaminophen-cysteine concentrations over time in the obese group (upper line graph) and in the non-obese group (lower line graph).

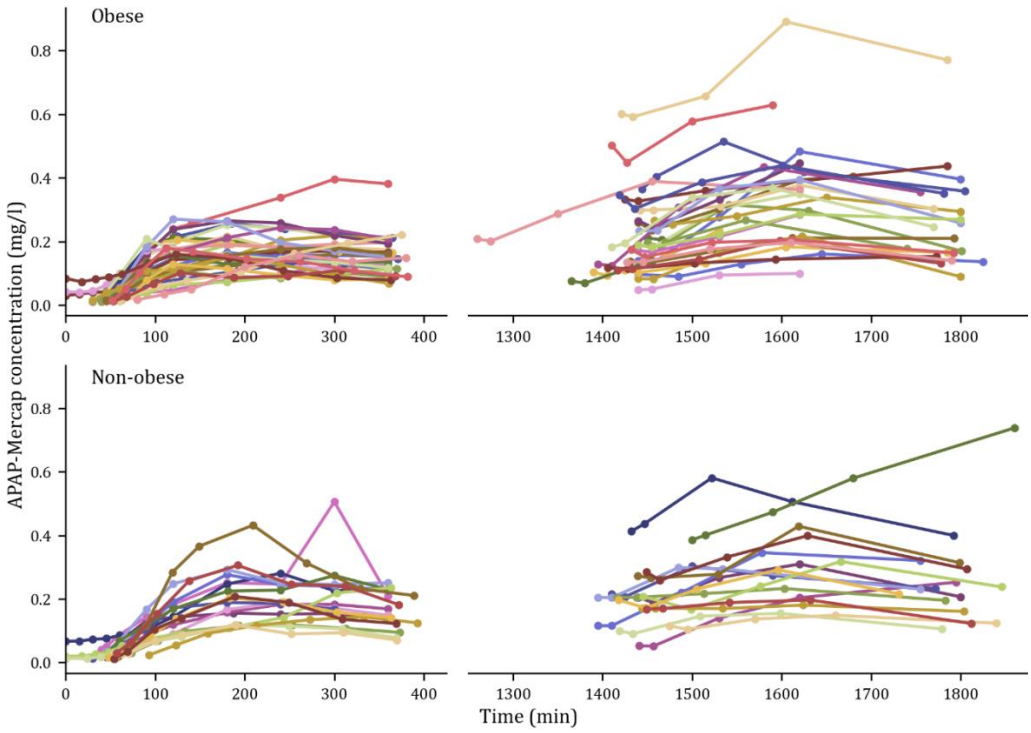

Fig. 6 — Acetaminophen-mercapturate concentrations over time in the obese group (upper line graph) and in the non-obese group (lower line graph).

Table VI. — Linear mixed model analysis for the assessment of the correlation between acetaminophen-cysteine (APAP-Cyst) concentrations and obesity.

APAP-Cyst							
Variable					df	F	Sig.
Intercept					1	119.337	<.001
Measurements					4	51.105	<.001
Obesity					1	0.878	0.353
Obesity*Measurements					4	0.997	0.412
	Estimate	SE	df	t	Sig.	95% CI	
						UB	LB
Intercept	0.846	0.075	75.069	11.321	<.001	0.697	0.995
Measurements	-0.53	0.129	133.126	-4.101	<.001	-0.786	-0.274
Obesity	-0.043	0.124	73.15	-0.343	0.732	-0.289	0.204
Obesity*Measurements	-0.313	0.172	132.391	-1.819	0.071	-0.654	0.027

Table VII. — Linear mixed model analysis for the assessment of the correlation between acetaminophen-mercapturate (APAP-Mercap) concentrations and obesity.

APAP-Mercap							
Variable					df	F	Sig.
Intercept					1	159.685	<.001
Measurements					4	39.772	<.001
Obesity					1	0.479	0.492
Obesity*Measurements					4	1.027	0.396
	Estimate	SE	df	t	Sig.	95% CI	
						LB	UB
Intercept	0.28	0.022	93.521	12.632	<.001	0.236	0.324
Measurements	-0.177	0.047	136.484	-3.793	<.001	-0.269	-0.085
Obesity	-0.019	0.037	90.058	-0.527	0.6	-0.092	0.053
Obesity*Measurements	-0.061	0.062	135.237	-0.98	0.329	-0.184	0.062

Table VIII. — Linear mixed model analysis for the assessment of the correlation between acetaminophen-cysteine (APAP-Cyst) concentrations and metabolic syndrome (MS).

APAP-Cyst							
Variable					df	F	Sig.
Intercept					1	136.076	<.001
Measurements					4	58.119	<.001
MS					1	2.537	0.117
MS*Measurements					4	1.155	0.334
	Estimate	SE	df	t	Sig.		95% CI
						LB	UB
Intercept	0.717	0.082	68.262	8.7	<.001	0.553	0.882
Measurements	-0.671	0.135	127.547	-4.988	<.001	-0.938	-0.405
MS	0.186	0.113	67.672	1.646	0.104	-0.039	0.411
MS*Measurements	0.113	0.166	127.49	0.677	0.5	-0.217	0.442

Table IX. — Linear mixed model analysis for the assessment of the correlation between acetaminophen-mercapturate (APAP-Mercap) concentrations and metabolic syndrome (MS).

APAP-Mercap							
Variable					df	F	Sig.
Intercept					1	161.996	<.001
Measurements					4	41.407	<.001
MS					1	0.001	0.98
MS*Measurements					4	0.613	0.654
	Estimate	SE	df	t	Sig.		95% CI
						LB	UB
Intercept	0.276	0.026	90.531	10.583	<.001	0.224	0.328
Measurements	-0.234	0.056	131.429	-4.165	<.001	-0.345	-0.123
MS	-0.011	0.036	89.7	-0.296	0.768	-0.081	0.06
MS*Measurements	0.061	0.069	131.312	0.876	0.383	-0.077	0.198

Table X. — Linear mixed model analysis for the assessment of the correlation between acetaminophen-cysteine (APAP-Cyst) concentrations and: sex, total body weight (TBW), lean body mass (LBM) calculated by using the Janmahastian formula and age.

APAP-Cyst							
Variable					df	F	Sig.
Intercept					1	7.599	0.007
Sex					1	3.92	0.052
Measurements					4	2.34	0.059
TBW					1	9.095	0.004
LBM					1	7.192	0.009
Age					1	0.096	0.758
Sex*Measurements					4	3.198	0.016
Measurements*TBW					4	3.608	0.008
Measurements*LBM					4	2.83	0.028
Measurements*Age					4	1.511	0.203
	Estimate	SE	df	t	Sig.		95% CI
						LB	UB
Intercept	1.003	0.528	68.743	1.899	0.062	-0.051	2.056
Sex	0.163	0.363	66.227	0.448	0.656	-0.562	0.887
Measurements	1.967	1.298	120.367	1.515	0.132	-0.604	4.538
TBW	0.014	0.007	69.745	1.901	0.062	-0.001	0.028
LBM	-0.032	0.02	67.369	-1.547	0.126	-0.073	0.009
Age	0.005	0.005	74.877	1.155	0.252	-0.004	0.014
Sex*Measurements	2.761	0.814	120.179	3.392	<.001	1.149	4.372
Measurements*TBW	0.043	0.014	119.792	3.092	0.002	0.016	0.071
Measurements*LBM	-0.123	0.044	120.101	-2.777	0.006	-0.21	-0.035
Measurements*Age	-0.018	0.01	120.487	-1.864	0.065	-0.038	0.001

Table XI. — Linear mixed model analysis for the assessment of the correlation between acetaminophen-mercapturate (APAP-Mercap) concentrations and: sex, total body weight (TBW), lean body mass (LBM) calculated by using the Janmahastian formula and age.

APAP-Mercap							
Variable					df	F	Sig.
Intercept					1	2.9	0.092
Seks					1	1.526	0.22
Measurements					4	0.619	0.65
TBW					1	3.154	0.08
LBM					1	2.169	0.145
Age					1	0.059	0.809
Sex*Measurements					4	1.321	0.266
Measurements*TBW					4	2.035	0.094
Measurements*LBM					4	1.305	0.272
Measurements*Age					4	2.133	0.081
	Estimate	SE	df	t	Sig.		95% CI
						LB	UB
Intercept	0.153	0.168	85.449	0.916	0.362	-0.18	0.487
Seks	0.046	0.115	82.053	0.405	0.686	-0.181	0.274
Measurements	0.646	0.508	123.449	1.271	0.206	-0.36	1.651
TBW	0.004	0.002	87.681	1.65	0.103	-0.001	0.008
LBM	-0.007	0.006	83.996	-1.144	0.256	-0.02	0.005
Age	0.003	0.001	94.356	2.133	0.035	0	0.006
Sex*Measurements	0.629	0.319	123.128	1.974	0.051	-0.002	1.259
Measurements*TBW	0.008	0.005	122.464	1.444	0.151	-0.003	0.019
Measurements*LBM	-0.025	0.017	122.998	-1.466	0.145	-0.06	0.009
Measurements*Age	-0.008	0.004	123.64	-2.124	0.036	-0.016	-0.001

Table XII. — Analysis of liver function markers in obese patients and non-obese patients, before and 30 hours after the 2 g intravenous acetaminophen loading dose and subsequent scheduled doses. At 30 hours a total of 6g acetaminophen has been administered in all patients.

Liver function markers	Obese patients, n= 35		p-value
	T = 0h	T = 30h	
AST [U/L]	26 (± 14)	45 (± 55)	0.065
RR: 0 – 37			
ALT [U/L]	39 (± 31)	60 (± 93)	0.545
RR: 7 – 40			
GGT [U/L]	41 (± 26)	38 (± 22)	0.039
RR: < 64			
Bilirubin [mg/dL]	0.5 (± 0.3)	0.6 (± 0.2)	0.008
RR: 0.2 - 1.3			
PT [%]	100 (± 13)	89 (± 11)	<.001
RR: 70 - 120			
INR	0.99 (± 0.08)	1.05 (± 0.08)	0.005
RR: 0.9 - 1.1			

Liver function markers	Non-obese patients, n= 18		p-value
	T = 0h	T = 30h	
AST [U/L]	24 (± 8)	59 (± 90)	0.056
RR: 0 – 37			
ALT [U/L]	22 (± 9)	52 (± 77)	0.551
RR: 7 – 40			
GGT [U/L]	28 (± 36)	29 (± 36)	0.916
RR: < 64			
Bilirubin [mg/dL]	0.5 (± 0.3)	0.6 (± 0.2)	0.452
RR: 0.2 - 1.3			
PT [%]	100 (± 11)	79 (± 15)	<.001
RR: 70 - 120			
INR	0.95 (± 0.06)	1.14 (± 0.16)	0.001
RR: 0.9 - 1.1			

Discussion

Our study demonstrates no effect of obesity on the CYP2E1 activity compared to the cohort of non-obese and overweight patients.

Our results are in contrast with the observations published by Van Rongen et al[8]. Our study applied the same exclusion criteria. Their study used the median AUC 0–8h value of APAP protein adducts (APAP-Cyst + APAP-Mercap) to APAP ratio to demonstrate the differences in APAP metabolism in obese and non-obese patients. This study showed a significant increased formation of APAP-Cyst in obese patients. Our study only analyzed the plasma concentrations of APAP-Cyst and APAP-Mercap at five distinct points in time.

Our results could be explained with the review of Begriche et al. stating the relationship between obesity and hepatotoxicity is not always reported and is ultimately a balance between protective and detrimental metabolic effects[9]. Protective factors such as a downregulation of CYP2E1 activity, a reduced CYP3A4 and CYP1A2 activity, an increased APAP glucuronidation can all potentially reduce NAPQI formation.

Our study did not assess the GSH status by measuring preoperative serum GSH levels. Choramanska et al. found reduced plasma GSH in obese patients compared to lean individuals. Additionally, this effect was age and gender-dependent with lower levels in male and older obese subjects[29]. The fact that we did not find higher protein adducts in the obese cohort might indicate a preserved GSH status in our patients. This might be attributed to the fact that all our bariatric patients undergo a multi-disciplinary screening and workout before being eligible for surgery with optimization of their preoperative condition by exercise and diet advice.

APAP induced hepatotoxicity in obese patients is linked to MASLD. We did not screen our patients for the presence of liver steatosis by using ultrasound of biopsy. However to gain an impression we screened our patients for metabolic syndrome using the criteria of the U.S. National Cholesterol Education Program Adult Treatment Panel III as this is still the most widely-used clinical definition. Detrimental metabolic factors such as lower basal levels of GSH, mitochondrial dysfunction and impairment of other antioxidant defenses have been reported in patients with MASLD[4,16,18,19]. Our analysis did not demonstrate a significant impact of metabolic syndrome on the formation of APAP protein adducts.

In our study, the protein adduct concentrations were above the suggested cutoff value of 1.0 µmole/L for APAP induced liver injury in patients admitted with acute liver failure[24,30]. We found no reports relating early and increased formation of CYP2E1-mediated metabolites to acetaminophen hepatotoxicity. Our therapeutic dosing regimen of 2g APAP loading dose followed by 1g per 6 hours did not show a significant difference of liver function markers in both groups. None of our patients developed hepatotoxicity.

Limitations of the study

Protein adduct concentrations during therapeutic dosing of APAP are known to peak after approximately 4 days and plateau after 6 days. Most of our study patients were in a fast-track program and were discharged the second day after surgery, leaving only an observation window of 30 hours.

During the analysis of the measurements on study day 2, we detected many potential protocol violations: doses of APAP were not correctly registered in the electronic patient data management system, administered too early, too late, or not traceable. This might have affected the measurements on study day 2.

Conclusions

Our data did not confirm a significant impact of obesity and metabolic syndrome on CYP2E1 activity. Although liver function markers were significantly different in obese patients, this was not clinically relevant. Further research with longer observation and sampling periods even after stopping APAP are needed before any sound recommendations can be made on the safety of higher dosage of APAP in patients with obesity.

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