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Cysteine-lowering treatment with mesna against obesity: Proof of concept and results from a human phase I, dose-finding study

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Abstract

Aim: To investigate whether mesna—sodium-2-mercaptoethane sulfonate) can reduce diet-induced fat gain in mice, and to assess the safety of single ascending mesna doses in humans to find the dose associated with lowering of plasma tCys by at least 30%.

Methods: C3H/HeH mice were shifted to a high-fat diet ± mesna in drinking water; body composition was measured at weeks 0, 2 and 4. In an open, phase I, single ascending dose study, oral mesna (400, 800, 1200, 1600 mg) was administered to 17 men with overweight or obesity. Mesna and tCys concentrations were measured repeatedly for a duration of 48 hours postdosing in plasma, as well as in 24-hour urine.

Results: Compared with controls, mesna-treated mice had lower tCys and lower estimated mean fat mass gain from baseline (week 2: 4.54 ± 0.40 vs. 6.52 ± 0.36 g; week 4: 6.95 ± 0.35 vs. 8.19 ± 0.34 g; $P_{overall}$ = .002), but similar lean mass gain. In men with overweight, mesna doses of 400-1600 mg showed dose linearity and were well tolerated. Mesna doses of 800 mg or higher decreased plasma tCys by 30% or more at nadir (4h post-dosing). With increasing mesna dose, tCys AUC_{0-12h} decreased (P_{trend} < .001), and urine tCys excretion increased (P_{trend} = .004).

Conclusions: Mesna reduces diet-induced fat gain in mice. In men with overweight, single oral doses of mesna (800-1600 mg) were well tolerated and lowered plasma tCys efficiently. The effect of sustained tCys-lowering by repeated mesna administration on weight loss in humans deserves investigation.

KEYWORDS
antiobesity drug, body composition, clinical trial, mouse model, pharmacodynamics, phase I study

1 | INTRODUCTION

Cysteine is a semi-essential sulphur-containing amino acid obtained from diet or synthesized endogenously from the essential amino acid methionine. Evidence suggests that elevated plasma total cysteine (tCys) predisposes to human obesity.[1] In population studies, plasma tCys is independently associated with body mass index (BMI),[2] atherogenic plasma lipid profile,[3] fat mass[4] and insulin resistance,[5] but not lean mass.[4] Longitudinal studies conducted for a duration of 6 years show that change in tCys is prospectively associated with corresponding changes in BMI[6] and fat mass,[4] after controlling for baseline tCys and BMI. Cystine, the disulphide form of cysteine, enhances adipogenesis and lipid accumulation in murine and human preadipocytes,[7,8] and is positively associated with fat mass in humans.[8,9] Transgenic and dietary animal studies also suggest that high cysteine availability promotes fat gain. Mice fed a high cystine diet had lower energy expenditure and increased central adiposity and worsened glucose tolerance.[10] Conversely, restricted intake of cysteine and its precursor, methionine, results in a lean, hypermetabolic and insulin-sensitive phenotype in rats.[11–15] Importantly, such antiobesity effects are reversed by cysteine supplementation.[14,16] Further, knockout models of cystathionine beta synthase (CBS), which catalyses the first irreversible step in cysteine synthesis, feature low fat mass with comparatively preserved lean mass,[17,18] and reduced fat mass is common in patients with homozygous CBS deficiency.[19,20] Weight loss on a diet restricted in cysteine and methionine has also recently been shown in an open-label trial.[21] Furthermore, major weight loss postbariatric surgery had a minimal effect on tCys, indicating that the cysteine–obesity association is not attributable to reverse causality.[22]

Based on consistent evidence from animal and human studies that decreased tCys attributed to dietary or genetic causes results in low body weight, we hypothesized that pharmacological lowering of plasma tCys may facilitate weight loss. Mesna—sodium-2-mercaptoethane sulfonate—is a thiol-reducing agent used to prevent urothelial toxicity of oxazaphosphorine metabolites in cancer patients treated with ifosfamide and cyclophosphamide.[23] Intravenous mesna received US Food and Drug Administration approval in 1988, and oral mesna in 2002.[24] In circulation, mesna is rapidly oxidized to its disulphide, dimesna, or forms disulphides with circulating thiols including cysteine.[25,26] Because of the thiol–disulphide exchange of mesna with endogenous thiols, mesna increases the urinary excretion of cysteine.[26,27] Mesna lowers plasma tCys when used within its licensed indication in cancer patients and in healthy volunteers.[25–27]

3The effect of the plasma tCys-lowering effect of mesna on body adiposity is not known. In a cross-sectional population of 5000 adults, ~30% lower plasma tCys (250 vs. 350 μM) corresponded to 7.5 kg lower fat mass.[4] In a preclinical proof-of-concept study in mice, we tested whether plasma tCys-lowering by mesna can reduce fat gain in a model of diet-induced obesity. We then conducted a phase I, non-randomized, single ascending dose study to establish the mesna dose that lowers plasma tCys by at least 30% in men with overweight.

2 | METHODS

2.1 | Study 1: A proof-of-concept study in mice

This study investigated whether mesna could limit the increase in fat mass gain caused by consuming a calorie-dense (high-fat) diet in mice.

2.1.1 | Animal husbandry

This pilot proof-of-concept study was conducted at the MRC Mary Lyon Centre and Harwell Mammalian Genetics Unit. The study was approved by the Medical Research Council Harwell Institute Animal Welfare and Ethical Review Board (MRC AWERB), and all procedures were carried out within project licence restrictions (PPL 30/2642) under the UK Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 (SI-4-2012/3039) and following the ARRIVE guidelines. Male C3H/HeH mice were used, under controlled light (12-hour light–dark cycle), temperature (21 ± 2°C) and humidity (55% ± 10%) conditions, with food and water provided ad libitum. The mice were weaned on a standard diet (SDS Rat and Mouse No.3-Breeding diet [RM3]; 3.36 gm% fat, 22.45 gm% protein and 71.21 gm% carbohydrates) and shifted to a high-fat diet (HFD) at age 11 weeks.

2.1.2 | HFD and mesna administration

At 11 weeks of age, mice were shifted to a HFD (D12451, Open-Source Diets) with or without mesna in drinking water at a concentration of 6 g/L for 4 weeks to achieve a dose of 1 g mesna per kg body weight per day. The HFD provided 45% of calories from fat, 20% from protein and 35% from carbohydrates. Food intake was measured in metabolic cages at week 2 over a 24-hour period. After 4 weeks, fasting blood was collected from the retro-orbital sinus under deep terminal anaesthesia to measure plasma tCys and fractions and free fatty acids, as previously described.[28] The mice were then culled by exsanguination and the liver was harvested and homogenized for tCys measurement.[28]

2.1.3 | Body composition measurement

Body composition was measured at baseline (week 0), and at weeks 2 and 4 by an EchoMRI whole body composition analyser (Echo Medical System, Houston, TX).

2.1.4 | Statistical analysis

Body composition at baseline, week 2 and week 4 was analysed using linear mixed model regression. The dependent variable in each of these models was the change in body composition from baseline.

FIGURE 1 A, Estimated marginal mean (SEM) change in body weight and composition from baseline in adult male C3H/HeH mice 2 and 4 weeks after shifting to a high fat diet with (Mesna group; $n = 22$) or without (control group; $n = 24$) Mesna in drinking water. Data are from linear mixed models adjusted for the baseline value of the outcome. P value is for the overall effect of the intervention over time. B, Terminal plasma total cysteine (tCys), reduced cysteine (rCys), and cystine, and liver tCys. C, Terminal plasma total glutathione (tGSH) and reduced glutathione (rGSH), liver tGSH, and plasma free fatty acids (FFA) in the Mesna and control groups. Plasma and liver data are mean (SEM); *P <.05, **P < .001 by an independent samples t test.
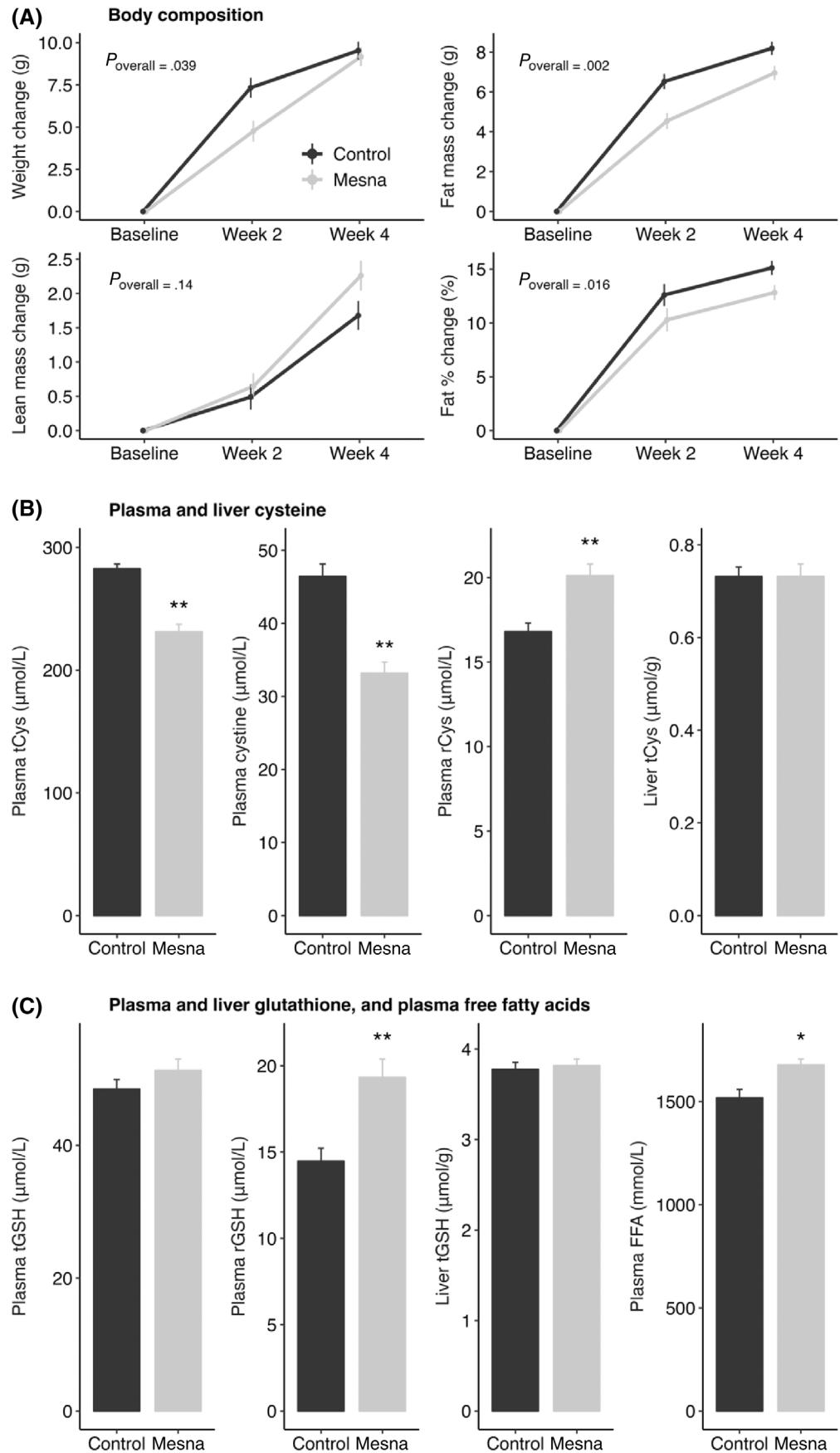
Models included the grouping variable, the time variable, their interaction terms to estimate the marginal mean change from baseline at each time point, and the baseline value of the outcome being assessed to account for any differences. The P value for the overall effect was derived from a model including the grouping variable and the baseline value of the outcome.[29] A random term for each animal was included to account for within-animal correlation. Repeated measures data are presented as estimated marginal means (EMMs) ± SEM. Terminal plasma and liver measurements are reported as mean ± SEM and were analysed using an independent samples t-test.

2.2 | Study 2: A phase I, non-randomized, single-centre, single ascending dose study in healthy men with overweight or obesity

2.2.1 | Study population

This study included men with overweight or obesity, but who were otherwise healthy. Inclusion criteria were age 18-55 years, no apparent health problems (as determined by medical history, physical examination, 12-lead ECG and laboratory tests), BMI 27-40 kg/m^2, male gender and capability of giving informed consent. Exclusion criteria included chronic disease, chronic drug use, past or intended use of over-the-counter or prescription medication including herbal medications within 14 days prior to dosing, veganism, strenuous physical activity at least three times/week and smoking.

2.2.2 | Study protocol

This was an open, non-randomized, phase I, single ascending dose study. Mesna was administered as a single oral dose to $N = 6$-7 participants at each dose level, with ascending single doses of 400, 800, 1200 and 1600 mg, using film-coated 400 or 600 mg tablets (Uromitexan, Baxter AS) (Figure 1). Study volunteers were allowed to participate at more than one dose level after a washout period of at least 14 days and re-screening. The participants were recruited through social media channels of the Faculty of Medicine and Department of Nutrition, University of Oslo. Participation in the study required three study visits to the Clinical Research Unit, Section of Clinical Pharmacology, Oslo University Hospital Rikshospitalet. The participants met in the morning after an overnight fast. At the first visit (day 1), a venous catheter was inserted into an arm vein for serial blood sampling for the measurement of pharmacokinetic (PK), safety and pharmacodynamic (PD) variables. Then mesna tablet(s) were administered with one glass of water. Blood samples were drawn at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours after mesna administration. A fasting blood sample was subsequently collected 24 and 48 hours after administration. During the 12-hour study visit on day 1, participants received standardized meals to minimize diet-induced variations in urine tCys. On days 2 and 3, participants were encouraged to continue on their habitual diet. For assay of mesna and tCys

excretion, 24-hour urine was collected on day 1. Details of blood and urine sampling, and calculation of fractional tCys and mesna excretion, are provided in the supporting information.

2.2.3 | Safety laboratory assessments

Safety laboratory assessments were performed at the Department of Medical Biochemistry (Oslo University Hospital Rikshospitalet, Oslo, Norway), and included platelet count, red blood cell (RBC) count and indices, haemoglobin, haematocrit, white blood cell (WBC) count, iron variables (iron, ferritin, transferrin), blood urea nitrogen, creatinine, fasting glucose, insulin, C-peptide, potassium, sodium, calcium, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase (GGT), lactate dehydrogenase (LD), creatine kinase (CK), bilirubin, C-reactive protein, total protein and lipids, as assessed by colorimetric and/or enzymatic methods (Cobas c702 analyser, Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Urine pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite and leukocyte esterase were measured by an automated urine test strip reader (UC-3500 Sysmex, Kobe, Japan); urine RBC and WBC counts were assessed by a urine particle analyser (UF-5000 Sysmex, Kobe, Japan).

2.2.4 | Assays of mesna, cysteine and related sulphur metabolites

Plasma and urine mesna and tCys, and plasma concentrations of cysteine fractions and sulphur metabolites, were measured by liquid chromatography–tandem mass spectrometry, as described previously[30,31] and summarized in the supporting information.

2.2.5 | Pharmacokinetic and pharmacodynamic evaluations and statistical analysis

All statistical analyses and plots were performed in R version 4.2.2 in R Studio (R for Statistical Computing, Vienna, Austria) using the *drm* and *Pmetrics* packages.

Pharmacokinetic evaluations
Details of the PK assessments are outlined in the supporting information. In brief, non-compartmental analyses were performed and dose linearity was assessed by a linear regression analysis of dose versus $AUC_{0\text{-inf}}$.

Pharmacodynamic evaluations
Individual and mean curves for tCys concentration–time profiles were plotted using linear concentration scales. For the PD endpoint, nadir tCys concentration and the time to nadir following a single dose of mesna were determined. Percentage differences in tCys from baseline were calculated. The half maximal effective concentration (EC_{50}) of

mesna for tCys reduction at nadir was derived by fitting a four-parameter log-logistic dose–response curve. Log_{10}-transformed mesna was plotted against the percentage of maximal change in tCys at nadir.

For exploratory analyses across dose levels, AUC_{0-12h} for tCys and its fractions were calculated using the trapezoidal method. P_{trend} was calculated using linear regression, with $tCys_{AUC0-12h}$ as the dependent variable and dose level as the independent variable. Similar analyses were performed for the other sulphur amino acids.

3 | RESULTS

3.1 | Study 1: A proof-of-concept study in mice

3.1.1 | Effect of mesna on plasma tCys, body weight gain and composition

This study tested whether tCys-lowering by mesna can reduce diet-induced fat gain in mice.

Shifting the mice onto a HFD resulted in significant increases in body weight, fat mass, lean mass and body fat% in both the mesna and control groups by week 4 relative to baseline. At baseline, the mesna group by chance had significantly higher fat mass than the control group (Table S1), therefore analysis of the effect of mesna on body composition was adjusted for baseline measures (Figure 1). Although there were no differences between the groups after 4 weeks, mesna-treated mice appeared to gain weight at a slower pace compared with controls (EMM ± SEM at week 2: 4.76 ± 0.62 vs. 7.33 ± 0.59 g; week 4: 9.17 ± 0.55 vs. 9.53 ± 0.53 g; $P_{overall}$ = .039). Mesna-treated mice gained ~15% less fat compared with controls after 4 weeks (6.95 ± 0.35 vs. 8.19 ± 0.34 g; $P_{overall}$ = .002), but had similar lean mass gains ($P_{overall}$ = .14; Figure 1A). As a result, by week 4, mice receiving mesna had a lower rise in body fat% versus controls ($P_{overall}$ = .016; Figure 1A). Food intake did not differ between the groups (0.08 ± 0.02 vs. 0.07 ± 0.01 g/g body weight/24-h in the mesna and control groups, respectively; P = .56).

Terminal plasma tCys was significantly lower in the mesna group (mean ± SEM: 231 ± 9 μmol/L) than controls (283 ± 5 μmol/L; P < .001; Figure 1B). Cystine disulphide was also lower in mesna mice than in controls, whereas the reduced fractions of both cysteine and glutathione were higher (all P < .001). Liver tCys and glutathione did not differ between the groups (Figure 1B,C). Most individual free fatty acids were significantly increased in terminal plasma from mesna mice versus controls (Table S2), and the sum of free fatty acid concentrations was 11% higher in the mesna group than in controls (P = .027; Figure 1C).
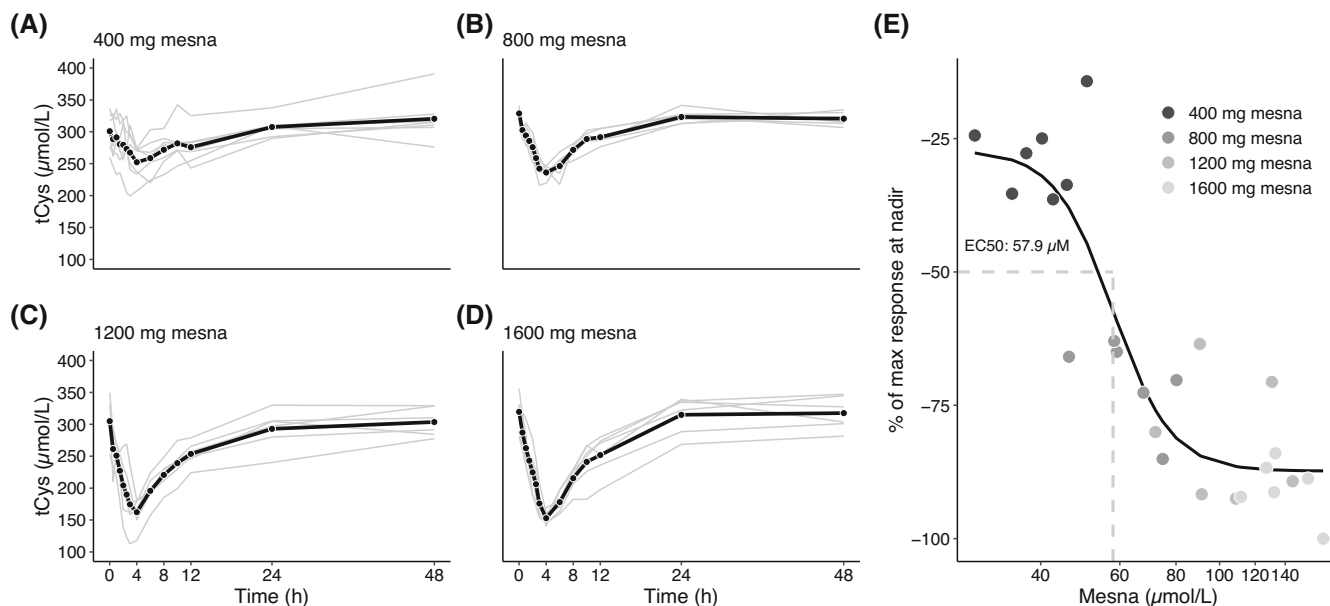
3.2 | Study 2: A phase I, non-randomized, single-centre, single ascending dose study in healthy men with overweight or obesity

3.2.1 | Characteristics

A total of 25 mesna dosages were administered to 17 men, of whom five participated at more than one dose level. The participants were healthy, as determined by physical examination and clinical laboratory tests (Table S3). Mean ± SD age was 43.5 ± 8.4 (range 28-55) years, weight was 107 ± 12.6 (range 81.3-133) kg and BMI was 32.7 ± 3.0 (range 27.4-37.3) kg/m^2. Mean ± SD plasma tCys before mesna dosing was 307 ± 26.2 (range 253-356) μmol/L.

3.2.2 | Pharmacokinetic variables

The plasma mesna pharmacokinetic variables are listed in Table 1. Mesna showed dose linearity in the range 400-1600 mg (R^2 = 0.92, beta = 0.15, P < .001) (Figure S1), and AUC_{0-inf} increased from mean ± SD 60 ± 13 mg*h/L (400 mg dose) to 238 ± 31 mg*h/L (1600 mg dose). The terminal half-life was 7.0 ± 2.1 hours for the 400 mg dose and 9.4 ± 0.3 hours for the 1600 mg dose. Figure S3 shows the mean individual plasma concentration versus time plots for each dose level.

TABLE 1 Plasma pharmacokinetic variables at four different single oral doses of mesna

	Oral mesna			
	400 mg	800 mg	1200 mg	1600 mg
C_{max} (mg/L)	9.3 (1.6)	19.5 (2.1)	28.8 (3.6)	34.3 (5.1)
C_{max} (μmol/L)	44.5 (9.8)	96.7 (12.7)	144.8 (21.8)	165.7 (30.8)
T_{max} (h)	3.6 (0.6)	2.7 (0.4)	2.8 (1.0)	3.0 (0.6)
$T_{1/2}$ (h)	7.0 (2.1)	7.7 (1.3)	9.3 (1.5)	9.4 (0.3)
AUC_{0-inf} (mg*h/L)	60 (13)	114 (23)	185 (10)	238 (31)
AUC_{0-inf} (μmol*h/L)	368 (82)	697 (143)	1127 (61)	1447 (192)
CL/F (L/h)	6.9 (1.5)	7.3 (1.7)	6.5 (0.3)	6.8 (1.0)
Vd/F (L)	67 (16)	81 (23)	87 (12)	93 (13)

Note: Values are mean (SD).
Abbreviations: AUC_{0-inf}, area under the plasma concentration–time curve from time 0 to infinity; CL/F, total clearance of the drug from plasma after oral administration; C_{max}, maximum (peak) plasma drug concentration; $T_{1/2}$, elimination half-life; T_{max}, time to reach maximum (peak) plasma concentration following drug administration; Vd/F, apparent volume of distribution.

FIGURE 2 Concentration–time profiles of the change in plasma total cysteine (tCys) up to 48 hours after a single dose of mesna, with four ascending doses from 400 to 1600 mg. Black curves represent the mean values within each dose level, and the grey curves are individual concentration–time profiles. A, 400 mg, $n = 7$; B, 800 mg, $n = 6$; C, 1200 mg, $n = 6$; and D, 1600 mg, $n = 6$. E, The half maximal effective concentration (EC50) of mesna for the percentage of max response in tCys-lowering at nadir. The dose–response curve was fitted using a log-logistic model. The various grey circles represent the individual responses within each dose level.

3.2.3 | Pharmacodynamic variables

Plasma tCys

Figure 2 shows the mean and individual plasma concentration profiles of tCys by ascending mesna doses (Figure 2A–D), and the half maximal effective concentration (EC50) of mesna. Mesna produced a rapid, dose-dependent decease in plasma tCys. With increasing dose, the total decrease in plasma tCys was larger and more sustained, till approximately 12 hours postdosing, before returning to baseline levels after 24 hours. Nadir tCys was reached at 4 hours at all dose levels. Nadir mean ± SD% difference in tCys from baseline ranged from 16.2% ± 4.5% at 400 mg to 52.0% ± 3.2% at 1600 mg. A tCys-lowering of 30% or more at nadir was achieved by 800 mg or higher doses, but not by the 400 mg dose (Figure 2, Table S4). The tCys-lowering of 30% or more was sustained for 6 hours after the 800 and 1200 mg doses, and for 8 hours following the 1600 mg dose. Mean tCys AUC_{0-12h} decreased with increasing dose levels (Figure 3) (P_{trend} across dose levels < .001). In summary, there was a dose–response lowering of plasma tCys at nadir from 400-1600 mg mesna, with more sustained lowering at 1600 mg.

Plasma tCys fractions

Similar to tCys, mesna induced a dose–response lowering of the plasma tCys fractions, protein-bound cysteine, free total cysteine and free oxidized cystine (Figure 3). The largest effect was observed for free oxidized cystine, where mean AUC_{0-12h} decreased across dose levels (P_{trend} < .001). The effect of mesna on free reduced cysteine was different to the remaining fractions, with an increase in reduced cysteine coinciding with the nadir tCys time point (4 hours) and a gradual decline thereafter.

Cysteine-related metabolites

The AUCs for methionine, S-adenosylmethionine, S-adenosylhomocysteine and cystathionine, as well as for total homocysteine and total glutathione and their fractions across mesna doses, are shown in Table S5. Mean AUC_{0-12h} increased for methionine ($P_{trend} = .033$) and cystathionine ($P_{trend} = .018$) across dose levels, and decreased for S-adenosylhomocysteine ($P_{trend} = .029$). Although there was no significant trend across dose levels for the other thiols besides tCys, the AUCs for total homocysteine (tHcy) and homocystine followed the same pattern as tCys and cystine, and were lower at the three upper doses than at the lowest dose. No clear patterns were found for glutathione (GSH) or its fractions.

3.3 | Cumulative and fractional renal excretion, and renal clearance of tCys, mesna and creatinine

The cumulative and fractional excretion and clearance of tCys, mesna and creatinine in 24-hour urine are shown in Figure 4 and Table S5. With increasing mesna dose, cumulative excretion of both tCys ($P_{trend} = .004$) and mesna ($P_{trend} = .007$) increased. There was increased tCys clearance across dose levels ($P_{trend} = .002$), while fractional excretion and clearance of mesna showed no trend across doses (Table S6). The estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI equation)[32] was within the normal range for all participants (median [range] eGFR [mL/min/1.73m^2] 116 [99.0-127] for 400 mg; 106 [89.5-118] for 800 mg; 107 [93.5-118] for 1200 mg; and 103 [91-119] for 1600 mg mesna).
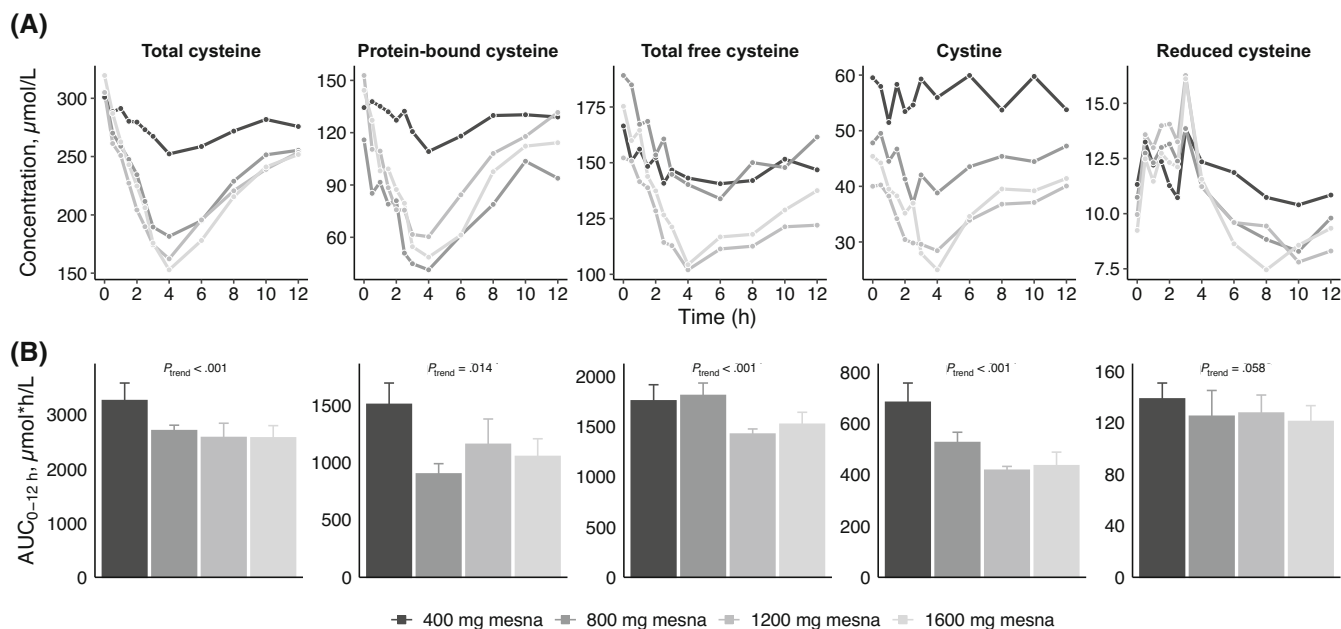
FIGURE 3 A, Concentration–time profiles of the change in plasma total cysteine (tCys) and its fractions including protein-bound cysteine, total free cysteine, free oxidized cystine and free reduced cysteine up to 12 hours after a single dose of mesna. The grey curves represent the mean concentration–time profile within each dose level, with four ascending doses from 400 to 1600 mg mesna. B, The bars are the mean (SD) estimated area under the plasma concentration–time curve time from 0 to 12 hours (AUC$_{0-12h}$) for tCys and its fractions after the four oral mesna doses.

FIGURE 4 Mean urinary excretion rates at the four oral mesna doses. The bars represent mean (SD) values for cumulative excretion (μmol and mg) of A, Mesna, B, tCys, and C, Creatinine at each dose. tCys, total cysteine.
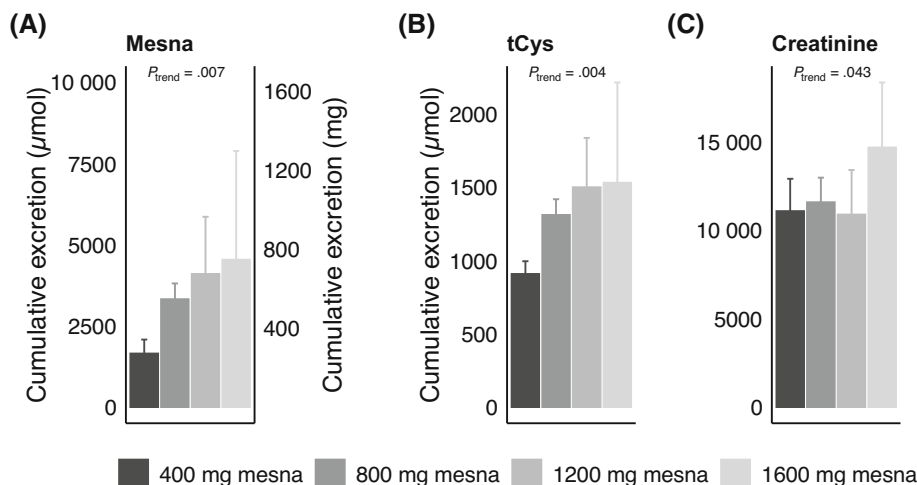
3.4 | Adverse events

In general, all mesna doses were safe and well tolerated. One participant experienced a moderate headache and mild tiredness within 12 hours of 400 mg mesna administration, which were not considered to be related to the study drug, but possibly to caffeine withdrawal. Laboratory adverse events that were possibly related to the drug occurred in 36% of participants in samples taken at 24 or 48 hours postdosing. These included elevated leukocytes in urine (n = 2, one after 400 mg mesna, one after 1200 mg mesna), traces of blood in urine (n = 5, one after 1200 mg mesna, four after 1600 mg mesna) and traces of protein in urine (n = 2, after 1600 mg mesna). These laboratory changes were not considered to be clinically significant.

4 | DISCUSSION

Building on consistent experimental and epidemiological associations of plasma tCys/cystine with obesity,[1–5,7,8,14,33] the current study shows that pharmacological lowering of plasma tCys by mesna administration in mice limits diet-induced fat gain. In healthy men with overweight or obesity, four ascending single doses of mesna were well tolerated and induced a dose–response increase in urinary tCys excretion, as well as lowering of plasma tCys and cystine. In previous cross-sectional population-based studies, 30% lower plasma tCys corresponded to approximately 7.5 kg lower average fat mass[4] and 2.3 kg/m² lower BMI.[2] In the current study, 30% lowering of tCys was achieved 4-8 hours postadministration of 800, 1200 and

1600 mg mesna, but not by 400 mg mesna. These findings provide the basis for testing whether long-term mesna administration can facilitate weight loss in humans.

The current study is the first to show that mesna in mice lowers plasma tCys and cystine, and protects against diet-induced fat mass gain. Consistent with epidemiological data that tCys is independently associated with fat mass but not lean mass,[4] the 4-week fat mass gain in mice fed a HFD was ~20% lower with mesna than without, without a reduction in lean mass gain. The overall improvement in body composition with mesna is attractive for the prospect of pharmacological weight loss in humans, where lean mass loss is a significant concern.[34] Importantly, this improvement occurred without lowering liver cysteine or glutathione; thus tissue cysteine availability appears to be preserved for its vital functions, including glutathione synthesis. Plasma-reduced cysteine and glutathione were higher in mesna mice than controls, suggesting a favourable shift in redox state.

The observed reduction of fat gain by mesna is consistent with experimental evidence that decreased cysteine availability leads to a lean phenotype. In human and animal studies, reduced cysteine and methionine intake results in weight loss.[11–15,21,35] CBS-deficient mice have low plasma cysteine and decreased fat mass, but preserved lean mass.[17,18] In early studies of mature adipocytes, lower extracellular cysteine was associated with greater lipolysis.[36] In the current study, plasma free fatty acids were increased in mesna-treated mice, suggesting increased lipolysis. This finding needs to be further investigated, and more work is needed to identify both the gross and molecular mechanisms underlying mesna effects on body adiposity. Animal dietary and transgenic studies reviewed in the Introduction collectively suggest that decreasing cysteine availability increases energy expenditure, and induces extensive remodelling of lipid metabolism that overall favours energy dissipation over storage[11–14,37]; this remains to be tested in relation to mesna-induced cysteine-lowering. The main molecular target mediating cysteine effects on fat mass remains elusive, but studies to date suggest key roles for the lipogenic enzyme stearoryl coenzyme-A desaturase-1[14,38,39] and the amino acid-sensing metabolic regulator fibroblast growth-factor-21.[35,40,41]

The human trial sought to determine an appropriate mesna dose to be tested for weight loss in humans, based on ability to lower plasma tCys. In a single-arm, open-label, dose-finding study, we evaluated nadir plasma tCys at ascending mesna doses in healthy men with overweight or obesity. Mesna doses of up to ~10 g/d have been used to limit the toxicity of chemotherapy in patients with cancer.[23] Some evidence across different studies suggested a dose–response effect of mesna in healthy normal-weight volunteers, where 1200 mg oral mesna (equivalent to 17 mg/kg in an individual with a body weight of 70 kg) lowered plasma tCys by 50%,[26] whereas a lower dose (10 mg/kg) decreased tCys by 25%.[25] The current study is the first to show a dose–response tCys-lowering effect of mesna in a homogeneous group of healthy men with overweight or obesity.

Based on data from cross-sectional studies showing substantial fat mass and BMI differences across a 30% difference in plasma tCys,[2,4] a 30% lowering of tCys at nadir was considered the primary endpoint in the current trial. A 30% tCys-lowering was achieved by each of the 800, 1000 and 1200 mg doses of mesna, and was sustained for 6-8 hours. At 12 hours, tCys was approximately 16%-21% lower than at baseline for these three doses, and returned to baseline 24 hours after dosing. The optimal mesna dosing regimen for sustained lowering of tCys has yet to be investigated, where there may be a cumulative benefit from repeated daily dosing. Conversely, when cysteine is depleted by diet or drugs, compensatory mechanisms may be activated to maintain plasma tCys.[42,43] In the current trial, mesna increased plasma cystathionine in a dose–response manner, suggesting a compensatory increase in transsulphuration and hence cysteine synthesis. However, in the animal study, plasma tCys was low after 4 weeks of mesna treatment. To determine whether cysteine-lowering treatment has clinical implications for human weight loss, the effect of repeated mesna dosing over several weeks on safety, plasma tCys and obesity-related variables warrants investigation.

Secondary trial endpoints included the effects of mesna on circulating tCys fractions, and on sulphur-related metabolites. Free cystine disulphide followed the same pattern of decline in postmesna administration as tCys, which is attractive in the light of studies showing that cystine is the main fraction associated with fat mass in humans[8,9] and enhances adipogenic differentiation and lipid accumulation in animal and human preadipocytes in vitro.[7,8] Free total cysteine (comprising the disulphide and reduced sulphydryl fractions) increased for up to 3 hours after mesna administration, and then decreased. The initial increase in free cysteine is probably explained by this fraction being liberated by mesna, before excretion in urine and/or uptake by cells. The reduced cysteine fraction exhibited a smaller total decrease than tCys or cystine, and in fact, initially increased at 4 hours, corresponding with nadir tCys. The rise in reduced cysteine is in line with the known thiol-reducing action of mesna,[25,26,44] and with the higher plasma reduced cysteine in mice treated with mesna than in controls in the current study. Urinary cysteine excretion increased in a dose-dependent fashion following mesna administration, documenting one mechanism of cysteine-lowering by mesna. Based on one early study reporting increased intracellular cysteine 30 minutes postmesna infusion in two subjects,[26] we anticipate that mesna may additionally affect translocation of cysteine across cellular and extracellular compartments, but this warrants further investigation.

Mesna has an adverse effect profile that is similar to other thiol compounds, including the potential for severe skin reactions and other hypersensitivity reactions.[23] The risk of other adverse effects for a target population of otherwise healthy individuals with obesity was considered low, as mesna is usually co-administered with ifosfamide, and at much higher doses than in the current study. We did not observe any serious adverse effects in the current study. However, one outlier with a high increase in creatinine excretion was observed at the 1600 mg mesna dose. This finding did not represent a consistent pattern at the highest dose, and eGFR was within the normal range for all participants, suggesting that the outlier's increase in creatinine was not associated with a clinically relevant decline in renal function.

The strengths of this trial include the rich postdose sampling providing 13 points for PK/PD investigation, and measurement of

the different thiol fractions in plasma that was immediately acid-precipitated following withdrawal, to preserve the relative concentrations of free and protein-bound cysteine forms. Strict inclusion criteria and administering a standardized diet on the first day minimized confounding caused by subject characteristics and diet. The inclusion of men only further prevented confounding caused by sex or by the possible effect of cyclic variation in female sex hormones on the outcomes measured, but the effect of mesna on cysteine-lowering in women will need to be shown. As per the standard study design in this type of study, the comparatively small sample size is associated with large variations in the assessment of PK and PD variables.

To the best of our knowledge, this is the first study to show that oral mesna lowers plasma tCys in healthy men with overweight or obesity in a dose-dependent manner. Further testing of mesna for multiple dosing is required before a randomized, placebo-controlled trial to determine whether mesna can aid weight loss.

AUTHOR CONTRIBUTIONS

KJV, TO, RDC, HR, KR, and AE conceptualized and designed the study. KJV, TO, HKZ, NEB, ES, AFD, RDC, and AE collected data. KJV, TO, AÅ, and AE were responsible for data analysis. KJV, TO, and AE drafted the manuscript. All authors contributed to interpretation of the results, preparation and review of the manuscript, and approval of the final manuscript for publication.

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CONFLICT OF INTERESTS STATEMENT

The authors declare that they have no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.