STUDY TITLE: *The effect of intraoperative N-acetylcysteine on hepatocellular injury during laparoscopic bariatric surgery. A randomised controlled trial.*

INVESTIGATIONAL MEDICINAL PRODUCT: *N-acetylcysteine*

INDICATION: *intraoperative hepatocellular protection*

A single blinded randomised controlled trial of intraoperative N-acetylcysteine given to morbidly obese patients undergoing laparoscopic weight loss surgery

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This study was conducted in compliance with Good Clinical Practice.

FINAL CLINICAL STUDY REPORT 1ST NOVEMBER 2012

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1. LIST OF ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index (in kg/m^2)
CK-18	cytokeratin 18
CRF	case report form
CRP	C-reactive protein
ELISA	enzyme linked immunosorbent assay
FasL	Fas Ligand
GBP	Gastric Bypass (roux-en-Y)
GPX	glutathione peroxidase
HA	hyaluronic acid
HOMA-IR	Homeostasis Model Assessment of insulin resistance
ICF	Investigation Consent Form
IL-10	interleukin 10
IL-6	interleukin 6
IMP	investigational medicinal product
INR	International Normalised Ratio of prothrombin time
LFTs	Liver Function Tests
LSG	laparoscopic sleeve Gastrectomy
NAC	n-acetylcysteine
NAFLD	non alcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	non alcoholicsteatohepatitis
NICE	National Institute of Clinical Excellence
PIS	Patient Information Sheet

PLT	Platelets
RNA	RiboNucleic Acid
SAE	Severe Adverse Event
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
TNFα	tumour necrosis factor α
TRAIL	TNF-related apoptosis-inducing ligand
WCC	White Blood Cell Count
WLS	Weight Loss Surgery (ie bariatric surgery)

2. ETHICS

2.1 Ethics Committee Review

The study protocol was reviewed by the King's College Hospital Research Ethics Committee. Subsequent protocol revisions were also reviewed by the same committee.

2.2 Statement of Ethical Conduct of Study

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and are detailed in Good Clinical Practice (GCP).

2.3 Patient Information and consent

All study participants were included after taking fully informed consent at the time of pre-screening and before any study-specific interventions were undertaken.

A copy of the Patient Information Sheet and Consent Form are provided in Appendix B.

3. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Chief Investigator/Principal Investigator: Mr Ameet Patel Consultant Surgeon

Co-ordinating Investigator: Mr Ajay Belgaumkar, Clinical Research Fellow

Co-investigators: Ms Kirstin Carswell, Clinical Research Fellow

Laboratory co-investigators: Dr RagaiMitry

Dr Robin Hughes

Professor Anil Dhawan

Trial Steering Committee Members: Mr Ameet Patel

Mr Ajay Belgaumkar

Ms Kirstin Carswell

KHPClinical Trials Office Monitors Ms Hannah Mason

Ms Ingrid Brumarescu

KHP Clinical Trials Office Quality Manager Ms Jackie Pullen

4. INTRODUCTION

4.1 Clinical Question

Can intraoperative administration of N-acetylcysteine improve patient outcomes after laparoscopic bariatric surgery by reducing hepatocellular damage and post-operative inflammatory responses?

4.2 Hypotheses

i) Administration of N-acetylcysteine before and during the period of liver retraction will prevent or decrease the degree of liver damage, due to ischaemia-reperfusion injury during laparoscopic bariatric surgery.

ii) Reduced hepatocellular damage may decrease systemic inflammatory response, as assessed by serum markers, and post-operative complications.

NAC improves the liver's tolerance to surgical trauma as demonstrated at a cellular level, including processes involving apoptosis, inflammation, fibrosis and oxidative stress.

4.3 Background

Bariatric Surgery

Weight-loss (bariatric) surgery involves changes to the anatomy of the digestive tract, limiting the amount of food intake and decreasing the absorption of the digested food into the bloodstream. Morbid obesity (body mass index > 35 kg/m2) is associated with diabetes mellitus, hypertension, hyperlipidaemias and fatty liver disease, comprising the metabolic syndrome [1]. The development of resistance within the peripheral tissues to the effects of insulin is a key factor leading to metabolic syndrome [2]. Depending on type of surgery and patient compliance, expected sustained weight loss could range from 30 to 80% of excess body weight (as calculated with a body mass index, BMI, of 25 kg/m^2) at 2 years post-surgery [3]. This compares with medical treatments for obesity, which are expected to produce 5 to 10% reductions in excess body weight. Surgery can be offered to patients with a BMI over 40 kg/m^2 or in patients with a BMI over 35 kg/m^2 who have associated complications, such as diabetes, hypertension, sleep apnoea and life-limiting arthritis[4].

Over 120 operations are performed annually at King's College Hospital. These operations are performed laparoscopically (keyhole). The main types of surgery performed at King's are:-

- adjustable gastric banding, where an inflatable band is placed around the top of the stomach, restricting the amount of food that can be eaten
- Roux-en-Y gastric bypass, where the volume of the stomach is reduced, restricting intake, with a Roux loop of small intestine is joined to the small stomach pouch, bypassing the duodenum and upper small intestine and reducing the length of bowel available for nutrient absorption
- sleeve gastrectomy, where the stomach is fashioned into a narrow tube by excising the greater curve, restricting intake

Laparoscopic surgery has been shown to provoke less post-operative inflammatory changes than open surgery [5]. Post-operative systemic inflammatory response has multiple aetiologies [6]. Surgical tissue trauma, with a local inflammatory response, has an important role in generating a systemic response. The systemic inflammatory response can lead to organ dysfunction, including respiratory, renal and immune dysfunction. Surgery in patients with morbid obesity is associated with a higher rate and severity of complications [7]. Even so, bariatric surgery is recognised as an effective

route to weight loss, leading to a regression of the features of metabolic syndrome. Bariatric surgery is recommended in the UK by the National Institute of Clinical Excellence (NICE) [4].

Fatty Liver Disease

Over 80% of patients undergoing bariatric surgery have fatty liver disease, compared with up to 15% of the non-obese population [8]. The condition is related to insulin resistance [9]. Non-alcoholic fatty liver disease (NAFLD) is characterised by hepatocyte accumulation of fat, with or without associated inflammation and fibrosis, in patients who do not consume more than 20 grams per day of alcohol or have viral hepatitis or metabolic storage disease, such as haemochromatosis. Diagnosis of NAFLD is made histopathologically, with liver biopsy. There is no consistent relationship between serum liver function tests and extent of fatty infiltration, fibrosis and inflammation. The severity of liver damage ranges from simple steatosis (bland fatty infiltration) to inflammation (steatohepatitis, NASH), fibrosis and liver cirrhosis, sometimes requiring liver transplantation. Up to 37% of obese individuals will have features of NASH [8]. Of these, a fifth may progress to liver cirrhosis. The damage to the liver is believed to be a "two-hit" phenomenon [10], where the fat deposits in the liver cells cause oxidant-mediated damage, leading to production of cytokines which cause inflammation and then fibrosis. Fatty liver is more vulnerable to damage from toxins, ischaemia and infections. These insults may trigger further liver injury, precipitating liver failure.

Liver injury during bariatric surgery

During laparoscopic surgery, the left lobe of the liver is retracted to expose the stomach. Obese patients have especially large livers. Liver retraction squeezes the blood supply, which causes the liver to be partially starved of oxygen (ischaemia). When the retractor is released at the end of surgery, the restoration of a normal oxygenated blood supply can also cause tissue damage (ischaemia-reperfusion injury). The combination of mechanical trauma and reperfusion injury is sufficient to cause derangement of post-operative liver transaminases, with up to a six-fold rise in aspartate transaminase and alanine transaminase from baseline levels [11]. Serum liver enzyme levels return to baseline within 72 hours.

The consensus is that bariatric surgery can improve fatty liver disease [12]. However, it is possible that liver damage may worsen transiently when the liver suffers ischaemia

intra-operatively. This injury can trigger a systemic inflammatory response and jeopardise the recovery of the patient. The directly attributable clinical effect of intraoperative hepatocellular damage on post-bariatric surgery outcomes has not been quantified. Clinical outcomes may include post-operative pyrexia, renal failure, acute respiratory distress syndrome and hypotension. Up to 10% of bariatric surgery patients may have clinically significant post-operative complications [3], leading to additional allocation of health resources and an increase in hospital stay. Obesity and steatosis is an independent risk factor in the development of complications after liver resection[13] and transplantation [14]. In the longer term, there are case reports of acute liver failure occurring following bariatric surgery, in patients with NASH [15] and cirrhosis [16]. Follow-up studies with post-operative liver biopsies have confirmed resolution of steatosis, but with varying changes in the degree of inflammation and fibrosis [12].

Assessment of liver injury

Ischaemia and ischaemia-reperfusion injury mediate their effects through the production of reactive oxygen species, leading to direct cellular damage, oxidative stress and production of pro-inflammatory stimuli [17]. In turn, this can trigger the systemic inflammatory response. Hepatocellular injury leads to a local response as well, creating a pro-inflammatory, pro-fibrogenic environment (see Figure 1). Both systemic and local changes can be detected in the serum [18], using biomarkers for inflammation (eg. interleukins 6 and 10, tumour necrosis factor alpha, C-reactive protein), fibrosis (eg. tumour growth factor beta, hyaluronic acid), oxidative stress (eg lipid peroxidation status, nitric oxide) and apoptosis (programmed cell death, eg cytokeratin 18, Fas ligand). The expression of genes within the liver controlling these processes of inflammation, fibrosis, oxidative stress and apoptosis can be analysed using molecular biology techniques, including quantitative real-time polymerase chain reaction and microarray technology [19].

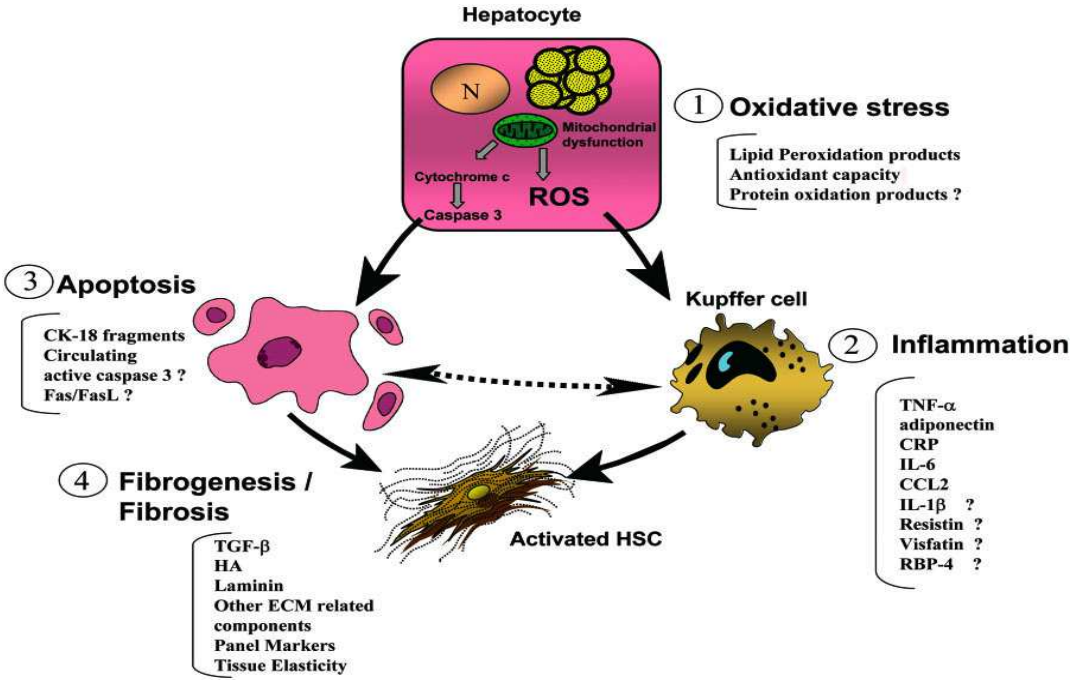


Figure 1. The cellular processes involved in the liver injury that leads to non-alcoholic steatohepatitis. Hepatocyte steatosis is a prerequisite for the subsequent events that lead to liver injury and fibrosis. Many of the biomarkers mentioned are still experimental. Reproduced from Wieckowska et al, Hepatology 2007[18]

N-acetylcysteine

N-acetylcysteine (NAC) is a pharmacological agent, approved for use in the prevention of radiological contrast induced nephropathy and the management of paracetamol overdose. It is a derivative of the amino acid cysteine and acts as a precursor to glutathione, an important antioxidant [20]. It also acts directly as a free radical scavenger [21]. It is used widely as a treatment for paracetamol poisoning, where intracellular glutathione is depleted, leading to hepatocellular necrosis and liver failure. Its effects as an anti-oxidant have been established in vitro and in animal studies [21]. It has also been shown to cause an increase in hepato-splanchnic blood flow [22]. N-acetyl cysteine (NAC) has been shown to improve the metabolic function of hepatocytes when given as an infusion in fatty livers rejected for orthotopic transplantation[23]

There have been a number of studies looking at the effect of N-acetylcysteine in critical care settings, including randomised controlled trials in severe sepsis[24], coronary artery bypass grafting[25], liver transplantation[26] and acute pancreatitis[27]. These studies have not shown a significant beneficial clinical effect. However, these small trials have not

been performed specifically in an obese population. Moreover, only severely ill patients, with corresponding poor prognoses, have been recruited into these trials. Given the multiple variables within this severely ill population, the beneficial effect of NAC is likely to be small and therefore, the studies have been underpowered. No sub analyses looking specifically at obese patients have been possible.

Why perform this randomised trial?

No study has specifically looked at the role of N-acetylcysteine in a morbidly obese population. These patients have a propensity to develop an exaggerated inflammatory response to surgical trauma[7] and are more vulnerable to oxidative stress[10]. With an increasing proportion of the population being obese, the prevalence of obesity-related post-operative complications will escalate. N-acetylcysteine's role as an anti-oxidant and free radical scavenger may have a protective effect within this population, ameliorating the intra-operative insult to the fatty liver. In vitro data demonstrate a significant benefit from N-acetylcysteine for hepatocytes derived from fatty livers. In vivo, the effect of NAC may reduce immediate post-operative complications and also prevent further deterioration long term in the subset of patients with NASH. The use of NAC may be extended to other obese patients undergoing liver surgery, reducing the burden of post-operative complications on the health service.

4.4 Aims and Expected Outcomes

Primary outcome measures

To quantify the decrease in extent of hepatocellular damage and its clinical effect in NAC as measured by: -

- Difference in liver function tests from before and after surgery, specifically AST and ALT as markers of hepatocellular damage (ALT is more specific to liver)
- Extent of hepatocellular damage, as assessed by histopathological criteria
- Decrease in post-operative morbidity and length of hospital stay

Secondary outcome measures

To elucidate the mechanism by which NAC decreases hepatocellular damage using

- Genetic (molecular biology) analysis
- Serum biomarkers of inflammation, fibrosis, apoptosis and oxidative stress

5. METHODOLOGY

5.1 Details of Trial Protocol

5.1.1 Trial design

This is a prospective randomised controlled trial investigating the effects of N-acetylcysteine.

5.1.2 Study groups

1: Treatment Arm - NAC

2: Control Arm

5.1.3 Recruitment

Patients were seen in the Multidisciplinary Morbid Obesity Clinic or Pre-operative Assessment Clinic at King's College Hospital, who are being referred for morbid obesity surgery.

5.1.4 Inclusion Criteria:

Male or Female

Age range 18 to 75 years inclusive

Patients must meet the criteria set out by NICE for morbid obesity surgery, that is they must have BMI >40kg/m2 or >35kg/m2 with obesity-related complications, and are undergoing either Laparoscopic Adjustable Gastric Banding or Roux-en-Y Gastric Bypass or Laparoscopic Sleeve Gastrectomy

Fully informed, written consent obtained prior to enrolment into this study.

5.1.5 Exclusion Criteria:

Patients undergoing Duodenal Switch

Patients undergoing OPEN bariatric surgery (a small minority of total patients)

Pregnancy

History of chronic liver disease, including viral hepatitis, haemochromatosis, alcoholic liver disease or known alcohol intake 28 units per week

Previous liver surgery, eg resection, orthotopic transplantation

History of active psychiatric illness, including severe depression, bipolar disorder, schizophrenia and eating disorders

Bleeding tendency or anticoagulant medications

Known allergies to N-acetylcysteine or related compounds

5.1.6 Randomisation

To NAC or non-NAC using a sequential sealed envelope method. The research team opened the allocation envelope a minimum of 2 hours before surgery to allow time for Pharmacy to dispense the NAC infusion.

5.1.7 Blinding

The operating surgeon and anaesthetist were aware if the patient receives NAC infusion as it is easily distinguished due to its bright colour and pungent odour.

The patients were blinded to the intervention.

5.1.8 Trial interventions:

Subjects randomised to the intervention received N-acetyl cysteine infusion, at a standard 150mg/kg in 200ml 5% dextrose over 15mins at induction of anaesthesia followed by an infusion of 50mg/kg in 500mls of 5% dextrose during surgical retraction of liver for a maximum of 4 hours, together with standard anaesthetic medications.

The maximum dose was limited to 16.5g for loading dose and 5.5g for infusion, making a maximum total dose of 22g per study participant.

The Investigational Medicinal Product (IMP), N-acetylcysteine was sourced as

Acetylcysteine 200mg/ml and supplied from Aurum Pharmaceuticals Ltd, Bampton Road, Harold Hill, Romford, Essex RM3 8UG Product License/ UK Marketing Authorisation: 12064/0026.

IMP was handled according to the Medicines for Human Use (Clinical Trial) Regulations 2004 and amended in 2006.

The control subjects received standard anaesthetic medications alone.

See Sample Collection 5.1.10 for details of tissue samples taken intra- and peri-operatively.

5.1.9 Standard Treatment of patients

Patients were treated as per routine practice. Although the inclusion criteria included adjustable gastric banding and Roux-en-Y gastric bypass, only patients undergoing Laparoscopic Sleeve Gastrectomy were included in this study. This was because a concurrent study in the unit was recruiting patients undergoing gastric bypass, so none could be recruited before the closure of the study.The LSG was performed by a single experienced surgeon (Ameet Patel, CI) for all patients. Six trocars were utilised and LSG was performed with standard techniques using a 38-Fr bougie. A 12mmHg carbon dioxide pneumoperitoneum was created. A liver biopsy from the left lateral section of the liver was taken using a Tru-Cut cannula, taking 2 or 3 passes in order to obtain cores of liver tissue at a minimum of 1cm in length. A fixed Nathanson liver retractor was placed under the left lateral section of the liver to facilitate clear visualisation of the hiatus. After devascularisation of the greater curvature using an ultrasonic coagulating dissector, the LSG commenced 4–6 cm proximal to the pylorus on the greater curvature and continued towards the angle of His. The completed staple line was reinforced with absorbable sutures. A further liver biopsy from the left lateral section was taken before release of pneumoperitoneum. Haemostasis was ensured before the operation was completed. Blood loss was recorded by measuring volume of effluent in the suction lavage apparatus. Post operatively, all patients received anticoagulation therapy with low molecular weight heparin and wore compression stockings for embolism prophylaxis.

Gastrograffin meal contrast radiography was performed for all patients and methylene blue dye tests were completed on patients with post-operative drains in situ to detect gastric leak. Post-operative dietary education was provided by qualified dieticians. Post-operative diet consisted of fluids only for 4 weeks, puréed consistency foods for a further 2–4 weeks, followed by soft foods for an additional 4 weeks, then the gradual return to normal foods in reduced portions.

5.1.10 Sample Collection:

1) Serial serum samples taken (i) before surgery, (ii) at end of surgery, (iii) at 24, 48, 72 and 96 hours post-operatively, (iv) at outpatient follow-up 6 weeks after surgery, (v) at outpatient follow up 6 months after surgery.

Blood samples are taken routinely at these time points for standard biochemical and haematological testing.

A further 10 mls of blood will be taken as part of the study protocol - serum will be prepared from whole blood and then aliquots will be stored in cryotubes and immediately stored at –80^{o}C in a secure fridge within Institute of Liver Studies for future analysis. These samples will be analysed using ELISA techniques for serum biomarkers, including CK-18, IL-6, IL-10 and iNOS. Aliquots of serum will also be sent to the biochemistry laboratory for standard liver function tests.

2) Liver biopsies – Trucut, 16G from left lobe of liver taken (i) intraoperatively before onset of liver retraction, (ii) 10 minutes after the release of liver retraction.

Needle biopsy of the liver is performed routinely as part of our standard practice to ascertain the extent of fatty liver disease and to diagnose steatohepatitis (NASH). A further biopsy will be taken at the end of surgery as part of the study protocol.

The samples will be divided into three:- (1) sample fixed immediately in formalin for histopathology evaluation, (2) sample snap frozen to –80^{o}C, (3) sample fixed on a TissueTek gel plate and snap frozen to -80^{o}C.

All of the samples were stored in a secure freezer within the Institute of Liver Studies, according to the guidelines set out in the Human Tissue Act (2004).

5.2 Discussion of Aspects of Study Design

5.2.1 Choice of Control groups

This was a straightforward decision as we merely chose patients given standard perioperative care as the control, to assess if the IMP had any efficacy.

5.2.2 Selection of Study Population

The trial design was deliberately pragmatic and we sought to allow inclusion of as great a proportion of our adult bariatric surgery population as was practicable. NICE guidelines dictate which patients are eligible for bariatric surgery funded by the NHS and therefore it was necessary to stipulate that this was the main inclusion criterion. Given that the main outcome measure concerned assessment of liver function and damage, it was natural that patients with pre-existing diagnoses of liver disease were excluded. As described in the introduction, NAFLD is defined by the absence of other causes of hepatocellular injury and so these diagnoses were also included as exclusion criteria. The practicalities of recruitment to a clinical trial meant that patients with active psychiatric illness were excluded. Nevertheless, many patients with morbid obesity have a history of psychiatric problems, especially eating disorders, depression and the sequelae of previous psychological abuse. It was therefore necessary not to exclude all patients with any psychiatric illness.Theimportance of minimizing any potential unforeseen harm from the administration of the IMP (however unlikely) or from the second liver biopsy meant that elderly patients (defined here as >65 years), pregnant women and those with allergies were naturally excluded. Of course, it is unlikely that any pregnant women would undergo a non-life saving major operation until after delivery.

5.2.3 Removal of patients from therapy or assessment

Most patients undergoing WLS are being treated for ongoing medical problems, often the complications of morbid obesity and the metabolic syndrome. Therefore, it was important both clinically and from the point of setting the trial in real-world conditions, that any regular medications were continued as per routine practice. Patients taking anticoagulant medications were excluded from recruitment.

5.2.4 Method of assigning patients to treatment groups

Patients were randomized in blocks of 10 to the two study groups. This was chosen in order to maintain relatively balanced recruitment to each throughout the planned trial, in case the trial had to be stopped before the full number of patients had been recruited. The sequential sealed envelope method was employed as this was the easiest practical method, as well as being the cheapest. There was no specific funding available to use a third-party remote allocation system. Great care was taken to ensure that the envelopes were kept safely. The drawbacks of sequential sealed envelope randomisation are well recognized. Randomisation took place a few hours before surgery or on the last weekday before surgery. Our initial protocol had stated it would take place when the patient entered the anaesthetic room. However, there was a requirement for the IMP to be dispensed from the main Pharmacy department after the necessary checks and labelling issues were dealt with by the Trials Pharmacy team. It became apparent that this process did take some time and therefore the initial plan was not practical.

5.2.5 Selection of doses in the study

We used the standard dosage schedule for adult patients being given their first loading dose and subsequent dose of NAC, as described in the Summary of Product Characteristics and British National Formulary. We surmised that this dosage schedule had a long and identifiable track record for safety and efficacy for the recognised indications of NAC therapy, so was most appropriate for this trial.

5.2.6 Selection and timing of dose for each patient

In this study, the timing of the injury to the liver was intraoperative. We would expect that with release of both liver retraction and pneumoperitoneum, the direct traumatic and ischaemic insult to the liver, due to compression and then oxidative stress, would cease. Therefore we decide to the design the trial to look for the efficacy of a one-off intraoperative NAC infusion, rather than continuing NAC therapy for a sustained time period post-operatively. We concede that other trials looking at the effect of NAC have used longer dosage schedules as the investigators had postulated that the oxidative stress insult would exist for an extended time. However, we felt this could be assessed in subsequent studies, depending on our findings here.

5.2.7 Blinding

As stated in the protocol, we did not believe multi-level blinding was practical for this trial or essential in preventing bias. In the first instance, blinding the operating team would have required either an active placebo infusion, with a similar appearance and pungent smell to the IMP or for the infusion to be administered in some "secret" fashion, which is not possible within the operating theatre environment.In any case, the outcome measures were objective, numerical values derived in a laboratory setting and therefore not particularly susceptible to observer bias.

In ideal circumstances, this would have been a double-blind trial but we did not have the resources or wherewithal to perform such a study.

5.2.8 Prior and concomitant therapy

As stated above, this trial was designed pragmatically to include a representative sample of patients undergoing WLS. Most morbidly obese patients have concomitant medical diagnoses, and the development of these diseases (as part of the associated metabolic syndrome) is often the precipitant for the these patients to seek WLS. Therefore, we did not wish to stop patients from taking their regular medications, most often for diabetes, chronic pulmonary disease, cardiac ischaemia and hypertension, unless indicated as part of our standard perioperative practice.

5.2.9 Treatment compliance

IMP was administered whilst patients were anaesthetised by a Consultant Anaesthetist, and therefore there was no question of patients being unable to comply with taking NAC.

5.3 Efficacy Variables

The following patient specific data were collected as part of the study:-

5.3.1 Patient Demographics and medical history

Age, gender and ethnicity

Complete medical history was recorded in the patient's medical notes.

Clinical data

Pre-operative: weight, height, BMI, Drug History, physical examination and vital signs

Operative: type of operation, time of retraction, operating time, blood loss, anaesthetic and operative complications

Post-operative: Length of stay, complications

Clinical observations, including haemodynamic parameters, urine output

Follow-up: weight, change in medications, complications(see Appendix B), changes in physical examination and vital signs

Although all of the above data was collected in the Case Report Forms, at the time of analysis it was felt that the most important outcome variables in analysis of trial efficacy related to change in BMI, operative details, length of stay and post-operative complications. Concomitant medications were recorded carefully in case of any obvious patterns of adverse events or drug interactions. However, none of these were evident so a detailed analysis of concomitant medications is not provided herein.

5.3.2 Specimen data

Serum and Plasma

- Biochemistry LFTs, CRP, Lipid profile, Renal function

  Insulin resistance (fasting insulin X fasting glucose/ 22.5 - HOMA index)

- Haematology Full Blood Count

The above were performed according to standard techniques by the King's College Hospital Biochemistry Department, on an automated multi-analyser, the Advia 2400 (Siemens Healthcare Diagnostics, Camberley, Surrey, UK).

Although the above were recorded in the CRF, the most important markers of hepatocellular damage and inflammatory change included ALT, AST, CRP, White cell count and platelet count. These outcomes are analysed herein. Changes in insulin resistance, measured by HOMA index, are also described. Other elements of liver function, such as bilirubin, albumin and INR, were not analysed in detail as they were not appropriate outcome measures in this study.

- Biomarkers inflammation: CRP (see above), IL-6, IL-10, TNFalpha,

  Adipocytokines

  Apoptosis: CK-18 fragments, TRAIL and FASligand

  Oxidative stress: TBARS, SOD, GPX, protein carbonyls

  Fibrosis: hyaluronic acid

These markers were measured using commercially available assays, according to the manufacturer's instructions. All samples assayed were run in duplicate and their concentrations were determined, with the aide of the manufacturer's standards, provided with each kit.

IL-6, IL-10, adiponectin, resistin and leptin: Fluorokine® MultiAnalyte Profiling Kit (R&D Systems, Minneapolis, USA) and the results were read on a Luminex® Analyzer (Luminex B.V., Oosterhout, The Netherlands).

CK-18 M65 and M30 ELISA (Peviva AB, Sweden)

Oxidative Stress Markers – ELISA and assay kits (Alexis Biochemicals, USA)

Hyaluronic Acid ELISA (TECOmedical, Switzerland).

The above markers were chosen as appropriate measures of inflammation, apoptosis and oxidative stress.

5.3.3 Liver tissue

- Histopathology NAFLD score

Biopsies were paraffin fixed, embedded and sections were stained with hae- matoxylin and eosin, reticulin, Orcein and Perl's. A diagnosis of NAFLD was made by a hepato-histopathologist on the basis of typical histological findings in the appropriate clinical setting and following exclusion of other liver disease. The pathologist also classified the biopsy as consistent with NASH or as NAFLD without evidence of NASH.

Liver biopsy scoring

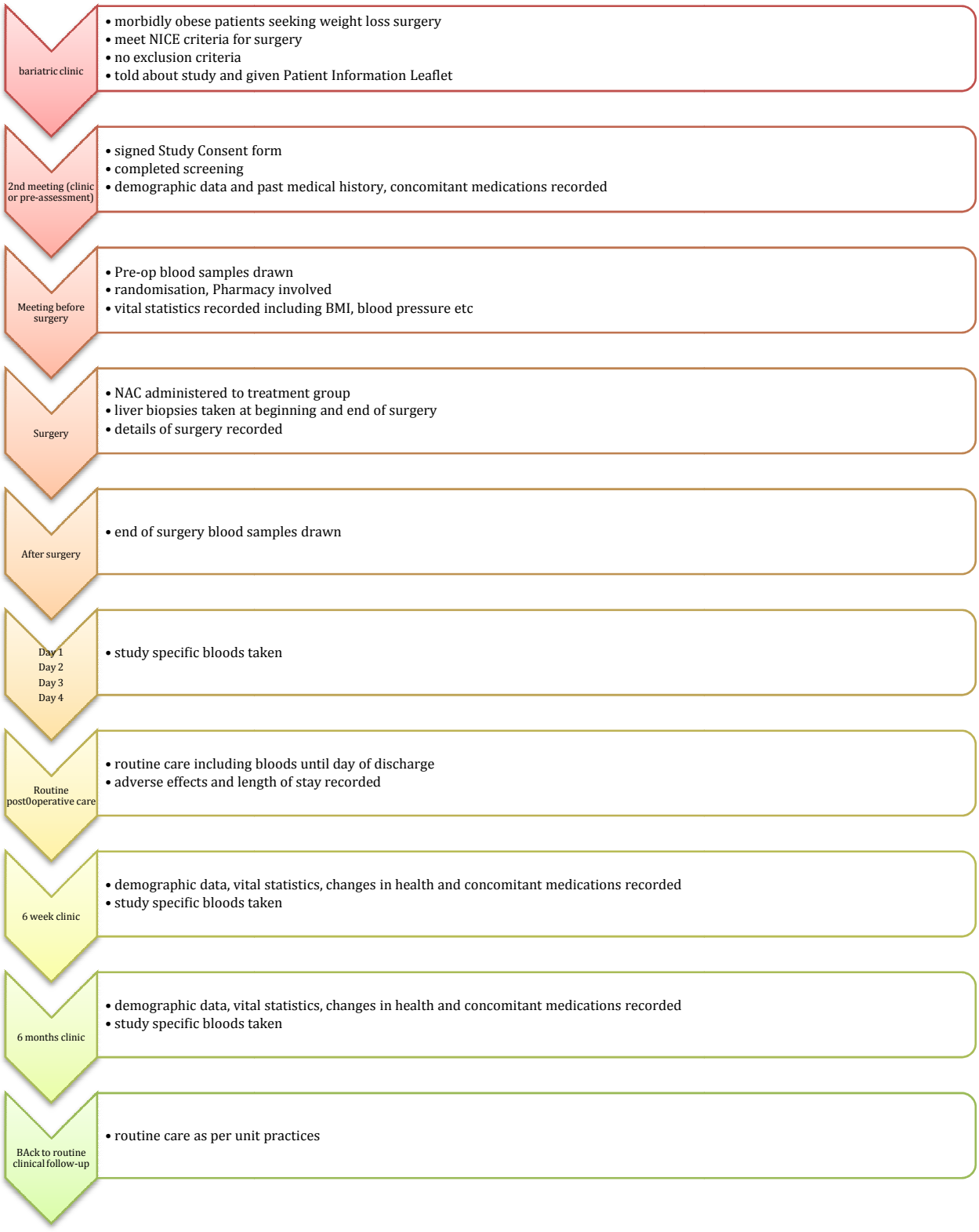
In order to differentiate between differing severity of disease, histological specimens were scored according to NAFLD activity score (NAS) and also a modified Suzuki score, indicating evidence of cellular trauma.

- Genetic Analysis

Gene expression profiling of microRNAs

RNA was extracted from liver tissue using miRNAeasy Extraction Kit (Qiagen®, USA). The RNA was hybridised onto Genechip® miRNA Arrays (Affymetrix®, USA). Unfortunately, the high costs of these materials and microArrays meant that we were unable to complete this part of the study and therefore no relevant results are presented in this report.

Figure 2: Flow sheet of Study specific interventions



5.4 Safety Variables

Participants were looked after intra-operatively by an experienced Consultant Anaesthetist for the duration of their general anaesthetic. They had continuous monitoring of vital signs, including cardiorespiratory parameters, together with the clinical status of the patient. Medications to be used for general anaesthesia included remifentanil, sevofluorane, fentanyl and morphine, rocuronium, glycopyrrolate and propofol.

Post-operatively, participants were nursed on either a High-dependency Unit or General Surgery Ward by an experienced medical and nursing team, under the supervision of a Consultant General Surgeon in King's College Hospital. There was round-the-clock full access to a range of support services, including radiologists and critical care physicians.

Patients' recovery was continuously supervised by the ward nursing team, with continuous access to in-house medical teams. Daily morning ward rounds were conducted, as well as ad-hoc reviews as appropriate. Daily monitoring of renal and liver function and haematological indices was performed as part of standard care.

Where adverse events occurred, the supervising Consultant Surgeon was made aware of any problems and directly involved in the response to these issues, as and when they arose.

Adverse events have been defined in advance in the protocol and are listed below. Please note that that post-operative pain was not defined to be an adverse event in advance of the start of the study. Although pain has been recorded as an adverse event, it is in fact an expected and unavoidable consequence of any type of abdominal surgery, and as such, it lacks any significance in terms of study-specific outcomes.

5.5 Adverse events that did not require reporting

5.5.1 Related to N-acetylcysteine

N-acetylcysteine has a good safety profile. Hypersensitivity reactions, including transient rash and bronchospasm, are recognised. Participants in the treatment will be under general anaesthetic when they receive N-acetylcysteine infusion.

Transient hypersensitivity reactions lasting less than 2 hours, which require no significant changes in patient treatment, involving anaesthetic medications, intravenous fluids and vasoactive drugs (eg adrenaline), will not be classified as Adverse Events unless they fulfil the criteria for an SAE.

5.5.2 Related to Operation

Weight loss surgery carries a risk of complications (approximately 10%). The commonest complications include post-operative chest infection, wound site infection, haemorrhage and anastomotic leak. See Appendix Afor full list.

These will be recorded as Adverse Events. They will not be reported as SAEs unless they fulfil the criteria for SAEs.

5.5.3 Treatment Stopping Rules

Individual participants, who developed a serious adverse reaction to N-acetylcysteine during the infusion, had the infusion terminated. This occurred in one patient, whose demographic data is included in the final analysis of the trial, but whose other laboratory data is not included as she did not undergo any of the other interventions.

5.5.4 Appropriateness of Measurements and Primary efficacy variables

The primary outcome measures were defined as ALT and AST levels in the protocol. These routine laboratory tests are used widely as measures of overall liver function, by measuring signs of hepatocellular damage. The levels were measured in the Clinical Biochemistry department using their automated processing machines and standard protocols, which are regularly recalibrated and validated. We also recorded clinical outcomes. Histopathological scoring of the liver biopsies was undertaken with the help of our Hepatobiliary specialist

Histopathology department using a modified scoring system. This is a non-validated score and its limitations are discussed in the results and conclusions.

Secondary outcome measurements looked at accepted measures of liver damage and oxidative stress. As well as cytokeratin-18 measurements, we also looked at TRAIL and FASligand as these are increasingly accepted as measures of liver cell apoptosis and injury. Oxidative stress can be measured in a number of ways and all methods act as indirect measures of the presence of free radicals and depletion of natural anti-oxidants within the liver and serum. We used TBARS, superoxide dismutase, protein carbonyls and glutathione perioxidase to measure oxidative stress. We had hoped to assess changes in gene expression within the perioperative liver biopsy samples, but unfortunately only had sufficient funds to perform array experiments on a subset of the biopsies taken at the beginning of surgery. We had hoped to perform further tests on the remaining samples but have not been able to so far. Therefore, these results are not presented in this report.

5.5.6 Drug concentration measurements

This was not performed due to its complexity and because there was no clinical or scientific rationale for doing so, when the study was designed. The dose of NAC was varied according to body weight as per the Summary of Product Characteristics.

5.5.7 Data Quality Assurance

Outcome measures were recorded where appropriate in the case report forms. Laboratory values of biomarkers and other measures were recorded on Excel Spreadsheets using anonymised patient identification numbers and timepoints. All recordings were made contemporaneously. Cross checking of data points with the original machine outputs in the laboratory was performed in conjunction with laboratory colleagues. No external auditing process or data verification was undertaken as it was felt that the overall volume of data was of a small and manageable scale. In any case, there was no funding source for such third party involvement.

6. STATISTICAL ANALYSIS

Initial plans stated that comparative statistical analysis and multiple logistic regression using quantitative data would be performed, between pre and post-operative data in NAC- versus controls. Statistical analysis were performed using SPSS 20 (SPSS, USA).

A planned interim analysis was performed after 2 years, at which point it became apparent that various methodological flaws in the design of the trial meant that a meaningful result would be unlikely, even if more patients were enrolled. The reasons for this conclusion are discussed below.

Following early closure of the trial and interim analysis, it became apparent that the data required only simple statistical tests, mostly non-parametric comparisons. Multiple logistic regression was not performed.

6.1. Data Handling and Storage

Data was stored on Microsoft Excel Spreadsheet.

Personal data was stored securely on password-protected workstations within the office of the Principal Investigator at King's College Hospital and was only accessible to the research team. All data were anonymised before analysis. No publications will contain information that will be able to identify individual participants.

Patients' samples were stored in a coded, anonymised form within the Institute of Liver Studies, according to the guidelines set out by the Human Tissue Act (2004).

6.2 Sample size calculation

The relevant section of the protocol is reproduced below:

Comparative statistical analysis will be performed, with a null hypothesis stating that there is no difference between the two trial study groups.

Sample Size Calculation

In a previous study, the AST increased significantly from baseline (24+/-6) and peaked at 24 hours after laparoscopic (152 SD= 102 U/L) and open (231 SD= 518 U/L) GBP but there was no significant difference in AST levels between s [Nguyen et al, AJS 2003[11]].

If we design the study to have 80% power to detect a 50% reduction in the post-operative rise in AST in relation to placebo in the population of laparoscopy patients, assuming an increase in AST level of 128 U/L and a pooled standard deviation of 100 U/L, the 50% reduction corresponds to an effect of size 0.64 which yields a sample size of 40 patients in each of the two s

i.e. 40 patients in control and 40 NAC-infused patients.

6.3 Trial Statistician

Dr A Nora A Donaldson

King's College Hospital

2nd Floor Weston Education Centre

Bessemer Road

London

SE5 9RS

Unfortunately Dr Donaldson left her post after the start of the study and no specific Trial Statistician was employed subsequently. The investigators took statistical advice from the King's College London Statistical Advice Service on an ad hoc basis, who advised that simple non parametric and parametric comparisons were sufficient to analyse the data, depending on specific data distribution patterns.

The power calculation was determined using results from a study of gastric bypass operations.

This operation takes, on average, much longer than sleeve gastrectomy and produces much larger rises in post-operative liver transaminases (the primary outcome measures ALT and AST). Nevertheless, we believed the overall target of 80 patients was realistic at the outset.

7. PROTOCOL CHANGES

7.1 1st Substantive Protocol change:

Protocol amendment: dated 1st April 2009

Old Protocol version: Version Final 1.0 (01May08)

New Protocol version: Version 2.0 (01Apr09)

7.1.1 Reason for Amendment

1. Major change to inclusion/exclusion criteria.

Sleeve gastrectomy operation was included in the study as this had become an increasingly common operation performed on patients in the unit over the preceding year.

2. Minor clarification of one exclusion criterion.

The exclusion criterion of psychiatric illness was clarified to specify exclusion of patients with active illness. Many morbidly obese patients have a history of psychiatric illness, either as a causative factor or a sequel of obesity. It was obviously not practical to include patients with ongoing active psychiatric symptoms, as the validity of the assessment of their autonomy and ability to consent would have been in question.

3. Correction of inconsistencies between text and schedule of investigations in the protocol.

Various transcription errors between various parts of the protocol were corrected.

4. Change to AE reporting guidelines in the protocol.

It was stipulated that common surgical post-operative complications would be recorded as AEs but not necessarily SAE, unless they fulfilled the SAE criteria. Indeed, this part of the protocol probably should have been revised further as some post-operative issues, such as

wound pain and nausea, are universal and classification of them as AEs was probably not appropriate.

5. Change to blinding procedure.

Text changes were as follows:

Version 1: The researcher will be blinded to the intervention until after data collection and sample analysis has occurred. This blinding will occur by marking the data and samples with a pre-arranged identification code, which will be contained within the envelope for randomisation.

After data collection and sample analysis has occurred, the research will be made aware of study allocation, so that comparative statistical analysis can be undertaken.

Version 2: After data collection and sample analysis has occurred, the researcher can check the randomisation schedule and will be aware of study allocation, so that comparative statistical analysis can be undertaken.

This is discussed above. Briefly, it became apparent that Pharmacy would require some time to dispense the IMP. Also, organising the appropriate per protocol dispensation of IMP would have to be the Research Fellow's responsibility due to the clinical commitments of all other parties, including Operating Surgeon and Anaesthetist.

6. Correction of typos/inconsistencies in protocol.

7.2. 2nd Substantive Protocol change

Protocol Change: dated 26th July 2009

Old Protocol version: Version 2.0 (01Apr09)

New Protocol version: Version 3.0 (26Jul09)

7.2.1.Reasons for Amendment

1. Change to inclusion criteria (upper age limit)

The upper age limit was increased to 75 from 65 years in line with the evolving clinical practice of the Unit.

2. Change to unit of measurement of alcohol intake in exclusion criteria

This was changed to "units" as defined as being approximately 8-10g of alcohol, corresponding to 1 glass of wine, a 25ml measure of whisky or approximately half a pint of lager.

3. Extra study-specific blood test to be taken 96hrs post-surgery

It had always been the Investigators intention to take study-specific blood tests up to 4 days after surgery but this was left out in error during previous protocol versions.

4. Clarification of when participants are approached about the study

Text changes were as follows:

Version 2: Patients seen in the Multidisciplinary Morbid Obesity Clinic at King's College Hospital, who are being referred for morbid obesity surgery, will be eligible for inclusion

They will have until the time of their admission (usually 10-12 weeks) to make their decision. They will have a second opportunity to discuss the study further at the Pre-operative Assessment Clinic, approximately 1 week prior to admission.

Version 3: Patients seen in the Multidisciplinary Morbid Obesity Clinic or Pre-operative Assessment Clinic at King's College Hospital, who are being referred for morbid obesity surgery, will be eligible for inclusion.

They will have until the time of their admission to make their decision. There will be another opportunity to discuss the study at the Pre-operative Assessment Clinic.

This change was required as the timings between initial assessment and allocation of the date on the waiting list became more unpredictable due to organisational changes in practice, NHS targets and the ad hoc availability of extra operating lists. Nevertheless, the importance of giving potential participants multiple opportunities to discuss the study and adequate time to

make decisions regarding their consent to participate was still pre-eminent.

5. Clarification about running time of 2nd NAC infusion

Version 2: Subjects randomised to the intervention will receive N-acetyl cysteine infusion, at standard 150mg/kg in 200ml 5% dextrose over 15mins at induction of anaesthesia followed by an infusion of 50mg/kg in 500mls of 5% dextrose during surgical retraction of liver for a maximum of 4 hours, together with standard anaesthetic medications.

Version 3: Subjects randomised to the intervention will receive N-acetyl cysteine infusion, at standard 150mg/kg in 200ml 5% dextrose over 15mins at induction of anaesthesia followed by an infusion of 50mg/kg in 500mls of 5% dextrose during surgical retraction of liver for a maximum of 4 hours, together with standard anaesthetic medications. *If surgery lasts for less than 4 hours, the infusion will be continued until it finishes.*

The last sentence was added as the length of operating became increasingly shorter over the preceding year or so (as a natural consequence of increasing operative experience). Therefore this stipulation was required so that patients received the same dose of IMP.

6. Clarification of when concomitant medication information will be collected

Having not been stipulated clearly before, the following was added: Information shall be collected regarding all concomitant medications (including over the counter vitamins and supplements) that participants are taking from the time of screening to the end of their participation in the trial (Month 6 post-operation visit).

7. Removal of PIS, ICF and GP letter from protocol appendix

This was done to bring the protocol in line with the standard template.

8. DISPOSITION OF PATIENTS AND PROTOCOL DEVIATIONS

20 patients were enrolled in the study. The details of protocol deviations are as follows:

001 and 002: The first two patients were enrolled under Protocol version 2 (dated 1 April 2009). Both patients had study-specific blood samples taken on post-operative day 4, although such an intervention was not stated in the protocol. This was an administrative error and oversight from the previous protocol amendment. The patients had routine blood testing on post-operative day 4 and they had given their consent at the time. When the error was noted, a protocol amendment was made before further recruitment.

004: 1 patient completed the study in its entirety. However, following surgery, the patient received a blood transfusion on the evening of surgery.Therefore, although study-specific bloods were taken on post-operative days 1-4 as per protocol, it was felt that it would be a waste of resources to perform non-routine biomarker assays on these samples, given the changes to circulating markers after transfusion. Primary outcome measures, ALT and AST, were still analysed on intention-to-treat basis.

008: 1 patient completed the study in its entirety but had a post-operative haemorrhage requiring repeat surgery on the same evening as the first operation. Therefore, although study-specific bloods were taken on post-operative days 1-4 as per protocol, it was felt that it would be a waste of resources to perform non-routine biomarker assays on these samples, given the additional operative intervention and liver damage incurred. Primary outcome measures, ALT and AST, were still analysed on intention-to-treat basis.

015: 1 patient developed respiratory difficulties and bronchospasm during initiation of anaesthesia. A clinical decision was taken not to proceed with surgery. The patient recovered quickly and left hospital the same evening. This reaction was reported as an SAE, although it may have been unrelated to the administration of IMP. Although her demographic details are included in the analysis, all other details are excluded from analysis, as she did not undergo surgery.

010: 1 patient enrolled in the study and completed all study-specific events until the first post-operative review. Subsequently, she decided to transfer her ongoing care to another hospital so did not attend the 6 month follow-up appointment. Her results have been included up to the point of withdrawal.

The other 14 patients completed the study as per Protocol Version 3.0 (dated 26.07.09).

9. STOPPING THE TRIAL

After a period of 1 year, only 20 patients had been recruited. Laboratory analysis of the collected samples along with the assessment of the clinical data was performed before an interim analysis could take place, as planned in the protocol. The results are presented below. Both clinical and laboratory markers displayed a wide variation in data, with large confidence intervals and standard deviations. Such variance is likely to be due to multiple factors but included deficiencies in study design. It was noted that large differences in the timing of liver retraction and its apparent effect on the liver function were also evident. The intraoperative insult to the liver was not standardized, in terms of pressure exerted on the liver tissue or extent of tissue hypoxia induced. This was because there are no clinically available instruments to measure intraparenchymal oxygen tension or mechanical pressure at this time. Thus, the investigators felt that it was unlikely that continuation of the study to full recruitment would lead to a more reliable and valid conclusion. Therefore the study was terminated. The investigators may in future design similar studies to answer a similar clinical question with more reproducible conditions.

10. RESULTS

10.1 Demographics

20 patients participated in this study. Their baseline characteristics, including age, BMI and co-morbidities were similar. All recruited patients underwent Laparoscopic Sleeve Gastrectomy. By the time recruitment to the study had started, the majority of laparoscopic gastric banding operations were performed as day-case surgery. Any such operations performed as inpatient surgery were for patients with high-risk co-morbidities, not suitable for trial recruitment. Gastric Bypass patients were being recruited to another study in the unit and therefore we were only able to recruit LSG patients. Their BMIs were in the "super" morbid obese range, with an overall mean BMI 60.6 kg/m^2. Ethnic and gender mix were not significantly different. All patients had minimal regular alcohol intake, with 1 patient having given up heavy alcohol intake more than a decade previously. Only 1 patient was a smoker.

Table 1: Baseline Demographic Characteristics of study population

Treatment Group		N	Mean	Standard Deviation	Standard Error of Mean	p value (2-tailed)
Age years	NAC	10	48.20	11.631	3.678	0.2
	control	10	40.70	13.483	4.264	
Height cm	NAC	10	164.82	29.201	9.234	0.578
	control	10	171.87	26.429	8.357	
Weight kg	NAC	10	166.800	12.8651	4.0683	0.826
	control	10	167.900	8.7996	2.7827	
BMI	NAC	10	60.4200	12.26302	3.87791	0.871
	control	10	61.2400	9.96909	3.15250	

Figure 3: Boxplot of Baseline Demographic Characteristics demonstrating similarities between treatment and control groups

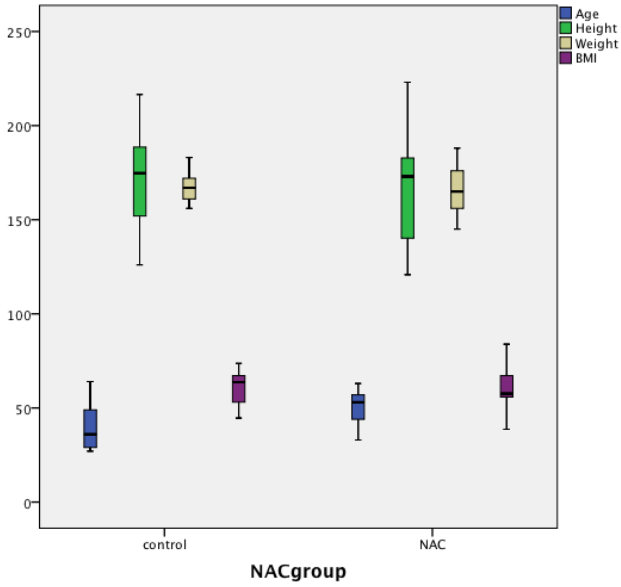


Table 2: Baseline Qualitative characteristics of study population

	08:02	10:00	0.14
Diabetes	5	5	1
OSA	7	8	0.61
Depression	4	4	1
Poor Mobility	2	4	0.33
Hypertension	7	7	1
Asthma	3	5	0.36
Cardiac	2	4	0.33
cholesterol	5	6	0.65

10.2 Clinical Outcomes:

10.2.1. Serious Adverse Events

Of 20 patients, 10patients had significant complications, meeting criteria for Serious Adverse Events. 1 patient had a post-operative haemorrhage requiring further surgery on the evening of LSG, followed by readmission and further surgery for a staple line failure on post-operative day 13. He was treated in-hospital for a further 8 weeks to allow the leakage and intra-abdominal infection to resolve. 1 patient required a 2-unit blood transfusion post-operatively, after a fall in haemoglobin on the evening after surgery. 1 patient was treated in with non-invasive ventilator support on the high dependency unit for post-operative respiratory difficulties. This was predicted pre-operatively given the patient's history of obesity hypoventilation syndrome and severe sleep apnoea and resolved within 48 hours without significant sequelae. 4 more patients were readmitted within 30 days of discharge. 1 patient became dehydrated following an episode of diarrhoeal infection and was readmitted for rehydration. 2 patients were also readmitted over concerns about poor oral intake and were rehydrated intravenously. 1 patient fainted on post-operative day 22 and was readmitted as a precaution. Pre-operatively, that patient had been taking three antihypertensive medications and these were stopped following readmission.

1 patient was readmitted 3 months following surgery with poor oral intake. Following a period of rehydration, she was discharged but was subsequently admitted two weeks later to a hospital elsewhere with confusion and leg weakness and was diagnosed with Wernicke's encephalopathy, brought on by poor nutritional intake.

2 further patients were reported as Serious Adverse Events, although they did not suffer complications per se. 1 patient was admitted to a different hospital to have an ovarian cystectomy 4 months post LSG. Another patient fell pregnant 5 months post LSG and went on to have an uncomplicated pregnancy and gave birth to a healthy baby.

1 patient in the treatment group developed respiratory difficulties after intubation, with evidence of high airway pressures and possible laryngospasm. A decision was taken to terminate the procedure immediately and she recovered with sequel and left hospital the same evening. This was reported as an Adverse Drug Reaction as such respiratory complications might occur after NAC infusion, although the attending anaesthetic team was not completely convinced this was due to NAC. The patient underwent LSG (outside of the study) 6 months afterwards without complication.

10.2.2. Adverse Events

As expected in a group of morbidly obese patients undergoing major abdominal surgery, there was a high rate of adverse events without any important clinical significance. Most patients had post-operative pain and nausea, along with transient episodes of tachycardia, pyrexia and

signs of basal atelectasis.

1 patient developed meralgia paraesthetica diagnosed following discharge. It was felt that compression of the lateral cutaneous nerves of the thighoccurred whilst recumbent during the initial post-operative recovery period. The patient required treatment with neuromodulating analgesia and recovered over the next few months.

Lengths of stay were similar, although 1 patient from each group did have prolonged readmissions within the reporting period, as detailed above.

10.3 Intraoperative Characteristics:

Operating time, blood loss and liver retraction times were similar between groups.

Table 3: Intraoperative characteristics of study groups

	Treatment Group	Mean	Std. Deviation	Std. Error Mean	p value
Liver Retraction	NAC	86.11	25.231	8.41	0.591
(time in min)	Control	94.6	39.797	12.585	
Operation Time	NAC	121.89	26.483	8.828	0.543
(time in min)	Control	134.3	54.394	17.201	
Blood Loss	NAC	216.67	106.066	35.355	0.353
(mls)	Control	165	127.039	40.173	
Hospital Stay	NAC	7	3	1	0.934
(days)	Control	7.1	2.183	0.69	

Table 4: Adverse Events and SAE patient by patient

TrialNo	NACGroup	Adverse Events 1	2	3	4		SAE
1	1	pyrexia	tachycardia	pain	nausea		
2	1	pain	nausea				
3	0	pyrexia	pain	poor air entry at lung bases			
4	0	haemorrhage post-op requiring transfusion	pain	poor air entry at lung bases	loose stools		
5	0	pain	nausea	poor air entry at lung bases	pyrexia		
6	1	tachycardia	pain	poor air entry at lung bases	pyrexia	toe infection w	readmission for diarrhoeal illness
7	1	tachycardia	pain	meralgia paraesthetica			respiratory failure due to obesity hypoventilation
8	1	pain	pyrexia	poor air entry at lung bases			2: post op haemorrhage, Post op anastomotic leakage
9	0	angina	pain	exacerbation of neuropathic pain in legs			
10	0						(2) readmission with persistent vomiting, leg weakness & confusion Wernicke's
11	1	pain	nausea				
12	1	skin fold erythema					
13	1						
14	0	heartburn					ovarian cyst operation 4 months post LSG
15	1						Adverse Drug Reaction: increase in airway pressure at induction of anaesthesia
16	0	staple line required further intraoperative reinforcement	pain	pyrexia	tachycardia		
17	0	pain	heartburn	ankle pain after fall	loose motions	pyrexia	readmission with poor oral intake and heartburn
18	1	staple line required further intraoperative reinforcement					readmission with faint due to excess antihypertensive medication
19	0	abdominal pain	skin fold erythema				readmission with poor oral intake
20	0	pyrexia					pregnant 5 months post-LSG

10.4 Efficacy Evaluation

The main aim of the study was to assess the effect of NAC in reducing hepatocellular damage. Thus the main outcome measure stipulated in the protocol was ALT (and AST, which is less liver-specific). Although data was collected at 6 weeks and 6 months timepoints and recorded in the CRF, these data are not analysed herein as they do not significantly contribute to an understanding of the efficacy of the IMP.

10.4.1 Main outcome measure – ALT:

There were no significant differences at baseline (p=0.575.) Following surgery, ALT rises, peaking at postoperative day 1. However there were no significant differences between treatment groups. There was a wide variation between subjects in both groups in the extent of the ALT rise post-operatively and the standard deviations and confidence intervals are correspondingly large. The data appeared to have a normal distribution and so we employed Student's t test to test for significance. However, for additional clarity, we have also given the median values to demonstrate the lack of difference between treatment groups more clearly.

Table 5: Change in ALT between treatment groups – mean values

	Treatment Group	N	Mean	Std. Deviation	Std. Error Mean	p value
ALTpre	NAC	9	29.89	11.581	3.86	0.575
	Control	10	33	12.083	3.821	
ALTend	NAC	9	152.33	68.242	22.747	0.469
	Control	10	190.5	140.237	44.347	
ALTday1	NAC	8	196.63	121.107	42.818	0.46
	Control	10	276.5	276.414	87.41	
ALTday2	NAC	9	213.22	178.891	59.63	0.543
	Control	10	274.6	242.858	76.798	

ALTday3	NAC	9	151	111.349	37.116	0.561
	Control	10	188.9	159.56	50.457	
ALTday4	NAC	9	104.67	61.762	20.587	0.465
	Control	10	135.1	107.121	33.875	

Table 6: Change in ALT median values

	Treatment Group	Median	Min	Max
ALTpre	Control	31	9	50
	NAC	25	13	48
ALTend	Control	146	47	391
	NAC	123	74	269
ALTday1	Control	127	55	737
	NAC	151	102	464
ALTday2	Control	152	50	688
	NAC	127	69	599
ALTday3	Control	108	40	458
	NAC	92	52	344
ALTday4	Control	77	35	303
	NAC	73	41	218

Figure 3:

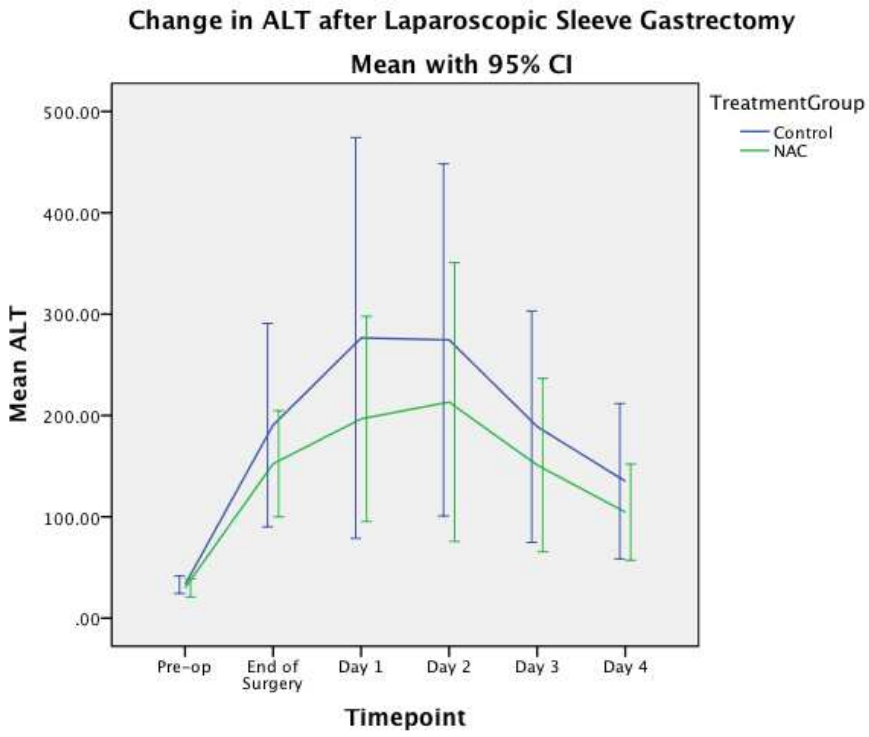
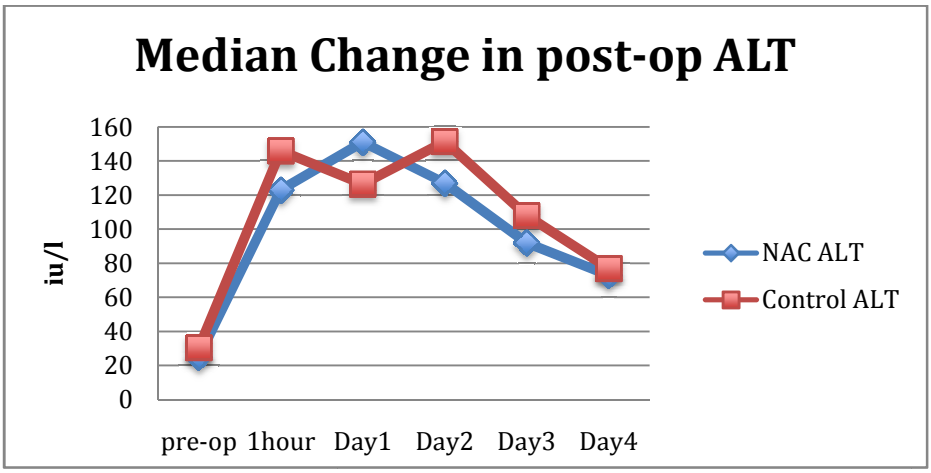

Change in ALT after Laparoscopic Sleeve Gastrectomy
Mean with 95% CI
TreatmentGroup
Control
NAC
Mean ALT
500.00
400.00
300.00
200.00
100.00
.00
Pre-op
End of Surgery
Day 1
Day 2
Day 3
Day 4
Timepoint

Figure 4


Median Change in post-op ALT
160
140
120
100
80
60
40
20
0
iu/l
pre-op
1hour
Day1
Day2
Day3
Day4
NAC ALT
Control ALT

10.4.2 Other clinical markers of liver damage and inflammation

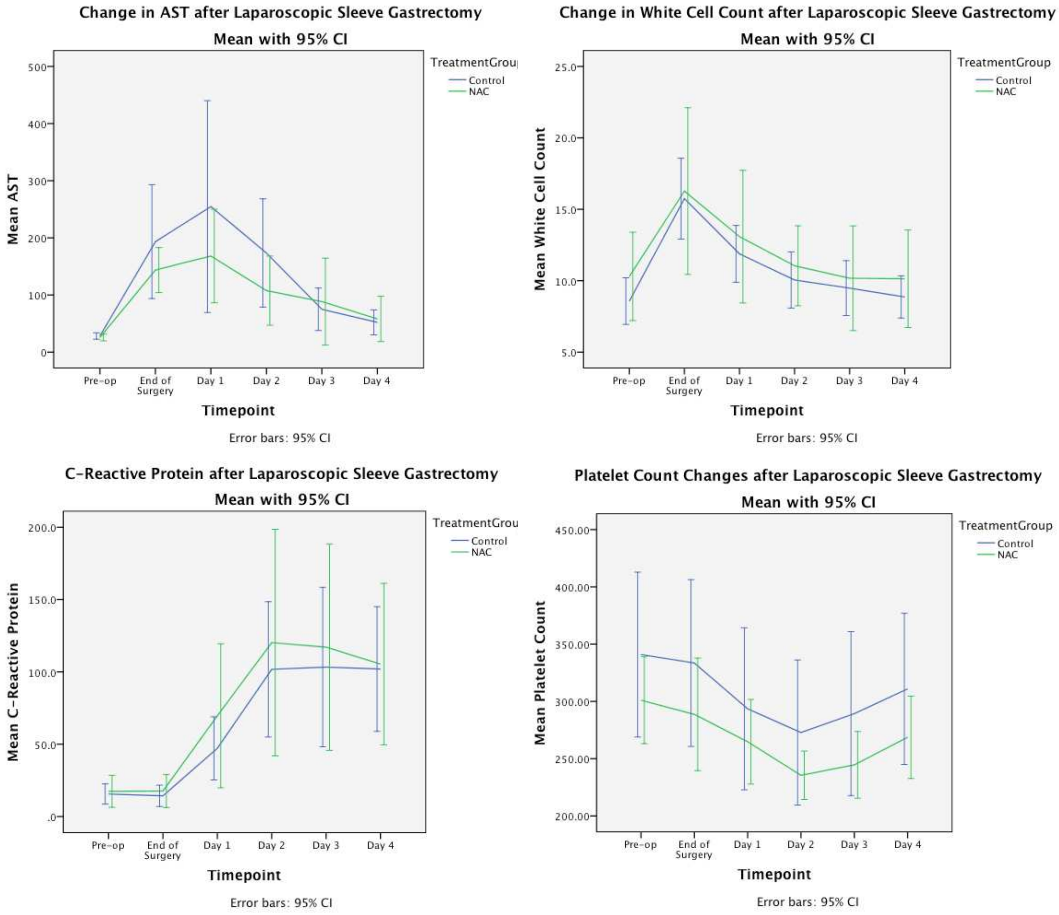
Data are also presented for changes in AST, WCC, CRP and Platelets. Again, these demonstrate a lack of any significant differences between groups, but with a large change post-operatively. The AST and WCC also peak by the 1st day. CRP lags and the peak are around the 2nd day, where it then reaches a plateau, before falling off more slowly. Platelet account appears to be depressed slightly, although this is unlikely to have any clinical significance.

Table 7: Changes in WCC, CRP, Platelets and AST after surgery

	NAC	Control	significance
ASTpre	25.7 ±2.6	28.2±2.5	0.49
ASTend	143.7 ±17.1	193 ±43.2	0.3
AST day 1	168.3 ±34.6	254.7 ±81.2	0.39
AST day 2	107.7±26.3	173.5 ±41.9	0.21
AST day 3	88.4 ±33.0	75.0 ±16.4	0.71
AST day 4	58.1 ±17.2	52.1 ±9.7	0.76
WCC pre	10.3 ±1.3	8.6 ±0.7	0.26
WCC end	16.3 ±2.5	15.7 ±1.3	0.85
WCC day 1	13.1 ±2.0	11.9 ±0.9	0.56
WCC day 2	11.0 ±1.2	10.1 ±0.9	0.51
WCC day 3	10.2 ±1.6	9.5 ±0.8	0.7
WCC day 4	10.1 ±1.5	8.9 ±0.7	0.43
CRP pre	17.5 ±4.8	15.6 ±3.1	0.74
CRP end	17.6 ±5.0	14.4 ±3.3	0.58
CRP day 1	69.7 ±21.1	47.2 ±9.7	0.31
CRP day 2	120.3 ±34.0	101.8 ±20.7	0.64
CRP day 3	117.1 ±30.9	103.3 ±24.4	0.73

CRP day 4	105.4 ±24.2	102.0 ±19.1	0.91
PLTpre	301 ±16	341 ±32	0.3
PLTend	289 ±21	334 ±32	0.27
PLT day 1	265 ±16	294 ±31	0.46
PLT day 2	235 ±9	273 ±28	0.24
PLT day 3	245 ±13	289 ±32	0.23
PLT day 4	269 ±16	311 ±29	0.23

Figure 5:


Change in AST after Laparoscopic Sleeve Gastrectomy
Mean with 95% CI
Mean AST
Timepoint
Pre-op
End of Surgery
Day 1
Day 2
Day 3
Day 4
TreatmentGrou
Control
NAC
Error bars: 95% CI
Change in White Cell Count after Laparoscopic Sleeve Gastrectomy
Mean with 95% CI
Mean White Cell Count
Timepoint
TreatmentGroup
Control
NAC
Error bars: 95% CI
C-Reactive Protein after Laparoscopic Sleeve Gastrectomy
Mean with 95% CI
Mean C-Reactive Protein
Timepoint
TreatmentGrou
Control
NAC
Error bars: 95% CI
Platelet Count Changes after Laparoscopic Sleeve Gastrectomy
Mean with 95% CI
Mean Platelet Count
Timepoint
TreatmentGroup
Control
NAC
Error bars: 95% CI

10.4.3 Markers of Apoptosis

There is a large rise in CK-18 post-operatively, indicating significant cellular damage. Although there are no significant differences between treatment groups, these data do demonstrate that a significant degree of cell death occurs following surgery. Both necrosis and apoptosis occurs. TRAIL and FasL are not affected by NAC infusion and these data suggest they may not play a significant role in the cellular damage that occurs in this scenario.

Table 8

	Treatment Group	N	Mean	Std. Deviation	Std. Error Mean	p value
M65pre	NAC	9	378.11	247.271	82.424	0.542
	Control	10	315.8	188.074	59.474	
M65day1	NAC	8	1039.13	729.705	257.99	0.118
	Control	8	2103.25	1655.998	585.484	
M65day2	NAC	8	994.5	932.814	329.799	0.458
	Control	9	1493.22	1626.474	542.158	
M65day4	NAC	8	531.38	236.875	83.748	0.575
	Control	9	451.11	326.319	108.773	
M30pre	NAC	9	187	64.915	21.638	0.522
	Control	10	223.2	153.877	48.66	
M30end	NAC	5	242.6	114.4	51.161	0.34
	Control	5	305.2	77.125	34.491	
M30day1	NAC	8	443.75	387.468	136.99	0.104
	Control	8	1522.88	1711.157	604.985	
M30day2	NAC	8	319.5	264.781	93.614	0.091
	Control	9	818.11	738.825	246.275	
M30day4	NAC	8	194.5	81.963	28.978	0.933
	Control	9	198.44	103.944	34.648	

Figure 6

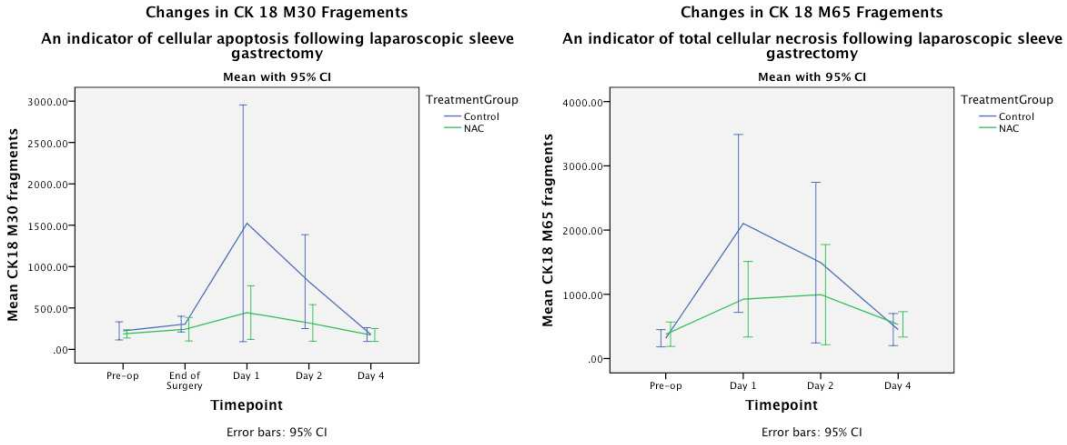


Table 9

	Treatment group	N	Mean	Std. Deviation	Std. Error Mean	p value
TRAILpre	NAC	9	90.19	33.63	11.21	
	Control	10	100.84	27.758	8.778	0.46
TRAILend	NAC	9	102.59	36.509	12.17	
	Control	8	94.08	25.606	9.053	0.591
TRAILday1	NAC	8	34.96	19.917	7.042	
	Control	7	56.46	34.5	13.04	0.157
FASLpre	NAC	9	92.5	23.97	7.99	
	Control	10	90.8	34.638	10.954	0.904
FASLend	NAC	9	94.24	32.318	10.773	
	Control	8	98.29	35.973	12.718	0.81
FASday1	NAC	8	79.18	40.963	14.483	
	Control	7	81.96	43.989	16.626	0.901

10.4.4 Markers of Oxidative Stress

The wide variance in these data, with large confidence intervals and standard deviations indicate the difficulties in accurately assessing oxidative stress changes in plasma and serum. A number of systematic biases may exist as slight variations in technique of sample collection and processing, such as minor differences in time from collection to freezing, may have had a large impact on the assay results. In the short term, no significant differences in the markers of lipid peroxidation (TBARS) and protein damage occur. Superoxide dismutase levels do rise following surgery but are not affected by NAC.

Figure 7

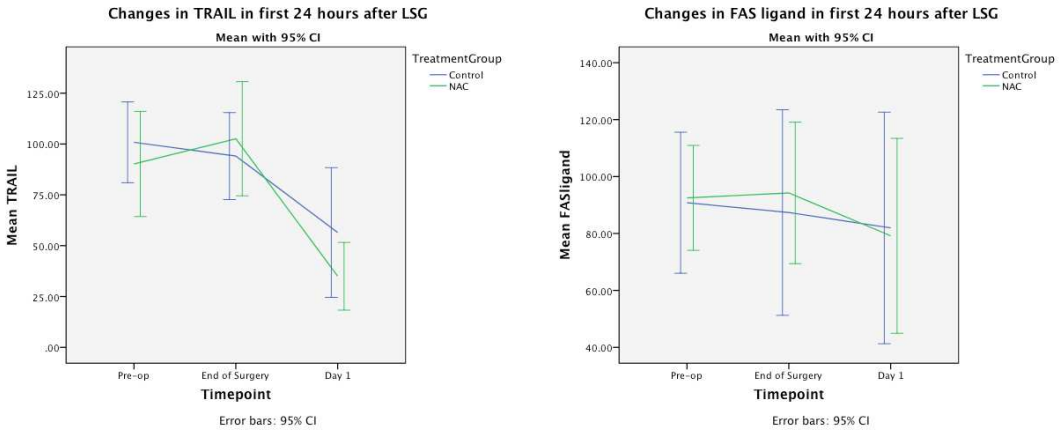
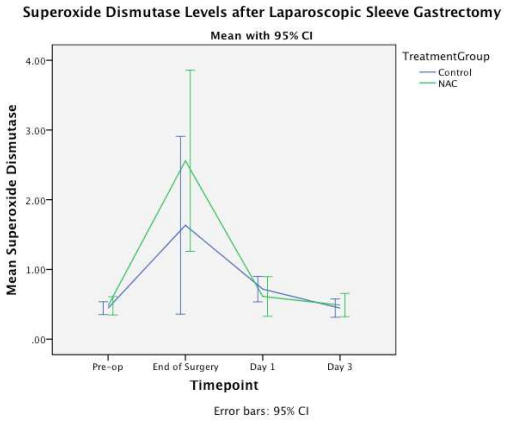


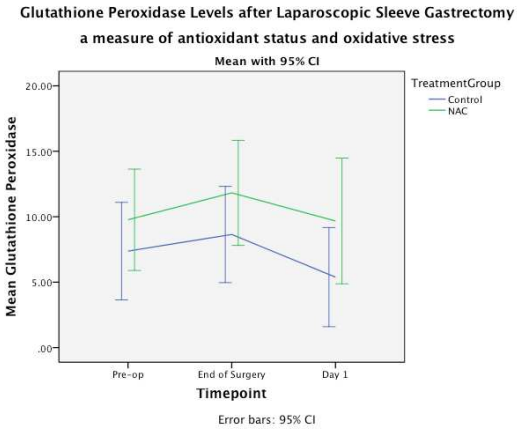
Table 10

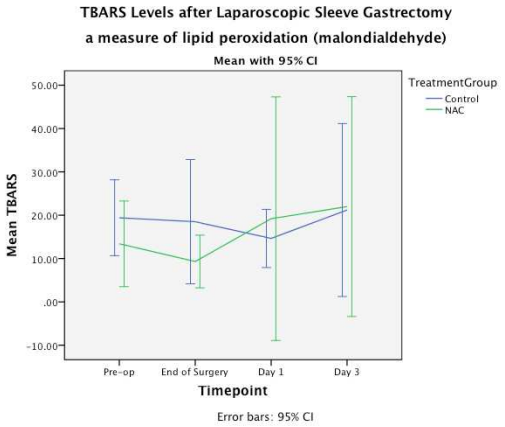
	Treatment group	N	Mean	Std. Deviation	Std. Error Mean	p value
SODpre	NAC	8	0.4775	0.15786	0.05581	0.626
	Control	10	0.444	0.12842	0.04061	
SODend	NAC	9	2.558	1.69015	0.56338	0.264
	Control	10	1.6336	1.78303	0.56384	
SODday1	NAC	8	0.6126	0.34105	0.12058	0.464
	Control	10	0.7178	0.25506	0.08066	
SODday3	NAC	8	0.4889	0.20036	0.07084	0.636
	Control	10	0.4451	0.18351	0.05803	
TBARSpre	NAC	9	13.3967	12.85799	4.286	0.312
	Control	10	19.404	12.24774	3.87308	
TBARSend	NAC	9	9.3167	7.91737	2.63912	0.138
	Control	9	20.5544	20.10288	6.70096	
TBARSday1	NAC	8	19.1937	33.6102	11.883	0.7
	Control	9	14.6467	8.7305	2.91017	
TBARSday3	NAC	8	21.99	30.3467	10.72918	0.954
	Control	9	21.1933	25.94577	8.64859	
GPXpre	NAC	9	9.7675	5.04254	1.68085	0.324
	Control	10	7.3725	5.21028	1.64764	
GPXend	NAC	9	11.8225	5.22205	1.74068	0.199
	Control	10	8.6443	5.14069	1.62563	
GPXday1	NAC	8	10.8865	5.4415	1.92386	0.078
	Control	9	5.9844	5.24442	1.74814	

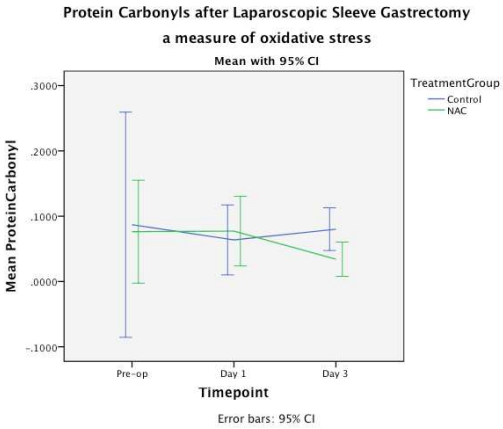
PCpre	NAC	8	0.0669	0.08328	0.02944	0.928
	Control	5	0.0716	0.1	0.04472	
PCday1	NAC	7	0.0993	0.06215	0.02349	0.439
	Control	9	0.0711	0.07555	0.02518	
PCday3	NAC	6	0.0342	0.02504	0.01022	0.034
	Control	9	0.0801	0.04259	0.0142	

Figure 8


Superoxide Dismutase Levels after Laparoscopic Sleeve Gastrectomy
Mean with 95% CI
TreatmentGroup
Control
NAC
Mean Superoxide Dismutase
4.00
3.00
2.00
1.00
.00
Pre-op
End of Surgery
Day 1
Day 3
Timepoint
Error bars: 95% CI


Glutathione Peroxidase Levels after Laparoscopic Sleeve Gastrectomy
a measure of antioxidant status and oxidative stress
Mean with 95% CI
TreatmentGroup
Control
NAC
Mean Glutathione Peroxidase
20.00
15.00
10.00
5.00
.00
Pre-op
End of Surgery
Day 1
Timepoint
Error bars: 95% CI


TBARS Levels after Laparoscopic Sleeve Gastrectomy
a measure of lipid peroxidation (malondialdehyde)
Mean with 95% CI
TreatmentGroup
Control
NAC
Mean TBARS
50.00
40.00
30.00
20.00
10.00
.00
-10.00
Pre-op
End of Surgery
Day 1
Day 3
Timepoint
Error bars: 95% CI


Protein Carbonyls after Laparoscopic Sleeve Gastrectomy
a measure of oxidative stress
Mean with 95% CI
TreatmentGroup
Control
NAC
Mean ProteinCarbonyl
.3000
.2000
.1000
.0000
-.1000
Pre-op
Day 1
Day 3
Timepoint
Error bars: 95% CI

10.4.5 Inflammatory Cytokines

As expected, there are large changes in cytokines following surgery, with peak changes occurring within an hour of surgery in IL-6 and IL-10. Changes in adipocytokines are more difficult to interpret and there are no significant differences within 24hours of surgery or between treatment groups.

Table 11

	Treatment group	N	Mean	Std. Deviation	Std. Error Mean	p value
IL6pre	NAC	9	7.4944	4.5149	1.50497	0.177
	Control	10	5.216	2.29041	0.72429	
IL6end	NAC	9	41.9344	29.44484	9.81495	0.319
	Control	10	30.056	20.71458	6.55053	
IL6day1	NAC	8	31.3675	30.62858	10.82884	0.611
	Control	9	24.3811	24.86427	8.28809	
IL10pre	NAC	9	0.7356	0.35837	0.11946	0.599
	Control	10	0.981	1.32967	0.42048	
IL10end	NAC	9	51.8578	67.78066	22.59355	0.65
	Control	10	66.63	71.20327	22.51645	
IL10day1	NAC	8	4.615	3.52434	1.24604	0.094
	Control	9	2.2478	1.75969	0.58656	
LeptinPre	NAC	9	195741.1811	139328.5765	46442.85884	0.511
	Control	10	158718.396	99991.26231	31620.0135	
LeptinEnd	NAC	9	157742.5711	92625.90365	30875.30122	0.491
	Control	10	131147.127	71723.5907	22680.9908	

				2	6	
LeptinDay1	NAC	8	212755.1663	114639.8726	40531.31566	0.757
	Control	9	192511.0244	146147.7581	48715.91936	
ResistinPre	NAC	9	10003.7456	3805.34997	1268.44999	0.667
	Control	10	11057.049	6247.06966	1975.49688	
ResistinEnd	NAC	9	17269.4711	7992.06855	2664.02285	0.51
	Control	10	14678.796	8705.8072	2753.01796	
ResistinDay1	NAC	8	13105.21	5307.78231	1876.58443	0.953
	Control	9	12914.3222	7504.21006	2501.40335	
AdiponectinPre	NAC	9	10006633.55	6073140.609	2024380.203	0.467
	Control	10	12231441.98	6871139.317	2172845.036	
AdiponectinEnd	NAC	9	9977003.406	6051405.519	2017135.173	0.902
	Control	10	10326382.81	6145746.82	1943455.788	
AdiponectinDay1	NAC	8	8509492.574	5417245.459	1915285.5	0.876
	Control	9	8176498.583	3021590.702	1007196.901	

Figure 9

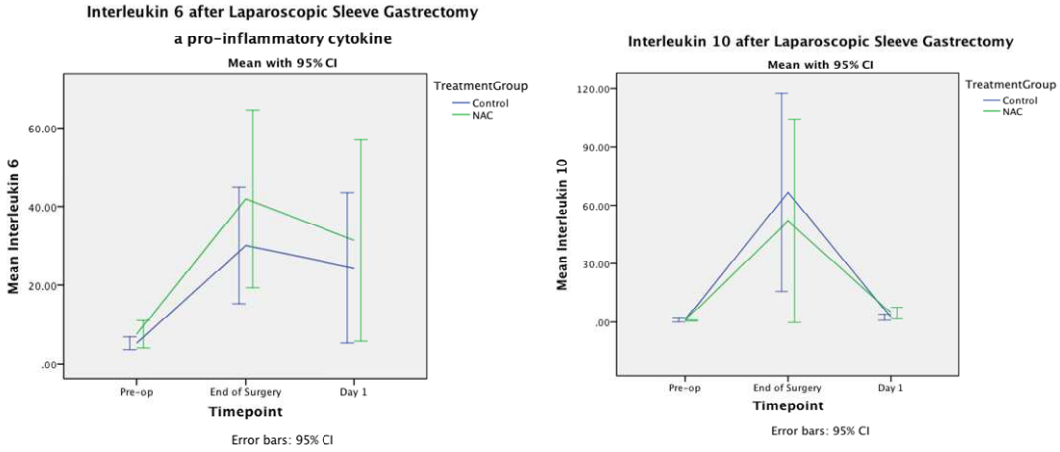
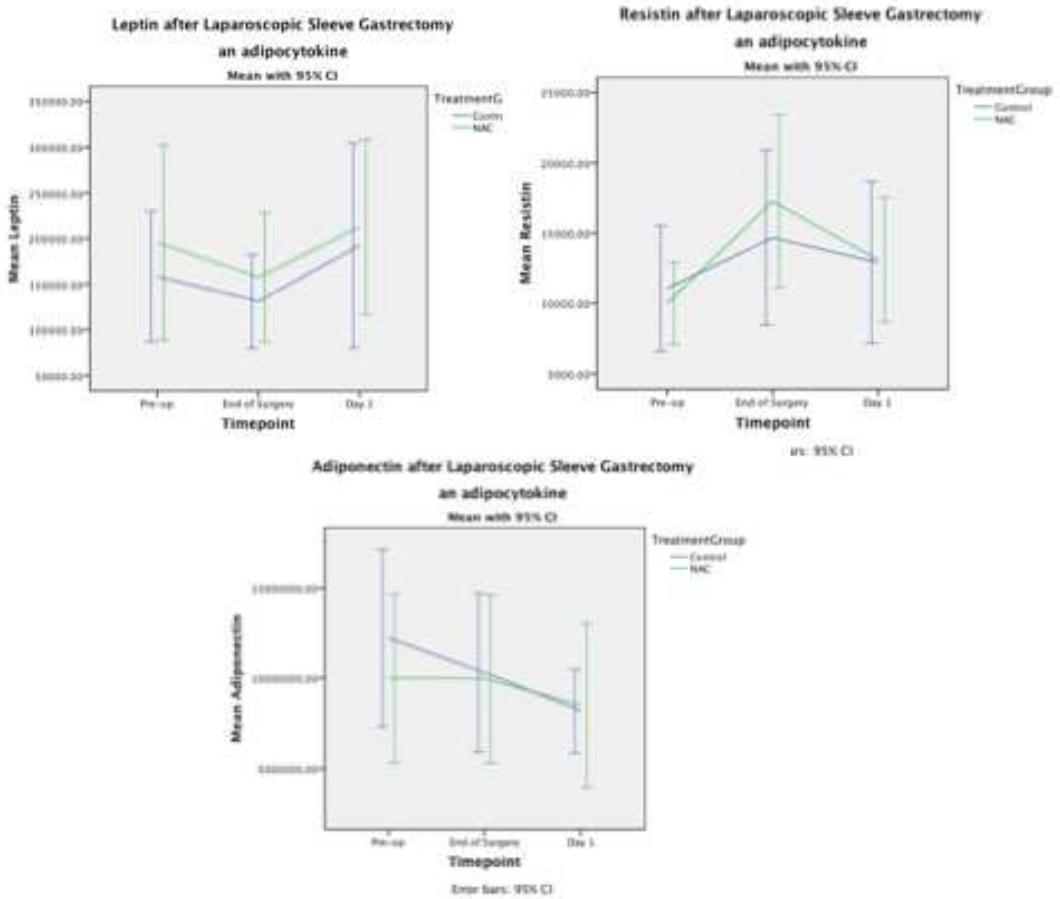


Figure 10



11. CONCLUSIONS

The main aim of this study was to establish if an intraoperative infusion of n-acetylcysteine might reduce the extent of liver damage during laparoscopic bariatric surgery. Due to deficiencies in study design, the study was terminated prematurely before the full sample of participants had been recruited. The results of the study show no statistically significant differences between treatment groups of both primary and secondary outcome measures. The small study size, along with a wide variability of the data (wide confidence intervals and large standard deviations), mean that a definite conclusion about the efficacy of NAC in this clinical setting cannot be made. This is therefore a "failed" trial.

Nevertheless, some interesting observations were made about the extent of cellular damage that occurs following surgery, including a large increase in circulating CK-18 fragments, indicating the occurrence of cellular necrosis and apoptosis. Similarly large rises in inflammatory markers also occurred in the first 24 hours after surgery. N-acetylcysteine was selected for its antioxidant properties. Measurement of oxidative stress was difficult to perform accurately. The greatest confounding factor was probably the minute variations in sample collection and processing between patient and between timepoints, leading to changes in markers of oxidative stress. It was also not possible for financial reasons to perform more assays at all selected timepoints in order to get a more accurate trend in changes over time. Oxidative stress may be affected by various factors, including obstructive sleep apnoea, presence of subclinical infections. The administration of low molecular weight heparin has also been shown to affect TBARS[28] and all patients in this study received routine thromboprophylaxis.

A major drawback of the study was a lack of a standardised, reproducible toxic insult to the liver during intraoperative liver retraction. The Nathanson liver retractor was placed during surgery to allow adequate visualisation of the hiatus for surgery to proceed safely. The pressure applied to liver varied from patient to patient depending on their body habitus, intraabdominal dimensions and size and texture of the liver. There are no routine clinical methods of measuring tissue oxygen tension or pressure within the liver. Some experimental methods do exist, including insertion of microdialysis catheters to allow continuous monitoring of metabolites, such as lactate, or of tissue pO_2 and pCO_2. It is also possible to use a surface oxygen saturation probe[29], although this does not have approval for routine clinical use and would itself have required new MHRA(UK) approval for use as a medical device.

12. APPENDICES

Appendix A Definition of Complications

Appendix B Participant Consent Form and Information Leaflet

Appendix C Bibliography

Appendix A

Definitions of Complications occurring within 30 days of surgery

Major

Anastomotic leak – objective evidence of leakage (either on contrast radiography or visual confirmation at re-operation) with or without clinical signs and symptoms.

Fluid collection - CT scan or ultrasound presence of fluid collection at least 5 cm in diameter with or without clinical relevance.

Hemorrhage- Requirement of at least 3 units of packed Red blood cells (1000 mL) within 24 h after the operation.

Chest Infection – signs of sepsis or septic shock accompanied by clinical signs and symptoms of chest infection

Sepsis – pulse >90bpm, temperature <36^{o}C or >38^{o}C, respiratory rate >20 or paO_2, White cell count <4 or >12000cells/mm^3 in presence of proven infection

Renal Failure – Creatinine> 150 or doubling of pre-operative value, persisting for at least 72 hours

Myocardial infarction – relevant ECG changes accompanied by clinical symptoms and signs, with a raised Troponin I

Pulmonary embolism – relevant clinical signs and symptoms with corresponding appearance on Computed Tomography (CTPA) or Ventilation/Perfusion scanning

Delayed gastric emptying: failure to resume oral liquid intake by postoperative day 10, and/or emesis over 500 ml on or after postoperative day 5, and/or continued nasogastric drainage >500 ml on or after postoperative day 5.

Cardiac Arrhythmias – ECG-proven new onset arrhythmias, eg.atrial fibrillation

Minor

Wound infection – erythema, purulent discharge and pain at wound site persisting for more than 48 hours

Post-op pyrexia – temperature above 38^{o}C lasting less than 48 hours with no progression to sepsis or accompanying clinical signs of local infection

Deep vein thrombosis – relevant clinical symptoms with sonographic evidence of thrombosis in the absence of signs of suspected pulmonary embolism

Appendix B

Participant Consent Form **(Final version 1.0, 01.05.2008 (1 page)**

Patient Identification Number for this trial:

CONSENT FORM

Randomised Trial of N-acetylcysteine in Laparoscopic Bariatric Surgery

Name of Researchers: Mr A Belgaumkar, Mr AG Patel (Consultant Surgeon)

Please initial box

1. I confirm that I have read and understand the information sheet dated 1st May 2008 (final version 1.0) for the above study. I have had the opportunity to consider the information and ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed by letter of my participation in the study ☐

5. I agree to take part in the above study. ☐

6. I agree to genetic tests being performed on the samples and these have been explained to me. ☐

7. I agree to my samples to be stored for future studies. I understand I have the right to withdraw my consent in the future. ☐

_____	_____	_____
Name of Patient	Date	Signature

_____	_____	_____
Name of Person taking consent	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

Participant Information Leaflet (final version 1.0, 01.05.2008 (6 pages)

Study Title:

A Randomised Trial of N-acetylcysteine in laparoscopic bariatric surgery

King's College Hospital NHS Foundation Trust

After consultation with your surgeon, you are now on the waiting list to have Weight Loss Surgery. We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully.

Talk to others about the study if you wish.

(Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study).

Ask us if there is anything that is not clear or if you would like more information.

Take time to decide whether or not you wish to take part.

Purpose of the study

The aim of this research is to find out if giving patients a drug, N-acetylcysteine (NAC), reduces the complications of laparoscopic weight loss surgery by protecting the liver in patients with morbid obesity.

Why are we doing this study?

N-acetylcysteine has been used for more than 30 years to protect people who have paracetamol poisoning from liver failure. We also know from other laboratory experiments that NAC will improve the health of individual liver cells. Patients with morbid obesity have a high rate of fatty liver. These patients adapt less well to damaging actions on their liver compared with patients without fatty liver disease. During keyhole surgery, we have to retract the liver out of the way to provide enough space to perform the surgery. This squeezes the liver and causes damage severe enough to temporarily make liver blood tests abnormal for a few days. We also believe this liver damage may contribute to other post-operative complications, such as fevers, chest infections and kidney problems.

We would like to find out if we can help patients having keyhole surgery. We want to know if N-acetylcysteine will protect the liver and lead to less post-operative complications.

Why have I been invited?

All the patients who are planning to have weight loss surgery at King's College Hospital will be invited to take part in this study.

Do I have to take part?

It is completely up to you. This information sheet will describe the study and if you feel that you would like to participate, you will then be asked to sign a consent form. At any time, you are free to withdraw from the study. This will not affect the standard of your care.

What will happen to me if I take part in the study?

You will take part in a "Randomised Trial". In order to find out if a new treatment is effective, we need to compare it with standard treatment. We put people into two groups. One group will receive N-acetylcysteine during their surgery; one group will not receive the treatment. The results are then compared to see which group did better. In order to make sure the groups are the same to start with, each patient is put into a group by chance (randomly).

You have a 1 in 2 chance of receiving NAC infusion during surgery.

During the surgery, we usually take a needle sample (biopsy) of the liver at the beginning of the operation as part of standard treatment. This is analysed in the laboratory for features of liver disease. If there are signs of liver disease, our patients are referred to the Hepatology (Liver) specialists for further advice and follow-up.

If you participate in this study, we would like to take a second biopsy at the end of operation.

During your admission, you will usually have blood samples taken for routine blood tests before and after surgery until discharge. You will also have blood taken when you attend follow-up outpatient clinic.

If you participate in this study, we would like to take some additional blood samples (one extra vial, about 10mls, or two teaspoons) for additional tests.

The rest of your care will be unchanged. We will not make any changes to your regular medications. You do not have to make any additional visits to the hospital. Patients who have had weight loss surgery have regular blood tests and outpatient appointments, so you will be seen regularly as part of your routine follow-up.

What is N-acetylcysteine (NAC)?

It is a drug made from the amino acid cysteine. Amino acids are building blocks of proteins. It has been used for many years as an antidote to paracetamol poisoning and also to reduce the secretions in the lungs of patients with cystic fibrosis. It works as an antioxidant and is

source of a substance called glutathione, which in the liver binds up toxic compounds to prevent liver failure. It is sometimes sold in tablet form in health food shops.

What are the side effects of N-acetylcysteine?

Some people can shows signs of allergy to N-acetylcysteine, such as a rash or wheeze. This is quite unusual. As you will be under General Anaesthesia, the anaesthetist can treat any problems at the time, using any medications as appropriate to counteract the effects of the wheezing. We will also stop the N-acetylcysteine infusion and you will not receive any more.

As with all medications, *very rarely* you can have a serious allergic reaction. This will be treated during the anaesthetic as appropriate.

PLEASE TELL US IF YOU HAVE EVER HAD A PROBLEM WITH THIS MEDICATION BEFORE.

What are the risks and disadvantages of taking part?

You may get an allergic reaction to the N-acetylcysteine during surgery. This is rare but can be serious. It is usually easily treatable during your anaesthetic.

Participants in this study will be asked to give some extra blood samples each time they have blood taken. This will be 1 extra vial (approximately 10mls or two teaspoons) each time. Usually blood is taken before and on each day after surgery.

Participants will also have an extra liver biopsy sample at the end of the operation. All our patients have a liver biopsy at the beginning of the operation. Liver biopsy does have a small risk of causing bleeding (1 in 300) or damage at the tip of the needle biopsy instrument. This should be less of a problem during surgery as the surgeon can see where the needle is going and stop any bleeding if there is any.

As the participants will be allocated at random, you may not receive N-acetylcysteine. Only trial participants are currently receiving N-acetylcysteine as we are not sure whether or not it is helpful. If you do not wish to participate in this study, your treatment will not be affected and you will not receive N-acetylcysteine.

Any complications resulting from the interventions in this study will be treated by the clinical team as appropriate. The information will be passed on to the committees who have authorised this study for further scrutiny. Participation in this study does not affect your rights regarding formal complaints or legal procedures.

What are the benefits of taking part?

We cannot guarantee that you will benefit from taking part in this study. Although N-acetylcysteine is an effective drug treatment in other medical conditions, we do not know if it is useful in this setting. This is the reason for us to perform this research study. By participating, you may help other people in the future who have the same operation.

What happens after the research study finishes?

Participating in this study does not change the way you are treated after your operation. You will attend outpatient clinic at regular intervals.

What happens if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my personal information be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be anonymised. The details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART2 Participant Information Leaflet

What happens when the research project stops?

Your follow-up will continue as agreed by you and your surgeon. Usually you are seen 6 weeks after your operation. Subsequent appointments are arranged every 6 months to yearly, but can be earlier if needed.

What happens if I want to withdraw from the study?

You are free to withdraw from the study at any time. Obviously if you have already had surgery, this will only affect the additional blood samples that you give us. We will use the data up to your withdrawal. If you wish, we will destroy any remaining samples that we still have.

What happens if new information about the research medicine comes along?

We actively monitor the results of obesity surgery continuously. If any particular problems occur, we will amend or stop the study. Because we see you regularly in the clinic anyway, you will have an opportunity to find out if any new information comes to light.

What if there is a problem or something goes wrong?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact number 02032993065). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure . Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against King's Healthcare NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Will anyone else know I'm doing this?

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the research team. They may also be looked at by representatives of regulatory authorities and by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

The data will be stored securely in files and on a computer within a locked office in King's College Hospital. Access will be limited to authorised persons only.

We will inform you GP, if you wish, that you are participating in this trial by letter.

What will happen to any samples I give?

The samples collected from you will be labelled with an identification code. They will be stored in a secure storage facility, which conforms to strict regulations of the Human Tissue Authority.

A number of specialised tests will be performed, looking for indicators of liver damage within the blood and the liver tissue.

There may be some blood or liver tissue left over. We would like to continue to store this for future use in other research projects. We can inform you of these projects, if you wish. At that time, you can ask for your samples to be destroyed if you wish.

Will there be any Genetic tests?

Yes. Some of the tests performed on the samples look at the activity of particular genes controlling the processes of inflammation, scarring and cell damage within the liver tissue. These tests do not currently have any uses in the medical treatment of an individual. They do help us further understand the way the body tissues work.

These tests do not help to identify particular information about you, such as the risk of particular inherited diseases. The information has no use to insurance companies or employers. They are not the same tests used in "DNA fingerprinting" by the police.

If you would like further detailed information about these tests, then let us know.

Who is organising and funding the research?

The research is organised by the Department of Surgery and Institute of Liver Studies at King's College Hospital. Funding for the research is from the Research budget at King's College Hospital.

The salary of the researchers is paid for by King's College Hospital and is for clinical work within the hospital.

Your surgeon and research staff are not paid for specifically for performing this trial.

This research project forms part of a research degree thesis (MD Res) being undertaken by Mr A Belgaumkar, Clinical Research Fellow, for submission to the University of London.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and approved by King's College Hospital Research Ethics Committee. The Medicines and Healthcare products Regulatory Agency (a national organisation responsible for licensing all medications) have given permission for us to use N-acetylcysteine within the trial setting.

Who will be told the results of this study?

The results of this study will be communicated to the wider medical and scientific community. This could be in the form of oral presentations, poster presentations and scientific articles published in relevant scientific journals. Occasionally, the non-scientific press may publicise the results.

None of the data presented will include personal identifying information.

If you want to know the results of the study, we can discuss them with you when you attend your usual follow-up outpatient appointments. We can also provide you with copies of any written articles.

Further Information

If you would like more information or would like to discuss the trial further, please contact us. We can arrange an appointment to see you. Alternatively, we can meet you again when you attend Pre-operative Assessment Clinic.

CONTACT: Mr A Belgaumkar, Research Fellow, Tel 02032993065

Participant Information Leaflet

KEYFACTS:

Participation is completely VOLUNTARY

If you do not participate, it makes no difference to your care.

You may or may not benefit individually from participation in this trial.

No extra visits to the hospital are required.

Half of the participants will be RANDOMLY allocated to receive N-acetylcysteine during surgery, half will not receive NAC.

All the participants will have ONE EXTRA Liver biopsy at the end of surgery.

All the participants will give ONE EXTRA VIAL of blood each time they have samples taken before and on each day after surgery.

A small extra risk of bleeding from the second liver biopsy exists.

A small risk of allergic reaction to N-acetylcysteine exists.

Tests on the blood and liver samples include genetic analysis.

You can change your mind at anytime if you don't want to participate.

You can ask any further questions or have more details as you wish.

Please contact the researchers on 02032993065 for further information.

Appendix C

Bibliography

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