

BMJ Open Body composition, mitochondrial oxygen metabolism and metabolome of patients with obesity before and after bariatric surgery (COMMITMENT): protocol for a monocentric prospective cohort study

Charles Neu,^{1,2} Kornel Skitek,^{1,2} Hermann Kissler,³ Philipp Baumbach,^{1,2} Utz Settmacher,³ Ricardo Esper Tremel,^{1,2} Sina M Coldewey ^{1,2,4}

To cite: Neu C, Skitek K, Kissler H, *et al.* Body composition, mitochondrial oxygen metabolism and metabolome of patients with obesity before and after bariatric surgery (COMMITMENT): protocol for a monocentric prospective cohort study. *BMJ Open* 2022;**12**:e062592. doi:10.1136/bmjopen-2022-062592

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2022-062592>).

Received 03 March 2022
Accepted 16 May 2022



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For numbered affiliations see end of article.

Correspondence to

Professor Sina M Coldewey;
sina.coldewey@med.uni-jena.de

ABSTRACT

Introduction Obesity, defined as a body mass index ≥ 30 kg/m², is one of the most prevalent health conditions worldwide. It is part of the metabolic syndrome, which encompasses arterial hypertension, dyslipoproteinaemia and diabetes. Obesity is viewed as a systemic disease with pathophysiological mechanisms on the molecular level. Dysfunction of the mitochondrion and systemic low-grade inflammation are among the proposed causes for the metabolic changes. In severe cases of obesity, laparoscopic sleeve gastrectomy, a bariatric operation, can achieve the desired weight loss and has been associated with clinical outcome improvement. Hitherto, the influence of patients' body composition on mitochondrial function and concomitant metabolic changes has not been fully understood. This study aims to quantify the patient's body composition before and after laparoscopic sleeve gastrectomy and to correlate these findings with changes in mitochondrial oxygen metabolism, metabolome and immune status.

Methods and analysis In this prospective monocentric cohort study, patients undergoing laparoscopic sleeve gastrectomy (n=30) at Jena University Hospital (Germany) will be assessed before surgery and at four time points during a 1-year follow-up. Body composition will be measured by bioimpedance analysis. Non-invasive assessment of mitochondrial oxygen metabolism using protoporphyrin IX-triplet state lifetime technique (PPIX-TSLT) and blood sampling for, among other, metabolomic and immunological analysis, will be performed. The primary outcome is the difference in relative fat mass between the preoperative time point and 6 months postoperatively. Further outcomes comprise longitudinal changes of PPIX-TSLT and metabolic and immunological variables. Outcomes will be assessed using paired t-tests, Wilcoxon signed-rank tests and regression analyses.

Ethics and dissemination The study was approved by the Ethics Committee of Friedrich Schiller University Jena (2018-1192-BO). Written informed consent will be obtained from all patients prior to enrolment in the study.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study employs a novel device for the non-invasive measurement of mitochondrial function in patients with obesity before and after laparoscopic sleeve gastrectomy.
- ⇒ The study will assess concomitant metabolomic, immunological and clinical outcomes.
- ⇒ Due to its monocentric design and moderate sample size, the generalisability of results may be limited.

The results will be published in peer-reviewed journals and presented at appropriate conferences.

Trial registration number DRKS00015891.

INTRODUCTION

Background

Obesity represents one of the most prevalent health conditions and it places an economic burden on healthcare systems worldwide.¹ It is defined as a body mass index (BMI) of equal or above 30 kg/m². Patients with obesity have an increased risk of developing cardiovascular disease, diabetes and dyslipoproteinaemia.² A clustering of these conditions is referred to as metabolic syndrome.³ Obesity can be treated conservatively with lifestyle interventions such as dietary restriction, physical exercise and recently with liraglutide.⁴ In severe cases, bariatric surgery can achieve the desired weight loss.⁵ One such operation is the laparoscopic sleeve gastrectomy, where part of the stomach is excised, leaving a sleeve with only little capacity for food.⁶ Within the first year, sleeve gastrectomy elicits a mean loss of excess body weight

of 72%.⁷ Bariatric surgery has also been shown to improve obesity-associated conditions in meta-analyses.^{8–10}

In accordance with recent research, the metabolic syndrome is regarded as a severe systemic condition with pathophysiological interactions at the molecular level.¹¹ Studies of the metabolome of patients with obesity have shown changes in lipid metabolism.¹² A monocentric study demonstrated that changes in the metabolome of hepatocytes were partially reversible after bariatric surgery.¹³ The mitochondria—organelles responsible for, among other things, cellular energy homeostasis via ATP production—may play a role in the pathophysiology of the metabolic syndrome and in obesity-related diseases.^{14–16} Currently, one of the proposed pathophysiological mechanisms for the development of the metabolic syndrome and mitochondrial dysfunction in patients with obesity is inflammation^{17 18} Adipokines, a class of pro-inflammatory cytokines produced by adipose tissue, may contribute to this low-grade inflammatory state. A pilot study with 20 participants found improved mitochondrial respiration, in skeletal muscle biopsies and in peripheral blood monocytes after bariatric surgery.¹⁹ Thus far, analyses of mitochondrial function and concomitant metabolic changes in obesity have been conducted using *ex vivo* methods.

Mik *et al* developed a device enabling the non-invasive measurement of mitochondrial oxygen metabolism in the human skin.²⁰ The measurement is based on the protoporphyrin-IX triplet state lifetime technique. In brief, a small patch of the patient's skin is pretreated with 5-aminolevulinic acid (5-ALA), a precursor of the haem molecule. A sensor emitting green laser light detects the delayed fluorescence which correlates with the oxygen

partial pressure in the mitochondrion. The CE-certified cellular oxygen monitor (COMET) has been tested in healthy volunteers and is currently being employed in a study of septic cardiomyopathy²¹ where it was deemed feasible for use in patients with sepsis.²² Main variables are the mitochondrial oxygen tension (mitoPO₂), mitochondrial oxygen consumption (mitoVO₂) assessed by the decrease of mitoPO₂ during inhibition of the micro-circulation by applying pressure on the skin and mitochondrial oxygen delivery (mitoDO₂) as increase of the mitoPO₂ after releasing the pressure.^{20 23} Hitherto, the influence of patients' body composition on their metabolome and mitochondrial oxygen metabolism has not been fully understood. Addressing this research question could yield new insight into the pathophysiology of obesity and the mechanisms behind the therapeutic effects of bariatric surgery.

Aim

The aim of this prospective monocentric cohort study is to accurately quantify the patient's body composition before and after laparoscopic sleeve gastrectomy and to correlate these findings with changes in the patient's metabolome, mitochondrial oxygen metabolism, immune status and epigenetic modifications of immune cells.

METHODS AND ANALYSIS

Study design

This study is a monocentric prospective longitudinal study of patients with obesity scheduled for gastric sleeve surgery. Patient recruitment commenced in April 2018 aiming to include 30 patients. The last follow-up is

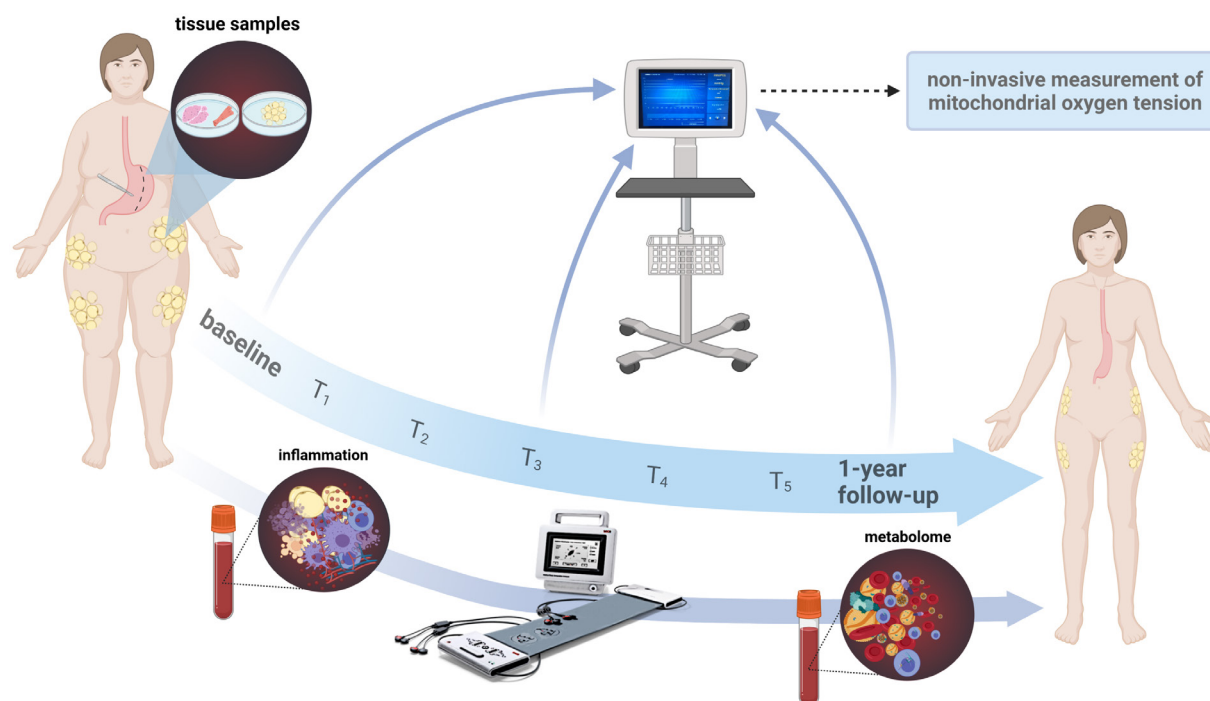


Figure 1 Graphical abstract of the overall design and employed methods of the study. Created with BioRender.com.

planned for December 2022. [Figure 1](#) displays in graphical form the design and methods employed in the study.

Study setting

Patients will be recruited from the outpatient facilities of the Department of General, Visceral and Vascular Surgery of the Jena University Hospital, Jena, Germany. Follow-up assessments will take place in outpatient facilities of the Department of Anaesthesiology and Intensive Care Medicine of Jena University Hospital.

Study population and sample size

The study population consists of one group of approximately 30 patients with obesity receiving laparoscopic sleeve gastrectomy.

Patient inclusion and exclusion criteria

Patients are eligible to participate in the study if they suffer from class III obesity with a BMI ≥ 40 kg/m², have reached at least stage 2 of the Edmonton Obesity Staging System and are scheduled for gastric sleeve surgery. Patients must be at least 18 years of age and provide written informed consent.

Patients with contraindications for one of the measurement methods, pregnant or breastfeeding patients and patients with participation in an interventional trial or prior participation in this study are excluded from the study.

Study outline

[Table 1](#) shows an overview of the scheduled clinical and laboratory analyses for patients.

Clinical and laboratory assessments

The patient's history and clinical data will be obtained from patient interviews, medical documents and hospital databases. It will be gathered preoperatively (T₀) and updated during the follow-up assessments at 3±1 months (T₂), 6±1 months (T₃), 9±1 months (T₄) and 12±1 (T₅) after surgery. During the gastric sleeve operation (T₁), tissue biopsies are obtained for future analyses of mitochondrial oxygen metabolism. It is planned to assess mitochondrial respiration using high-resolution respirometry (Oxygraph-2k, Oroboros, Innsbruck, Austria).

Blood samples are taken at T₀ and T₂ through T₅ for the analysis of, among other, the metabolome, lipidome

(including specific bioactive lipid mediators), proteome and surrogate parameters of organ and endothelial function and immune status (including epigenetic modifications of immune cells) via *i.a.* targeted and untargeted liquid chromatography–mass spectrometry, fluorescence-activated cell sorting, ELISA and multiplex assay analyses.

Non-invasive measurement of mitochondrial oxygen metabolism will be conducted using the COMET measurement system (Photonics Healthcare BV, Utrecht, Netherlands).²⁴ To this end, a medical plaster containing 5-ALA (Alacare, photonamic, Wedel, Germany) will be applied to a small patch of skin on the thorax at least 5 hours before measurement. The measurement will yield mitochondrial oxygen partial pressure, mitochondrial oxygen consumption²⁰ and mitochondrial oxygen delivery.²³

To assess body composition, particularly body fat, total body water and fat-free mass, the mBCA 525 (seca, Hamburg, Germany) will be employed in bioelectrical impedance analysis.²⁵

Outcome measures

The primary outcome measure of this study is the difference in relative fat mass between the preoperative time point (T₀) and 6 months postoperatively (T₃). The secondary outcome measure is the difference in mitoVO₂ between T₀ and T₃.

Furthermore, the following research questions will be addressed:

- Differences in mitochondrial oxygen metabolism (mitoPO₂, mitoVO₂ and mitoDO₂) between T₀, T₃ and T₅.
- Explorative analysis of the association between BIA variables and mitoPO₂ as well as mitoVO₂ at T₀, T₃ and T₅.
- The influence of bariatric surgery on the metabolome, immune status and epigenetics of immune cells.

Sample size calculation and statistical analysis

The primary endpoint will be tested using a paired t-test. We expect a medium to large sized reduction of the relative fat mass (Cohen's $d \leq -0.5$). To achieve a statistical power of 0.8 on a one-sided α -level=0.05 about 27 patients have to be included in the study. We aim to include 30 patients to allow a loss to follow-up rate of 10%. The secondary and most of the further outcomes will also be analysed with paired tests

Table 1 Study outline for patients

Item	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Medical history and clinical data	X		X	X	X	X
Skeletal muscle, gastric and fat tissue biopsies		X				
Mitochondrial oxygen metabolism measurement	X			X		X
Bioimpedance analysis	X		X	X	X	X
Blood sampling	X		X	X	X	X

T₀ preoperatively, T₁ day of operation, T₂ 3±1 months after the operation, T₃ 6±1 months after the operation, T₄ 9±1 months after the operation, T₅ 12±1 months after the operation.

(t-tests or Wilcoxon signed-rank test in case of non-normally distributed variables). For the explorative analysis on associations between BIA variables and COMET variables, regression models (eg, generalised estimation equations) will be used.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

ETHICS AND DISSEMINATION

Informed consent

Designated study physicians will obtain written informed consent from patients prior to enrolment in the study.

Ethics approval, study registration and data management

The study in its current version (1.2, 23 February 2021) is in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Friedrich Schiller University Jena (2018-1192-BO, 9 November 2018, current version approved 9 March 2021). Any changes to the study protocol are submitted to and approved by the Ethics Committee of the Friedrich Schiller University Jena. All data are saved associated with pseudonymised patient identification numbers. Data will be stored on servers of Jena University Hospital.

Dissemination

The results will be published in peer-reviewed journals and presented to the scientific community at appropriate conferences. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) criteria²⁶ will be observed when publishing the results.

Author affiliations

¹Department of Anaesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany

²Septomics Research Centre, Jena University Hospital, Jena, Germany

³Department of General, Visceral and Vascular Surgery, Jena University Hospital, Jena, Germany

⁴Centre for Sepsis Control and Care, Jena University Hospital, Jena, Germany

Contributors Principal investigator: SMC; conception of the study: SMC; contribution to study design: SMC, CN, KS, HK and PB; on-site implementation: RET, KS, HK, US and SMC; drafting of the case report forms: KS; planning of sample size and statistical analysis: PB; funding acquisition and grant holder: SMC; drafting of the manuscript: CN and SMC; revision of the manuscript prior to submission: CN, KS, HK, PB, US, RET and SMC.

Funding This study is an investigator-initiated study of the Department of Anaesthesiology and Intensive Care Medicine and the Department of General, Visceral and Vascular Surgery at Jena University Hospital, Jena, Germany. This study was funded by the Federal Ministry of Education and Research within the Centre for Innovation Competence Septomics (Translational Septomics, grant 03Z22JN12 to SMC).

Disclaimer The funding source had no role in the design of the study and will not have any role in its execution, analyses or interpretation of the data or the decision to publish results.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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ORCID iD

Sina M Coldewey <http://orcid.org/0000-0002-7130-0006>

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