Impaired mitochondrial oxidative phosphorylation (OXPHOS) in liver tissue has been hypothesized to contribute to the development of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease (NAFLD). It is unknown whether OXPHOS capacities in human visceral (VAT) and subcutaneous adipose tissue (SAT) associate with NAFLD severity and how hepatic OXPHOS responds to improvement in NAFLD. In biopsies sampled from 62 patients with obesity undergoing bariatric surgery and nine control subjects without obesity we demonstrate that OXPHOS is reduced in VAT and SAT while increased in the liver in patients with obesity when compared with control subjects without obesity, but this was independent of NAFLD severity. In repeat liver biopsy sampling in 21 patients with obesity 12 months after bariatric surgery we found increased hepatic OXPHOS capacity and mitochondrial DNA/nuclear DNA content compared with baseline. In this work we show that obesity has an opposing association with mitochondrial respiration in adipose- and liver tissue with no overall association with NAFLD severity, however, bariatric surgery increases hepatic OXPHOS and mitochondrial biogenesis.
Non-alcoholic fatty liver disease (NAFLD), which is strongly linked to obesity and metabolic dysregulation such as type 2 diabetes (T2DM), has emerged as one of the most frequent causes of chronic liver disease across most of the world. In NAFLD, fat accumulates within the hepatocytes but the disease spectrum of the condition is broad and gives rise to a variety of phenotypes, from the relatively benign non-alcoholic fatty liver (NAFL), to the aggressive non-alcoholic steatohepatitis (NASH), which may eventually progress to cirrhosis. Despite extensive research, the pathophysiology driving the different phenotypes of NAFLD remains poorly understood. Yet, hepatic mitochondrial dysfunction has been hypothesised to be involved in the progression of NAFL to NASH and fibrosis and reduced mitochondrial oxidative phosphorylation (OXPHOS) capacity has previously been reported in liver tissue sampled from patients with obesity and NASH when compared with OXPHOS in those with NAFL.

Visceral- (VAT) and subcutaneous adipose tissue (SAT) -inflammation, adipokine dysregulation and -insulin resistance are important defining features of obesity and major contributors to the development of obesity-related metabolic complications including NAFLD. For instance, release of free fatty acids from insulin resistant adipose tissue is the primary source of the lipids accumulating in the liver in NAFLD and adipose tissue inflammation has previously been associated with NAFLD severity.

Studies in humans and rodents have previously indicated suppressed mitochondrial biogenesis, downregulation of genes that encode mitochondrial respiratory complex products and decreased respiration/OXPHOS in adipose tissue in obesity.

Suboptimal mitochondrial function may be connected directly to both the adipose tissue dysregulation and the development of metabolic disease. Moreover, data from mice even suggest a link between impaired adipocyte mitochondrial function and decreased adiponectin (a recognised hepatoprotective and anti-fibrotic adipokine) levels, decreased adiponectin synthesis, as well as hepatic steatosis.

If adipose tissue OXPHOS reflects adipose tissue metabolic dysregulation, the level of mitochondrial respiration could in turn influence NAFLD severity. Moreover, SAT and VAT do not present with the same mitochondrial respiratory capacity, as VAT has a lesser respiratory reserve capacity compared with SAT. Thus, a decreased capacity to oxidise substrates in VAT may indirectly negatively influence the liver, because of potential lipid peroxidation, and cell damage and subsequent release of inflammatory cytokines/adipokines to the liver via the portal vein. In addition, a diminished capacity to oxidise lipids may result in increased release of FFA into the portal vein, thereby facilitating hepatic steatosis. Yet, no studies have measured VAT and SAT mitochondrial respiratory capacity together with hepatocyte mitochondrial respiratory capacity in patients with NAFLD. Also, the effect of major weight loss induced by bariatric surgery on hepatic OXPHOS is unexplored.

We investigated OXPHOS patterns using high-resolution respirometry (HRR) analyses in VAT, SAT, and liver tissue sampled from subjects with obesity and with varying stages of NAFLD who underwent bariatric surgery and in control study subjects without obesity (CON).

We predicted that mitochondrial respiration in VAT and SAT was mirrored in liver tissue and reflected NAFLD severity, with the lowest OXPHOS in all three tissues be measured in those with NASH.

In addition, we investigated the effects of bariatric surgery on hepatic OXPHOS capacities 12 months after surgery.

Here, we report no impact of NAFLD severity on hepatic OXPHOS in individuals with obesity, an obesity driven decrease in adipose tissue respiration but a marked increase in hepatic OXPHOS and hepatic mitochondrial biogenesis 12 months after bariatric surgery.

Results

Subjects characteristics at baseline (Table 1). Weight, BMI and waist-hip ratio were similar across the NAFLD (NAFL+, NASH+) and CON groups. Subjects in the NASH group were more likely to have T2DM (P < 0.05) and median (interquartile range, IQR) HOMA-IR was higher in NASH (7.6 (6.2–10.0) vs. NAFL+ - 4.0 (2.9–5.4), P < 0.001) and NASH+ (5.3 (4.4–6.4), P = 0.057). Alanine aminotransferase (ALT) levels were higher in the NASH group (32 U/L (30–52) vs. NAFL− group (26 U/L (20–31)), P < 0.05) and NAFL− group (27 (22–39), P < 0.05).

Mitochondrial content in the hepatic biopsies and in the adipose tissue was not different across CONs and NAFLD groups (Table 2). Figure 1 depicts the schematic study set-up.

Oxygen fluxes in liver tissue (Figs. 2a–f, 4a+b and Supplementary Data S1). In general, both mass-specific (Fig. 2a), citrate synthase (CS) activity (Fig. 2c) and mtDNA/nDNA -corrected oxygen fluxes (Fig. 2e) in liver tissue in substrate-inhibitor protocol 1 (SUIT P1) followed a specific pattern, with NAFL+ presenting with the highest numerically respiratory rates across all substrate steps, NASH and NAFL+ with intermediate flux rates, and CON with the lowest oxygen flux rates. However, this was non-significant between the four groups.

First, we corrected mass-specific hepatic fluxes for variation in CS levels (Table 2), which did not alter the respiratory- or significance patterns in SUIT P1 (Fig. 2c). Respiratory rates remained non-significant between groups (Fig. 2c).

When adjusting for mtDNA/nDNA (Fig. 2e, Table 2 and Supplementary Data S1) OXPHOSmax pr. mtDNA/nDNA (GMOsD), the step representing maximal coupled oxidative phosphorylation, was significantly increased by 55% in NAFL+ when compared to CON (P < 0.05) and by 23% and 18% (albeit not significantly) compared with NASH− and NASH+, respectively. No significant differences between groups were observed for the maximal-uncoupled respiration (FCCP) (P = 0.054), GM2 capacity (P = 0.047 but significance was lost in the pairwise comparison with correction for multiple comparison) or GMOsD capacity (P = 0.070) between the NAFLD groups.

The P/E (Fig. 4a) and RCR (Fig. 4b) were also similar in the four groups.

In regression analyses, none of the chosen eight predictors (BMI, HOMA-IR, steatosis grade, ballooning grade, VAT OXPHOSmax, plasma leptin, plasma adiponectin and alanine aminotransferase) were found to be significantly associated with liver OXPHOSmax per CS.

BMI was the only significant predictor (out of the above eight mentioned) of liver OXPHOSmax per mtDNA/nDNA (unstandardised β: 0.0013; 95% CI: 0.0004–0.0021, adjusted R² = 0.128, P < 0.01). In the multiple linear regression of hepatic OXPHOSmax using the four independent variables from the simple linear regressions with the lowest P-values (BMI, leptin (P = 0.050), ALT (P = 0.081) and steatosis grade (P = 0.05)), all were positively associated with hepatic OXPHOSmax but only BMI proved to be a significant predictor (unstandardised β: 0.0013; 95% CI: 0.0004–0.0021, adjusted R² = 0.127, P = 0.004). In post hoc multiple linear regression analyses neither sex (P = 0.634), T2DM status (P = 0.258) status or age (P = 0.214) were significantly associated liver OXPHOSmax per mtDNA/nDNA and BMI remained statistically significant (P = 0.002) (Supplementary Information).
respectively) (Fig. 2f). With the addition of Antimycin A (blocking of electrons from complex III) the mtDNA adjusted fluxes were again markedly higher in NAFL+ when compared with NASH (P < 0.05).

### Table 1 Clinical, anthropometrical and biochemical characteristics at baseline in patients with obesity (stratified by NAFLD severity) and normal weight control subjects undergoing bariatric surgery and cholecystectomy, respectively.

| Baseline | Patients with obesity according to histology | Controls |
|----------|---------------------------------------------|----------|
|          | NAFL− (n = 16)                              | NAFL+ (n = 15) | NASH (n = 9) | CON |
| Liver histology |                                            |                        |                |        |
| NAFLD activity score | 2 (2-3)                                    | 3 (3-4)                  | 5 (5-5)***      | 1 (0.5-15)***      |
| Steatosis | 0 (0-0)                                     | 1 (1-1)***               | 2 (1-2)***      | 0 (0-0)***        |
| Inflammation | 1 (1-2)                                    | 1 (1-1)                  | 2 (1-2)†        | 1 (0.5-1)         |
| Ballooning | 1 (1-1)                                    | 1 (1-1.75)               | 2 (2-2)†       | 0 (0-0.5)***      |
| Age (years) | 44 (36-51)                                  | 45 (40-52)               | 44 (41-53)     | 39 (34-43)        |
| Female (%) | 25 (8)                                      | 8 (50)                   | 8 (53)         | 7 (78)           |
| RYGB (%)/SG | 12 (39)/19                                  | 12 (75)/4                | 5 (33)/10      | 0 (0)           |
| Diabetes (%) | 2 (5)                                       | 5 (31)                   | 8 (53)‡      | 0 (0)           |
| Hypertension (%) | 8 (26)                                      | 6 (37)                   | 5 (33)       | 0 (0)           |
| Dyslipidemia (%) | 5 (16)                                      | 6 (38)                   | 6 (40)       | 0 (0)           |
| Weight (kg) | 121 (105-134)                               | 136 (118-168)            | 127 (110-132)  | 70 (62-80)***     |
| BMI (kg/m²) | 41.6 (37-45.2)                              | 45.6 (39-51.2)           | 41.6 (35-47.1) | 24.5 (22.3-26.4)*** |
| Waist-hip ratio | 0.83 (0.8-0.94)                             | 0.90 (0.87-0.95)         | 0.97 (0.90-1.04) | 0.84 (0.75-0.90)† |
| ALT (U/L) | 26 (20-31)                                  | 27 (22-39)               | 32 (30-52)††    | 19 (18-27)        |
| AST (U/L) | 23 (20-28)                                  | 21 (18-30)               | 27 (20-30)†     | 20 (17-26)        |
| Fasting glucose (mmol/L) | 5.8 (5.5-6.1)                             | 6.4 (5.5-8.4)            | 6.4 (6.2-7.0)† | 5.3 (5.2-6.2)‡‡ |
| C-peptide (pmol/L) | 1050 (922-1270)                           | 1280 (1060-1410)         | 1655 (1270-2112) | 863 (650-921)‡‡‡ |
| Fasting insulin (pmol/L) | 108 (84-143)                                | 116 (103-172)            | 191 (147-231)†‡ | 64 (47-96)*** |
| HbA1c (mmol/mol) | 35.0 (33-38.0)                              | 37.0 (35.0-50.0)         | 37.5 (35.4-40.0) | 33 (29-34.5)‡‡ |
| HOMA-IR | 4.0 (2.9-5.4)                               | 5.3 (4.4-6.4)            | 7.6 (6.2-10.0) #|| 21.1 (6.3-5.5)‡‡‡ |
| hHuCRP (mg/L) | 4.4 (1.6-7.4)                               | 6.8 (4.2-12.4)           | 3.6 (2.3-7.8)  | 0.9 (0.7-2.9)‡‡‡ |
| Adiponectin (ng/mL) | 6463 (4850-8781)                           | 4936 (4478-6056)         | 5255 (2943-6307) | 9327 (4021-12630) |
| Leptin (ng/mL) | 50 (27-99)                                  | 50 (31-80)               | 26 (18-60)     | 13 (8-21)‡‡‡ |
| sCD163 (ng/mL) | 687 (571-758)                               | 651 (592-705)            | 759 (664-915)  | 556 (492-618)‡‡‡ |
| sCD206 (ng/mL) | 325 (241-424)                               | 394 (229-497)            | 378 (315-440)  | 256 (214-510) |
| IL-1β (ng/mL) | 0.05 (0.02-0.07)                           | 0.07 (0.03-0.10)        | 0.02 (0.01-0.04) | 0.02 (0.02-0.05) |
| IL-6 (ng/mL) | 0.94 (0.60-1.38)                           | 1.07 (0.86-1.50)        | 1.00 (0.91-1.45) | 0.62 (0.25-1.18) |
| TNF-α (ng/mL) | 1.97 (1.59-2.34)                           | 2.12 (1.77-2.50)        | 1.97 (1.59-2.10) | 1.71 (1.16-2.43) |

Data are presented as medians (IQR). P-values (2-sided) are Kruskal-Wallis with correction for multiple comparison or Chi Square test. NAFLD non-alcoholic fatty liver disease, RYGB Roux-en-Y gastric bypass, SG sleeve gastrectomy, BMI body mass index, ALT alanine aminotransferase, AST aspartate aminotransferase, HbA1c glycated haemoglobin, HOMA-IR Homeostatic Model Assessment for Insulin Resistance, hHuCRP high-sensitivity C-reactive protein, sCD163 soluble cluster of differentiation 163, sCD206 soluble cluster of differentiation 206. †, ††, ††† denotes statistical significance (P < 0.05, 0.01, 0.001, respectively) compared with NAFL−. ‡, ‡‡, ‡‡‡ denotes statistical significance (P < 0.05, 0.01, 0.001, respectively) compared with NAFL+. ††, †††, ††††, †††‡ denotes statistical significance (P < 0.05, 0.01, 0.001, respectively) compared with NASH. *P = 0.052 compared with NASH. **P = 0.0027 compared with NAFL+. *Denotes significantly more study subjects with type 2 diabetes in the NASH group.

### Table 2 Citrate synthase activity and mtDNA/nDNA count in liver-, visceral adipose- and subcutaneous adipose tissue.

| Baseline | NAFL− | NAFL+ | NASH | CON |
|----------|-------|-------|------|-----|
| Liver tissue | Citrate synthase activity (µmol g⁻¹ min⁻¹) | 10.5 (7.5-12.4) | 10.9 (7.8-13.8) | 11.0 (7.7-12.6) | 13.2 (9.9-14.5) |
|          | mtDNA/nDNA count | 470 (421-520) | 479 (436-519) | 508 (463-531) | 536 (417-709) |
| Visceral adipose tissue | mtDNA/nDNA count | 268 (248-308) | 267 (254-302) | 259 (218-287) | 292 (201-378) |
|          | Subcutaneous adipose tissue | mtDNA/nDNA count | 235 (197-276) | 263 (215-285) | 239 (198-281) | 254 (229-276) |

In substrate-inhibitor protocol 2 (SUIT P2) (Figs. 2b, d, f and Supplementary Data S1) respiratory fluxes (mass-specific, CS and mtDNA/nDNA adjusted) were similar across groups. However, with the addition of rotenone (inhibitor of complex I respiration) NAFL+ displayed slightly higher mtDNA/nDNA adjusted fluxes when compared with both NAFL− and NASH (P < 0.01 and P < 0.01, respectively) (Fig. 2f). With the addition of Antimycin A (blocking of electrons from complex III) the mtDNA adjusted fluxes were again markedly higher in NAFL+ when compared with NASH (P < 0.05).
**Fig. 1 Depiction of the experimental study set-up.** Liver tissue, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were sampled from 62 patients undergoing surgery—Roux-en-Y gastric bypass (n = 29) or sleeve gastrectomy (n = 33)—and stratified into three groups: 31 patients without liver steatosis (NAFL−); 16 patients with liver steatosis (NAFL+); and 15 patients with NASH (NASH). Nine normal weight control subjects without hepatic steatosis and who underwent planned laparoscopic cholecystectomy served as a control group (CON). Twenty-one patients with obesity underwent repeat liver biopsy 12 months after bariatric surgery. Fresh tissue from both timepoints underwent ex vivo, high-resolution respirometry (HRR) measurements in an Oxygraph, which measures tissue oxygen consumption and hence can determine mitochondrial respiratory chain complexes’ (I-IV) respiratory rates and maximal electron transport system capacity. Two separate sequential substrate-inhibitor (SUIT) protocols were applied in the tissue. SUIT P1, panel A: (1) Adding of glutamate and malate (GM) to reveal leak respiration through complex I (2) Adding of ADP (GMD) to initiate complex I linked oxidative respiratory process (3) Addition of a lipid (the fatty acid octanoyl carnitine) to assess complex II lipid respiratory capacity (GMOD) (4) Addition of the powerful stimulator rotenone followed by (1) addition of succinate to assess specific complex II respiration. For further details of SUIT protocols, please see Methods and Supplementary Information. This figure was created with BioRender.com.

Subcutaneous adipose tissue (Fig. 3b+d, Fig. 4a, b and Supplementary Data S1). In SAT, mass-specific OXPHOSmax and FCCP) when compared with CON (Fig. 3a). No differences among the NAFLD groups were found. Although mtDNA copy numbers were not statistically different between groups (P = 0.624) (Table 2), when correcting fluxes for mtDNA/ nDNA content, respiratory fluxes were no longer significantly different (Fig. 3c).

sCD163, a plasma macrophage marker, was the factor most strongly and negatively associated with VAT OXPHOSmax but it was neither significant in the univariable model (P = 0.042, R² = 0.055) nor in the multivariable model with BMI, sCD163, adiponectin and HOMA-IR (P = 0.048, R² = 0.054) with the significance level set to <0.006.

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Patients with and without T2DM had comparable respiratory fluxes in both VAT and SAT (Supplementary Data S3) and T2DM, age and sex were insignificant predictors of VAT/SAT OXPHOS in post hoc regression models (Supplementary Information). In logistic regression models neither OXPHOSmax nor maximal-uncoupled respiration (FCCP) in VAT (P = 0.449, \(P = 0.515\)) or SAT (0.445, 0.713) predicted the presence of NASH in liver tissue in patients with obesity.

Twelve months follow-up (Figs. 5 and 6, Table 3 and Supplementary Data S2). Twelve months after surgery most clinical and biochemical parameters had improved. BMI dropped from 43.3 kg/m² (37.9−45.7) to 32.5 kg/m² (27.3−37.5) (P < 0.001). HOMA-IR (baseline: 4.4 (3.5−7.2), 12 months: 1.9 (1.4−2.4) (P < 0.01) decreased, and the plasma insulin (Table 3) NAS decreased by a median of two points (0.5−2), with significant reductions in steatosis, inflammation and ballooning (Table 3).

Hepatic maximal electron transport capacity had increased markedly 12 months after surgery (P < 0.01), as evidenced by the uncoupled respiratory capacity. The increase in OXPHOSmax was not significant (P = 0.099) (Fig. 5a), but overall resulted in a significant reduction in the P/E (P < 0.05) (Fig. 5e), indicating an increased hepatic respiratory reserve capacity 12 months after bariatric surgery, which was not different from the capacity in the CON group.

Specific complex II and IV, but not complex I, activity was significantly increased (P < 0.05) but only when fluxes were corrected for mtDNA and not CS.

Our data suggest that mitochondrial respiratory function in human hepatic tissue is not affected by NASH and also not clearly augmented in those with NAFLD. This finding is overall not in line with conclusions reached in previous studies, but there is little consensus in the literature on this topic. It has been proposed that hepatic energy metabolism including mitochondrial respiration increases with the development of steatosis, while decreasing markedly once NASH develops. Koliaki et al. drew this latter conclusion from their CS corrected flux data obtained in a cohort that was similar to the present, albeit including fewer patients in the NASH group.

Our conclusion that mitochondrial respiration is not decreased in liver tissue in patients with NASH stands in contradiction to the conclusion reached by Koliaki et al., who report significantly decreased intrinsic (respiration per CS) respiration in those with NASH compared with both NAFLD and NAFL+. Of note, respiration in those with NASH was not decreased when compared with their CON group. The authors speculate, however, that the decreased intrinsic respiration in NASH relative to NAFLD and NAFL− is due to reduced ‘metabolic adaptiveness’ in the NASH liver tissue and reflective of mitochondrial dysfunction.

Some discrepancies appear between our study and the study by Koliaki and coworkers, the most important being that they recorded a 30−50% higher mitochondrial mass (measured by mode of CS per mg protein) in those with NASH compared with their CON, NAFLD− and NAFL+. Thus, the diminished respiration in the NASH group in the study by Koliaki et al. is entirely driven by the reported increased mitochondrial content. In the present study, we did not at all observe a difference in mitochondrial mass when measured by mode of CS (Table 2). In particular, we found similar levels of CS within the NAFLD subgroups. To validate our findings of similar mitochondrial mass, we also quantified mtDNA/nDNA content and again found no significant differences in mitochondrial mass between groups (Table 2). Consequently, neither adjustment for CS nor mtDNA/ nDNA changed our results and conclusion (Fig. 2e, f).

The second major finding was the decreased respiratory rates in VAT and SAT in NAFLD groups compared with CON.
Fig. 3 Mitochondrial respiratory rates in visceral- and subcutaneous adipose tissue (VAT and SAT, respectively) at baseline. a Mass-specific substrate-inhibitor protocol 1 (SUIT P1) visceral adipose tissue (VAT) fluxes. b P1 VAT fluxes adjusted to mtDNA/nDNA. c P1 VAT fluxes adjusted to mtDNA/nDNA. d P1 SAT fluxes adjusted to mtDNA/nDNA. e Adipocyte count per counting frame (0.4 mm²) in VAT and SAT, respectively. f Number of CD68+ macrophages per 100 adipocytes in VAT and SAT. Data are median (horizontal line), interquartile range (boxes) and 10–90% percentile (error bars). P-values (2-sided) are Kruskal–Wallis pairwise comparison with correction for multiple comparison. Grey boxes; CON, blue boxes; NAFL− (boxes) and 10–90% percentile (error bars). P-values (2-sided) are Kruskal–Wallis pairwise comparison with correction for multiple comparison. Grey boxes; CON, blue boxes; NAFL−, yellow boxes; NAFL+, red boxes; NASH. VAT: NAFL− n = 30, NAFL+ n = 12, NASH n = 14, CON n = 9. SAT: NAFL− n = 29, NAFL+ n = 13, NASH n = 14, CON n = 9. Source data are provided as a Source Data file. Specific n’s for each substrate and group are provided in the Source Data file.

Fig. 4 P/E and RCR in tissue. a P/E (OXPHOSmax/FCCP) in liver tissue, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT); b Respiratory control ratio (RCR), (GMD/GM) in liver tissue, VAT and SAT. P/E and RCR were significantly higher (P/E: P < 0.001, RCR P = 0.003) in VAT and SAT when compared with liver tissue. No significant differences in P/E and RCR were found among the four groups in the three tissues. Data are median (horizontal line), interquartile range (boxes) and 10–90% percentile (error bars). P-values (2-sided) are Kruskal–Wallis pairwise comparison with correction for multiple comparison. Grey boxes; CON (n < 10), blue boxes; NAFL−, yellow boxes; NAFL+, red boxes; NASH. Source data are provided as a Source Data file. Specific n’s for each tissue and group are provided in the Source Data file.

(Fig. 3a–d), and for SAT this difference remained significant with adjustment to the mtDNA/nDNA ratio. This points towards a reduced mitochondrial capacity in SAT, that is not only explained by quantitatively-fewer mitochondria but also by qualitatively-impairment in mitochondria function.

Our group has previously compared oxidative properties by mode of HRR in whole tissue VAT and SAT from humans with obesity and reported similar results as the present29.

We are not aware of other AT mitochondrial respiration studies conducted among NAFLD patients that are available for comparison. In studies involving individuals with obesity data have primarily been obtained in SAT32–34 and studies have assessed mitochondrial function by mode of mitochondrial gene expression profiles20,33,34 with reports of impaired mitochondrial biogenesis and decreased expression of, e.g., genes encoding electron transport chain products. A few studies have compared VAT with SAT at a transcriptional level20 and at a functional level in isolated mitochondria using enzymatic-based approaches21,35. In combination with data obtained in animal and experimental models of obesity, the evidence that obesity may be associated with a reduction in adipose tissue mitochondrial oxidative capacity and mitochondrial biogenesis is quite robust. However, it is an ongoing debate whether the changes in mitochondrial function/reduced oxidative capacity are causative or secondary to the metabolic consequences (insulin resistance, T2DM) connected to obesity.

In relation to this debate, some interesting notions in our study deserve attention. Firstly, NAFLD severity did not appear to influence VAT or SAT intrinsic respiratory capacity (flux per mtDNA/nDNA) despite evidence of otherwise increasing metabolic deterioration as witnessed for example by the high HOMA-IR in the NASH group. Secondly, we did not prove any correlation between AT OXPHOS and liver OXPHOS. Thirdly, nor did we find significant difference in OXPHOS when study subjects were stratified for presence of T2DM (Supplementary Data S3) nor when T2DM was a variable in the multiple linear regression analyses (Supplementary Information). Hence, the culprit of decreased AT respiration appears to be associated with obesity rather than with NAFLD or the systemic/tissue-specific metabolic deterioration such as insulin resistance, which NAFLD is reflective of.

This is in line with the findings of similar intrinsic mitochondrial respiratory capacity measured by mode of HRR in skeletal muscle39 and hepatic tissue30 from insulin resistant patients with T2DM compared with healthy controls. The findings are also in agreement with the conclusions reached by Chattopadhyay et al.32 who found diminished respiratory chain activities (measured enzymatically) in isolated mitochondria from SAT sampled from individuals with obesity without any additional effect of T2DM.

Contrary to our initial predictions the largest discrepancies in mitochondrial respiration between CON and NAFLD subgroups were surprisingly found in SAT and not VAT, the latter of which is traditionally recognised as the fat depot with the strongest associations to metabolic disease including NAFLD40–42. Also, levels of CD68+ macrophages in both VAT and SAT in the NAFLD subgroups were indeed increased compared with CON, suggesting on-going adipose tissue inflammation, but macrophage counts were similar among both NAFLD groups and tissues (VAT vs. SAT) and degree of histological adipose tissue
Hepatic mass specific flux (P1)

![Box plot of Hepatic mass specific flux (P1)]

Hepatic mass specific flux (P2)

![Box plot of Hepatic mass specific flux (P2)]

Hepatic mass specific flux per mtDNA (P1)

![Box plot of Hepatic mass specific flux per mtDNA (P1)]

Hepatic mass specific flux per mtDNA (P2)

![Box plot of Hepatic mass specific flux per mtDNA (P2)]

P/E before and after surgery

![Box plot of P/E before and after surgery]

RCA before and after surgery

![Box plot of RCA before and after surgery]
Fig. 5 Mitochondrial respiratory rates in liver tissue obtained from 21 patients with obesity during Roux-en-Y gastric bypass surgery or sleeve gastrectomy (baseline) and again by Tru-Cut percutaneous liver biopsy twelve months later. a Mass-specific substrate-inhibitor protocol 1 (P1) fluxes, b mass-specific protocol 2 (P2) fluxes; (GMd/GM). c P1 fluxes adjusted to mtDNA/nDNA. d P2 fluxes adjusted to mtDNA/nDNA. e P/E (OXPHOS\textsubscript{max}/FCCP). f Respiratory control ratio (RCR), (GMd/GM) This patient cohort consisted of NAFL+ (\(n = 6\)), NAFL− (\(n = 13\)) and NASH (\(n = 2\)) (based on their baseline liver biopsy). CONs (baseline) are also shown. No statistically significant differences were found between CONs and OBE 12 months after surgery. P1 and P2 were performed as described in Fig. 1 and Methods. Data are median (horizontal line), interquartile range (boxes) and 10–90% percentile (error bars). P-values (2-sided) are Wilcoxon-signed rank test. Orange boxes; OBE baseline, green boxes; OBE 12 months after surgery, grey boxes (dimmed); CON (\(n < 10\)) at baseline. Source data are provided as a Source Data file. Specific \(n\)'s in each protocol and for each substrate, group and timepoint are provided in the Source Data file.

![Fig. 6 Mitochondrial DNA/nuclear DNA content in hepatic tissue from the 21 patients with obesity at baseline and 12 months later. a spaghetti plot. b median mtDNA/nDNA at the two timepoints. CONs (baseline) are also shown. No statistically significant differences were found between CONs and OBE 12 months after surgery. Data are median (horizontal line), interquartile range (boxes) and 10–90% percentile (error bars). P-values (2-sided) are Wilcoxon-signed rank test. Orange boxes; OBE baseline, green boxes; OBE 12 months after surgery, grey boxes (dimmed); CON (\(n < 10\)) at baseline. Specific \(n\)'s in each protocol and for each substrate and timepoint are provided in the Source Data file.](image-url)

inflammation was not significantly associated with low VAT or SAT OXPHOS\textsubscript{max} in regression models. Thus, and contrary to our initial hypotheses, we did not find a connection between decreased adipose tissue OXPHOS on one side and adipose tissue inflammation or NAFLD severity including presence of NASH on the other side.

It is a matter of speculation whether decreased adipose tissue OXPHOS is primarily due to increased adipocyte cell size, which is an undisputed observation in obese VAT and SAT sampled from individuals with obesity\textsuperscript{10,43,44} and also reported in the present study (Fig. 3e). Consequently, fewer cells in AT per mg of wet tissue in the OBE vs. CON could lead to fewer mitochondria available for respiration. The size of adipocytes have been reported to both affect\textsuperscript{45} and not affect\textsuperscript{41} VAT and SAT mitochondrial OXPHOS capacities. However, adjustment of AT respiratory fluxes by mtDNA/nDNA content, which was similar between groups, approximates the respiration ‘per cell’ and in SAT respiration remained significantly lower in NAFLD groups. We therefore conclude that the decreased respiration is indeed intrinsically or quantitatively decreased in SAT.

The third major finding is that crude complex II respiration as well as both crude and mtDNA/nDNA-corrected complex IV respiration as well as ETS (FCCP), increased markedly 12 months after bariatric surgery (Fig. 5). Furthermore, P/E decreased significantly after 12 months (Fig. 5c), meaning that the hepatic mitochondria had gained extra reserve capacity during the 12 months after bariatric surgery. In fact, the P/E approached that seen in CON (Fig. 5e), even though OBE were still individuals with obesity compared with CON (BMI 32.5 kg/m\(^2\) vs. 24.5 kg/m\(^2\), respectively). The reported increase in hepatic respiration appears to be driven by the marked increase in hepatic mtDNA/nDNA content of ~30%, suggesting that substantial and augmented mitochondrial biogenesis has taken place between baseline and 12 months after bariatric surgery.

We are not aware of any human studies for comparison. In mice/rat models using methodological approaches different to our own to assess mitochondrial function, RYGB and subsequent weight loss have been shown to increase mitochondrial respiratory chain capacity\textsuperscript{46}, CS activity\textsuperscript{47}, gene expression of respiratory chain complexes\textsuperscript{46} and decrease oxidative stress\textsuperscript{46,47} but not significant increase hepatic mtDNA\textsuperscript{46}.

The increase in hepatic respiration was irrespective of both mode of surgery, change in NAS and degree of weight loss, however, with only 21 study subjects we cannot exclude an effect of one or more of these parameters in a larger study cohort. Of note, the lack of association between weight loss and histological improvement on the one hand and increase in respiration on the other hand could also partly be explained by the uniformity of the data (the same degree of weight loss and same histological NAFLD improvement in all subjects) rather than the rather low number of study subjects 12 months after surgery. In our previously published paired data on liver histology in 40 individuals, 12 months after bariatric surgery we also could not conclude on the predictors for NAFLD improvement\textsuperscript{48}.

While the cross-sectional data comparison has many potential confounders (e.g., Table 1), the prospective paired comparison after 12 months has obvious strengths that makes the conclusion quite robust namely that following bariatric surgery there is an increase in hepatic mitochondrial respiratory capacity and mtDNA/nDNA content. As both mass-specific hepatic fluxes and
not the same, but Layec et al. have shown the same directional
system by use of nuclear magnetic resonance. The readouts are
obtained with the HRR. For skeletal muscle a crude comparison
methods available by which mitochondrial respiration can be
mitochondrial activity in paired tissue biopsies from the same study
adipose tissue samples from 71 study subjects and re-evaluation
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our data are indicative of substantial metabolic
extensive re-generative potential of the human liver as we here
human liver in response to bariatric surgery. The clinical
consumption by dynamic oxygen 17 (17O) nuclear magnetic
changes in the readouts of the two methods.

Table 3 Clinical and biochemical characteristics at baseline vs. follow-up 12 months after bariatric surgery in 21 OBE study subjects.

| Characteristic                  | Baseline (n = 21) | Follow-up (n = 21) | P-value |
|--------------------------------|------------------|--------------------|---------|
| NAFLD activity score           | 3 (2-3.5)        | 1 (0.5-2)          | <0.001  |
| Steatosis grade                | 0 (0-1)          | 0 (0-0)            | 0.08    |
| Inflammation grade             | 1 (1-1)          | 1 (0.5-1)          | 0.035   |
| Ballocromide index             | 1.7 (2)          | 0.0 (0-0)          | <0.001  |
| NAFLD / NASH                   | 13/6/2           | 18/3/0             |         |
| Age (years)                    | 44 (37-48)       | NA                 |         |
| Sex (female/male)              | 13/4             | NA                 |         |
| Type of surgery (SG/RYGB)      | 13/8             | NA                 |         |
| Weight (kg)                    | 105 (167-137)    | 90 (86-100)        | <0.001  |
| BMI (kg/m²)                    | 43.3 (37.9-45.7) | 32.5 (27.3-37.5)  | <0.001  |
| Waist hip ratio                | 0.86 (0.82-0.92) | 0.82 (0.78-0.88)  | <0.001  |
| %EWL                           | NA               | 60 (47-84)         |         |
| Total weight loss (kg)         | NA               | 32.7 (12.3-40.9)   |         |
| Systolic blood pressure (mmHg) | 126 (19-138)     | 112 (103-129)      | 0.005   |
| Diastolic blood pressure (mmHg)| 84 (77-92)       | 71 (65-82)         | <0.001  |
| HR (bpm)                       | 71 (66-83)       | 59 (50-70)         | <0.001  |
| ALT (U/L)                      | 31 (21-76)       | 23 (17-36)         | 0.254   |
| AST (U/L)                      | 23 (18-34)       | 24 (22-36)         | 0.631   |
| Fasting glucose (mmol/L)       | 5.9 (5.5-6.4)    | 5.3 (5.0-5.3)      | <0.001  |
| C-peptide (pmol/L)             | 160 (79-1360)    | 779 (652-846)      | <0.001  |
| Fasting insulin (pmol/L)       | 120 (101-178)    | 59 (41-72)         | 0.002   |
| HOMA-IR                        | 4.4 (3.5-7.2)    | 2.1 (1.4-2.4)      | <0.001  |
| HsCRP (mg/L)                   | 4.4 (1.6-7.1)    | 0.9 (0.4-2.0)      | <0.001  |
| Adiponectin (ng/mL)            | 5842 (4732-7695) | 11.97 (2827-13.89) | <0.001  |
| Leptin (ng/mL)                 | 45 (33-71)       | 16 (7-33)          | <0.001  |
| sCD163 (ng/mL)                 | 668 (450-729)    | 592 (552-630)      | 0.013   |
| sCD206 (ng/mL)                 | 338 (26-479)     | 272 (28-433)       | 0.018   |
| IL-1β (ng/mL)                  | 0.05 (0.03-0.08) | 0.04 (0.02-0.06)   | 0.210   |
| IL-6 (ng/mL)                   | 0.16 (0.03-1.30) | 0.59 (0.34-0.84)   | <0.001  |
| TNF-α (ng/mL)                  | 1.75 (1.47-2.28) | 1.93 (1.44-2.41)   | 0.970   |

Data are presented as medians (IQR). P-values (2-sided) are Wilcoxon-signed rank test.
NAFLD non-alcoholic fatty liver disease, SG sleeve gastrectomy, RYGB Roux-en-Y gastric bypass, BMI body mass index, %EWL percentage excess body weight loss, mmH/ml millimetres of mercury, HR heart rate, bpm beats per minute, ALT alanine aminotransferase, AST aspartate aminotransferase, HbA1c glycated haemoglobin, HOMA-IR Homoeostatic Model Assessment for Insulin Resistance, HsCRP High-sensitivity C-reactive Protein, CD68 soluble cluster of differentiation 163, sCD163 soluble cluster of differentiation 206, sCD206 interleukin 1 beta, sIL-6 interleukin 6, TNF-α tumour necrosis factor alpha.

resonance spectroscopy (NMR). However, the resolution is not near the resolution one can obtain with the ex vivo technique.

The main limitation of this study is its cross-sectional design at baseline, which does not allow for conclusions about the causality of the observed flux rates and their associations. Furthermore, the 8% mandatory weight loss required by the Danish Health Authorities for surgery could have impacted our results at baseline as our subjects were not weight stable. Yet, previously we have not found evidence of effect of the 8% diet induced weight loss on mitochondrial respiration in SAT50.

There are also some limitations to the paired data as the first liver biopsy was obtained as a wedge biopsy shortly after the introduction of anaesthesia, while the percutaneous biopsy was obtained by needle under local anaesthesia (lidocaine) and penetrates deeper into the tissue. The influence of general anaesthesia on mitochondrial respiration is unknown. However, in a supplementary experiment in a pig liver (Supplementary Fig. S1), we could not demonstrate marked differences in respiration in relation to the site of biopsy or by mode of sampling (wedge vs. percutaneous). If anything, the respiratory rates were slightly lower in the percutaneous biopsy compared with the wedge biopsy, which therefore only strengthens the finding of increased respiratory rates after weight loss. Another limitation is the lack of re-sampling of adipose tissue at follow-up. Finally, it should be noted that mature adipocytes constitute between 31% and 57% of the cell types in human adipose tissue samples as recently shown by the use of spatially resolved transcriptional profiling with single-cell RNA sequencing51. Therefore, the respiratory data reported here pertain to adipose tissue, and not necessarily specifically to adipocytes.

In conclusion, we report no evidence of impaired hepatic mitochondrial respiration in patients with obesity and NASH. However, in VAT and in SAT, especially, intrinsic respiration was lowered but the reduction was irrespective of NAFLD status; rather, obesity appeared to be the common denominator affecting respiration in a similar manner in both VAT and SAT. Thus, compromised adipose tissue respiration is not likely to significantly impact NAFLD severity. Whereas OXPHOS in liver tissue was generally increased in the individuals with obesity, OXPHOS was simultaneously decreased in AT, which point towards a differentiated respiratory adaptiveness in liver and AT in obesity but with little additve effect of increased metabolic strain. Moreover, our results of enhanced hepatic OXPHOS and increased hepatic mitochondrial biogenesis 12 months after bariatric surgery expand upon previous data that suggest the human liver is an extremely adaptable organ both during and after major weight loss and remission of NAFLD.

Methods

Ethics. The study protocol was approved by the Scientific Ethics Committee in Capital Region of Denmark (H-16030784 and H-16030782). The Danish Data Protection Agency approved the collection, handling and storage of data (P-2019-514 and P-2020-606). Informed oral and written consent was obtained from all study participants. The study was conducted according to the Declaration of Helsinki.

Study participants. The respirometry data presented in the current study are based on 71 subjects in total: 62 patients with severe obesity (OBE) (41 females, 21 males, median age 44 years (IQR 39–57), undergoing laparoscopic bariatric surgery (either Roux-en-Y gastric bypass (RYGB) (n = 29) or sleeve gastrectomy (SG) (n = 33)) and nine normal weight control subjects (CON) (7 females, 2 males, median age 39 years (IQR 24–43). Subjects were enrolled between December 2016 and September 2019 at Copenhagen University Hospital Hvidovre. OBE fulfilled the existing criteria for bariatric surgery issued by the Danish Health Authorities, including a mandatory weight loss of 8% before surgery. Study-specific exclusion criteria were concurrent or previous alcohol consumption of >2.5 units/day for men and >1.5 units/day for women, preexisting liver disease other than NAFLD, pre-existing disease in the lipid metabolism and acute or chronic inflammatory disease,
or an ethnic origin other than North European. Mode of surgery (RYGB or SG) was decided by the endocrinologists at the Endocrinology Department. Additional information on details of groups for bariatric surgery in Denmark can be found in Supplementary Information.

CON consisted of patients with a BMI between 18 and 27 kg/m² undergoing laparoscopic cholecystectomy due to gallbladder stones. CONs had to be otherwise healthy with no current or former comorbidities and zero intake of prescribed medication other than medication for mild allergies, migraine and/or contraception. CONs received a fee of 1500 DKK (corresponding to 200 euros) before surgery (taxes of ~37% were applied).

### Study design and anthropometrics

Blood samples were collected in the morning on the day of surgery (baseline) and after 12 months on the day of the percutaneous liver biopsy, on both occasions after a minimum of 10 h of fasting (Fig. 1). Study participants were phenotypically and anthropometrically characterised (Table 1).

During surgery (bariatric or cholecystectomy) a Wedged liver biopsy and a specimen of VAT was sampled from the greater omentum. All biopsies were immediately after surgery placed and stored in BIOs. A flowchart of study flow is available (Supplementary Fig. S2).

At baseline the biopsies were immediately divided into smaller pieces of 50–100 mg, placed in separate tubes containing a chilled mitochondrial preservation buffer (BIOsP)29, snap-frozen in liquid nitrogen and stored at −80 °C for later mitochondrial DNA (mtDNA) analysis or immersed and fixed in 2% paraformaldehyde for paraffin embedment and histological evaluation.

At 12 months, the percutaneous liver biopsies were placed in BIOs for HRR measurements) and paraformaldehyde (histology) or snap frozen for biopsy analysis (mtDNA analysis).

Tissues for measurements of mitochondrial respiratory capacity were transported on wet-ice and reached the laboratory 45–60 min after sampling.

### Mitochondrial respirometry protocols

HRR allows direct, ex vivo measurement of mass-specific mitochondrial respiratory capacity. In brief, HRR measures the use of oxygen during electron flow through the complexes of the mitochondrial electron transport chain in response to the addition of substrates and inhibitors. Each tissue biopsy was carefully trimmed while in fresh, chilled BIOs and washed in respiration medium. Between 2 and 4 mg of liver tissue and 40 mg of both VAT and SAT were transferred to the 37 °C chamber, (liver tissue: hyperoxygenated (200–2000 nmol/mL, AT, normaloxygenated (200–100 nmol/mL)) in the respirometer (Oroboros Instruments, Innsbruck, Austria. Software Oroboros DatLab 6.0) as described previously29,30 80 g digitoxin was added for permeabilization of adipose tissue. All tissues were studied in duplicate at baseline and liver tissue in singlicates after 12 months. Oxygen consumption was corrected for tissue wet weight and given as oxygen flux expressed as pmol mg wet weight−1 s−1.

Two separate substrate-inhibitor (SUIT) protocols were performed.

AT and hepatic tissue were studied in substrate-inhibitor (SUIT) protocol 1 (P1). State 2 leak respiration (GM) was measured after the addition of 10 mM glutamate and 2 mM malate and electron flow through complex I. With no addition of diphosphate (ADP) yet added, this step measured the oxygen consumption used for compensating the naturally occurring electron leak through the inner mitochondrial membrane. State 3 complex I respiration (GM3) was obtained after adding ATP + MgCl2 (5 mM + 3 mM). When ATP is added the oxidative respiratory process is initiated and ATP is produced. State 3 complex I and II lipid respiratory capacity was measured after the further addition of 1.5 mM of the medium chain fatty acid octanoyl carnitine (GMO3), which stimulated mitochondrial β-oxidation and electron transport through complex I and subsequently fed electrons to complex II via the electron shuttle ubiquinone.

Subsequent addition of 10 mM of the tricarboxylic acid cycle (TCA) substrate succinate (S3) to the maximal coupled respirometry substrate (OXPHOSmax) through both complex I and complex II. Testing of mitochondrial outer membrane integrity was carried out with supplementation of 10 μM cytochrome C (CytC). A CytC response with a less than 10% increase from the OXPHOSmax step was deemed acceptable and used as an indicator for preserved outer mitochondrial membrane not damaged from the preparation procedures. Lastly, by titrating the uncoupling agent p-trifluoromethoxyphenoxyhydroazone (FCCP step) in steps of 0.2 μM we assessed maximal-uncoupled respiration (also called maximal electron transport system capacity (ETS)).

Hepatic tissue was also studied using substrate-inhibitor SUIT protocol 2 (P2). Firstly, glutamate was stimulated as described in P1 by addition of malate (2 mM) and glutamate (10 μM) and then, and ADP + MgCl2 (5 mM + 3 mM) (GM3), to yield complex I oxygen flow. By subsequently adding rotenone (0.5 μM), an inhibitor of complex I, and then the complex II substrate succinate (10 mM), specific complex II oxygen flow was assessed. By blocking electron flow through complex II with antimycin A (5 μM) and subsequently stimulating complex IV (the cytochrome-c oxidase, COX) with the COX-specific electron donor N,N,N′,N″-tetramethyl-p-phenylenediamine (TMPD) (0.5 μM) and ascorbic acid (2 mM), specific complex IV activity could be measured. Figure 1 provides a schematic overview of the study set-up and SUIT P1 and P2.

### Histological evaluation of liver and adipose tissue

#### Liver tissue

Sections of formalin-fixed, paraffin embedded liver biopsies were stained with Hematoxylin & Eosin (HE) and NAFLD activity score (NAS) was calculated using standard guidelines52. All biopsies were evaluated by three expert liver pathologists with specific experience in NAFLD and who were blinded to clinical details. Consensus was sought in the event of disagreement among scores.

#### Adipose tissue

VAT and SAT were fixed in formalin and embedded in paraffin. From the paraffin blocks, 3 μm sections were cut and mounted onto coated slides (FLEX IHC slides K8020, Dako, Glostrup, Denmark). The sections were dried at 60 °C for 20 min. The slides were then placed on the OMNIS Platform (Agilent), deparaffinized on-board and submitted to heat induced epitope retrieval (HIER) in Flex TRS high pH (EnVision Flex Target Retrieval solution, GV804) for 30 min at 97 °C. Following the primary antibody for CD68 (Monoclonal mouse antibody, clone KPI, Ready-to-use, GA609, Dako, Glostrup, Denmark) was applied for 25 min at 32 °C and endogenous peroxidase blocking (EnV Perox GV800/GV823). After a wash in buffer the visualisation system, OptiView DAB (EnV HRP-labelled polymer with GV 823) was applied. The slides were finally developed with DAB (EnV FLEX Substrate Working Solution, GV825) and counterstained with hematoxylin (GC808). Slides were scanned using NanoZoomer (Hamamatsu, Hamatsu, Japan) and evaluated digitally with the NDP.view2 viewing software (Hamamatsu, Hamamatsu, Japan) on a 2 K screen at ×20 magnification. Adipocytes and macrophages were counted in eight randomly chosen areas (each representing a region of 418,660 μm²) per slide using an unbiased counting frame. Counting was performed blindly by one observer. Macrophages are expressed as number of CD68+ cells normalised to 100 adipocytes.

Based on liver histology sampled at baseline (during surgery), we stratified the OBE into three groups:

1) NAFL minus (NAFL−): 31 patients without liver steatosis and with a median NAS of 2;
2) NAFL plus (NAFL+): 16 patients with liver steatosis and a median NAS of 3;
3) NAS: 15 patients with a NAS of 5 or above and all with points from all NAS subcategories (steatosis, inflammation and ballooning).

NAFL− had zero hepatic steatosis and a median NAS of 1.

In total, 21 OBE with repeat liver biopsies 12 months after surgery were available for our paired experiment. All 21 had valid P1 HRR runs at both timepoints (baseline and 12 months). The 21 OBE consisted of 15 with NAFL− at baseline and six with NAFL+ at baseline and two with NAS (21 OBE were comprised of 15 with NAFL− at baseline and six with NAFL+ at baseline and two with NAS).

Phenotypically, anthropometrically, biochemically and in terms of OXPHOS and median NAS the 21 OBE with paired HRR analyses did not separate statistically significant from the 41 OBE who only participated in the cross-sectional analysis at baseline.

### Plasma biomarkers

Routine metabolic and liver markers were analysed immediately after blood sampling. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), plasma-glucose and triglycerides were measured using the Roche/Hitachi Cobas e 8000 system (Roche Diagnostics GmbH, Mannheim, Germany) with Cobas calibrators and reagents, according to the manufacturer’s instructions. Serum insulin and c-peptide concentrations were measured by immunoassay Cobas e 602. HbA1c was measured in plasma with the Tosoh TSKgel G8 Variant Hs on the Tosoh Automated Glycohemoglobin Analyzer HLC–723G8 (Tosoh Corporation, Tokyo, Japan). All markers were measured in the fasting state.

#### Plasma inflammatory markers

Frozen plasma was stored in the −80 °C freezer until analysis of a panel of selected inflammatory markers (Table 1). Adiponectin (Cat. No. DRP00, R&D Systems, McKinley Place NE, Minneapolis) and sCD206 (Cat. No. DC1630, R&D Systems, McKinley Place NE, Minneapolis) were measured by sandwich ELISA (Cat. No. ELH–MMR, RayBiotech, Norcross, Georgia). IL-1β, IL-6 and TNF–α were measured by customised electropheroimmunolucence (ECL) assay (Cat. No. K1S1AH-91, Mesoscale (MD), Rockville, Maryland). sCD163 was measured in Hep-Plasma and the remaining inflammatory markers in cooled EDTA plasma. All markers were measured in the fasting state.

### Quantification of mitochondrial activity in VAT, SAT and liver tissue

Citrate synthase (CS) activity: In liver tissue we measured CS activity and adjusted for tissue wet weight. We used the same procedures as for oxidative respiratory process is initiated and ATP is produced. State 3 complex I. We used the same procedures as for oxidative respiratory process is initiated and ATP is produced. State 3 complex I.
analysing the slope with Lightcycler 480 Software (Roche Life Science). Efficiencies were determined to be 1.938 (93.8%) for ggcttt.

Quantification of mitochondrial DNA. In VAT, SAT and liver tissue we quantified mitochondrial DNA (mtDNA) content, which is presented as mtDNA/nuclear DNA (nDNA) ratio. Respiratory rates were adjusted accordingly.

To isolate DNA, 5–20 mg of adipose or liver tissue was disrupted in 250 μL of alkaline lysis solution (25 mM NaOH and 2 mM EDTA) using a QIAGEN TissueLyser II bead homogeniser with two consecutive one-minute rounds at 30 hertz. After homogenisation, samples were heated for one hour at 96 °C, and then neutralised with 250 μL of neutralisation buffer (40 mM Tris-HCL (pH 8.0)). After centrifugation at 12,000 x g, the supernatant was transferred to a new tube and used for semi-quantitative PCR (CPCR). mtDNA/nDNA data were analysed as described19, with the addition of primer efficiency correction. Two sets of primers were used to amplify mtDNA at the mitochondrially encoded cytochrome C oxidase II (mtCO2) and mitochondrially encoded cytochrome B (mtCYTB) loci, while primer sets in the peroxisome proliferator-activated receptor gamma (PPARG) and mitochondrial protein gene 1 (UCP1) gene loci were used to amplify nuclear DNA. Primer sequences: mtCO2 F: ttcagaaatgccttgcagtg, mtCO2 R: ggtgtg, nUCP1 F: ggcaatgcttccgctc, nUCP1 R: ttagcacttggattttat, nPPARG F: ttcagaattgcctcggag, nPPARG R: acatttctgcatattt

P/E was calculated as OXPHOSmax(GMOSD)/state u(FCCP). The P/E expresses how close the measured OXPHOSmax is to the maximal electron transport system capacity (ETS) in the uncoupled state.

Respiratory control ratio (RCR) was calculated as Gm/KGM. The RCR was used as a general measure of mitochondrial function, i.e., the ability of the mitochondria to increase respiration in response to ADP supplementation.

The Homoeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated according to the formula [last ing glucose (mg/dL) x insulin (mU/L)]/21. The percentage excess body weight loss (%EBWL) at 12 months was calculated as [(baseline BMI – follow-up BMI)/(baseline BMI-25)] x 100%.

Statistical analyses. The criteria for normal distribution were not met. Comparisons between the four groups were made using the Kruskal–Wallis test with adjustment for multiple comparison or the Wilcoxon-signed rank test for paired data (baseline vs. 12 months follow-up).

A simple linear regression was used to explore eight predictors; these were selected by the author group based on presumed clinical importance for mitochondrial respiration (OXPHOSmax) at baseline (liver tissue; BMI, HOMA-IR, steatosis grade, ballooning grade, VAT OXPHOSmax, plasma leptin, plasma adiponectin and ALT; VAT/SAT; BMI, HOMA-IR, plasma leptin, plasma adiponectin, HbA1c, plasma sCD163, plasma sCD206, VAT/SAT macrophage count. To correct for multiple testing in the regression analyses, the Bonferroni correction was used and a P-value of 0.05/8 = 0.006 was considered significant. The coefficient estimates are given with 95% confidence intervals (CI). The four most significant variables for each tissue were tried in multiple linear regression models with forward selection and model control, including collinearity testing and log transformation when necessary. Simple logistic regression was used to explore if VAT and SAT adipose tissue OXPHOS influenced the presence of NASH. In post hoc analyses, sex and type 2 diabetes status were tried alongside the four significant variables in each of the three tissues at baseline. Furthermore, in simple regression analysis total weight loss, delta NAS and type of surgery were tried as predictors for delta respiration per mtDNA in liver tissue.

Study participant characteristics are presented as medians with an interquartile range (IQR) or as frequencies. P < 0.05 (two-sided) is considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics 25.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Source Data to figures are provided with this paper. Other data that support the findings of this study are available upon reasonable request from the corresponding author [Julie Steen Pedersen, contact information: julie.steen.pedersen@regionh.dk] on the condition that individual approval can be provided by the Danish Data Protection Agency. The timeframe for research requests is dependent on the case processing time at the Danish Data Protection Agency. Some data are not publicly available due to restrictions set by the Danish Data Protection Agency as public sharing and sharing of large sets of metadata compromise research participant privacy/consent and hence could violate our Data Agreement with the Danish Data Protection Agency.

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