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Short Communication

Acute and diet-induced hepatic steatosis differentially alter mitochondrial electron transfer in female rats



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Introduction

Hepatic steatosis develops when lipid delivery and synthesis exceed oxidation and export. The resulting accumulation of triacylglycerols (TAGs), cholesterol and other lipids can disrupt hepatocyte metabolism and may progress to inflammatory and fibrotic liver injury. Mitochondria are central to this process because they integrate fatty-acid oxidation, the tricarboxylic acid cycle and oxidative phosphorylation. Importantly, recent evidence shows that changes in individual electron transfer system complexes are heterogeneous across fatty-liver models rather than uniformly decreased [1].

Ethionine provides a rapid model of steatosis by disrupting methyl-group metabolism and phosphatidylcholine-dependent lipoprotein export, whereas prolonged dietary lipid exposure produces a slower phenotype. We therefore compared acute ethionine-induced steatosis with a 12-week olive-oil-enriched diet model to identify mitochondrial changes that were shared or model-specific. Female rats were selected because ethionine responses are sexually dimorphic, with greater susceptibility of females to ethionine-associated inhibition of hepatic protein synthesis and triglyceride accumulation [2]. Sex-dependent hepatic lipid responses to olive-oil feeding have also been reported experimentally [3].

The study was designed to survey mitochondrial electron transfer rather than prespecify a single affected complex. Hepatic lipid measurements, blue native polyacrylamide gel electrophoresis (BN-PAGE), and enzymatic assays were combined to define the bioenergetic phenotype of the two steatosis models.

Methods

Thirteen female Sprague-Dawley rats were assigned to control chow ($N = 5$), diet-induced fatty liver (D-FL; $N = 5$), or ethionine-induced fatty liver (E-FL; $N = 3$). D-

FL rats received standard chow soaked in extra-virgin olive oil for 12 weeks after weaning. E-FL rats received four intraperitoneal injections of 3 mL D,L-ethionine (17 mg·mL^{-1} in isotonic saline) at 3 h intervals, beginning 24 h before euthanasia. The study was approved by the Scientific Research Ethics Committee of Birzeit University and conducted in accordance with NIH animal-care guidance.

Livers were examined macroscopically and used for lipid extraction and mitochondrial isolation. Lipids were separated by thin-layer chromatography using hexane-diethyl ether-formic acid (80:20:2, by volume); TAG species were assessed by tandem mass spectrometry and cholesterol by the Liebermann-Burchard reaction. Liver mitochondria were isolated by differential centrifugation in mannitol-sucrose-HEPES buffer and standardized to 2 mg·mL^{-1} protein.

Intact oxidative phosphorylation complexes were separated by BN-PAGE and assessed by in-gel activity staining [4]. Solution assays measured citrate synthase, rotenone-sensitive Complex I, Complex II, NADH:cytochrome *c* reductase (Complexes I+III), succinate:cytochrome *c* reductase (Complexes II+III), and Complex IV using established substrate-inhibitor combinations [5]. Complex I activity was calculated from matched measurements in the absence and presence of rotenone; one unmatched control trace was excluded from the specific-activity calculation.

For inferential analysis, repeated assay runs were not counted as additional independent observations. One value per independent mitochondrial preparation was used (N = 3 per group for citrate synthase, Complexes I, II, I+III and IV). Data are mean (SD). Because of small sample size and unequal variances, group effects were assessed by Welch's one-way ANOVA followed by Games-Howell comparisons with control. Complexes II+III were reported descriptively because only two E-FL preparations were available. $P < 0.05$ was considered statistically significant.

Results and discussion

Both interventions produced visible hepatic lipid accumulation. D-FL and E-FL livers were paler than controls and showed increased neutral-lipid signals by thin-layer chromatography; several TAG species increased in both models. Hepatic cholesterol was also higher in steatotic livers, supporting successful induction of hepatic lipid accumulation.

Mitochondrial responses were complex-specific (Table 1). Citrate synthase differed among groups (Welch $P = 0.023$); E-FL was lower than control (Games-Howell $P = 0.022$), while the D-FL comparison did not reach significance ($P = 0.063$). Rotenone-sensitive Complex I activity showed large numerical reductions in both steatosis models, but high inter-preparation variability prevented a significant group effect (Welch $P = 0.209$). Thus, the present dataset does not support a statistically established selective Complex I defect.

Complex II showed the clearest model-dependent response (Welch $P = 0.0001$): activity was lower in D-FL than control ($P = 0.008$) but higher in E-FL than control ($P = 0.017$). NADH-linked Complex I+III activity was approximately halved in both models, but the overall Welch test was borderline ($P = 0.054$) and post-hoc

comparisons did not reach P < 0.05. Complex IV was unchanged (Welch P = 0.516). Succinate-linked Complex II+III activity was similar in control and D-FL preparations and was higher in the two available E-FL preparations, but this endpoint was not tested inferentially because E-FL *N* = 2.

These quantitative data refine our initial interpretation. Rather than a single shared Complex I lesion, the two models produce distinct mitochondrial signatures, with strong evidence for divergent regulation of Complex II and large but underpowered reductions in NADH-linked electron transfer. This is consistent with the broader fatty-liver literature, in which the direction and magnitude of respiratory-complex changes depend on model, disease stage and metabolic context [1,6,7]. The diet and ethionine interventions therefore should not be treated as bioenergetically interchangeable despite producing hepatic lipid accumulation.

The study remains limited by small biological sample numbers and the use of female animals only; the findings should therefore be regarded as female-specific until replicated in males. Future work should increase biological replication and directly measure mitochondrial fatty-acid oxidation, oxygen-consumption rates, ATP production, reactive oxygen species, and OXPHOS complex abundance and assembly. These experiments will help determine whether altered substrate oxidation is a cause or consequence of hepatic lipid overload.

Table 1. Mitochondrial enzyme activities in control and steatotic liver mitochondria.

Activity	Control	D-FL	E-FL	Welch P
Citrate synthase	116.6 (6.6) *N*=3	85.1 (12.7) *N*=3; P=0.063	90.1 (7.3) *N*=3; P=0.022	0.023
Complex I, rotenone-sensitive	103.1 (62.8) *N*=3	-1.0 (28.5) *N*=3; P=0.162	8.7 (6.2) *N*=3; P=0.211	0.209
Complex II	73.8 (3.7) *N*=3	43.2 (0.5) *N*=3; P=0.008	89.1 (1.8) *N*=3; P=0.017	0.0001
Complexes I+III	246.1 (54.9) *N*=3	131.9 (5.5) *N*=3; P=0.121	107.6 (13.9) *N*=3; P=0.077	0.054
Complexes II+III	126.2 (1.9) *N*=3	137.4 (15.7) *N*=3	234.5 (29.6) *N*=2	not tested
Complex IV	53.8 (5.4) *N*=3	47.6 (11.0) *N*=3; P=0.689	48.9 (3.4) *N*=3; P=0.469	0.516

Values are mean (SD), nmol·min^{-1}·mg^{-1} mitochondrial protein. P values within D-FL and E-FL columns are Games-Howell comparisons with control. D-FL, diet-induced fatty liver; E-FL, ethionine-induced fatty liver.

Conflicts of interest: All authors declare no conflict of interest.

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Author contributions: JS designed the study and obtained funding. BMI, RJ, YA and JS performed data collection and evaluation. YAH provided lipidomic analysis. BMI drafted the full manuscript; JS revised it. All authors reviewed and approved this short communication.

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