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Liver fat response to two days fasting and two days isocaloric high-carbohydrate refeeding in lean and obese women

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ABSTRACT

Background and aims: Prolonged fasting, which leads to the mobilization of fat from adipose tissue, can result in the development of hepatosteatosis. However, it is not yet known whether the accumulation of fat in the liver after fasting can be affected by concurrent obesity. Therefore, this study aimed to assess how excessive adiposity influences changes in liver fat content induced by fasting and subsequent refeeding.

Methods and results: Ten lean women and eleven women with obesity (age: 36.4 ± 7.9 and 34.5 ± 7.9 years, BMI: 21.4 ± 1.7 and 34.5 ± 4.8 kg/m^2) underwent a 60-h fasting period followed by 2 days of isocaloric high-carbohydrate refeeding. Magnetic resonance spectroscopy (MRS) examinations of liver were conducted at baseline, after 48 h of fasting, and at the end of refeeding period. Hepatic fat content (HFC) increased in lean women after fasting, whereas no statistically significant change in HFC was observed in women with obesity. Additionally, fasting led to significant reductions in liver volume in both groups, likely attributable to glycogen depletion, with subsequent restoration upon refeeding. Notably, changes in hepatic fat volume (HFV) rather than HFC inversely correlated with baseline liver fat content and HOMA-IR.

Conclusion: We demonstrated that prolonged fasting results in accumulation of fat in the liver in lean subjects only and that this accumulation is inversely related to baseline fat content and insulin resistance. Moreover, the study underscored the importance of evaluating hepatic fat volume rather than hepatic fat content in studies that involve considerable changes in hepatic lean volume.

1. Introduction

The accumulation of liver fat is considered to be one of the cornerstones in the development of obesity, insulin resistance and metabolic syndrome [1,2] and the excessive accumulation of fat in the liver then represents the initial stage of metabolic dysfunction-associated steatotic liver disease (MASLD), one of the major metabolic health problems over the world [3]. Interventions designed to improve metabolic health in obese and diabetic patients frequently include various forms of caloric restriction including fasting of various length. However, the consequences of such interventions might not be always necessarily favorable. It has been repeatedly demonstrated that prolonged fasting induces accumulation of fat in the liver in mice [4]. In humans, 36–48 h fasting results in an increase of hepatic fat content (HFC) in healthy men [5,6] although no changes in HFC were observed in healthy women after 48 h fast [6].

Therefore, it is important to know whether people with obesity are at risk of further worsening liver steatosis while undergoing prolonged fasting and subsequent refeeding. To address this, we analyzed the impact of adiposity on HFC in response to fasting and subsequent refeeding in women with obesity and young lean women.

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2. Methods

2.1. Design of the study

The subjects included in the study were a subgroup of a larger group of women enrolled in a project DELISA (Clinical Trials NCT 04260542) studying the response of adipose tissue metabolism to prolonged fasting followed by isocaloric high-carbohydrate refeeding. In this study, lean and obese women fasted for 60 h and subsequently received an isocaloric high-carbohydrate diet for 2 days (Fig. 1). Twenty-two out of 43 women from DELISA project participated in this study that included three magnetic resonance (MR) examinations: a) baseline measurement – before fasting period, b) fasting measurement – in the evening of the second day of fasting (48 h without food), and c) refeeding measurement – after 48 h of high-carbohydrate refeeding. Baseline blood samples were obtained approximately a week before fasting period, at the end of fasting (60 h without food), and after refeeding period. At all the timepoints, the blood collection was performed in the morning after overnight fast.

The women also underwent additional examinations focused on adipose tissue metabolism including body composition determination using dual energy X-ray absorptiometry (DEXA).

2.2. Subjects

The inclusion criteria for subject selection were age 25–40 years, BMI 20–25 kg/m^2 for lean and BMI 30–40 kg/m^2 for obese subjects. The groups were matched for age and physical activity status evaluated by the IPAQ questionnaire.

The exclusion criteria were: diagnosed cancer, diabetes, liver and renal diseases, major cardiovascular event, bariatric surgery, allergy to lidocaine, positive serology for hepatitis (B and C) and HIV, smoking above 10 cigarettes/day, alcohol consumption above 66 g/day, sleep apnoea, poor venous status, weight-change more than 3 kg in last 3 months, untreated hyper- or hypo-thyroidism and long term use of medication and/or steroids and inability to undergo the MR examination. Shift workers and individuals with abnormal sleep/wake pattern were also excluded.

Eleven lean and eleven obese women were included in this study. One of the lean women was unable to undergo the last MR session due to nausea and was then excluded from the study. Demographics of the study population is summarized in Table 1. The study was approved by the Ethics Committee of Kralovske Vinohrady University Hospital and by the Joint Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital. All participants gave written informed consent prior to inclusion in the study. All work was conducted in compliance with the principles of the Declaration of Helsinki.

2.3. MRI and MRS examination

MR protocol consisted of MR imaging and proton MR spectroscopy (^{1}H MRS) of the liver for quantification of hepatic fat content (HFC) and liver volume (every MR session) and MR volumetry of subcutaneous and visceral adipose tissue at the level of the 3rd lumbar vertebra (only during the first MR visit).

MR examination of the liver was performed in the supine position, all sequences were applied in a held exhalation to maximize the reproducibility of all measurements. Whole-body 3T system Siemens Vida (Healthineers, Germany) equipped with 30-channel surface and spine coil was used. Imaging part of measurement consisted of the HASTE (half-Fourier acquisition single-shot turbo spin-echo) localizer in three orthogonal planes and VIBE (Volumetric Interpolated Breath-hold Examination) sequences in transversal plane for volumetry of the liver (VIBE e-Dixon sequence: repetition time/echo time (TR/TE) = 3.97/1.29 ms, resolution 1.2 × 1.2 × 3 mm, flip angle = 9°, 80 slices, acceleration factor 2 × 2 caipirinha, automatic segmentation routine of Siemens). The automatic liver segmentation was checked and manually corrected if necessary.

Hepatic fat fraction (fat signal/fat and water signal) was measured by LiverLab engine consisting single voxel spectroscopic technique – HISTO (STEAM sequence with parameters: TR = 3000 ms, voxel size 40 × 30 × 25 mm, bandwidth 1200 Hz, 5 spectra during one breath-hold with TE = 12, 24, 36, 48, 72 ms) [7]. Automatic and manual shimming were combined to reach a line halfwidth below 50 Hz. Voxel size of our volume of interest (VOI) was set at 40 × 30 × 25 mm and the VOI position in the liver segments V/VIII was carefully controlled during the second and third follow-up examinations in each subject by two experienced MR specialists.

For MR volumetry of adipose tissue, T2-weighted HASTE (Half Fourier Acquisition Single shot Turbo spin Echo) sequence with TR/TE = 1800/91 ms, base resolution = 512 was applied at the level of the 3rd lumbar vertebra.

Table 1
The biochemical and anthropometric characteristics of lean and obese women.

	Lean women	Obese women
n	10	11
Age [years]	36.4 ± 7.9	34.5 ± 7.9
Weight [kg]	60.6 ± 5.6	94.4 ± 19.3[b]
BMI [kg/m^2]	21.4 ± 1.7	34.5 ± 4.8[b]
Waist circumference [cm]	69 (65–72)	98 (96–103)[b]
Android/gynoid ratio	0.67 ± 0.11	1.11 ± 0.09[b]
Fat mass [kg]	16.6 (15.4–18.8)	38.4 (37.8–45.8)[b]
Fat mass [%]	28.1 ± 4.0	44.9 ± 3.4[b]
Visceral fat - area content L3 [cm^2]	49 ± 19	170 ± 55[b]
Subcutaneous fat - area content L3 [cm^2]	104 (70–110)	346 (308–476)[b]
Total cholesterol [mmol/l]	4.23 ± 1.00	4.41 ± 0.52
HDL [mmol/l]	1.51 ± 0.31	1.23 ± 0.49
LDL [mmol/l]	2.35 ± 0.73	2.56 ± 0.77
Triglycerides [mmol/l]	0.69 ± 0.23	1.39 ± 0.44[b]
Fasting glucose [mmol/l]	4.90 ± 0.19	5.16 ± 0.28[a]
Fasting insulin [mIU/l]	5.5 (2.8–7.4)	19.3 (12.0–25.3)
HOMA-IR index	1.20 ± 0.81	5.31 ± 2.96[b]
ALT [μkat/l]	0.28 (0.24–0.30)	0.35 (0.29–0.57)[a]
AST [μkat/l]	0.25 ± 0.05	0.33 ± 0.09[a]
ALP [μkat/l]	0.87 ± 0.17	1.11 ± 0.24[a]
GGT [μkat/l]	0.20 ± 0.07	0.36 ± 0.12[a]
Hepatic fat content (HFC) [%]	0.7 (0.5–0.9)	5.2 (2.1–11.3)[b]

Data are mean ± SD or median (Q1-Q3).
[a] $p < 0.05$.
[b] $p < 0.001$.

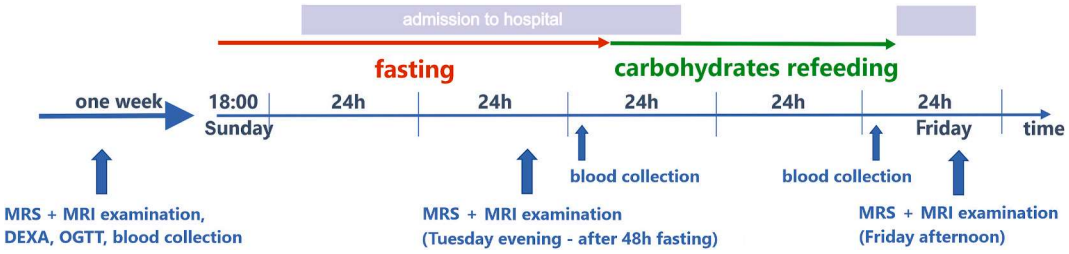

Fig. 1. Design of the study.

The entire MR examination took approximately 40 min.

2.4. MR measurements processing

To calculate hepatic fat volume (HFV) and hepatic lean volume (HLV) the following steps were taken: First, hepatic fat fraction from the HISTO protocol was converted to a volume fraction of lipids, known as the hepatic fat content (HFC) according to Longo [8]. This HFC was then multiplied by the hepatic volume (HV) to obtain the HFV. Finally, HLV was determined by subtracting the HFV from the HV.

Subcutaneous and visceral adipose tissue was segmented manually in ITK-SNAP from the HASTE sequence from a single slice precisely located in the middle of the 3rd lumbar vertebra in the transversal plane.

2.5. Biochemistry

Non-esterified fatty acids (NEFA) were measured by colorimetric assay (Randox), β-hydroxybutyrate (3HB) was determined by capillary electrophoresis with contactless conductivity detection, the other biochemical parameters were obtained in certified laboratories by standard methods. Very low density lipoproteins (VLDL) for VLDL-TG determination were isolated by ultracentrifugation [9].

2.6. Statistical analysis

Data are presented as mean ± SD or as median (Q1-Q3). According to the results of the Shapiro-Wilk normality test the data were logarithmically transformed if necessary. The statistical analyses were then carried using Prism 10 and OriginPro 2021b software. Differences between lean and obese subjects were compared using *t*-test and the effect of interventions was evaluated using two-way mixed-design ANOVA test. The Sidak test was used as a post hoc test. A p-value <0.05 was considered as statistically significant. The association between variables was evaluated using Pearson's correlation analysis.

3. Results

3.1. Baseline characteristics

Women with obesity differed from lean women in the weight and all the other anthropometric parameters associated with adiposity (Table 1). They had slightly elevated triglyceride levels and were more insulin resistant as documented by HOMA-IR. Five of them even fulfilled the criteria of metabolic syndrome according to ATP III criteria [10]. ALT, AST, GGT, and ALP were also increased in women with obesity compared to lean subjects but still in the physiological laboratory range. Hepatic fat content was several times increased in women with obesity and five of them had liver fat content above the upper limit of reference range 5.56 % for hepatic steatosis [11] – approximately corresponding to histopathology Kleiner's steatosis score 2. Four out of five obese women with steatosis had also metabolic syndrome and obese subjects with metabolic syndrome had higher HFC that their obese counterparts without metabolic syndrome (3.8 % vs 16.2 %, respectively). However, the difference was not statistically significant ($p = 0.13$) and there was no other difference between obese subjects with and without metabolic syndrome. For this reason, the group of obese women was analyzed as a whole and not further subdivided. All of the lean women had a normal HFC. For all subjects combined, HFC (after logarithmic transformation) correlated significantly with weight ($r = 0.698$, $p < 0.001$), BMI ($r = 0.789$, $p < 0.001$), waist circumference ($r = 0.707$, $p < 0.001$), android/gynoid fat ratio ($r = 0.749$, $p < 0.001$), total fat mass ($r = 0.675$, $p < 0.001$), area of subcutaneous fat ($r = 0.739$, $p < 0.001$), and area of visceral fat ($r = 0.692$, $p < 0.001$), plasma insulin ($r = 0.800$, $p < 0.001$), HOMA-IR ($r = 0.827$, $p < 0.001$), plasma TG ($r = 0.547$, $p = 0.010$), and VLDL-TG ($r = 0.615$, $p = 0.003$), but not with NEFA ($r = 0.157$, $p = 0.496$) and glucose ($r = -0.613$, $p = 0.954$).

3.2. Response of hepatic fat and hepatic volume to fasting and refeeding

After 48 h of fasting, HFC in lean women increased by 1.3 % to nearly three times the baseline values (Fig. 2A). On the other hand, no statistically significant change of HFC after fasting was observed in women with obesity. However, response to fasting was extremely variable in this group ranging from 2.6 % decrease to 3.1 % increase. No further changes in HFC were induced by two days refeeding with diet rich in carbohydrate in either group.

Importantly, fasting for 48 h resulted in a marked change of hepatic volume (HV) which decreased by 17 % in women with obesity (−320 ± 150 ml) and by 18 % in lean women (−230 ± 60 ml) and the response of HV did not differ between both groups (Fig. 2C). HV returned back to baseline after refeeding in both obese and lean subjects. These changes appeared to be driven mainly by changes of hepatic lean volume (HLV) which decreased by 280 ± 140 ml in women with obesity and by 250 ± 70 ml in lean women after fasting and then returned back to baseline after refeeding in both groups (Fig. 2D).

Given such pronounced changes in HV, we further analyzed the changes in hepatic fat volume (HFV), which should better reflect quantitative changes in liver fat. After fasting HFV increased by 13.4 ± 10.3 ml in lean women. No statistically significant change of HFV was observed in obese women (Fig. 2B) although the response varied from a decrease of 187 ml to an increase of 33 ml. No changes in HFV were induced by two-day refeeding in both groups. Importantly, changes in HFV induced by fasting were strongly inversely correlated with HFV at baseline ($r = -0.791$, $p < 0.001$) (Fig. 3A) and baseline HOMA-IR ($r = -0.778$, $p < 0.001$) (Fig. 3B). Corresponding inverse correlation was also found for relationship between change of HFC and baseline HFC ($r = -0.513$, $p = 0.017$) but not for relationship between change of HFC and baseline HOMA-IR ($r = -0.358$, $p = 0.110$).

3.3. Response of metabolic parameters to fasting and refeeding

After 60 h of fasting, the concentration of NEFA increased threefold in lean women and almost twofold in obese women and then returned to the baseline after refeeding; the response to dietary intervention did not differ between both groups (Table 2). Plasma TG rose in lean women after fasting and then dropped during refeeding whereas did not change in obese women. On the other hand, the concentration of VLDL-TG was not affected by fasting and rose in both groups after refeeding. Fasting stimulated a rise in β-hydroxybutyrate (3HB), which was much more pronounced in lean subjects, the levels of 3HB then returned back to baseline after refeeding. Glucose concentration dropped after fasting and then returned back to baseline after refeeding, whereas concordant changes in insulin concentration were found only in obese women; insulin concentration was not affected in lean women. Accordingly, HOMA-IR did not change in lean women during the intervention, while it decreased after fasting and returned to baseline in women with obesity (Table 2).

4. Discussion

In summary, 48-h fasting resulted in an accumulation of liver fat indicated by changes in both hepatic fat content (HFC) and hepatic fat volume (HFV) in healthy lean women. In contrast, no statistically significant change in liver fat was observed in the group of women with obesity. Detailed analysis of the data suggested that the change of HFV due to fasting was inversely related to the degree of insulin resistance assessed as HOMA-IR and to HFV at baseline. Obese insulin-resistant women with the highest liver fat even lost its significant proportion during fasting.

Such a result seems to be in a contrast with findings of previous study that found no change in HFC after a 48-h fasting in women [6]. However, the women in that study had a mean BMI 27 kg/m^2, which is much higher that BMI of lean women in our study and it cannot be ruled out

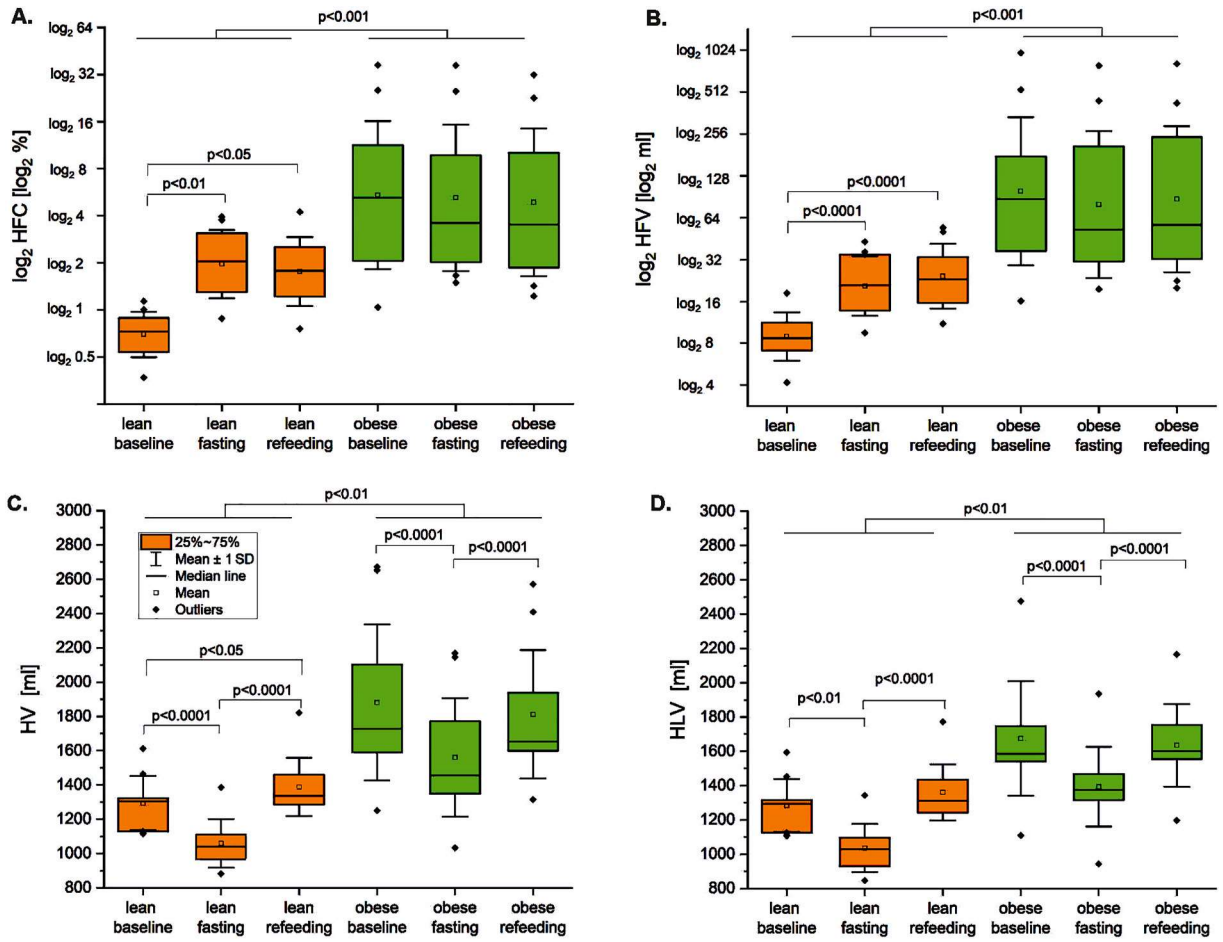

Fig. 2. A. Hepatic fat content (HFC), B. hepatic fat volume (HFV), C. hepatic volume (HV), and D. hepatic lean volume (HLV) in lean and obese women at baseline, after 48h fasting and the end of refeeding.

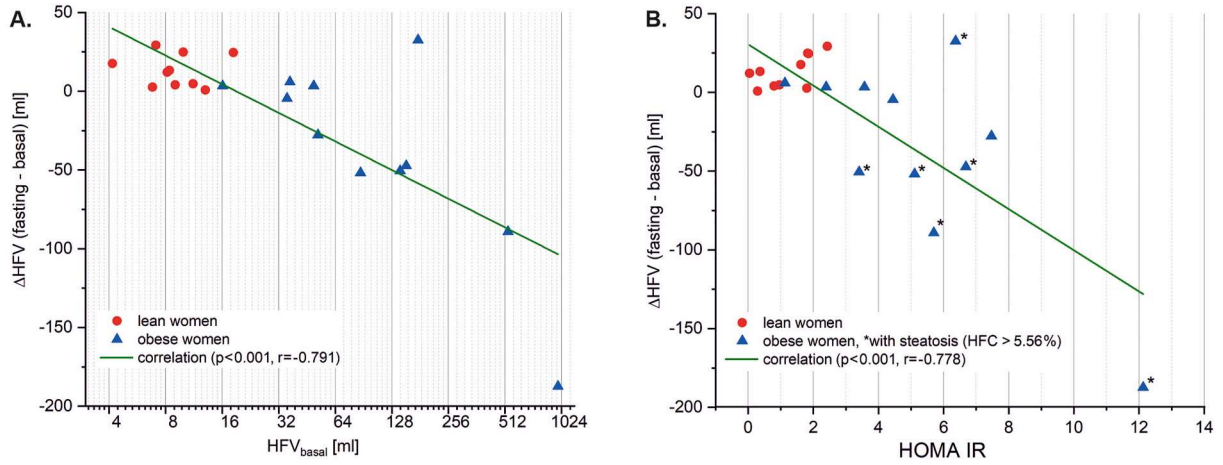

Fig. 3. Correlation between changes in HFV induced by fasting and baseline HFV (A. $r = -0.791$, $p < 0.001$) and HOMA-IR (B. $r = -0.778$, $p < 0.001$).

that the results of both studies are actually in agreement. The findings that hepatic fat accumulates in male subjects after 36–48 h of fasting [5, 6] may be also compatible with our findings – both studies included lean men with rather low HFC.

Importantly, 48 h of fasting resulted in a profound reduction in HV (Fig. 2C). Such a reduction can be easily explained by a decrease in hepatic lean volume – with the exception of women with the highest HFC the changes in HFV are unlikely to make a significant contribution to such a change. The change of HLV is likely the result of depletion of glycogen stores - it has been documented that 48 h of fasting indeed results in almost complete depletion of liver glycogen stores [12]. The observation that HLV returns to a baseline after 48 h of refeeding suggests that liver glycogen stores are completely replenished during this time. Accordingly, a similar decrease in HV was also observed in a study of healthy overweight or obese subjects who were on a low-carbohydrate diet for 6 days, which also likely resulted in depletion

Table 2
Selected metabolic parameters determined at baseline (before the study), after 60 h of fasting and then after 48 h of high-carbohydrate refeeding.

	Baseline	Fasting	Refeeding	Intervention	Adiposity	Intervention x adiposity
NEFA [mmol/l]						
Lean	0.49 ± 0.19	1.54 ± 0.46$^{*,\$}$	0.46 ± 0.21	<0.001	n.s.	<0.001
Obese	0.59 ± 0.28	1.06 ± 0.21$^{*,\$,£}$	0.62 ± 0.28			
TG [mmol/l]						
Lean	0.77 ± 0.32	1.19 ± 0.36$^{*,\$}$	0.98 ± 0.31*	<0.001	0.008	0.010
Obese	1.45 ± 0.58$^{£}$	1.52 ± 0.52	1.56 ± 0.41$^{£}$			
VLDL-TG [mmol/l]						
Lean	0.25 (0.22–0.55)	0.35 (0.30–0.71)	0.54 (0.35–0.68)*	0.005	0.009	n.s.
Obese	1.01 (0.39–1.34)$^{£}$	0.91 (0.48–1.35)$^{£}$	1.18 (0.81–1.31)$^{*,£}$			
β-hydroxy-butyrate [μmol/l]						
Lean	81 ± 47	2970 ± 1000$^{*,\$}$	29 ± 15	<0.001	<0.001	<0.001
Obese	46 ± 20	1240 ± 541$^{*,\$,£}$	28 ± 14			
Glucose [mmol/l]						
Lean	4.90 ± 0.19	3.58 ± 0.46$^{*,\$}$	4.85 ± 0.19	<0.001	<0.001	0.045
Obese	5.16 ± 0.28	4.34 ± 0.47$^{*,\$,£}$	5.27 ± 0.28$^{£}$			
Insulin [μU/mL]						
Lean	5.5 (2.8–7.4)	4.5 (3.4–5.0)	8.0 (4.8–8.9)	<0.001	<0.001	0.01
Obese	19.3 (12.0–25.3)$^{£}$	7.2 (6.4–13.5)$^{*,\$}$	17.3 (10.9–18.0)$^{£}$			
HOMA-IR						
Lean	1.2 ± 0.81	1.00 ± 0.60	1.79 ± 0.88	<0.001	<0.001	0.003
Obese	5.31 ± 2.96$^{£}$	2.41 ± 1.51$^{*,\$,£}$	4.47 ± 01.57$^{£}$			

Data are mean ± SD or median (Q1-Q3). Two-way mixed-design ANOVA and Sidak Post hoc test: *) $p < 0.05$ vs baseline, $^{\$}$) $p < 0.05$ vs refeeding, $^{£}$) $p < 0.05$ obese vs lean.

of hepatic glycogen stores [13].

The observation that prolonged fasting is associated with marked changes of liver volume has also an impact on interpretation of quantitative changes of liver fat content. If HFC does not change during fasting but liver volume decreases by 20 %, then actual liver fat content, which can be assessed as HFV, will also decrease by 20 %. For this reason, changes in liver fat should be assessed as changes in HFV rather than HFC in all studies in which a marked decrease in liver glycogen and thus HLV can be expected. Consistent with such an idea, changes in HFV were more strongly correlated with baseline HFV than changes in HFC with baseline HFC. Moreover, an inverse correlation with HOMA-IR was found only for change in HFV and not for change in HFC.

It should be pointed out that the liver must adapt to prolonged fasting by shifting metabolic pathways to gluconeogenesis and to the extensive use of fatty acids for ketone bodies production and also for incorporation into VLDL-triglyceride to provide fatty acids to extrahepatic tissues. It has been found that the daily production of ketone bodies rises from 30 to 60 g up to 300 g after several days of starvation [14]. Additionally, approximately 25 g of TG is exported in VLDL each day [15]. Just to produce 300 g of β-hydroxybutyrate a day, 194 of TG (as tripalmitate) with a volume of 220 ml have to be used. Such an amount is much greater than that found in the liver of lean women suggesting that turnover of hepatic TG must be significantly increased during prolonged fasting.

To meet such metabolic demands of the liver for fatty acids, lipolysis in adipose tissue must be upregulated and it was indeed documented by an increase in plasma NEFA concentration. Interestingly, such an increase is much steeper in lean than in obese women, which is consistent with observations that mobilization of fatty acids from adipose tissue is likely restrained in obese and insulin resistant subjects [16].

As expected, ketogenesis dramatically increases after 60 h of fasting as documented by increasing β-hydroxybutyrate concentrations that were again more pronounced in lean women. Although there is no significant change in VLDL-TG concentration after prolonged fasting, the significant increase in total TG in circulation in lean women may indicate that VLDL-TG hydrolysis is upregulated and VLDL is converted to LDL faster. Triglycerides remaining in these LDL particles may not be catabolized fast enough. However, no such impact of fasting on plasma and VLDL-TG was observed in obese women. The increase in VLDL-TG in lean subjects after refeeding may reflect the increased VLDL production due to increased liver fat compared to baseline.

Combining these data with changes in HFV suggests that in lean women the marked increase of NEFA supply to the liver exceeds the capacity of the liver to metabolize fatty acids and they then accumulate in the liver and are stored as TG. On the other hand, insulin resistant obese women seem to efficiently use intrahepatic TG to cover the need for ketone body production. The question that remains to be addressed in the future is whether the observed restrained response of adipose tissue lipolysis to prolonged fasting is somehow related to the high content of liver fat.

Refeeding a high-carbohydrate diet for 48 h led in both groups to the return of glycemia, insulinemia as well as liver volume to the baseline suggesting that hepatic glycogen stores were fully replenished. A certain limitation of our study is the small number of subjects. Moreover, more frequent monitoring of metabolic changes and liver fat could contribute to a better understanding of the mechanisms involved in the response of hepatic fat to prolonged fasting.

To the best of our knowledge, this is the first study that analyzed the impact of obesity/insulin resistance on the changes in hepatic fat after prolonged fasting. The results of the study suggest that prolonged fasting may not be detrimental for the accumulation of fat in the liver especially in subjects with obesity. The results of the study also highlight the importance of the determination of hepatic fat volume instead of the percentage of hepatic fat in studies that can involve significant changes in hepatic volume (such as studies with ketogenic diets [17]).

In conclusion, our data suggest that the response of hepatic fat to a prolonged fasting is strongly related to insulin resistance and initial liver fat content. Accumulation of fat in the liver, as a substrate for production of ketone bodies, occurs only in lean women, while in women with obesity the amount of liver fat seems to be sufficient for ketone body and VLDL-TG production and does not change during prolonged fasting.

Author contributions

PŠ, TD, BŠ, DP and MH were responsible for MR examination and evaluation of MR data. LR, MŠ and VŠ were involved in women recruitment. VŠ, MK and JK conducted biochemical analysis. PŠ and MK performed statistical analysis. EK and JG carried out medical

supervision during fasting. The manuscript was written by PŠ, JK and MD. All authors read and approved the manuscript.

Ethical statement

The authors declare that all experiments on human subjects were conducted following the Declaration of Helsinki, and that all procedures were carried out with the adequate understanding and written consent of the subjects.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

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References

[1] Tessari P, Coracina A, Cosma A, Tiengo A. Hepatic lipid metabolism and non-alcoholic fatty liver disease. Nutr Metabol Cardiovasc Dis 2009;19:291–302. https://doi.org/10.1016/j.numecd.2008.12.015.

[2] Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, et al. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord 2022;22:63. https://doi.org/10.1186/s12902-022-00980-1.

[3] Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology 2023;77:1335–47. https://doi.org/10.1097/HEP.0000000000000004.

[4] Jensen TL, Kiersgaard MK, Sorensen DB, Mikkelsen LF. Fasting of mice: a review. Lab Anim 2013;47:225–40. https://doi.org/10.1177/0023677213501659.

[5] Moller L, Stodkilde-Jorgensen H, Jensen FT, Jorgensen JO. Fasting in healthy subjects is associated with intrahepatic accumulation of lipids as assessed by 1H-magnetic resonance spectroscopy. Clin Sci 2008;114:547–52. https://doi.org/10.1042/CS20070217.

[6] Browning JD, Baxter J, Satapati S, Burgess SC. The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. J Lipid Res 2012;53:577–86. https://doi.org/10.1194/jlr.P020867.

[7] Sedivy P, Dezortova M, Burian M, Dusilova T, Kovar J, Hajek M. Comparison of accuracy of magnetic resonance spectroscopic and imaging techniques for the liver steatosis assessment [Czech]. Chem Listy 2021;115:46–53.

[8] Longo R, Pollesello P, Ricci C, Masutti F, Kvam BJ, Bercich L, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. J Magn Reson Imag 1995;5:281–5. https://doi.org/10.1002/jmri.1880050311.

[9] Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345–53.

[10] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American heart association/national heart, lung, and blood Institute scientific statement. Circulation 2005;112:2735–52. https://doi.org/10.1161/CIRCULATIONAHA.105.169404.

[11] Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 2005;288:E462–8. https://doi.org/10.1152/ajpendo.00064.2004.

[12] Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI. Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. Science 1991;254:573–6. https://doi.org/10.1126/science.1948033.

[13] Bian H, Hakkarainen A, Lundbom N, Yki-Jarvinen H. Effects of dietary interventions on liver volume in humans. Obesity 2014;22:989–95. https://doi.org/10.1002/oby.20623.

[14] Balasse EO, Fery F. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes Metab Rev 1989;5:247–70. https://doi.org/10.1002/dmr.5610050304.

[15] Cohn JS, Patterson BW, Uffelman KD, Davignon J, Steiner G. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. J Clin Endocrinol Metab 2004;89:3949–55. https://doi.org/10.1210/jc.2003-032056.

[16] Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes 2011;60:2441–9. https://doi.org/10.2337/db11-0425.

[17] Yang Z, Mi J, Wang Y, Xue L, Liu J, Fan M, et al. Effects of low-carbohydrate diet and ketogenic diet on glucose and lipid metabolism in type 2 diabetic mice. Nutrition 2021;89:111230. https://doi.org/10.1016/j.nut.2021.111230.