Liver fat scores do not reflect interventional changes in liver fat content induced by high-protein diets

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Non-alcoholic fatty liver disease (NAFLD) is common in Metabolic Syndrome and type 2 diabetes (T2DM), driven by energy imbalance, saturated fats and simple carbohydrates. NAFLD requires screening and monitoring for late complications. Liver fat indices may predict NAFLD avoiding expensive or invasive gold-standard methods, but they are poorly validated for use in interventional settings. Recent data indicate a particular insensitivity to weight-independent liver fat reduction. We evaluated 31 T2DM patients, completing a randomized intervention study on isocaloric high-protein diets. We assessed anthropometric measures, intrahepatic lipid (IHL) content and serum liver enzymes, allowing AUROC calculations as well as cross-sectional and longitudinal Spearman correlations between the fatty liver index, the NAFLD-liver fat score, the Hepatosteatosis Index, and IHL. At baseline, all indices predicted NAFLD with moderate accuracy (AUROC 0.731–0.770), supported by correlation analyses. Diet-induced IHL changes weakly correlated with changes of waist circumference, but no other index component or the indices themselves. Liver fat indices may help to easily detect NAFLD, allowing cost-effective allocation of further diagnostics to patients at high risk. IHL reduction by weight-independent diets is not reflected by a proportional change in liver fat scores. Further research on the development of treatment-sensitive indices is required.

Trial registration: The trial was registered at clinicaltrials.gov: NCT02402985.

Abbreviations

ALT Alanine aminotransferase
AST Aspartate aminotransferase
BMBF Bundesministerium für Bildung und Forschung (German Federal Ministry for Education and Research)
BMEL Bundesministerium für Ernährung und Landwirtschaft (German Federal Ministry for Food and Agriculture)
BMI Body-mass index

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Non-alcoholic fatty liver disease (NAFLD) is a common metabolic disorder with increasing prevalence. Paralleling its epidemiological development with type 2 diabetes (T2DM), both manifestations of the Metabolic Syndrome are mainly driven by a low energy expenditure and non-proportionally high energy intake.

Hepatosteatosis may progress further to non-alcoholic steatohepatitis (NASH), hepatic fibrosis, hepatic cirrhosis, and primary hepatic malignoma, highlighting the need for prevention and therapy.

To detect early NAFLD, ultrasound sonography may be helpful, but requires experienced personnel and good imaging quality. Sonoeastography can amplify a first diagnosis by adding information about liver tissue density, but not all causes of liver fibrosis are necessarily linked to high liver fat content allowing weak to moderate performance as monitoring tools within low-fat lifestyle intervention trials. On the other hand, their usefulness within a low-carb diet could not be shown. Missing correlation between change of liver fat indices and change of actual IHL was attributed to the missing correlation between weight change and liver fat reduction. Similar effects need to be expected from glitazones, but also from dietary approaches with polyunsaturated fatty acids, which act independently of weight loss. A recent short-term trial on high-protein diets has demonstrated, that this approach is another way to reduce liver fat without relevant loss in body weight.

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As liver fat reduction does not require weight loss, more data is needed to evaluate, if liver fat scores can reflect changes of IHL content independently of the therapeutic approach.

Therefore, we investigate the statistical relation between changes in three liver fat scores in a human lifestyle intervention trial, featuring two isocaloric diets. For our analysis, assessments of liver fat content by \(^1\)H-MRS and liver fat scores on the basis of anthropometric measurements and fasted blood samples are available.

**Methods**

Data for this publication are extracted from the lifestyle intervention trial, registered at clinicaltrials.gov: NCT02402985 (submitted on 4th February 2015, first posted on 31st March 2015). This randomized parallel-designed trial compared two isocaloric six-week dietary interventions with either plant- or an animal-based high-protein diet in subjects with T2DM. The study was conducted in accordance with the Declaration of Helsinki. The ethics committee of the University of Potsdam approved the study protocol. Recruitment for this study started in September 2013 and was completed in March 2015. All subjects provided their informed consent prior to participation. Data from the trial, including a detailed section about the study protocol, was already published elsewhere.\(^{31,32}\)

At baseline, the participants of the study underwent fasting blood sampling, a mixed meal tolerance test, full anthropometry (body weight, height, abdominal circumferences, bio-impedance analysis) and medical examination. Study volunteers were also subjected to liver \(^1\)H-MRS on a 1.5 T whole body imager (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany) according to pre-published specifications.\(^{33}\) In brief, a single voxel STimulated Echo Acquisition Mode (STEAM) technique with short echo time (TE = 10 ms) and long repetition time (TR = 4 s) was applied and the volume of interest (30 × 30 × 20 mm\(^3\)) was placed in the posterior part of segment 7. Ratio of the integral of methylene and methyl resonances (lipids) and water + lipids was calculated

6 weeks. All assessments from the baseline visit were repeated after that time period.

Dietary intervention (supplementation with neutrally labelled high-protein food products of similar appearance) was conducted for two dietary regimes with comparable isocaloric nutrient distribution of 25–30% relative energy intake (EI%) of protein, 40 EI% of carbohydrates, 30–35 EI% of fat, but differing in their protein source: animal protein (dairy/meat) or legumes. Randomisation was done by non-clinical personnel by group matching for age, sex, body mass

As reported in our previous publication, liver fat reduction in these 31 subjects was very strong (6.7 ± 5.0%-pts.), but was hardly explained by the very limited weight loss of 2.1 ± 1.7 kg.\(^{31}\) In this interventional perspective there was no significant correlation between changes of IHL and changes of NAFLD-LFS or HSI, but we report a weak, trendwise association between change of IHL and change of FLI (Fig. 3).

**Results**

31 subjects of the study with full data on IHL and liver fat indices were selected for the presented data set. Baseline characteristics for the cohort are presented in Table 1.

NAFLD prediction and correlation at baseline. NAFLD prediction by FLI, NAFLD-LFS and HSI is comparable to their first publication,\(^{31,32}\) resulting in AUROC values of 0.731 for FLI, 0.752 for NAFLD-LFS and 0.770 for HSI in the entire cohort, respectively (Fig. 1A–C). Accordingly, correlations between IHL and each of the three liver fat indices were significant at baseline (Fig. 2A–C).

Correlations between change of index values and interventional \(^1\)H-MRS data. As reported in our previous publication, liver fat reduction in these 31 subjects was very strong (6.7 ± 5.0%-pts.), but was hardly explained by the very limited weight loss of 2.1 ± 1.7 kg.\(^{31}\) In this interventional perspective there was no significant correlation between changes of IHL and changes of NAFLD-LFS or HSI, but we report a weak, trendwise association between change of IHL and change of FLI (Fig. 3).

Correlations between changes of index parameters and \(^1\)H-MRS based IHL data. Correlation analysis between change of actual IHL content and change of single elements of the liver fat scores revealed just one significant correlation—for the reduction of waist circumference. The similarly weak correlation with weight loss failed to achieve statistical significance (Table 2).

Splitting any of the above-mentioned analyses by diet group did not lead to numerically different results (data not shown).
Table 1. Baseline characteristics. ALT alanine aminotransferase, AST aspartate aminotransferase, GGT gamma-glutamyltransferase, FLI fatty liver index, HSI Hepatosteatosis Index, NAFLD-LFS Non-alcoholic fatty liver disease-liver fat score; no significant differences between both groups.

| Parameter                                      | (n = 31)          |
|------------------------------------------------|-------------------|
| Age (years)                                    | 65 ± 6            |
| Sex (male/female)                              | 19 m/12 f         |
| Liver fat content (MR-S, %)                    | 15.4 ± 9.8        |
| FLI                                            | 74 ± 23           |
| NAFLD-LFS                                       | 0.54 ± 1.16       |
| HSI                                            | 40 ± 4            |
| Body weight (kg)                               | 89.4 ± 14.2       |
| BMI (kg/m²)                                    | 30.6 ± 3.7        |
| Waist circumference (cm)                       | 102.9 ± 10.9      |
| Fasting insulin (mU/L)                         | 9.48 ± 6.29       |
| Triglycerides (mmol/L)                         | 1.70 ± 0.59       |
| AST (U/ml)                                     | 25 ± 9            |
| ALT (U/ml)                                     | 28 ± 10           |
| AST/ALT ratio                                  | 0.9 ± 0.2         |
| GGT (U/ml)                                     | 44 ± 26           |

Figure 1. AUROC representation of NAFLD prediction by FLI (a), NAFLD-LFS (b) and HSI (c); the analyses show AUROC values of 0.731 (FLI), 0.752 (NAFLD-LFS) and 0.770 (HSI).

Figure 2. Correlation analysis between liver fat indices and IHL at baseline; FLI = fatty liver index, HSI = Hepatosteatosis Index, IHL = intrahepatic lipids, NAFLD-LFS = non-alcoholic fatty liver disease-liver fat score; (a) IHL ~ FLI; rho = 0.351, p = 0.049; (b) IHL ~ NAFLD-LFS; rho = 0.537, p = 0.002; (c) IHL ~ HSI; rho = 0.393, p = 0.032.
Discussion
Liver fat scores are an easy and cheap tool to assess NAFLD status in metabolic research, but also in clinical practice. These tools might save time, monetary personal and technical resources, as they are suitable for any kind of patient and do not require expensive body imaging.

However, previous data on the prediction quality of these scores show a limited predictive performance. Within the present study, there is additional support for the use of these indices in cross-sectional settings. Similar to a recent publication on a hypocaloric high-protein low-carb diet, the current analysis in subjects undergoing an isocaloric high-protein diet also reveals poor performance in a longitudinal approach. Liver fat reduction by this particular diet, independently of weight loss, is not reflected by any liver fat score with satisfying precision. IHL reduction in low-fat diets—with a consistent dependency of metabolic improvement on weight loss—seems to be the only dietary NAFLD treatment, that can be monitored by liver fat scores.

By correlation analysis we showed in our T2DM sample, that combined liver fat scores do not sufficiently reflect the actual change in IHL. While the FLI may provide a weak correlation in a larger data set, both NAFLD-LFS and HSI completely failed to mirror the changes in liver fat content. Apparently, the FLI outranks these indices as all FLI elements—body weight, waist circumference, triglycerides and GGT—are those single parameters which are most strongly connected to change of IHL. Among all liver enzymes, GGT is the most sensitive liver parameter for prediction of NAFLD and its sequelae. Insulin levels—being part of the badly performing NAFLD-LFS—may not necessarily reflect a metabolic improvement, especially in the context of T2DM patients with primarily reduced insulin secretion capacity, who often lack NAFLD. The HSI was developed for patients of Asian ethnicity, maybe limiting its application to Caucasian patients.

Most individual index parameters do not correlate with \( ^1H \)-MRS derived change of liver fat. The strongest connection between change of liver fat and other single anthropometabolic outcomes was seen with waist circumference. Apparently, in our high-protein setting, both visceral and hepatic fat depots are reduced simultaneously. However, change in body weight does not relevantly correlate with change in IHL in the present cohort. As measures of obesity are a major component of all liver fat scores, limited weight loss might be the crucial factor in missing correlation between change of liver fat scores and change of IHL. In our data set of the isocaloric LeguAN study, we observed a rather minor weight loss of \( 2.1 \pm 1.7 \) kg over an intervention period of six weeks. Most possibly, the same finding as ours could be present in isocaloric diets with improved dietary fat composition.
or in pharmaceutical treatments (glitazones, gliflozines), which have been shown to reduce liver fat independently of weight loss. As a strength of our analysis, the observed liver fat reduction is marked, especially given the very moderate weight loss. Thus, we can clearly show the dissociation of adiposity and NAFLD as metabolic outcomes of the study, but also as aspects of liver fat indices.

In comparison to a recently published analysis on IHL monitoring in a low-carb diet, correlations with several metabolic parameters in our cohort were at least similar in direction, if not magnitude. Especially changes in ALT and IHL reduction correlated under low-carb, but not low-fat conditions. High-protein diets seem to beneficially affect lipid levels, including triglycerides, and all transaminase levels. Missing correlation between these changes and change of IHL might indicate additional actions beyond mere steatosis. Possibly, transaminase levels in these patients do also reflect a certain degree of inflammation (NASH) or fibrosis, which is improved in parallel. The possibility of dietary effects even on high-stage fibrosis has been discussed recently.

New prediction scores might include parameters with higher prediction quality and specificity, such as ferritin.

Some limitations have to be addressed for the present study. Our data set provides a moderate statistical power compared to other studies on this issue. However, it is the first publication on subjects with overt T2DM. Further, we are unable to fully clarify the reasons for missing comparability of longitudinal correlations between liver fat scores and MR-based liver fat values in different diets. As shown in recent publications, liver fat reduction can be achieved in accompaniment of highly variable metabolic improvements, depending on type of diet. This diet-specific interaction also entails linkage of IHL reduction and total weight loss in low-fat diets, but not low-carb or high-protein diets.

Conclusively, with the present study, we underline the good predictive properties of FLI, NAFLD-LFS and HSI before a dietary intervention in subjects with overt T2DM by both AUROC analysis and Spearman correlation. However, liver fat reduction is not accompanied by correlating liver scores.

Liver fat indices need to be used cautiously, especially when assessing changes in different interventional settings. More research is needed to elaborate effects of specific diets on metabolic components and blood parameters that could be used for the design of a liver fat index.

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Author contributions

S.K. and M.M. wrote the paper, M.M. and S.S. conducted the experiments by dietary consultation, collected and interpreted according data, S.H. performed medical examinations and supervision for the participants, collected and interpreted according data, J.M., S.R. and J.H. collected and interpreted data with respect to specific laboratory analyses and MR spectroscopy, S.K. and M.M. performed the statistical analysis, S.H., O.P.R. and A.F.H.P. designed the study, all authors read and revised the manuscript, contributed to discussion and approved the final version of this paper. S.K. is responsible for the integrity of the work as a whole and serves as guarantor of this work.

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Competing interests

The authors declare no competing interests.

Additional information

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