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Changes in abdominal adipose tissue depots assessed by MRI correlate with hepatic histologic improvement in non-alcoholic steatohepatitis

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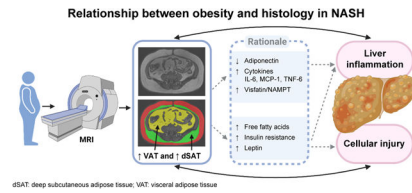[18]Virginia Commonwealth University, Richmond, VA, USA

Abstract

Background & Aims: Nonalcoholic steatohepatitis (NASH) is prevalent in adults with obesity and can progress to cirrhosis. As a secondary analysis of prospectively-acquired data from the multicenter, randomized, placebo-controlled clinical trial Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT), we investigated the relationship between reduction in adipose tissue compartment volumes and hepatic histologic improvement.

Methods: Adult participants in the FLINT trial with paired liver biopsies and abdominal MRI exams at baseline and end-of-treatment (72 weeks) were included (n = 76). Adipose tissue compartment volumes were obtained using magnetic resonance imaging (MRI).

Results: Treatment and placebo groups did not differ in baseline adipose tissue volumes, or in change in adipose tissue volumes longitudinally (P = 0.107 to 0.745). Deep subcutaneous adipose tissue (dSAT) and visceral adipose tissue (VAT) volume reductions were associated with NASH histologic improvement (i.e., nonalcoholic fatty liver disease activity score [NAS] reduction of two points, at least one point from lobular inflammation or hepatocellular ballooning, and no worsening of fibrosis) (P = 0.031, and 0.030, respectively). In a stepwise logistic regression procedure, which included demographics, treatment group, baseline histology, baseline and changes in adipose tissue volumes, MRI hepatic proton density fat fraction (PDFF), and serum aminotransferases as potential predictors, reductions in dSAT and PDFF were associated with NASH histologic improvement (regression coefficient = −2.001 and −0.083, P = 0.044 and 0.033, respectively).

Conclusions: In adults with NASH in the FLINT trial, those with greater longitudinal reductions in dSAT and potentially VAT volumes showed greater hepatic histologic improvements, independent of reductions in hepatic PDFF.

Graphical abstract

Relationship between obesity and histology in NASH

dSAT: deep subcutaneous adipose tissue; VAT: visceral adipose tissue

Keywords

central obesity; deep subcutaneous adipose tissue; visceral adipose tissue; liver histology

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is commonly seen in adults with obesity and can progress to cirrhosis, particularly in the subset of patients with nonalcoholic steatohepatitis (NASH) [1]. NAFLD and NASH are also associated with increased risks of cardiovascular disease and diabetes [2–5]. Although central obesity has been identified as a risk factor for obesity-related disorders including insulin resistance and cardiovascular disease [6, 7], the role of central obesity in NASH needs further clarification [8].

Once thought to be a stable energy storage depot, adipose tissue is now viewed as a diverse, hormone-secreting, metabolically active tissue [9]. Abdominal adipose tissue, especially visceral adipose tissue (VAT), is an important source of pro-inflammatory stimuli associated with inflammation and metabolic dysregulation [10–13] and may contribute to histologic alterations seen in NAFLD and NASH. Although previous studies have observed associations between increased VAT volume and NASH fibrosis stage [14], no study has examined whether a reduction in VAT volume predicts NASH histologic improvement. Further, most studies investigating the relationship between VAT volume and NASH or NAFLD have either used surrogate measures of VAT volume (i.e., anthropometrics or ultrasound), or performed only cross-sectional analysis of histologic data [14–19].

Subcutaneous adipose tissue (SAT), another important depot, is divided into two layers, superficial SAT (sSAT) and deep SAT (dSAT) [20–23] (Figure 1). Prior reports suggest that dSAT closely relates to VAT in function and may influence liver histology [20–23]. Increased macrophages and inflammatory factors were found in dSAT of NASH patients [20], and dSAT may be linked to NASH through its effect on insulin resistance [8, 24].

A phase 2b clinical trial entitled 'Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment (FLINT)' demonstrated that administration of obeticholic acid for 72 weeks in adults resulted in NASH histologic improvement [25]. Additionally, in the subset of participants who underwent abdominal magnetic resonance imaging (MRI), a 30% reduction in hepatic proton density fat fraction (PDFF) has been established as a quantitative imaging biomarker for NASH histologic improvement [26]. In the present analysis, without intent to develop a surrogate biomarker of NASH, we aimed to determine whether longitudinal reductions in abdominal adipose tissue depot volumes in the FLINT trial were independently associated with NASH histologic improvement.

EXPERIMENTAL PROCEDURES

Protocol and design

This was a retrospective, longitudinal, secondary ancillary analysis of data acquired prospectively in the FLINT trial (termed the 'parent study,' NCT01265498) that was conducted by the National Institute of Diabetes and Digestive and Kidney Diseases-sponsored NASH Clinical Research Network (NASH CRN). The FLINT trial was a multicenter, double-blind, placebo-controlled, randomized clinical trial conducted at medical centers in the USA in adult participants with non-cirrhotic NASH to assess efficacy of treatment with obeticholic acid for 72 weeks. Inclusion and exclusion criteria of the FLINT trial have been reported [25]. Histology slides were scored in consensus by a panel of expert pathology investigators of the NASH CRN [25, 27].

A subset of participants in the FLINT trial underwent MRI exams at baseline and after 72 weeks of treatment [28]. The MRI exams included pulse sequences for quantifying hepatic PDFF and volumes of adipose tissue depots, namely sSAT, dSAT, and VAT. Inclusion criteria for the MRI portion of the FLINT trial were willingness and ability to complete both the baseline MRI exam (before randomization, within 90 days of baseline biopsy), and the end-of-treatment MRI exam (within 90 days of end-of-treatment biopsy) [28]. MRI exam exclusion criteria were contraindication to MRI, extreme claustrophobia, pregnancy or trying to become pregnant, weight or girth exceeding MRI scanner capability, or any conditions or circumstances that in the opinion of the clinical trial site investigator would interfere with completion of the MRI exam. Participants in the FLINT trial who had paired liver biopsy and abdominal MRI at baseline and at end-of-treatment were included in this analysis (n = 76). We investigated whether changes in adipose tissue depot volumes, namely, sSAT, dSAT, and VAT, and changes in anthropometrics including weight, body mass index (BMI), and waist circumference in the treatment and placebo groups were related to NASH histologic improvement. In this secondary ancillary study, NASH histologic improvement was defined as a composite of improvement in the NAS by   2 points, with at least 1 point improvement in the scores for ballooning or lobular inflammation, and no worsening of the fibrosis stage, a variation of the original definition which did not specify reductions in specific scores [25, 29]. We additionally investigated whether changes in adipose tissue volumes are related to change in SAF-A score (the activity part of the Steatosis, Activity, Fibrosis [SAF] scoring system that incorporates scores for ballooning and inflammation), a recently used index as an eligibility criterion in a NASH clinical trial [30].

Participants in the FLINT trial provided written informed consent at the time of enrollment. The FLINT trial, including its MRI portion and this secondary ancillary analysis were approved by an Institutional Review Board at each participating clinical trial site and complied with the Health Insurance Portability and Accountability Act.

Magnetic resonance imaging

Seven of the eight participating FLINT clinical trial sites contributed MRI data to this analysis (1.5T at three sites, 3T at four sites). At each site, only a single MRI scanner was used to minimize longitudinal instrument-dependent measurement variability. As

described previously [28], the NASH CRN Radiology Coordinating Center (RCC) provided a standardized MRI protocol that included axial T1-weighted imaging through the abdomen and pelvis and axial PDFF imaging through the liver. Following acquisition, MRI exams obtained at each site were transmitted to the RCC for analysis.

Adipose tissue depots including sSAT, dSAT, and VAT (Figure 1) were semi-automatically segmented on T1-weighted images by an RCC image analyst (TID) under the supervision of an abdominal radiologist (GMC) (sliceOmatic 5.0, Tomovision, Montreal). Images were analyzed for sSAT, dSAT, and VAT volumes on six slices at the T12-L1, L1-L2, L2-L3, L3-L4, L4-L5, and L5-S1 inter-vertebral disk levels. The adipose tissue segmentation technique included threshold-based tools such as region growing (i.e., segmenting neighboring pixels of initial seed points and determining whether the pixel neighbors should be added to the region using a threshold range). Manual editing was applied when necessary. The fascia that separated sSAT and dSAT was manually traced when evident, or its location was inferred by image analysts to the best of their ability. Total sSAT, dSAT, and VAT volumes from T12-L1 to L5-S1 were calculated by interpolating between analyzed slices using the following formula:

$$V = (t + h_i) \sum_{i=1}^{6} A_i$$

where V was volume, A_i was the area selected on the i^{xh} analyzed slice, h_i was the interval between adjacent intervertebral disks at the ith analyzed slice, t was slice thickness, and 6 was the total number of analyzed slices [31]. The coefficient of variation for repeated acquisition (i.e., participant off table and repositioned) and analysis of adipose depots are 2.6% to 4.0% for the NASH CRN Radiology Coordinating Center [32].

Hepatic PDFF was analyzed at the RCC as previously described [28].

Statistical methods

The analysis sample was summarized overall and by parent study treatment group. Participant data were presented as the mean ± SD for continuous variables, and as numbers and percentages for categorical variables (Table 1). Treatment and placebo groups were compared on baseline characteristics and on changes over time using two-sample t-tests for continuous measures, or Fisher's exact tests for ordinal and categorical measures. Significance of changes was evaluated with paired t-tests or Wilcoxon-Mann-Whitney tests.

Spearman's correlations were computed between changes in weight, BMI, waist circumference, sSAT, dSAT, VAT, and hepatic PDFF, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). Spearman's correlations were also computed between changes in individual adipose depots and changes in ballooning, portal inflammation, and lobular inflammation. Relationships between changes in individual adipose depots and changes in histology were illustrated with boxplots.

A Bayesian information criterion (BIC)-based stepwise linear regression procedure was used to search for a model predicting NASH histologic improvement or SAF-A score. Of main

interest in the set of potential predictors were changes in sSAT, dSAT, and VAT volumes. Additionally, the potential predictor pool included age, sex, ethnicity, treatment group, baseline weight, baseline sSAT, dSAT, and VAT volumes, baseline hepatic PDFF, baseline histology, baseline alanine aminotransferase (ALT), baseline aspartate aminotransferase (AST), weight loss, change in hepatic PDFF, change in ALT and change in AST. The procedure uses both forward selection and backward elimination to select the best small model, using penalized goodness-of-fit (i.e., BIC) to choose between competing models.

A BIC-based stepwise linear regression procedure was also used to search for a model predicting NASH histologic improvement using anthropometric measures instead of MRI measures. The potential predictor pool included weight loss, BMI change, waist circumference change, age, sex, ethnicity, treatment group, baseline weight, baseline BMI, baseline waist circumference, baseline histology, baseline ALT, baseline AST, change in ALT, and change in AST.

The anthropometric model and the MRI model were compared using chi-square test of deviances.

Two-tailed ($\alpha = 0.05$) tests of significance were used.

Statistical analyses were carried out using R 3.5.1 statistical software (R Foundation for Statistical Computing, Vienna, Austria, 2018).

RESULTS

Descriptive statistics

Baseline characteristics of the study cohort are shown in Table 1 (age range 24 to 74 yrs; 28 men and 48 women). Most participants were white (n = 65, 86%). There were 39 participants in the treatment group and 37 participants in the placebo group. Almost all participants were overweight or obese, with BMI ranging from 24.8 to 45.4 kg/m^2 and weight ranging from 69 to 135 kg. sSAT volume ranged from 0.9 to 6.6 L, dSAT volume ranged from 0.9 to 4.2 L, and VAT volume ranged from 1.2 to 7.1 L. Treatment and placebo groups did not differ in baseline BMI, sSAT, dSAT, VAT, ALT, AST, histology scores, or hepatic PDFF (P = 0.177 to 0.745) (Table 1).

Mean BMI, sSAT, dSAT, and VAT volumes did not change from baseline to end-of-treatment in either group (paired t-test P = 0.062 to 0.840), and the treatment and placebo groups did not differ in changes of these parameters (P = 0.103 to 0.718). Although the treatment and placebo groups differed in changes in weight (-0.99 ± 5.19 kg, 1.36 ± 4.9 kg, P = 0.046), the change in weight in neither the treatment group (P = 0.242) nor the placebo group (P = 0.101) reached statistical significance. For individual participants, changes in adipose tissue volumes ranged from −1.1 to 1.0 L for sSAT, −0.8 to 1.1 L for dSAT, and −1.1 to 1.1 L for VAT. ALT and AST decreased in the treatment group (median, −10 U/L, Wilcoxon P < 0.001; −10 U/L, P = 0.001, respectively), but not in the placebo group (-3 U/L, Wilcoxon P = 0.152; −1 U/L, Wilcoxon P = 0.658, respectively). Hepatic PDFF decreased in the treatment group (−3.0 ± 8.6%, P = 0.036) , but not in the placebo group (−1.5 ± 9.2%, P =

0.326). However, there was no difference in hepatic PDFF reduction between treatment and placebo groups (P = 0.468). There were also no differences in lobular inflammation change, portal inflammation change, ballooning change, or fibrosis change between treatment and placebo groups (Wilcoxon P = 0.477 to 0.878). Therefore, in subsequent analyses, treatment and placebo groups were merged.

Univariate associations with histologic changes

Pairwise Spearman's correlations between changes in weight, BMI, waist circumference, sSAT, dSAT, VAT, and hepatic PDFF ranged from 0.17 to 0.65 (P < 0.001 to 0.134) (Table 2). The Spearman's correlation between HOMA-IR change and sSAT change, dSAT change, and VAT change were 0.06 (P = 0.609), 0.20 (P = 0.088), and 0.29 (P = 0.011).

As illustrated in the box plots (Figure 2A, 2B, and 2C), VAT change and dSAT change were significantly correlated with NASH histologic improvement (P = 0.031and P = 0.030, respectively), whereas sSAT change was not significantly correlated with NASH histologic improvement (P = 0.911).

As illustrated in the box plots (Figure 2D, 2E, and 2F), dSAT change was significantly correlated with ballooning change ($\rho = 0.28$, P = 0.015), and VAT change was correlated at a trend-level with lobular inflammation change ($\rho = 0.21$, P = 0.066). Adipose tissue volume changes did not significantly correlate with portal inflammation change (P = 0.190 to 0.789). sSAT change did not significantly correlate with lobular inflammation change, portal inflammation change, or ballooning change (P = 0.252 to 0.399).

Multivariable modeling of histologic changes

Summaries of the final model are presented in Table 3.

For NASH histologic improvement, the BIC-based search identified a model with baseline lobular inflammation, change in dSAT, and change in PDFF as the three predictors (Table 3). The relationship between baseline lobular inflammation and histologic improvement was positive: higher lobular inflammation at baseline was associated with histologic improvement. The relationship between change in dSAT and histologic improvement was negative: greater reduction in dSAT volume was associated with histologic improvement. Reduction in PDFF was associated with histologic improvement.

In the anthropometric model, change in waist circumference has been identified as the single predictor of NASH histologic improvement.

Histologic improvement =−1.189–0.069(waist circumference change) (Goodness of fit Chi-Square P=0.026)

The MRI model with adipose tissue depots was significantly better than the simple anthropometric model (Chi-square p-value for comparing goodness of fit = 0.006). The relationship between changes in weight, waist circumference, adipose tissue depots and NASH histologic improvement are further illustrated in Supplemental materials (Supplemental Figure 1).

For SAF-A score change, the BIC-based search identified the below model.

SAF-A score change=1.170+0.066(weight change)+0.13(AST change)–0.824(baseline lobular inflammation) (R^2=0.39, P<0.001).

DISCUSSION

This secondary analysis of prospectively-collected data from the FLINT trial found that in adults with NASH, those with greater longitudinal reductions in dSAT and VAT volumes were more likely to have histologic improvement. Our results also showed that body weight change is a predictor of change in SAF-A score; there is a trend that the participants with both body weight loss and dSAT or VAT change are more likely to have histologic improvement than participants with reduction in only weight, dSAT, or VAT (Supplemental Figure 1). Reduction in sSAT volume was unrelated to histologic improvement. Although previous studies have linked central adiposity to NASH [14] and weight loss to histologic improvement [33], the present analysis suggests that reductions in abdominal adipose tissue depot volumes may correlate with histologic improvement independent of change in hepatic fat content. As there was not a difference in the reduction in dSAT or VAT between the treatment and placebo groups, dSAT and VAT volume changes at the individual level observed in the present analysis are unlikely to be an effect of obeticholic acid treatment in the subset of patients included in this analysis. Instead, these changes may be driven by diet, behavioral, or other lifestyle modifications motivated by participation in this clinical trial.

Strengths of this study include longitudinal study design, volumetric assessment of SAT and VAT rather than a single-slice approach [34], central blinded reading of histology to determine improvement in specific NASH features, and central analyses of MRI exams. As previously shown, multi-slice MRI exams collected in the FLINT trial were better suited to detect small changes in SAT and VAT volumes than single-slice protocols that have been used in other studies [34]. The present analysis also takes advantage of MRI assessment of hepatic PDFF imaging that was acquired in the parent study [28]. Since hepatic PDFF provides more precise estimation of hepatic steatosis than biopsy [35], the inclusion of PDFF in this analysis allowed investigation of the relationship between abdominal adipose tissue depots and NASH histologic improvement independent of liver fat content. In this study, we adopted a definition of NASH histologic improvement that is different from that of the parent FLINT study by requiring at least 1 point improvement in ballooning or lobular inflammation scores. This more rigorous definition aligns with regulatory guidelines as they have evolved since the FLINT study was designed to exclude patients with improvement in just steatosis or inflammation from being classified as improved [36]. Previous studies used a similar definition by requiring at least a 1 point improvement in the NAS for ballooning [29], and we further included improved lobular inflammation as lobular inflammation improvement is independently associated with fibrosis improvement [29].

In this study we found that reduction in dSAT volume was associated with NASH histologic improvement, suggesting that dSAT might be related to exacerbation of NASH. Previous studies have linked increased dSAT volume to altered insulin resistance [21, 24, 37], which accelerates the development of NASH [38]. Insulin resistance promotes the accumulation

of fat in the liver and increases fatty acid oxidation through the influx of serum free fatty acids, augmenting intracellular oxidative stress and hepatocellular injury that contributes to the development and progression of NASH [39, 40]. Furthermore, SAT is one of the main secretors of leptin. A previous study reported strong correlation between leptin levels and NASH, which may imply that leptin plays a role in regulating insulin levels [41]. In FLINT trial participants, who had a higher BMI than the general population, it is possible that mechanism(s) for reduction in dSAT volume led to NASH histologic improvement through improvement in insulin resistance, as increased dSAT volume associates with insulin resistance [24].

VAT change did not enter the final model of NASH histologic improvement, which might be explained by the high correlation between dSAT and VAT (Spearman correlation coefficient of 0.614, P < 0.001). Univariate correlation between VAT and histologic improvement suggests that VAT may play a role in NASH histology, consistent with previous reports on potential associations between VAT, and NAFLD and NASH [14, 15, 18, 19]. VAT might influence liver histology and contribute to the development of NASH through metabolic dysregulation and by promoting inflammation through multiple pathways [10–13]. Increased VAT volume is linked to reduced release of protective adipokines such as adiponectin, a molecule with insulin-sensitizing, anti-inflammatory, and anti-fibrotic effects [42, 43], and elevated release of pro-inflammatory adipokines such as visfatin/nicotinamide phosphoribosyltransferase (NAMPT) which are thought to promote the development of NASH [44]. Previous studies demonstrated that the I148M polymorphism of Patatin-like phospholipase domain-containing 3 (PNPLA3) is related to NAFLD development and progression to hepatic fibrosis [45, 46]. More recent studies have reported that high VAT volume also might accentuate the role of the PNPLA3 in NAFLD [47]. Finally, the genes encoding arachidonic acid, sphingolipid and glycosphingolipid metabolism are upregulated in NASH and may contribute to VAT-mediated inflammation [48].

Although the present analysis showed that VAT volume change correlated with lobular inflammation improvement, and dSAT volume change correlated with ballooning improvement, liver inflammation and hepatocellular ballooning are pathologic processes that are themselves correlated [25]. It is likely that reduction in dSAT and VAT volumes both are associated with improvements in liver inflammation and cellular injury. Future studies are needed to clarify how VAT and dSAT interact in promoting NASH pathogenesis.

Our results highlight that reduction in central obesity, specifically dSAT and VAT, may be related to NASH histologic improvement. The findings from this analysis should increase awareness of the importance of lifestyle intervention in NASH. Future studies and clinical practice may design interventions that assess the reduction of dSAT and VAT volumes as outcome measures, rather than simply weight reduction. Plausibly, reduction of dSAT and VAT volumes may benefit both NASH histologic improvement, and the mitigation of cardiovascular complications accompanying NASH.

Our analysis was limited by sample size (i.e., only one-third of the participants of the parent study consented to MRI). The small sample size in the placebo group (n=37) did not permit examination of how adipose tissue depot volume changes may influence the natural course

of NASH without drug treatment. Because of the nature of the parent study, the present analysis was not designed to investigate drug effects on adipose tissue depots. Although we theorized how adipose tissue depot volumes might influence liver inflammation and cellular injury, our data cannot determine causality and we do not have adipokine (e.g., adiponectin and leptin) or inflammatory cytokine (e.g., IL-1, IL-6, and tumor necrotic factor alpha) data available. In addition, the parent study did not collect diet or exercise data, so assessments of contributions from diet and exercise on adipose tissue volume reduction and NASH histologic improvement were not possible. Larger independent studies are needed to further validate these results and to define the mechanism(s) by which changes in dSAT or VAT volumes are associated with reductions in inflammation and cell injury. Finally, our analysis sample is largely comprised of non-Hispanic Caucasian individuals. Whether our findings can be reproduced in other racial and ethnic groups remains to be determined.

In conclusion, our analyses of data acquired in the FLINT trial found that in adults with NASH, longitudinal reductions in dSAT and potentially VAT volumes were associated with histologic improvement, independent of reduction in hepatic PDFF. Reduction in sSAT volume alone did not relate to NASH histologic improvement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Conflict of interest statement

Dr. Middleton reports consultation to Alimentiv, Arrowhead, Glympse, Kowa, Median, and Novo Nordisk; lab service agreements under auspices of UCSD from Alexion, AstraZeneca, Bristol-Myers Squibb, Celgene, Enanta, Galmed, Genzyme, Gilead, Guerbet, Intercept, Ionis, Janssen, Janssen, NuSirt, Organovo, Pfizer, Roche, Sanofi, Shire, Synageva, and Takeda; stockholder Pfizer; and co-founder Quantix Bio.

Dr. Fowler reports personal consultation for Epigenomics, GE, Bayer and grant support from GE, Siemens, Philips, Bayer, FNIH, Gilead, and Pfizer. Unpaid position in advisory board of Quantix Bio.

Dr. Trout received research grants from Canon Medical Systems and Siemens Medical Solutions

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Dr. Loomba serves on the Steering Committee of the REGENERATE Trial funded by Intercept Pharmaceuticals. In addition, he has received research grants from Siemens Diagnostics and GE. He has research collaborations with AMRA and Antaros. He serves as a consultant or advisory board member for Arrowhead Pharmaceuticals, AstraZeneca, Bird Rock Bio, Boehringer Ingelheim, Bristol-Myer Squibb, Celgene, Cirius, CohBar, Conatus, Eli Lilly, Galmed, Gemphire, Gilead, Glympse Bio, GNI, GRI Bio, Intercept, Ionis, Janssen Inc., Merck, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Pfizer, Prometheus, Sanofi, Siemens, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Cirius, Eli Lilly and Company, Galectin Therapeutics, Galmed Pharmaceuticals, GE, Genfit, Gilead, Intercept, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, NuSirt, Pfizer, Prometheus, and Siemens. He is a co-founder of Liponexus, Inc.

Dr. Neuschwander-Tetri has been and advisor or consultant for Alimentiv, Allergan, Allysta, Alnylam, Amgen, Arrowhead, Axcella, Boehringer Ingelheim, BMS, Coherus, Cymabay, Enanta, Fortress, Genfit, Gilead, High Tide, HistoIndex, Innovo, Intercept, Ionis, LG Chem, Lipocine, Madrigal, Medimmune, Merck, Mirum, NGM, NovoNordisk, Novus Therapeutics, pH-Pharma, Sagimet, Target RWE, 89Bio; Stock options: HepGene; Institutional research grants: Allergan, BMS, Cirius, Enanta, Genfit, Gilead, Intercept, Madrigal, NGM

Dr. Sanyal is President of Sanyal Bio and has ownership interests in Genfit, Exhalenz, Tiziana, Inversago, Durect. He has served as a consultant to Astra Zeneca, Pfizer, Merck, Bristol Myers Squibb, Gilead, Boehringer Ingelhiem, Eli Lilly, Novo Nordisk, Hanmi, Intercept, Madrigal, Amgen, Regeneron, Blade, Genentech, Roche, Siemens, Glympse, 89Bio, Gelesis, Takeda, Salix and Malinckrodt. He gets royalties from Elsevier and Uptodate. His institution (VCU) receives grants from Pfizer, Astra Zeneca, Eli Lilly, Novo Nordisk, Hanmi, Gilead, Intercept, Boehringer Ingelhiem, Bristol Myers Squibb, Salix.

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Data Availability Statement:

Data described in the manuscript will be made available upon request, pending the FLINT study committee's approval.

Abbreviations:

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BIC	Bayesian information criterion
BMI	body mass index
dSAT	deep subcutaneous adipose tissue
FXR	Farnesoid X Receptor
FLINT	Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment

MRI	magnetic resonance imaging
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
NAFLD	nonalcoholic fatty liver disease
NAMPT	Nicotinamide phosphoribosyltransferase
NAS	nonalcoholic fatty liver disease activity score
NASH	nonalcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network
NASH CRN RCC	NASH Clinical Research Network Radiology Coordinating Center
PDFF	proton density fat fraction
PNPLA3	Patatin-like phospholipase domain-containing 3
SAF-A score	the activity part of the Steatosis, Activity, Fibrosis [SAF] scoring system that incorporates scores for ballooning and inflammation
sSAT	superficial subcutaneous adipose tissue
VAT	visceral adipose tissue

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Impact and implications

Although central obesity has been identified as a risk factor for obesity-related disorders including insulin resistance and cardiovascular disease, the role of central obesity in nonalcoholic steatohepatitis (NASH) needs further clarification. Our results highlight that reduction in central obesity, specifically deep subcutaneous adipose tissue and visceral adipose tissue, may be related to NASH histologic improvement. The findings from this analysis should increase awareness of the importance of lifestyle intervention in NASH for clinical researchers and clinicians. Future studies and clinical practice may design interventions that assess the reduction of deep subcutaneous adipose tissue and visceral adipose tissue as outcome measures, rather than simply weight reduction.

Highlights

- This is a secondary analysis of a subgroup of patients who had MRI exams in the FLINT trial.

- NASH patients with greater loss of deep subcutaneous adipose tissue showed greater levels of histologic improvement.

- NASH patients with greater loss of visceral adipose tissue probably also had greater levels of histologic improvement.
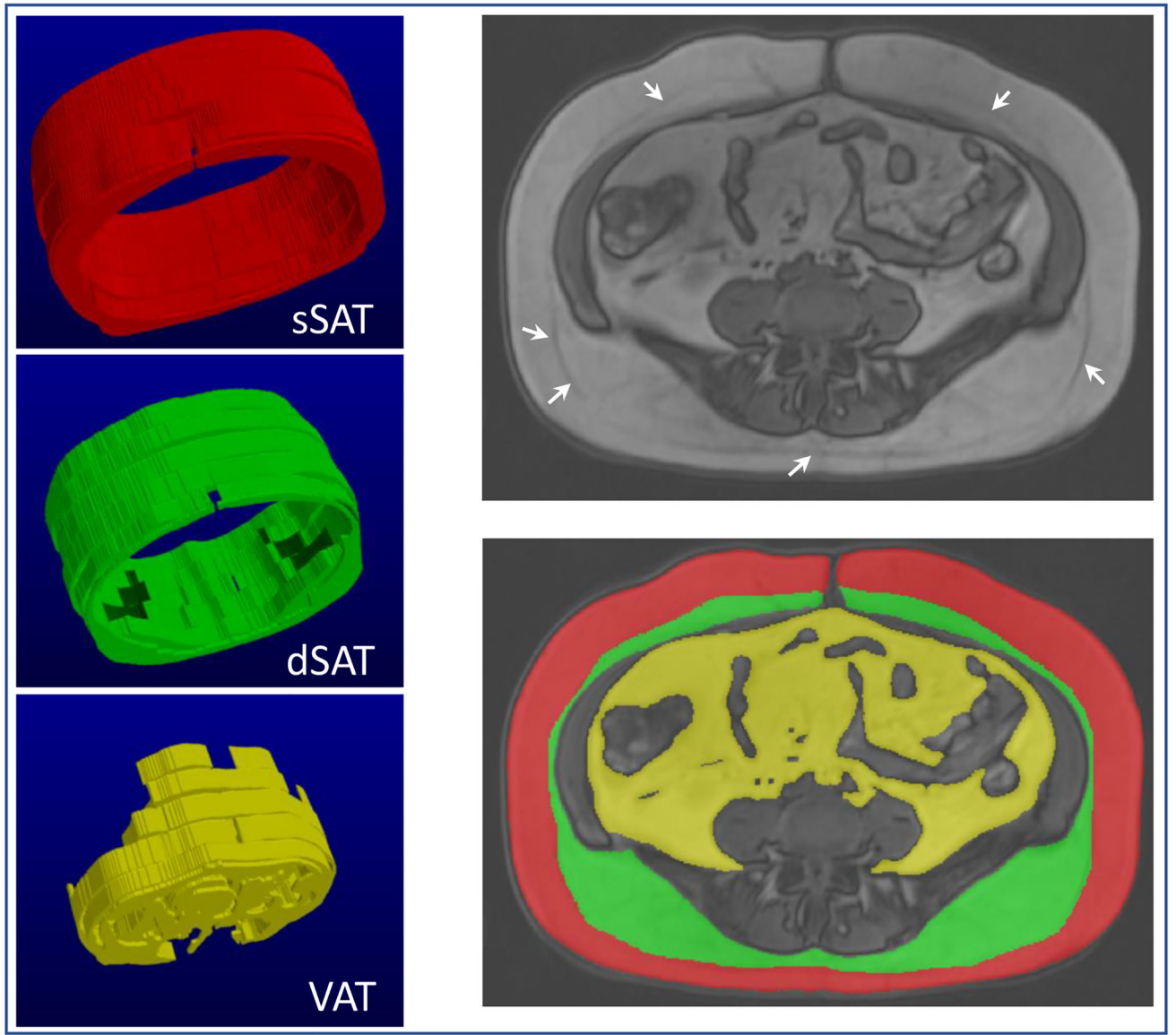
Fig.1. Sample adipose tissue depot segmentation.
Left panel shows 3D-reconstructed superficial subcutaneous adipose tissue (sSAT) (red, top left), deep subcutaneous adipose tissue (dSAT) (green, middle left), and visceral adipose tissue (VAT) (yellow, bottom left). Superficial and deep subcutaneous adipose tissue are divided by the fascia (indicated with white arrows) that separates the two adipose tissue depots. Right panel shows an MRI slice at L4-L5 level (top right) and segmentation of adipose tissue depots in the same colors overlaid on the MRI slice (bottom right).
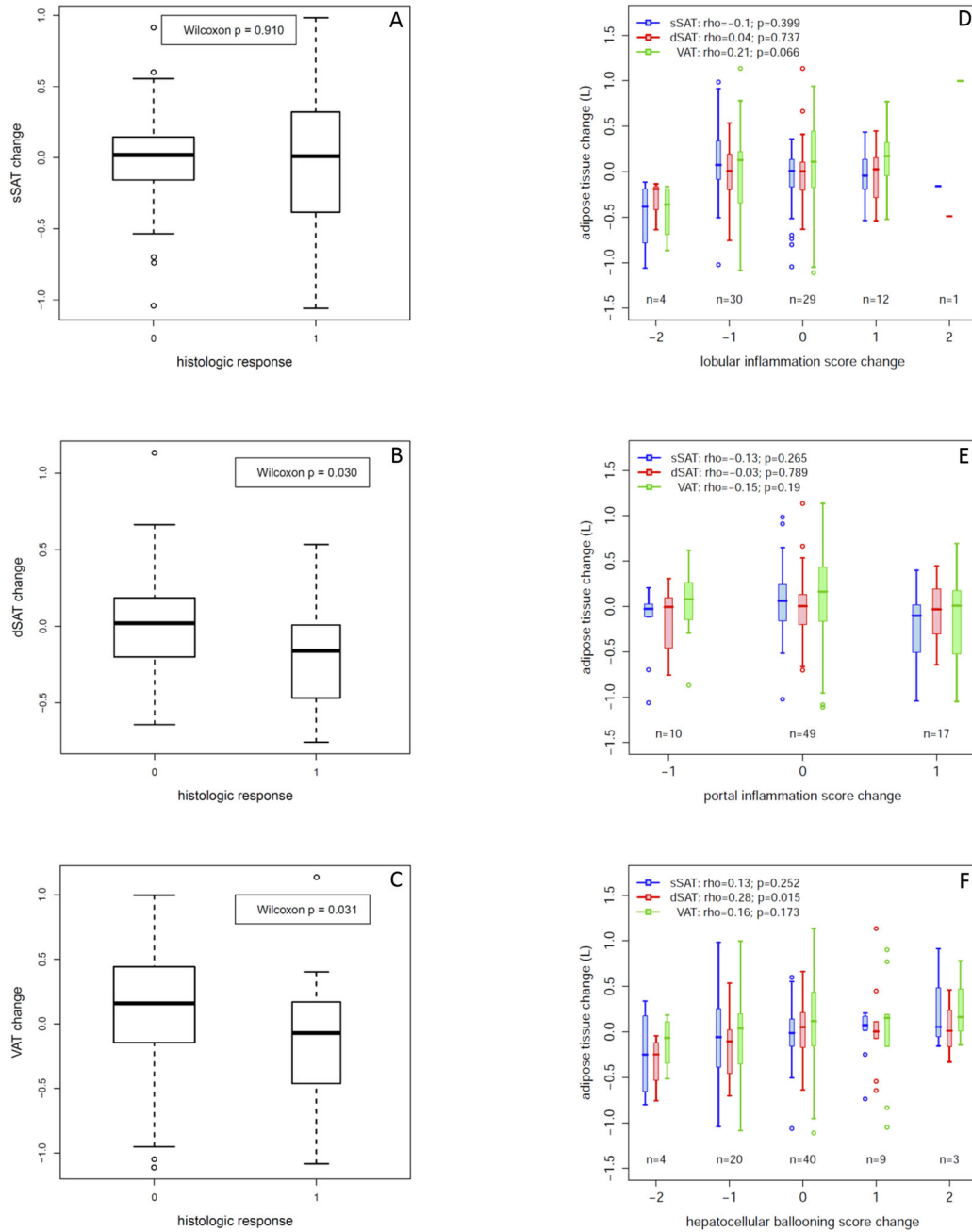
Fig. 2. Boxplots showing the relationship between changes in adipose depot volumes and histologic improvement in NASH.

Relationships (A) between change in sSAT and histologic improvement in NASH (P = 0.911, Wilcoxon test); (B) change in dSAT and histologic improvement in NASH (P = 0.030, Wilcoxon test); (C) change in VAT and histologic improvement in NASH (P = 0.031, Wilcoxon test); (D) change in sSAT, dSAT and VAT for different degrees of change of lobular inflammation score (Spearman's correlation $\rho = -0.1$, P = 0.399; $\rho = 0.04$, P = 0.737, and $\rho = 0.21$, P = 0.066, respectively); (E) change in sSAT, dSAT and VAT for different degrees of change of portal inflammation score (Spearman's correlation $\rho = -0.13$; P = 0.265, $\rho = -0.03$, P = 0.789, and $\rho = -0.15$, P = 0.19, respectively); (F) change in

sSAT, dSAT and VAT for different degrees of change of hepatocellular ballooning score (Spearman's correlation $\rho = 0.13$, P = 0.252; $\rho = 0.28$, P = 0.015, and $\rho = 0.16$, P = 0.173, respectively). dSAT, deep subcutaneous adipose tissue; NASH, non-alcoholic steatohepatitis; sSAT, superficial subcutaneous adipose tissue; VAT, visceral adipose tissue.

Table 1.

Baseline characteristics

	Entire sample n=76	Treatment group n=39	Placebo group n=37	P value
Male	28(37%)	14(36%)	14(38%)	1.000
Race				0.785
Asian	5(7%)	2(5%)	3(8%)	
Black or African American	1(1%)	0(0%)	1(3%)	
White	65(86%)	34(87%)	31(84%)	
Other	5(7%)	3(8%)	2(5%)	
Hispanic	13(17%)	7(18%)	6(16%)	1.000
Age (yrs)	52.4(10.9)	53.0(8.7)	51.8(12.8)	0.632
Body mass index (kg/m^2)	33.7(5.1)	34.4(5.5)	33.0(4.7)	0.242
Weight (kg)	95.1(16.3)	96.1(17.8)	93.9(14.8)	0.560
Waist circumference (cm)	110.2(12.1)	112.4(12.4)	107.9(11.5)	0.108
Definite steatohepatitis	60(79%)	31(80%)	29(78%)	1.000
Fibrosis stage	18(11)	19(11)	17(11)	0.491
0	13(17%)	5(13%)	8(22%)	
1	14(18%)	9(23%)	5(13%)	
2	23(30%)	10(26%)	13(35%)	
3	25(34%)	14(36%)	11(30%)	
4	1(1%)	1(2%)	0(0%)	
Total NAFLD activity score	5.2(1.3)	5.1(1.3)	5.4(1.3)	0.932
3	7(9%)	4(10%)	3(8%)	
4	17(22%)	10(26%)	7(19%)	
5	18(24%)	8(21%)	10(27%)	
6	22(29%)	12(31%)	10(27%)	
7	9(12%)	4(10%)	5(14%)	
8	3(4%)	1(3%)	2(5%)	
Hepatocellular ballooning score	1.4(0.7)	1.4(0.8)	1.4(0.7)	0.812
0	11(14%)	6(15%)	5(14%)	
1	24(32%)	11(28%)	13(35%)	
2	41(54%)	22(57%)	19(51%)	
Steatosis score	2.0(0.8)	1.9(0.8)	2.1(0.8)	0.767
0	1(1%)	1(3%)	0(0%)	
1	22(29%)	12(31%)	10(27%)	
2	32(42%)	17(43%)	15(41%)	
3	21(28%	9(23%)	12(32%)	
Lobular inflammation score	1.9(0.7)	1.8(0.7)	1.9(0.8)	0.795
1	25(33%)	13(33%)	12(33%)	
2	35(46%)	19(49%)	16(43%)	
3	16(21%)	7(18%)	9(24%)	
Portal inflammation score	1.2(0.6)	1.2(0.6)	1.2(0.6)	0.733

	Entire sample n=76	Treatment group n=39	Placebo group n=37	P value
0	7(9%)	4(10%)	3(8%)	
1	46(61%)	22(57%)	24(65%)	
2	23(30%)	13(33%)	10(27%)	
Alanine aminotransferase (U/L)	74(38)	79(41)	68(34)	0.212
Aspartate aminotransferase (U/L)	54(29)	57(33)	50(25)	0.298
Hepatic proton density fat fraction (%)	19(9)	18(8)	20(10)	0.359
Superficial subcutaneous adipose tissue (L)	2.9(1.3)	2.9(1.2)	2.8(1.4)	0.745
Deep subcutaneous adipose tissue (L)	2.3(0.8)	2.4(0.9)	2.3(0.8)	0.687
Visceral adipose tissue (L)	3.4(1.3)	3.6(13)	3.2(1.2)	0.177

Data are presented as n(%) or mean(SD); NAFLD, nonalcoholic fatty liver disease; SAT, superficial subcutaneous adipose tissue; dSAT, deep subcutaneous adipose tissue; VAT, visceral adipose tissue.

P values for comparisons between treatment and placebo groups are based on the Fisher's Exact test for ordinal and categorical variables and the two-sample *t*-test for continuous variables.

Table 2.

Spearman's correlations among change in anthropometrics and adipose depots (n=76)

	BMI	Waist circumference	sSAT	dSAT	VAT	Hepatic PDFF
Weight	0.88 (P<0.001)	0.71 (P<0.001)	0.65 (P<0.001)	0.55 (P<0.001)	0.62 (P<0.001)	0.32 (P=0.006)
BMI	-	0.68 (P<0.001)	0.55 (P<0.001)	0.49 (P<0.001)	0.65 (P<0.001)	0.30 (P=0.008)
Waist circumference	-	-	0.35 (P=0.002)	0.35 (P=0.002)	0.53 (P=0.002)	0.34 (P=0.003)
sSAT	-	-	-	0.378 (P=0.001)	0.512 (P<0.001)	0.306 (P=0.007)
dSAT	-	-	-	-	0.614 (P<0.001)	0.174 (P=0.134)
VAT	-	-	-	-	-	0.363 (P=0.001)

, change; BMI, body mass index; sSAT, superficial subcutaneous adipose tissue; dSAT, deep subcutaneous adipose tissue; VAT, visceral adipose tissue; PDFF, proton density fat fraction.

Table 3.

Regression model with NASH histologic improvement as the dependent variable.

Independent variables	Regression coefficients (P value)
Baseline lobular inflammation score	1.21 (P=0.011)
dSAT change	−2.00 (P=0.044)
Hepatic PDFF change	−0.08 (P=0.033)
Goodness of fit (deviance) test	P=0.0016

dSAT, deep subcutaneous adipose tissue; PDFF, proton density fat fraction.

A Bayesian information criterion-based stepwise linear regression procedure was used to search for a model predicting NASH histologic improvement. Potential predictors included age, sex, race, ethnicity, treatment group, baseline histology, baseline values and changes of weight, superficial subcutaneous adipose tissue, dSAT, visceral adipose tissue, hepatic PDFF, alanine aminotransferase and aspartate aminotransferase.