

Association of weight changes with changes in histological features and blood markers in non-alcoholic steatohepatitis

Short title: Changes in weight and histology in NASH

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Abbreviations

FLINT: Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment
NAFLD: Non-alcoholic fatty liver disease
NASH: Non-alcoholic steatohepatitis
T2D: Type 2 diabetes
PIVENS: Pioglitazone vs Vitamin E vs Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis

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Data Transparency Statement

Data are available through the NIDDK Central Repository.

Abstract

Background and aims

Weight loss is recommended for patients with non-alcoholic steatohepatitis (NASH) but the impact of weight change on disease activity remains unclear. We examined the association between weight change (gain/loss) and changes in biochemical and histological features of NASH.

Methods

This was an analysis of the PIVENS and FLINT trials in adults with NASH who had liver biopsies at baseline and at either 1.5 years or 2 years. Multivariable regression models examined how weight change was associated with changes in (a) blood liver markers, (b) NASH resolution with no fibrosis worsening, (c) fibrosis improving with no NASH worsening, and (d) individual histological features.

Results

The BMI of the 421 participants was 34.4kg/m² (SD:6.5) and their mean weight change was +0.4kg (SD:6.5). Weight change was independently and positively associated with changes in liver enzymes and the Fibrosis-4 score (all $p < 0.001$). Each kg of weight loss was associated with 7% (95%CI: 3-10%, $p < 0.001$) increase in odds of achieving NASH resolution with no fibrosis worsening and with 5% (95%CI: 1%-8%, $p = 0.01$) increase in odds of achieving fibrosis improvement with no NASH worsening. Weight gain was associated with worsening of disease activity. For every kg of weight lost, the odds of fibrosis improving were 5% (95%CI: 2-8%, $p = 0.001$). There was no evidence that the association between weight change and outcome depended upon pharmacological treatment, trial, body mass index, and baseline fibrosis.

Conclusions

102 Weight change was independently and monotonically associated with changes in
103 biochemical and histological features of NASH. Guidelines for NASH management should
104 incorporate recommendations for both avoidance of weight gain and support to lose weight.

105

106 **Keywords**

107 Non-alcoholic steatohepatitis, weight loss, weight gain, fibrosis, histology.

Introduction

Non-alcoholic steatohepatitis (NASH) is a chronic and progressive form of non-alcoholic fatty liver disease (NAFLD). NASH is estimated to affect 1%-6% of the population worldwide.¹ By 2030, cases of NASH are projected to rise by more than 40% in Europe, the USA, and China.² This is forecast to increase cirrhosis and liver cancer by 150%,³ leading to high burden for both patients and the healthcare systems.⁴ The hepatocellular injury and inflammation seen in NASH is associated with fibrosis,⁵ which is the strongest independent factor for worse long-term morbidity and mortality.^{6, 7} Therefore, improvements in NASH and, crucially, in fibrosis are targets of therapeutic agents.⁸

Many agents are under investigation, but no treatment has been approved yet. Instead, guidelines worldwide recommend that doctors offer advice for weight loss,⁹ as ≥70% of NASH patients have obesity and the disease is typically described as the hepatic manifestation of the metabolic syndrome.¹ This weight loss recommendation follows clear evidence from randomized trials primarily in early-stage NAFLD that modest weight loss (2-5kg) reduces blood liver markers and steatosis within 3-12 months.¹⁰ However, whether modest weight change is associated with histological changes in cohorts with more advanced disease is unclear.

A systematic review and meta-analysis showed a dose-response relationship between reductions in weight and improvements in blood liver markers and steatosis over a median of 6 months.¹¹ However, there was only limited and imprecise evidence of a relationship between weight loss and changes in the remaining histological features of NASH (ballooning, inflammation) as well as resolution of NASH over 1 year. Furthermore, the evidence of a relationship between weight loss and improvements in fibrosis over 1 year was inconsistent and imprecise. This is likely due to few studies with biopsy endpoints or because fibrosis, the main risk factor for long-term outcomes, progresses slowly over years and longer follow-up might be needed to observe significant changes.^{6, 7} Additionally, despite the recommendations for weight loss, more than a third of patients in routine care gain

weight,¹² and limited data exist on whether weight gain is associated with worsening in histological features of NASH.¹³

Therefore, this study aims to examine the association between changes in weight (both gain and loss) and changes in histological and biochemical features of NASH over 1.5 to 2 years as well as whether the impact of weight change differed between active treatment and placebo where participants also received standardized weight loss advice.

Methods

Design

This was a prospective longitudinal analysis of the Pioglitazone vs Vitamin E vs Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) and the Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) trials. Their design and main results have been reported.^{14, 15} The University of Oxford Medical Sciences Interdivisional Research Ethics Committee approved this study (Ref: R69921/RE001). All authors had access to the study data and reviewed and approved the final manuscript.

Participants

In PIVENS, 240 adults with histologically-proven NASH and without diabetes were randomized to pioglitazone, vitamin E, or placebo. Inclusion criteria were definite or possible steatohepatitis with a NAFLD activity score (NAS) ≥ 5 or definite steatohepatitis with NAS ≥ 4 . All patients had ballooning score ≥ 1 . In FLINT, 283 adults with histologically-proven NASH were randomized to obeticholic acid or placebo. Inclusion criteria were definite or borderline NASH with NAS ≥ 4 and ≥ 1 point in each of steatosis, ballooning, and lobular inflammation scores. Fifty-three percent had type 2 diabetes (T2D). Due to a protocol change for crossing the superiority boundary, 64 participants in FLINT did not undergo a follow-up biopsy. Cirrhosis, other liver diseases, and substantial alcohol consumption were exclusion criteria in both studies. All participants received standardized recommendations for weight loss,

healthy eating, and physical activity. NASH resolution was defined as a change in diagnosis from definite or borderline NASH at baseline to a diagnosis of fatty liver disease without steatohepatitis or no fatty liver disease at the end of treatment, according to pathology criteria.¹⁶ No worsening of NASH was defined as a no increase on any of ballooning, lobular inflammation, or steatosis.¹⁵ Fibrosis improvement was defined as reduction of ≥ 1 stage.

Statistical analysis

Participants with both baseline and end of treatment biopsies were included in this analysis. Baseline characteristics between the first and fourth quartiles of weight change were compared using independent t-tests or chi-square tests as appropriate.

We examined the association between changes in weight and changes in each histological feature using the Pearson correlation coefficient. We ran the following analyses of covariance and all models were adjusted for baseline BMI (continuous), baseline T2D status (yes/no), age, sex, and dichotomized treatment (placebo/active). For each histological feature (fibrosis, steatosis, inflammation, ballooning, and NAS), we ran a separate ordinal regression model examining the association between the follow-up histological score and weight change further adjusting for the baseline histological score. Ordinal regression models were chosen over linear regression ones, as the linear regression assumptions were violated.

Separate logistic regression models examined the association between weight change and each of the following categorical outcomes: (a) NASH resolution with no fibrosis worsening further adjusted for NAS and baseline fibrosis stage, (b) fibrosis improvement with no NASH worsening further adjusted for NAS and baseline fibrosis stage, at least 1-point improvement in each of (c) fibrosis, (d) steatosis, (e) inflammation, and (f) ballooning scores further adjusted for the baseline histological feature score, (g) at least 2-point improvement in the NAS further adjusted for baseline NAS.

For each blood marker (alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), γ -glutamyltransferase (GGT), the Fibrosis-4 (FIB-4)

score), we ran a separate generalized linear regression model examining the association between the follow-up log-transformed marker value and weight change further adjusting for the baseline marker value.

Moderation analyses for the ordinal and linear models were run with an interaction term between weight change and each of the following categorical moderators: treatment (placebo vs. any active treatment), trial (PIVENS vs. FLINT), BMI (below or at least 30 kg/m²), and baseline fibrosis stage (categorized as 0-1 or 2-3). Six participants with baseline fibrosis stage 4 were excluded from the last moderation analysis, as the small sample did not allow for a meaningful analysis. Sensitivity analyses excluded participants with a diagnosis of no steatohepatitis (n=28) at both baseline and follow-up biopsy. The level of significance was set at p<0.05. As the models were pre-specified, we did not correct for multiple testing.¹⁷ With the exception of a post-hoc logistic regression model of fibrosis improvement with no NASH worsening and a logistic regression model of fibrosis worsening (examining ≥1-stage fibrosis worsening vs. no worsening), we followed the pre-specified statistical analysis plan published before the analysis with no changes.¹⁸

Following peer-review, we conducted analyses (a) on the association between *percentage* weight change with the two composite outcomes (NASH resolution with no fibrosis worsening and fibrosis improvement with no NASH worsening), (b) with an interaction term between weight change and each type of treatment (obeticholic acid, pioglitazone, vitamin E, vs. placebo), (c) by excluding people without baseline fibrosis (n=51) from the models of fibrosis improvement, (b) on the association between weight changes and changes in glycated hemoglobin (HbA1c). Analyses were conducted in R v4.0.3.

Results

Table 1 presents baseline characteristics (n=221 for PIVENS and n=200 for FLINT). Participants were 63% female. The average (SD) age, weight, and BMI were 49 (12) years, 97.3kg (21.2) and 34.3kg/m² (6.5), respectively. Although average weight change was minimal (absolute: +0.5kg, percentage: +0.5%, there was substantial variation (SD: 6.5kg,

6.4%) (Figure 1). Comparing the first quartile of absolute weight change (-7.4kg (SD: 5.5), n=106) and with the fourth quartile (+7.9kg (SD: 4), n=105), there were no differences in baseline BMI, weight, waist circumference, and percentage body fat, blood pressure, lipids, liver histology, insulin, and most blood liver markers. However, participants who lost the most weight were significantly older ($p=0.03$), more likely to have T2D ($p<0.001$), had higher values in most blood glucose regulation markers (glucose $p<0.001$, HbA1c $p<0.001$, and HOMA $p=0.01$), and higher values in some liver markers (GGT $p=0.03$ and FIB-4 $p=0.048$) compared with those who lost the least weight. When the highest and lowest quartiles of percentage weight change were compared, these results remained largely unchanged (Table S1).

Weight change and blood markers

Weight loss was significantly positively associated with reductions in blood markers in the multivariable model. Every kg of weight change was positively associated with a change in follow-up ALT of 2% (95% CI: 1%-3%, $p<0.0001$), in AST of 2% (95% CI: 1%-2%, $p<0.0001$), in GGT of 2% (95% CI: 1%-3%, $p<0.0001$), and in FIB-4 score of 1% (95% CI: 0.4%-1%, $p<0.001$). There was no significant association with ALP (-0.1%, 95% CI: -0.5%-0.2%, $p=0.55$). The full model is available in Table S2. Every kg of weight change was also positively associated with a change in follow-up HbA1c of 0.22 mmol/mol (95% CI: 0.12 to 0.33, $p<0.001$).

Weight change and histology

At follow-up, 151 (36%) participants had NASH resolution with no fibrosis worsening and 114 participants (27%) had fibrosis improvement with no NASH worsening. Weight loss was associated with improved histological outcomes, whereas weight gain was associated with worsened histological outcomes. Each kg of weight gain was associated with lower odds of NASH resolving and fibrosis not worsening (OR: 0.93 (95%CI: 0.90-0.97, $p<0.001$)), or, equivalently, each kg of weight loss was associated with a 7% (95%CI: 3-10%) increased

odds of NASH resolution without worsening fibrosis. Similarly, each kg of weight gain was associated with reduced odds of fibrosis improving without NASH worsening (OR: 0.95 (95%CI: 0.92-0.99, $p=0.01$)), or equivalently, each kg of weight loss was associated with a 5% (95%CI: 1-8%) increase in the odds of fibrosis improvement (Tables S2-S3). Figure 2 shows the probabilities of achieving each outcome.

The change in each histological feature was associated with weight change (Figure 3). For every kg of weight lost in the multivariable ordinal regression models, the odds of fibrosis improving were 5% (95%CI: 2-8%, $p=0.001$), of steatosis improving 11% (95%CI: 8-15%, $p<0.0001$), of ballooning improving were 8% (95%CI: 5-12%, $p<0.0001$), of inflammation improving were 8% (95%CI: 4-11%, $p<0.0001$) and of NAS improving were 11% (95%CI: 8-14%, $p<0.0001$). Weight gain was associated with a lowering of the odds of improvement. Results from the multivariable logistic regression models of improvements of at least 1 point in each of fibrosis, steatosis, ballooning, and inflammation and of at least 2 points in NAS showed similar estimates (Tables S4-S8). Weight change was also positively associated with fibrosis worsening of ≥ 1 stage (OR 1.05, 95%CI: 1.01-1.10, $p=0.022$, Table S9).

Moderation analysis

There was no statistically significant interaction in the blood markers by treatment, study, weight status (BMI category), or baseline fibrosis stage with the exception of an interaction of weight status with ALT and GGT (Figure S1). Among the histological features, there was no significant interaction by trial and there was no significant interaction for achieving NASH resolution with no fibrosis worsening. Weight change was associated with greater change in probability of fibrosis improving with no NASH worsening among people with baseline fibrosis stage 0-1 compared with those with fibrosis 2-3 ($p_{\text{interaction}}=0.04$, Figure S2). The probability of steatosis worsening was higher with weight gain among people with baseline fibrosis stage 0-1 compared with those with fibrosis 2-3 ($p_{\text{interaction}}=0.02$). There was a significant interaction between weight status and inflammation ($p_{\text{interaction}}=0.006$) where

participants with BMI<30kg/m² had larger improvements in inflammation per kg of weight lost than participants with obesity. Participants receiving placebo had significantly greater changes in steatosis ($p_{\text{interaction}}=0.04$), ballooning ($p_{\text{interaction}}=0.02$), and NAS ($p_{\text{interaction}}=0.01$) with weight change than those in the active treatment groups (Figures S3-S7).

Sensitivity analysis

In sensitivity analyses excluding participants without NASH at both baseline and follow-up biopsies or excluding participants without fibrosis at baseline, the results did not materially change (Tables S10-S12). In a moderation analysis with the four treatment groups individually categorized, there were no significant interactions between treatments and weight change (all $p>0.05$) except for significant interactions between pioglitazone and weight change for NASH resolution with no fibrosis worsening ($p_{\text{interaction}}=0.02$), steatosis ($p_{\text{interaction}}=0.04$), and NAS ($p_{\text{interaction}}=0.02$) (Figures S1-S7). In models replacing absolute weight change with percentage weight change, the results did not differ (Figure S8).

Discussion

Weight loss was strongly associated with meaningful improvements in both histological features of NASH and fibrosis and non-invasive tests of NAFLD severity over 1.5-2 years. Weight gain was associated with disease worsening. These associations were consistent across trials, trial groups, BMI categories, and disease severity.

Our findings are broadly consistent with studies of behavioral interventions and bariatric surgery in NASH. The probabilities of NASH resolution with no fibrosis worsening for -10kg and -5kg were 47% (36-59%) and 39% (31-47%), respectively. The probabilities of fibrosis improvement with no NASH worsening for -10kg and -5kg were 31% (22-41%) and 26% (20-33%), respectively. A single-arm behavioral intervention study with 1-year paired biopsies showed the same direction of effect, but with larger effect estimates for improvements in each histological feature.¹⁹ This difference in the results might be due to inclusion of participants the less severe disease, as about 60% had no fibrosis at baseline

303 compared with 14% in the current cohort. Earlier-stage disease might be more readily
304 modified with weight change, as the estimates in our interaction analysis were slightly
305 attenuated in participants with moderate to advanced fibrosis (stages 2-3) at baseline.
306 Results are also consistent with a paired-biopsy (n=60) study in NASH 5 years after bariatric
307 surgery showing the rate of improvements in NASH and fibrosis was significantly lower in
308 patients with more severe NASH or fibrosis at baseline.²⁰ Bariatric surgery leads to
309 substantial weight loss that resolves NASH and improves fibrosis in most individuals.²⁰
310 However, our results suggest that improvements can be seen even with more modest weight
311 loss. Furthermore, the monotonic independent relationship between weight changes and
312 changes in NASH disease severity suggests that patients with NASH should avoid weight
313 gain. This is in line with small studies showing that weight gain was independently
314 associated with liver fibrosis progression. In the current analysis, T2D was not independently
315 associated with histological improvements. Although this might seem to contradict evidence
316 that T2D is associated with smaller improvements in NASH histology,¹⁹ participants with T2D
317 in this analysis lost more weight than those without T2D, because people with T2D who
318 received obeticholic acid lost more weight than people with diabetes in the FLINT trial.

319 These results have implications for changes in guidelines and clinical practice. The
320 lack of randomized controlled trials of behavioral weight loss interventions with the same
321 widely accepted endpoints applied in pharmacotherapy trials has led to only weak
322 recommendations for dietary change, physical activity, and weight loss advice as a first-line
323 treatment for NASH.⁹ Routine care primarily includes provision of simple advice,²¹ rather
324 than comprehensive behavioral support, such as referral to structured weight management
325 services which leads to greater weight loss.²² Qualitative data suggest that this frustrates
326 patients.²³ Embedding weight management services within the NASH care pathway could
327 reduce disease progression.

328 Future research should examine whether improvements in disease severity are
329 maintained despite expected slow and modest weight regain following behavioral weight
330 management programs. Despite this regain, the intensive diabetes prevention programs

have shown reductions of 27-32% in T2D incidence compared with simple advice over 13-15 years, so weight regain might not erode all benefits seen with weight loss.²⁴ Among people at risk of or with early-stage NAFLD, mean weight following a 6-month weight loss program remained lower than baseline after 2 years, and steatosis improvements were sustained at 2 years despite modest weight regain.²⁵ Long-term improvements in NASH and fibrosis should make weight management interventions both clinically and cost-effective.

Changes in ALT, AST, and GGT with weight change had consistent direction of effect with changes in histology, whereas ALP was not associated with weight change. These results are in line with reductions in ALT, AST, and GGT and no change in ALP seen in weight loss trials in early-stage NAFLD.¹⁰ Weight change was also positively associated with changes in FIB-4, a non-invasive fibrosis marker. Previous research found no evidence of changes in another non-invasive fibrosis marker, the Enhanced Liver Fibrosis (ELF) score, following weight loss in an unselected population with overweight and possible moderate fibrosis²⁶ but reductions in magnetic resonance measured liver stiffness with weight loss in NAFLD.²⁷ Identifying non-invasive biomarkers of NASH and fibrosis that are responsive to change is crucial for routine monitoring. These findings suggest changes in blood markers might be appropriate for monitoring changes in NASH and fibrosis during weight loss.

These results also have implications for the design of future pharmacotherapy trials. The beneficial effects of weight loss and negative effects of weight gain independent of the received treatment, together with the fact that structured weight loss programs lead to larger weight loss than advice alone²² suggest that trials should embed stronger behavioral support for weight loss in both placebo and active treatment. This will provide the best possible standard care to participants receiving placebo. It will also allow for examining the efficacy of agents as adjunct to such care. This is particularly applicable when the expected benefits of agents are modest, adherence might be low, and adverse events are common. Such trial designs will complement existing recommendations on weight stability before trial enrolment, standardized dietary and physical activity advice, and longitudinal diet and physical activity

measurement.²⁸ Such designs would examine the combined and separate effects of weight loss and pharmacotherapy on hard endpoints.

This analysis has several strengths, including the pre-registered statistical analysis plan, large number of paired biopsies with a follow-up of 1.5-2 years from a cohort with mostly moderate to advanced fibrosis at baseline, and the blinded interpretation of biopsies using the same standardized protocol. It also the first to report on the effect of weight loss in fibrosis improvement with no NASH worsening, one of the new NASH endpoints from the European Medical Agency. The analysis is limited by inclusion of the active treatment arms which have independent effects on liver histology as well as obeticholic acid leading to modest weight loss and pioglitazone leading to modest weight gain. However, the estimates remained robust in moderation analysis with mostly no significant interaction by active treatment or placebo. This aligns with an analysis of the FLINT trial with dichotomized weight change where a weight loss $\geq 2\%$ improved histology compared with weight loss $< 2\%$ and which showed no significant interaction between weight loss and treatment (active/placebo).²⁹ As this was an observational analysis, causality cannot be inferred. We focused on weight change and dietary changes or physical activity changes could have independent effects, but weight change does not occur without such changes. Although we adjusted for all major confounders, the possibility for residual confounding remains.

In conclusion, weight change was monotonically associated with changes in both blood liver markers and all liver histological features in NASH. Clinical guidelines should incorporate recommendations for both avoidance of weight gain and provision of structured support to lose weight in non-cirrhotic patients with NASH with or without fibrosis.

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468 Table 1: Sample characteristics in each trial, in the placebo groups, and by quartile of weight
469 change

Baseline characteristic	PIVENS	FLINT	Placebo groups	Quartiles of weight change			
				Q1	Q2	Q3	Q4
Total N	221	200	170	106	105	105	105
Sex, female, n (%)	131 (59.3)	133 (66.5)	104 (61.2)	75 (70.8)	65 (61.9)	61 (58.1)	63 (60)
Race, white, n (%)	187 (84.6)	165 (82.5)	140 (82.4)	90 (84.9)	82 (78.1)	87 (82.9)	93 (88.6)
Hispanic ethnicity, n (%)	28 (12.7)	26 (13)	20 (11.8)	10 (9.4)	12 (11.4)	21 (20)	11 (10.5)
Age, years	46.9 (11.9)	51.1 (11.4)	48.3 (11.9)	49.6 (11.2)	49.8 (13.4)	50.1 (10.2)	46.1 (12.1)
<u>Anthropometry</u>							
BMI, kg/m ²	34.1 (6.6)	34.6 (6.3)	34.2 (6.5)	35.8 (7.4)	32.6 (6)	33.1 (5.7)	35.9 (6)
Weight, kg	97.1 (22.2)	97.5 (20.2)	96.4 (18.9)	100.6 (21.4)	92.5 (20.8)	92.4 (18.9)	103.5 (21.6)
Change in weight, kg	1.9 (6.3)	-1.1 (6.3)	0.3 (5.7)	-7.4 (5.5)	-0.7 (0.9)	2.1 (0.8)	7.9 (4)
Change in weight, %	2 (6.3)	-1.1 (6.1)	0.4 (5.6)	-7.3 (5)	-0.7 (1.1)	2.4 (1)	7.8 (4)
Waist circumference, cm	107.7 (13.9)	110.7 (15)	108.2 (14)	112.5 (15.6)	106.6 (14.1)	105.8 (13.1)	111.7 (14)
Body fat, %	39.3 (8.9)	-	39.5 (9.4)	41.7 (9.2)	36.6 (8.2)	37.2 (8.5)	41.6 (8.6)
<u>Metabolic factors</u>							
Type 2 diabetes, n (%)	0 (0)	107 (53.5)	53 (31.2)	42 (39.6)	25 (23.8)	29 (27.6)	11 (10.5)
Fasting glucose, mmol/L	5.2 (0.7)	6.4 (2.1)	5.9 (2)	6 (1.6)	6 (2.1)	5.8 (1.8)	5.3 (0.9)
Fasting insulin, pmol/L	133 (108.9)	167.4 (183.9)	130.3 (108.2)	178 (219.6)	144.5 (133.2)	140.9 (123)	133.6 (90)
HOMA-IR, mmol/L x pmol/L / 22.5	31.6 (26.8)	50.4 (63.2)	35.7 (37.3)	50.8 (72.4)	40.8 (43.9)	38.2 (39.2)	32.1 (23.8)
HbA1c, mmol/mol	37.6 (5.9)	47.1 (11.6)	42.6 (10.1)	45.2 (11)	42.3 (10.6)	41.9 (11.1)	39 (6.7)
Hypertension, n(%)	92 (41.6)	123 (61.5)	88 (51.8)	60 (56.6)	59 (56.2)	50 (47.6)	46 (43.8)
Systolic BP, mmHg	132.2 (14.5)	132.7 (15.6)	132.3 (14.2)	132.8 (16.4)	133.8 (15.8)	130.6 (14.4)	132.5 (13.4)
Diastolic BP, mmHg	77.9 (10.2)	77 (10.3)	78.5 (9.9)	77 (11.1)	77.4 (10.1)	77.7 (10)	77.7 (9.8)
<u>Lipids</u>							
Hyperlipidaemia, n (%)	117 (52.9)	125 (62.5)	99 (58.2)	63 (59.4)	57 (54.3)	62 (59)	60 (57.1)
Total cholesterol, mmol/L	5.1 (1)	4.9 (1.2)	5.1 (1.2)	4.9 (1.1)	5.2 (1.2)	5 (1.1)	5 (1)
HDL, mmol/L	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.2 (0.4)	1.1 (0.3)
LDL, mmol/L	3.2 (0.9)	2.9 (1)	3.1 (1)	3 (1)	3.1 (1)	3 (0.9)	3.1 (0.9)
Triglycerides, mmol/L	1.9 (1.1)	2.1 (1.6)	2 (1.7)	1.9 (0.9)	2.4 (2.1)	1.8 (0.9)	1.9 (1.2)
<u>Blood liver markers</u>							
ALT, U/L	81.7 (47.1)	81.8 (48.5)	81.6 (49.2)	80.3 (48.6)	86.7 (48.9)	84.8 (45)	75.2 (48.2)
AST, U/L	55.7 (29.3)	59.9 (35.5)	55.5 (30.9)	58.8 (35.6)	59.2 (32.4)	61.1 (31.8)	51.6 (29.1)
GGT, U/L	58.9 (59.5)	71.3 (69.3)	65.3 (61.9)	69.3 (59.9)	70.4 (82)	65.7 (68)	53.8 (41)
FIB-4 score	1.4 (1.1)	1.5 (0.8)	1.3 (0.7)	1.5 (1.3)	1.5 (0.9)	1.5 (0.7)	1.2 (0.9)
<u>Liver histology</u>							
Total NAS	4.9 (1.4)	5.3 (1.3)	5 (1.4)	5.2 (1.4)	5.1 (1.3)	5.2 (1.4)	5 (1.4)
Steatosis score	1.9 (0.8)	2.1 (0.8)	2 (0.8)	2 (0.8)	1.9 (0.8)	2 (0.8)	2.1 (0.8)
Ballooning score	1.2 (0.8)	1.4 (0.7)	1.3 (0.7)	1.3 (0.8)	1.4 (0.8)	1.4 (0.7)	1.2 (0.8)
Inflammation score	1.7 (0.7)	1.8 (0.7)	1.7 (0.7)	1.8 (0.7)	1.8 (0.7)	1.8 (0.7)	1.7 (0.7)
Fibrosis stage	1.5 (1)	1.8 (1)	1.7 (1.1)	1.8 (1.1)	1.7 (1)	1.6 (1)	1.5 (1.1)

Data are presented as mean (SD) unless otherwise indicated. Total fat was not measured in the FLINT trial. HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, HbA1c: Glycated hemoglobin, BP: Blood pressure, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-glutamyltransferase, FIB-4: Fibrosis-4, NAS: Non-alcoholic fatty liver disease activity score

471 **Figure 1:** Weight change in each participant by treatment arm.

472

473 **Figure 2:** Predicted probabilities (95% CIs) of (a) NASH resolution with no fibrosis

474 worsening and (b) fibrosis improvement with no NASH worsening.

475

476 **Figure 3:** Change in histological features by weight change and correlation coefficients

477 between them. Boxplots represent median and interquartile range in weight change by score

478 change in each histological feature. Blue dots represent individual participant data.