Effects of tesamorelin on non-alcoholic fatty liver disease in HIV: a randomised, double-blind, multicentre trial

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Summary

Background Non-alcoholic fatty liver disease (NAFLD) is a substantial cause of comorbidity in people with HIV and there are no proven pharmacological treatments for the disease in this population. We assessed the effects of tesamorelin on liver fat and histology in people with HIV and NAFLD.

Methods This randomised, double-blind, multicentre study with identical placebo as a comparator was done in a hospital and a medical research centre in the USA. People with HIV infection and a hepatic fat fraction (HFF) of 5% or more by proton magnetic resonance spectroscopy were eligible. Participants were randomly assigned (1:1) to receive either tesamorelin 2 mg once daily or placebo once daily for 12 months, followed by a 6-month open-label phase during which all participants received tesamorelin 2 mg daily. The randomisation list was prepared by the study statistician using a permuted block algorithm within each stratum with randomly varying block sizes. The primary endpoint was change in HFF between baseline and 12 months. The primary safety endpoint was glucose. Analysis was by intention to treat using all available data. This trial is registered with ClinicalTrials.gov, number NCT02196831.

Findings 61 patients were enrolled between Aug 20, 2015, and Jan 16, 2019, of whom 30 received tesamorelin and 30 received placebo. Patients receiving tesamorelin had a greater reduction of HFF than did patients receiving placebo, with an absolute effect size of –4·1% (95% CI –7·6 to –0·7, p=0·018), corresponding to a –37% (95% CI –67 to –7, p=0·016) relative reduction from baseline. After 12 months, 35% of individuals receiving tesamorelin and 4% receiving placebo had a HFF of less than 5% (p=0·0069). Changes in fasting glucose and glycated haemoglobin were not different between groups at 12 months. Individuals in the tesamorelin group experienced more localised injection site complaints than those in the placebo group, though none were judged to be serious.

Interpretation Tesamorelin might be beneficial in people with HIV and NAFLD. Further studies are needed to determine the long-term effects of tesamorelin on liver histology.

Funding National Institutes of Health and National Institute of Allergy and Infectious Diseases.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined by excess storage of triglyceride in hepatocytes (steatosis) and is often accompanied by inflammation, cellular damage, and fibrosis. The development of ballooning hepatocellular injury shows progression to non-alcoholic steatohepatitis (NASH). NASH can progress to cirrhosis and is an increasingly important cause of end-stage liver disease in the general population. NAFLD can be more prevalent in people with HIV than in the general population, and studies suggest that progression of fibrosis is more likely in people with HIV.[1–3] Unlike many HIV-associated comorbidities that worsen with increased severity of HIV disease, NAFLD can occur more commonly in those with well treated HIV and higher CD4 T-cell counts and weight gain, often in association with central adiposity.[4–6] In people with HIV, weight gain, abdominal fat accumulation, and increases in visceral fat are common and seen even with newer antiretrovirals.[7] There are no proven therapies for NAFLD in people with HIV, nor is it known how strategies to reduce liver fat would affect progression of histological changes over time, and thus alter the natural history among people with HIV.

Growth hormone secretory dynamics are perturbed with reduced pulsatile growth hormone among people with HIV. The degree of perturbation closely parallels abdominal fat accumulation and weight gain.[8–10] Tesamorelin is a growth hormone-releasing hormone (GHRH) analogue that restores endogenous pulsatile growth hormone secretion and reduces visceral fat in individuals with HIV infection.[11–13] Tesamorelin is thought to stimulate lipolysis via increased endogenous growth hormone while maintaining feedback inhibition and limiting toxicity compared with growth hormone per se.[13] We previously showed that tesamorelin reduces liver fat content in a preliminary study of people with HIV chosen for abdominal obesity.[14] This current study was designed to substantially extend these data and address an important question for people with HIV. Our study assessed for the

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Research in context

Evidence before this study

We searched PubMed for literature published on or before July 15, 2019, without language restrictions using the terms "NAFLD and HIV" and "tesamorelin and HIV". Non-alcoholic fatty liver disease (NAFLD) is highly prevalent in people with HIV and can have a more aggressive natural history in this group of people than in those without HIV infection. No pharmacotherapies are currently approved for NAFLD in people with HIV. Growth hormone increases lipolysis and suppresses de novo lipogenesis in the liver. Tesamorelin, a growth hormone-releasing hormone analogue, increases endogenous pulsatile growth hormone production and reduces visceral fat in people with HIV. A 6-month study showed that tesamorelin reduces liver fat content in people with HIV and abdominal adiposity, but its effects on liver fat content and liver histopathology in people with HIV, chosen based on strictly defined NAFLD, are unknown.

Added value of this study

This study shows that tesamorelin reduces liver fat content in men and women with HIV and NAFLD. Furthermore, tesamorelin substantially attenuated the high rate of fibrosis progression that was seen in the placebo-treated group and was well tolerated. Tesamorelin improved systemic inflammation as measured by C-reactive protein, and reductions in liver fat with tesamorelin were associated with improvements in fibrosis.

Implications of all the available evidence

In individuals with HIV and NAFLD, tesamorelin decreases liver fat content and reduces progression of fibrosis. These data have numerous implications for research and clinical practice. This study suggests that a strategy mechanistically targeting liver fat reduction might provide a key long-term clinical benefit in terms of preventing fibrosis progression in HIV. Tesamorelin might be an important new strategy for people with HIV and NAFLD, simultaneously reducing liver and visceral fat, and improving important inflammatory indices in this population. Future studies are needed to further define the effects of tesamorelin on the histopathological features of steatohepatitis.

first time a therapeutic strategy specifically for people with HIV and NAFLD; and simultaneously assessed changes in liver histopathology, inflammatory, and metabolic indices, including specific indices of insulin sensitivity by euglycaemic hyperinsulinaemic clamp.

Methods

Study design and participants

We did a randomised, double-blind, multicentre trial at the Massachusetts General Hospital, Boston, MA, USA, and the National Institutes of Health, Bethesda, MD, USA. Participants were eligible for the study if they were between 18 and 70 years of age, had confirmed HIV infection, and hepatic steatosis as shown by a hepatic fat fraction (HFF) of 5% or more on proton magnetic resonance spectroscopy (MRS). Participants with heavy alcohol use (>20 g daily for women or >30 g daily for men) were excluded, as were participants with hepatitis B, active hepatitis C, α1 antitrypsin deficiency, Wilson's disease, haemochromatosis, or autoimmune hepatitis. Participants with a history of hepatitis C were required to have completed treatment more than 1 year before study entry and to have cure verified with hepatitis C virus with no viral load. Participants with known cirrhosis, stage 4 fibrosis on biopsy, or other severe chronic illness were also excluded. Participants with diabetes were eligible as long as their glycated haemoglobin (HbA_{1c}) was 7% or less, their antidiabetic drugs were stable for 6 months or more, and they were not using insulin or thiazoledinediones. Participants were also required to have a stable antiviral regimen for 3 months or more, stable use of any antihypertensives or lipid-lowering medications for 3 months or more, and, if applicable, stable use of vitamin E for 6 months or more before study entry. Participants using chronic systemic corticosteroids, methotrexate, amiodarone, tamoxifen, or growth hormone were excluded, as were participants with any active malignancy. Women aged 50 years or older were required to have a negative mammogram within 1 year of the baseline visit, and men with a history of prostate cancer were excluded. Participants with a history of hypopituitarism or other conditions known to affect the growth hormone axis were also not eligible. Other exclusionary criteria for safety reasons were haemoglobin less than 11 g/dL, CD4 count less than 100 cells per μL, HIV viral load more than 400 copies per mL, and prostate specific antigen more than 5 ng/mL. All participants provided written informed consent. The study was approved by the institutional review boards at the Massachusetts General Hospital and the National Institutes of Health.

Randomisation and masking

Participants were randomised (1:1) to receive tesamorelin 2 mg daily or placebo daily. The randomisation list was prepared by the study statistician using a permuted block algorithm within each stratum with randomly varying block sizes. Tesamorelin and placebo vials looked identical, and the randomization list was provided by the statistician only to pharmacy personnel. Both tesamorelin and placebo (mannitol) were provided in identical vials of lyophilised power, which patients reconstituted with sterile water before injection. Randomisation was stratified by site and vitamin E use, defined as consistent use of 400 international units or more daily. Potentially eligible participants at both sites

were identified through referrals from local physicians and local outreach, including advertisements on research websites. Eligible participants were enrolled by a co-investigator assigning a sequential randomisation number, which corresponded to a treatment assignment known only to the statistician and pharmacy staff. Thus all study staff remained blinded to all treatment assignments until the final participant had completed the 12 months of randomised, placebo-controlled treatment.

Procedures

Tesamorelin was administered at the US Food and Drug Administration (FDA)-approved dose of 2 mg subcutaneously daily for 12 months, followed by a 6-month open-label phase during which all participants received tesamorelin 2 mg daily. Participants were trained in reconstitution and self-injection at the baseline visit and administered the injections at home, returning used vials to assess compliance. Insulin-like growth factor 1 (IGF-1) Z scores were monitored throughout the study by an independent endocrinologist at the Massachusetts General Hospital otherwise unaffiliated with the study. A prespecified threshold of IGF-1 Z score of 3 or more was included in the protocol as a trigger to decrease the tesamorelin dose to 1 mg, along with a dummy dose reduction in the placebo group, but this was not required for any participant.

All participants received nutritional counselling from clinical research nutritionists at baseline, 6 months, and 12 months. Visits were done in the fasting state. The screening visit included history and physical examination, laboratory investigations for eligibility, MRS, and MRI, with the latter used to assess cross-sectional area of visceral adipose tissue (VAT) at the level of the fourth lumbar vertebra. The MRI and MRS, HbA_{1c}, CD4 cell count, and HIV viral load from the screening visit were used as the baseline measures. The baseline assessment included: an ultrasound-guided percutaneous liver biopsy; whole body dual energy x-ray absorptiometry (DXA); fasting assessment of liver function tests, lipids, serum inflammatory markers, and IGF-1; and bionutrition assessment including 4-day dietary record, Modifiable Activity Questionnaire, and anthropometric measures done in triplicate. These assessments, including a liver biopsy, were repeated at 12 months along with repeat MRI and MRS, HbA_{1c}, and immunological parameters. An interim MRI and MRS was done at the 6-month visit.

Histological scoring was done by a blinded central pathologist (DEK) for all liver biopsy samples using the Nonalcoholic Steatohepatitis Clinical Research Network (NAS CRN) scoring system.[15] The sum of grades for steatosis (grades 0–3), hepatocellular ballooning (0–2), and lobular inflammation (0–3) comprise the NAS score, and fibrosis is independently staged between 0 and 4.[15] Any presence of features of steatohepatitis, including borderline disease, was determined by histological review. Progression of fibrosis was considered any increase in fibrosis stage between baseline and 12 months. This included advancement from stage 1a to 1b. Cross-sectional MRI of the abdomen at the fourth lumbar vertebra was done using a T2-weighted half-Fourier-acquired single-shot turbo spin echo pulse sequence, with images read centrally by a blinded radiologist (MT). Visceral and subcutaneous adipose tissue areas were calculated using semi-automated pixel thresholding to separate each compartment and provide measures in cm^2.

Participants at Massachusetts General Hospital had a euglycaemic hyperinsulinaemic clamp (DeFronzo method) procedure to assess insulin sensitivity. After a 14 h overnight fast, a low-dose (insulin 20 mU/m^2 per min) clamp for 2 h was followed by a high-dose (insulin 80 mU/m^2 per min) clamp for 2 h.[16] Insulin stimulated glucose disposal was calculated during the last 20 min

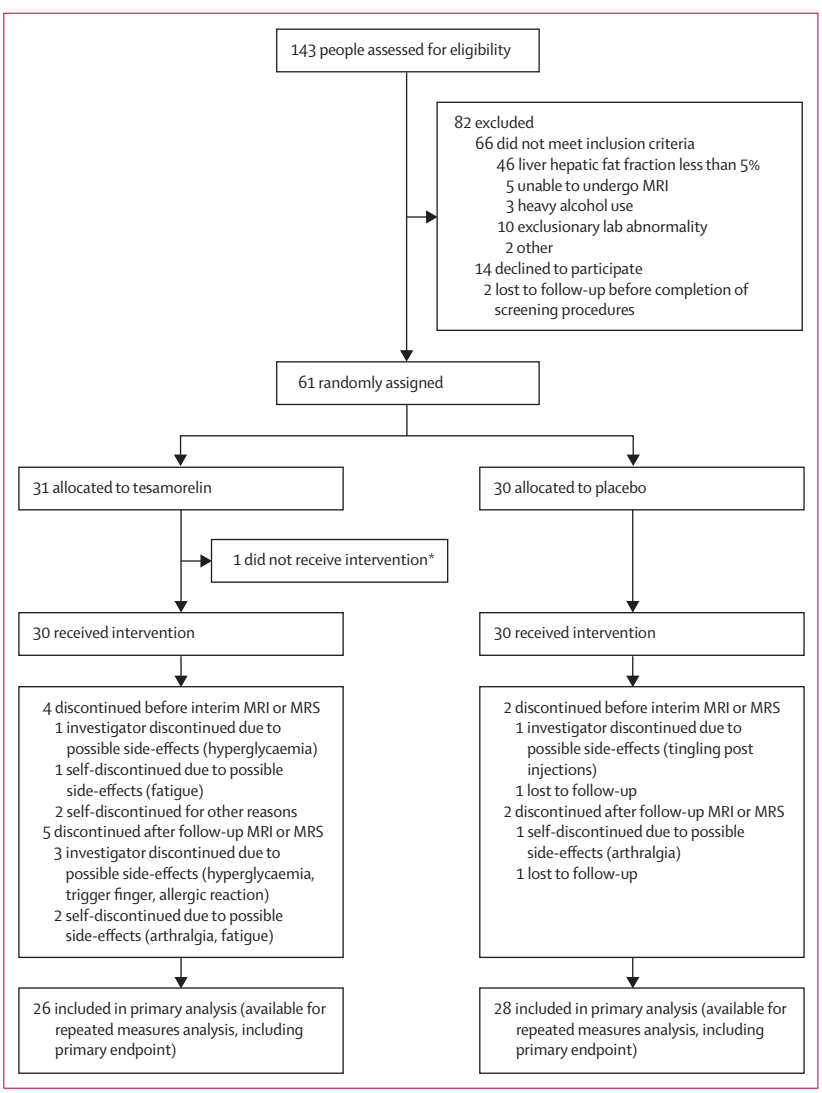

Figure 1: **Trial profile**
MRS=magnetic resonance spectroscopy. *Withdrew consent at baseline visit.

	Tesamorelin (n=31)	Placebo (n=30)
Sex		
Male	24 (77·4%)	24 (80·0%)
Female	7 (22·6%)	6 (20·0%)
Age (years)	52 (8)	54 (7)
Race		
White	21 (67·7%)	19 (63·3%)
Black	8 (25·8%)	10 (33·3%)
Other	2 (6·5%)	1 (3·3%)
Ethnicity		
Hispanic	6 (19·4%)	3 (10·0%)
Non-hispanic	25 (80·7%)	27 (90·0%)
Smoking status		
Never	13 (41·9%)	11 (36·7%)
Previously	14 (45·2%)	12 (40·0%)
Currently	4 (12·9%)	7 (23·3%)
Alcohol use (drinks per week)	0·3 (1·3)	0·9 (2·0)
Duration of HIV infection (years)	16 (9)	18 (8)
Current antiretroviral use		
NRTI	27 (87·1%)	29 (96·7%)
PI	9 (29·0%)	6 (20·0%)
NNRTI	12 (38·7%)	11 (36·7%)
Integrase inhibitor	21 (67·7%)	18 (60·0%)
Entry inhibitor	1 (3·2%)	0 (0%)
Type 2 diabetes		
Known diabetes	4 (12·9%)	4 (13·3%)
Current use of antidiabetics	3 (9·7%)	3 (10·0%)
Current use of metformin	3 (9·7%)	2 (6·7%)
No known diabetes	27 (87·1%)	26 (86·7%)
Current lipid-lowering medications		
Any current lipid-lowering medication use	13 (41·9%)	15 (50·0%)
Current statin use	10 (32·3%)	14 (46·7%)
No current lipid-lowering medication use	18 (58·1%)	15 (50·0%)
Current vitamin E use*		
Yes	2 (6·5%)	1 (3·3%)
No	29 (93·6%)	29 (96·7%)
Hepatic fat fraction	12·9% (7·7)	14·7% (9·0)
NASH†		
Histological NASH	10 (34·5%)	9 (31·0%)
No histological NASH	19 (65·5%)	20 (69·0%)
Fibrosis†		
Stage 0	15 (51·7%)	18 (62·1%)
Any fibrosis	14 (48·3%)	11 (37·9%)
Stage 1	4 (13·8%)	5 (17·2%)
Stage 2	6 (20·7%)	4 (13·8%)
Stage 3	4 (13·8%)	2 (6·9%)

Data are n (%) or mean (SD), unless otherwise stated. NASH=non-alcoholic steatohepatitis. NNRTI=non-nucleoside reverse transcriptase inhibitor. NRTI=nucleoside reverse transcriptase inhibitor. PI=protease inhibitor. *Vitamin E use defined as regular use of 400 international units or more daily. †Liver biopsy data were not available for two participants on tesamorelin one participant on placebo.

Table 1: **Baseline demographics and clinical characteristics**

using the DeFronzo method: low-dose clamp was used as the primary index to determine hepatic insulin sensitivity and a high-dose clamp to determine whole-body insulin sensitivity.

Laboratory analyses were done using standard methods. Clinical analyses were measured at the National Institutes of Health clinical laboratory, the Massachusetts General Hospital clinical laboratory, LabCorp, and Quest Laboratories. IGF-1 was measured centrally at Quest Laboratories. C-reactive protein (CRP) was measured using electrochemiluminescence (Meso Scale Discovery, Rockville, MD, USA), and adiponectin was measured using ELISA (R&D Systems, Minneapolis, MN, USA).

Outcomes

The prespecified primary endpoint was change in HFF between baseline and 12 months. HFF was measured with MRS in the morning after an 8-h fast. Fat fraction was calculated as the area under the spectroscopic lipid peak divided by the total area under the water and lipid peaks. Image acquisition followed a standard protocol at both centres, and liver fat content was quantified by blinded radiologists. The diagnostic accuracy of MRS for liver steatosis has an area under the receiver operating characteristic curve of 0·94 (95% CI 0·88–1·0) compared with assessment of liver biopsy by an experienced pathologist.[17]

Prespecified secondary endpoints were as follows: histological assessment of hepatic fibrosis using fibrosis stage; histological assessment of inflammation and cellular ballooning using the NAS score; alanine aminotransferase (ALT) and γ-glutamyl transferase concentrations; visceral fat area as measured with an MRI scan; total body fat and lean mass measured with DXA, and waist circumference by anthropometry; fasting lipids; CRP; and adiponectin. Prespecified endpoints related to safety included measures of glucose homoeostasis (fasting glucose, HbA_{1c}, and, at Massachusetts General Hospital, insulin-stimulated glucose uptake), as well as CD4 and CD8 cell counts and HIV viral load.

Statistical analysis

A sample size of 60 was chosen based on 80% power to detect a treatment difference of 0·85 or more SD change in HFF over 12 months, assuming a discontinuation rate of 25% (ie, 45 evaluable patients, at a two-sided α of 0·05).

For all endpoints, an intention-to-treat analysis was done using all available data; participants without any follow-up data for an endpoint were not included in the analysis of that endpoint. Per the prespecified analysis plan, change in HFF was assessed by random intercept mixed effects modelling for continuous repeated measures using restricted maximum likelihood to assess the effect estimate for the time×randomisation interaction. All available repeated measures data were used in the analysis, which was based on intention to

	Baseline		Change at 12 months		Treatment effect (95% CI)*	p value*
	Tesamorelin (n=31)	Placebo (n=30)	Tesamorelin (n=21)	Placebo (n=26)		
Primary liver endpoints						
Hepatic fat fraction (absolute change)	12·9% (7·7)	14·7% (9·0)	−4·7% (6·6)	−0·0% (4·1)	−4·1% (−7·6 to −0·7)	0·018
Hepatic fat fraction (relative change)	..	..	−32% (54)	5% (42)	−37% (−67 to −7)	0·016
Hepatic fat fraction at 12 months <5%	..	..	35%	4%	31% (8·5–53%)	0·0069
Secondary liver endpoints						
ALT (U/L)	33 (25)	26 (18)	−2 (11)	5 (15)	−7 (−15 to 1)	0·088
GGT (U/L)	55 (54)	65 (76)	−12 (31)	7 (46)	−19 (−43 to 4)	0·099
Adipose tissue (secondary endpoints)						
VAT (cm^2)	232 (91)	250 (104)	−21 (77)	14 (40)	−35 (−66 to −4)	0·026
SAT (cm^2)	290 (164)	333 (156)	24 (72)	12 (46)	11 (−19 to 41)	0·46
BMI (kg/m^2)	30·1 (6·0)	32·9 (6·2)	0·9 (2·3)	0·4 (1·1)	0·3 (−0·3 to 0·9)	0·37
Waist (cm)	107 (15)	114 (12)	0 (9)	1 (3)	−1 (−5 to 3)	0·59
Total body fat (kg)	30·3 (10·5)	34·4 (12·0)	0·5 (4·6)	1·2 (4·0)	−0·7 (−3·3 to 2·0)	0·61
Total lean body mass (kg)†	57·2 (10·2)	63·8 (10·6)	1·9 (3·7)	−0·0 (2·6)	1·9 (−0·0 to 3·8)	0·051
Metabolic indices (secondary endpoints)						
IGF-1 (ng/mL)	132 (43)	115 (43)	116 (84)	−1 (39)	117 (76 to 157)	<0·0001
Triglycerides (mg/dL)	151 (84)	128 (46)	19 (52)	−4 (48)	23 (−7 to 53)	0·12
HDL cholesterol (mg/dL)	47 (13)	45 (11)	2 (6)	−1 (6)	3 (−1 to 6)	0·17
LDL cholesterol (mg/dL)	113 (36)	102 (25)	−1 (28)	4 (27)	−5 (−21 to 11)	0·54
CRP (mg/L)	7·8 (9·9)	4·2 (3·5)	−3·3 (9·2)	1·4 (4·2)	−4·7 (−9·2 to −0·2)	0·04
Adiponectin (ng/mL)	2042 (1450)	1638 (875)	−118 (706)	−340 (798)	222 (−225 to 669)	0·32
Glucose homoeostasis and immunological parameters (safety endpoints)						
Fasting glucose (mg/dL)	96 (20)	97 (16)	7 (13)	4 (13)	4 (−5 to 13)	0·40
HbA_{1c}	5·7% (0·5)	5·8% (0·5)	0·2% (0·6)	0·1% (0·4)	0·2% (−0·1 to 0·5)	0·29
CD4 count (cells per µL)	733 (290)	798 (260)	−11 (141)	−28 (118)	17 (−60 to 95)	0·65
CD8 count (cells per µL)	865 (380)	967 (374)	−68 (165)	−49 (154)	−18 (−113 to 77)	0·70
HIV viral load (log_{10} copies per mL)	0·34 (0·59)	0·50 (0·74)	0·16 (0·72)	−0·02 (0·75)	0·18 (−0·25 to 0·62)	0·41

Data are n (%) or mean (SD). ALT=alanine aminotransferase. BMI=body-mass index. CRP=C-reactive protein. GGT=γ-glutamyl transferase. HbA_{1c}=haemoglobin A_{1c}. IGF-1=insulin-like growth factor 1. SAT=subcutaneous adipose tissue. VAT=visceral adipose tissue. *For continuous variables, treatment effect and p values are from repeated measures analysis for primary and secondary endpoints with interim measures (hepatic fat fraction absolute change, ALT, VAT, SAT, BMI, and glucose) and from between-group comparison of change at 12 months by Student's *t* test for other variables; for resolution of non-alcoholic fatty liver disease, p value by Pearson χ^2 test. †Tesamorelin and placebo group different at baseline.

Table 2: **Effects of tesamorelin on hepatic fat, metabolic, and immunological indices**

treat, including available interim data. In the model for the primary endpoint, residual plots appeared to have a normal distribution with homogeneity across groups, suggesting no violations of assumptions of linearity, normality, or homogeneity of variance. The same analysis was used for other endpoints measured at multiple timepoints during the double-blind period, including VAT, subcutaneous adipose tissue (SAT), ALT, body-mass index (BMI), and glucose. HFF, VAT, SAT, and ALT were available at baseline, 6 months, and 12 months. BMI and glucose were available at every visit. For secondary endpoints measured at only baseline and 12 months, a paired *t* test was done. Between-group comparisons at baseline were assessed using Student's *t* test for continuous variables and Pearson's χ^2 test for categorical variables. Pearson's correlation coefficient was used to assess relationships between continuous variables. Two data points, one baseline ALT value and one baseline CRP value, were excluded because they were more than 5 SDs above the sample mean. A two-sided α of 0·05 was the predefined threshold for statistical significance. Study data were collected and managed using Research Electronic Data Capture tools hosted at Partners HealthCare (Nashville, TN, USA). Sensitivity analyses were done to address missing data, which were assumed to be missing at random. Data augmentation for missing observations was done using the multiple imputation procedure (PROC MI) in SAS software (version 9.4). Multiple imputation was done for 100 iterations, discarding the first ten iterations. All data analysis was overseen by the study statistician (HL). Statistical analyses were done in SAS software (version 9.4) or in JMP software (version 12.0). This study is registered on ClinicalTrials.gov, number NCT02196831.

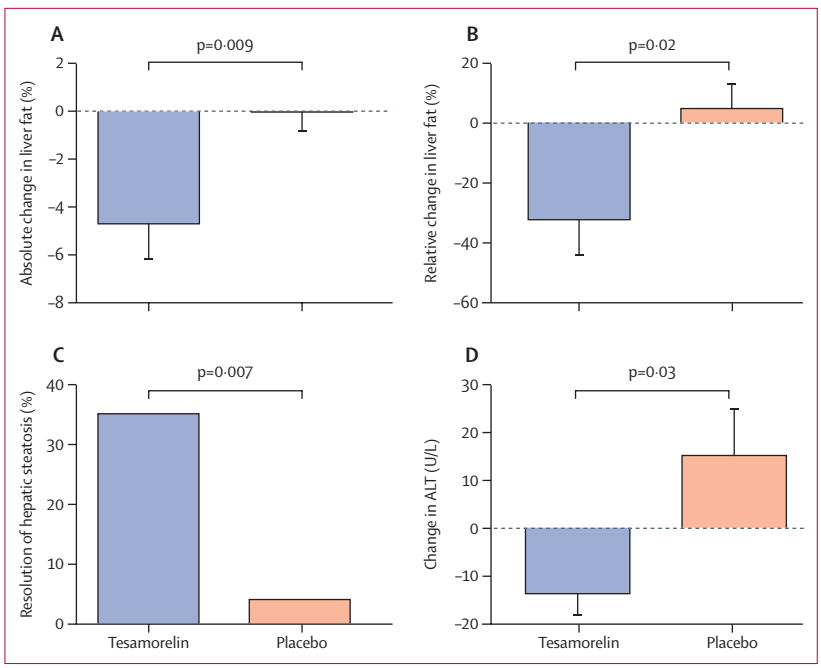

Figure 2: **Change in HFF, ALT, and resolution of steatosis between baseline and 12 months**
Change in (A) absolute and (B) relative HFF between baseline and 12 months. Data are mean (SE). (C) Percent resolution of steatosis, defined as 12-month hepatic fat fraction less than 5%, with p value for the Pearson χ^2 test. (D) Change in ALT for those with ALT greater than 30 U/L at baseline, with p value for *t* test comparing change between groups. Data are mean (SE). ALT=alanine aminotransferase. HFF=hepatic fat fraction.

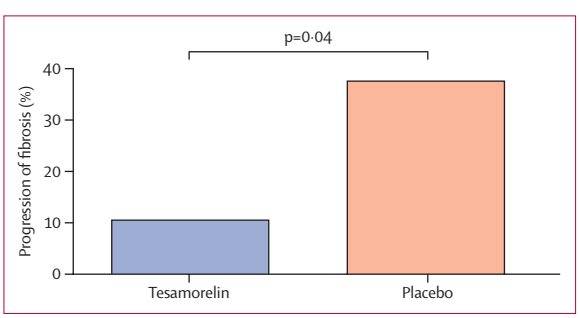

Figure 3: **Proportion of patients with any progression of fibrosis at 12 months**
p value is for the Pearson χ^2 test.

Role of the funding source

The National Institute of Allergy and Infectious Diseases, Division of AIDS, received monthly reports on study progress and safety and provided study monitoring. Additionally, the National Institute of Allergy and Infectious Diseases Division of Clinical Research data and safety monitoring board (DSMB) and Safety Office did a DSMB review every 6 months. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

See Online for appendix

Results

Of 143 participants screened, 61 were enrolled between Aug 20, 2015, and Jan 16, 2019 (figure 1). After one patient discontinued at the conclusion of the baseline visit, institutional review board permission was obtained to enroll another participant, resulting in 61 participants (figure 1).

15 participants were recruited at the National Institutes of Health and 46 at Massachusetts General Hospital. Four participants in the tesamorelin group and two in the placebo group discontinued before any follow-up imaging (figure 1). Five participants in the tesamorelin group and two in the placebo group discontinued after obtaining follow-up imaging that was used in the primary analysis. The overall discontinuation rate was not significantly different between the two groups (p=0·12).

Clinical characteristics (table 1) and measures of body composition and metabolism (table 2) were similar between groups at baseline. Mean HFF among the entire cohort was 13·8% (SD 8·4), with a range of 5·0% to 45·4%. At baseline, 33% of the cohort had a histological diagnosis of NASH. 43% of the cohort had fibrosis stage 1 or higher; per protocol, none had stage 4 fibrosis (cirrhosis) at baseline. These rates were similar in the treatment groups (table 1). Antiretroviral therapy (ART) regimen was similar between groups (table 1).

The mean adherence to daily injections by count of returned empty vials was similar between treatment groups: 87% (SD 16) for placebo and 80% (15) for tesamorelin (p=0·11). IGF-1 concentrations increased in the tesamorelin group (effect size 117 ng/mL, 95% CI 76–157, p<0·0001; table 2). No participants had IGF-1 Z scores over the prespecified dose-reduction threshold, but one participant received an institutional review board-approved dose reduction to 1 mg for symptoms potentially related to growth hormone. This individual self-discontinued from the study soon after the dose reduction.

Tesamorelin significantly reduced HFF compared with placebo (effect size –4·1%, 95% CI –7·6 to –0·7, p=0·018). There was no change in effect size or p value when adjusting independently for baseline measures of race, antiretroviral use, statin use, or smoking. The change between baseline and 12 months corresponded to a –37% (95% CI –67 to –7) relative change in HFF (figure 2). In the tesamorelin group, 35% of individuals had a reduction in HFF to less than 5%, whereas this occurred in 4% of individuals in the placebo group (p=0·0069; figure 2). Changes in HFF for each individual by group are shown in the appendix (p 2). Among individuals given tesamorelin, there was no significant relationship between change in IGF-1 over 12 months and change in HFF (r=0·10, p=0·67).

Tesamorelin prevented the progression of fibrosis during the treatment period, with two (10·5%) individuals showing progression in the tesamorelin group versus nine (37·5%) individuals in the placebo group (p=0·044; figure 3). However, tesamorelin did not improve existing fibrosis: among those with fibrosis stage 1 or higher at baseline, fibrosis improved in two individuals in the tesamorelin group and three individuals in the placebo

group (p=0·71). Changes in fibrosis during the study were positively associated with changes in NAS score (p=0·0003 for ANOVA). When compared with placebo, tesamorelin did not significantly change NAS score (effect size –0·3, 95% CI –1·0 to 0·5), lobular inflammation score (–0·3, –0·7 to 0·2), or hepatocellular ballooning (–0·1, –0·4 to 0·2). In exploratory analyses, the higher the baseline NAS score, more change was seen among the tesamorelin-treated individuals (r=–0·48, p=0·040), whereas a similar relationship was not observed in the placebo group (r=–0·14, p=0·52).

Compared with placebo, tesamorelin did not significantly reduce ALT or γ-glutamyl transferase over the treatment period, although both effect sizes suggest a modest reduction (table 2). Restricting the cohort to those with elevated ALT (>30 U/L) at baseline, tesamorelin did significantly decrease ALT after 12 months (effect size –29 U/L, 95% CI –55 to –3; figure 2) compared with placebo. Tesamorelin reduced CRP (effect size –4·7 mg/L, 95% CI –9·2 to –0·2; table 2) compared with placebo but did not have any effect on adiponectin.

In our study we did not see any effect of tesamorelin on LDL cholesterol, HDL cholesterol, or triglycerides (table 2). Tesamorelin did not significantly affect fasting glucose or HbA_{1c} during the treatment period (table 2). Fasting glucose at each study visit for each group is shown in the appendix (p 3). In the subset of patients at Massachusetts General Hospital who underwent euglycaemic hyperinsulinaemic clamp, tesamorelin did not affect the insulin-stimulated glucose uptake (M) during the low-dose clamp (treatment effect for low-dose M 0·0 mg/kg per min, 95% CI –1·1 to 1·1, p>0·99) nor the insulin stimulated glucose uptake during the high-dose clamp (treatment effect for high-dose M –0·9 mg/kg per min, 95% CI –2·4 to 0·7, p=0·27).

Tesamorelin significantly reduced VAT area compared with placebo, with no effect on SAT area (table 2). Tesamorelin modestly increased lean body mass by DXA, with no significant effect on total body fat mass (table 2). BMI and waist circumference did not change.

There were no significant changes in baseline and 12-month estimates of daily caloric and macronutrient intake by 4-day food record, self-reported alcoholic drinks per week, and hours of activity per week as assessed by the Modifiable Activity Questionnaire (appendix p 1).

Sensitivity analyses for the primary endpoint were done using multiple imputation for missing data. These data confirmed the primary results, with an estimated effect size of –3·8% (95% coverage interval –5·5 to –2·2) reduction in HFF.

A small number of serious adverse events were seen, which did not differ by treatment group, and were judged as unrelated to study drug (table 3). In the placebo group, two individuals were hospitalised with serious adverse events: one following a suicide attempt, and one following a haematoma after a liver biopsy. In the tesamorelin group, four individuals were hospitalised with serious adverse events: one with transient hemiplegia for which a cause was not found, one due to suicidal ideation, one for urosepsis, and one for pneumonia and separately for influenza.

	Tesamorelin (n=31)	Placebo (n=30)	p value*
Any adverse event	29	29	0·57
Serious adverse event	4	2	0·41
Event meeting criteria for discontinuation by investigator	4	1	0·17
Hyperglycaemia	12	11	..
Arthralgia	3	3	..
Myalgia	2	0	..
Paraesthesia	2	2	..
Injection site bruising	11	11	..
Injection site erythema	3	0	..
Injection site stinging	4	1	..
Other injection site complaints	10	1	..
Upper respiratory infection	5	5	..
Other infection	7	12	..
Other	25	24	..

The study was not powered to detect differences in adverse events, and p values are shown only for aggregate events. *p value for comparison of numbers of events by group by the Pearson χ^2 test.

Table 3: **Adverse events**

One individual in the placebo group and four in the tesamorelin group had events that met a-priori protocol criteria for investigator discontinuation (p=0·17), including two discontinuations for hyperglycaemia (2-week and 6-month visits) in the tesamorelin group. One had known diabetes at baseline. Other reasons for discontinuation are outlined in figure 1.

Discussion

Our data show that tesamorelin reduces liver fat content in people with HIV and NAFLD and further suggest it prevents a high rate of progression of fibrosis, and improves inflammatory indices, such as CRP. In HIV-positive individuals recruited for NAFLD, we saw a broad range of baseline NAS scores, and patients given tesamorelin with higher NAS scores at baseline had greater reductions in NAS score with tesamorelin. Additionally, in individuals with elevated ALT at baseline, ALT decreased with tesamorelin. Importantly, tesamorelin did not worsen insulin sensitivity. Collectively, these data suggest a significant benefit of tesamorelin among people with HIV and NAFLD.

In the larger context of comorbidities not related to AIDS, chronic liver disease causes substantial morbidity and mortality in people with HIV.[18] Although estimates of prevalence vary from 13% to 60%, NAFLD is likely to be present in 25% or more of people with HIV, with an increased prevalence in those with visceral or generalised obesity.[6,19,20] NAFLD is expected to soon

become the leading cause of liver-related morbidity and mortality in people with HIV.[21] In the general population, lifestyle modification leads to reduction in liver fat content and amelioration of steatohepatitis and is currently the cornerstone of therapy for NAFLD.[22] However, weight loss of up to 5–10% might be needed in this regard, and data from the general population might not be generalisable to the HIV population, in whom ectopic adipose tissue, dysfunctional subcutaneous adipose tissue, and excessive immune activation are all seen.[22,23] At present, there are no widely accepted pharmacological strategies to treat NAFLD and NASH. Vitamin E reduces ALT and improves histological features of inflammation, and some organisations recommend it for non-diabetic adults with NASH in the general population, but there is concern from meta-analyses that long-term use could modestly increase all-cause mortality.[22,24] Pioglitazone, a peroxisome proliferator-activated receptor γ agonist, also improves features of steatohepatitis, but use is typically accompanied by weight gain.[24] Obeticholic acid is a farnesoid X receptor agonist that improved steatohepatitis in earlier trials but also increased LDL cholesterol and decreased HDL cholesterol.[25] A phase 3 study's results are consistent with earlier trials, both in terms of improvement in steatohepatitis and increase in LDL cholesterol.[26] Other drugs with varying mechanisms of action are being investigated, but none have thus far been approved. Moreover, with few exceptions, these studies have been done in the general population, and data for the HIV population are insufficient.

Considering our results in the context of existing and emerging treatment strategies, tesamorelin is the only drug thus far to show efficacy in reducing liver fat and preventing fibrosis progression specifically in HIV. To our knowledge, the only other pharmacological strategy that has been specifically tested for NAFLD in people with HIV is the bile acid conjugate aramchol, which did not reduce liver fat in 50 people with HIV and lipodystrophy that were given the drug for 12 weeks.[27] Tesamorelin is FDA approved to reduce visceral fat in people with HIV with central adiposity. Our results now suggest that it might be beneficial among the larger group of people with HIV and NAFLD.

The mechanisms whereby liver steatosis in NAFLD progresses to fibrosis and steatohepatitis are not known, nor is the natural history of these changes well defined in longitudinal studies among people with HIV. The data from our study show for the first time that a strategy aimed mechanistically at reducing liver fat has a significant effect to prevent changes in liver histopathology in people with HIV. Tesamorelin can reduce liver fat via multiple mechanisms. One of the most important physiological effects of growth hormone is to increase the activity of hormone-sensitive lipase, an important lipolytic enzyme present in adipose tissue, liver, muscle, and other tissues. With the data available, it is unclear if increased lipolysis in the liver, adipose tissue, or both are responsible for net reductions in liver fat, and the tissue-specific mechanisms of tesamorelin require further study.

Fibrosis stage is the strongest predictor of mortality in patients with NAFLD, and FDA guidance on treatments for NAFLD stresses the importance not only of liver fat reduction, but also of prevention of fibrosis and reduction of inflammation.[28] We did liver biopsies before and after treatment and they showed that tesamorelin prevented progression of fibrosis. Moreover, reductions in liver fat were associated with reductions in fibrosis in a population with strictly defined NAFLD. Such an effect might prevent the development of cirrhosis in people with HIV. Future studies are needed to further explore clinical outcomes in the context of liver fat reduction and progression of fibrosis in this population. Our data suggest a large percentage (38%) of HIV patients with NAFLD in the placebo group show progression of fibrosis over 1 year. This is an important finding and highlights the crucial need for a therapy to prevent fibrosis progression in this population.

In this study, two individuals in the tesamorelin group, one with baseline diabetes, required discontinuation for worsening hyperglycaemia. However, among the entire cohort, tesamorelin was neutral to glucose homoeostasis over the 12-month study period, and clinically relevant hyperglycaemia events were well balanced between the placebo and tesamorelin groups. These results are consistent with our previous studies, which show a modest worsening of insulin resistance in the first few weeks to months of tesamorelin treatment, followed by a return to baseline over longer-term therapy.[14,29] In previous studies aggregating phase 3 clinical trial data, the degree of VAT reduction was associated with lowering of HbA_{1c},[29] suggesting that ongoing reduction in ectopic fat, as well as increasing lean body mass, can ultimately counterbalance initial increases in glucose. Nonetheless, caution should be used in patients with NAFLD and significant baseline dysglycaemia, and a minority of patients might not be able to continue tesamorelin due to hyperglycaemia. Clinically, tesamorelin has been safely used in the population with HIV for almost a decade, but patients with NAFLD can have more severe glucose derangements, and assessment of glucose early after intervention in such patients is prudent. Importantly, we did not test whether simultaneous antidiabetic strategies to lower glucose and insulin might be useful to allow the small subset experiencing dysglycaemia to continue with tesamorelin and maintain normoglycaemia. Future studies should address this issue, because of the potential value of reducing fibrosis progression in these patients.

Our study was randomised, placebo-controlled, and included gold standard imaging before and after biopsy sampling, as well the performance of euglycaemic

hyperinsulinaemic clamp. We recruited for NAFLD as prespecified in the study protocol, and the study was powered a priori to detect changes in liver fat. By contrast, the study was not designed as a therapeutic trial for NASH, not all patients had steatohepatitis, and there was a range of baseline NAS scores.

Therefore, we could not definitively assess effects on endpoints recommended by the US FDA as appropriate for late-stage studies of NASH therapeutics, namely improvements in histological evidence of fibrosis, or inflammation, or both. Further studies specifically recruiting for NASH are now indicated. Our data demonstrate an effect of tesamorelin to prevent progression of fibrosis among people with HIV and NAFLD, with strong relationships between reductions in liver fat and fibrosis. Importantly, this suggests that the strategy of tesamorelin, aimed mechanistically at reducing liver fat, might be useful to prevent liver fibrosis in people with HIV. Studies of individuals achieving visceral fat reduction with tesamorelin show that visceral fat reaccumulates after discontinuation of treatment,[30] and studies investigating the use of tesamorelin in NAFLD and NASH will need to investigate whether effects are durable beyond the treatment period.

In conclusion, our data show that tesamorelin robustly decreases liver fat, while preventing fibrosis progression, in association with improvement in indices of liver inflammation among people with HIV and NAFLD. The treatment is generally well tolerated, and future studies are needed to further define the clinical role of tesamorelin with respect to NAFLD in people with HIV, and potentially other populations.

Contributors

TLS, HL, CMH, and SKG were responsible for study conception and design. TLS, LTF, JP, IZ, CSP, JA, AK, MNF, CMH, SKG, and KB were responsible for study conduct and data collection. HL, TLS, LTF, SKG, and CMH were responsible for data analysis. TLS, LTF, MNF, JP, IZ, CSP, JA, CB, AK, KB, C-YL, HL, KEC, RTC, DEK, MT, CMH, SKG, and AT were responsible for approval of the final manuscript. CB, AT, MT, and C-YL were responsible for the MRI and MRS at Massachusetts General Hospital and assistance in its interpretation. KEC and RTC were responsible for assistance in interpretation of measures of NAFLD and NASH. HL was responsible for oversight of all statistical analyses and manuscript preparation. MT was the central reader for abdominal fat. DEK was responsible for histopathological assessment of all liver biopsy samples. TLS, LTF, CMH, and SKG were responsible for manuscript authorship.

Declaration of interests

TLS received funding from Novo Nordisk for an investigator-initiated grant unrelated to the current project. AK was an employee of the National Institutes of Health at the time of article submission. KC received grant funding from BMS and Boehringer Ingelheim and fees for consulting from Gilead and Novo Nordisk unrelated to the current project. RC received funding to the institution from Gilead, Merck, BMS, Janssen, Boehringer, and Roche unrelated to the current project. DEK and CMH are employees of the National Institutes of Health. SKG has served on a scientific advisory board for Theratechnologies, is a consultant to Theratechnologies, and has received research support for an investigator-initiated project unrelated to this project from Theratechnologies. SKG is the named inventor on a patent application on the effects of tesamorelin in the treatment of hepatic disease. All other authors declare no competing interests.

Data sharing

Research data, with all patient identifiers removed, will be available as per National Institutes of Health policy to other researchers through request to the principal investigator (SKG).

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