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### Authors

Ajmera, Veeral H  
Cachay, Edward  
Ramers, Christian  
[et al.](#)

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## Novel MRI assessment of treatment response in HIV-associated NAFLD: a randomized trial of an SCD1 inhibitor (ARRIVE Trial)

Veeral H. Ajmera<sup>1,2</sup>, Edward Cachay<sup>3</sup>, Christian Ramers<sup>4</sup>, Irine Vodkin<sup>2</sup>, Shirin Bassirian<sup>1</sup>, Seema Singh<sup>1</sup>, Neeraj Mangla<sup>1</sup>, Richele Bettencourt<sup>1</sup>, Jeannette L Aldous<sup>5</sup>, Daniel Park<sup>5</sup>, Daniel Lee<sup>3</sup>, Jennifer Blanchard<sup>3</sup>, Adrija Mamidipalli<sup>6</sup>, Andrew Boehringer<sup>6</sup>, Saima Aslam<sup>7</sup>, Olof Dahlqvist Leinhard<sup>8,9</sup>, Lisa Richards<sup>1</sup>, Claude Sirlin<sup>6</sup>, Rohit Loomba<sup>1,2,10</sup>

<sup>1</sup>NAFLD Research Center, Department of Medicine, La Jolla, California

<sup>2</sup>Division of Gastroenterology, Department of Medicine, La Jolla, California

<sup>3</sup>Division of Infectious Diseases, Owen Clinic, University of California San Diego, San Diego, California

<sup>4</sup>Family Health Center, San Diego, California

<sup>5</sup>San Ysidro Health, San Diego, California

<sup>6</sup>Liver Imaging Group, University of California, San Diego, La Jolla, California

<sup>7</sup>Division of Infectious Diseases and Global Public Health, University of California, San Diego, San Diego California

<sup>8</sup>AMRA Medical AB, Linkoping Sweden

Please address correspondence to: Rohit Loomba, MD, MHSc, ACTRI Building, 1W202, 9452 Medical Center Drive, La Jolla, CA 92037, Ph: 858-246-2201, Fax: 858-246-2255, roloomba@ucsd.edu, Web: <http://fattyLiver.ucsd.edu>.

### AUTHOR CONTRIBUTIONS

Veeral Ajmera: analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, approved final submission.

Edward Cachay: patient recruitment, critical revision of the manuscript, approved final submission

Christian Ramers: patient recruitment, critical revision of the manuscript, approved final submission

Irine Vodkin: patient recruitment, critical revision of the manuscript, approved final submission

Shirin Bassirian: data collection, critical revision of the manuscript, approved final submission.

Seema Singh: data collection, critical revision of the manuscript, approved final submission

Neeraj Mangla: critical revision of the manuscript, approved final submission.

Richele Bettencourt: data analysis, critical revision of the manuscript, approved final submission

Jeannette L Aldous: patient recruitment, critical revision of the manuscript, approved final submission

Daniel Park: patient recruitment, critical revision of the manuscript, approved final submission

Daniel Lee: patient recruitment, critical revision of the manuscript, approved final submission

Jennifer Blanchard: patient recruitment, critical revision of the manuscript, approved final submission

Adrija Mamidipalli: data analysis, critical revision of the manuscript, approved final submission

Andrew Boehringer: data analysis, critical revision of the manuscript, approved final submission

Saima Aslam: patient recruitment, critical revision of the manuscript, approved final submission

Olof Leinhard: data analysis, critical revision of the manuscript, approved final submission

Lisa Richards: patient visits, critical revision of the manuscript, approved final submission

Claude Sirlin: data analysis, critical revision of the manuscript, approved final submission

Rohit Loomba: study concept and design, analysis and interpretation of data, drafting of the manuscript, critical revision of the

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<sup>9</sup>Department of Medical and Health Sciences, Linköping University, Linköping, Sweden

<sup>10</sup>Division of Epidemiology, Department of Family and Preventive Medicine, University of California at San Diego, La Jolla, California

## Abstract

Aramchol, an oral stearoyl-coenzyme-A-desaturase-1 (SCD1) inhibitor, has been shown to reduce hepatic-fat content in patients with primary nonalcoholic-fatty-liver-disease (NAFLD), however, its effect in patients with HIV-associated NAFLD is unknown. The ARRIVE trial was a double-blind, randomized, investigator-initiated, placebo-controlled trial to test the efficacy of 12 weeks of treatment with aramchol versus placebo in HIV-associated NAFLD. Fifty patients with HIV-associated NAFLD, defined by MRI-proton-density-fat-fraction (PDFF)  $\geq 5\%$ , were randomized to receive either aramchol 600 mg daily (n=25) or placebo (n=25) for 12 weeks. The primary endpoint was a change in hepatic-fat as measured by MRI-PDFF in co-localized regions-of-interest. Secondary endpoints included changes in liver-stiffness using MR-elastography (MRE) and vibration-controlled-transient-elastography (VCTE) and exploratory endpoints included changes in total body fat and muscle depots on DXA, whole-body and cardiac MRI. The mean ( $\pm$ SD) of age and BMI were  $48.2\pm 10.3$  years and  $30.7\pm 4.6\text{kg/m}^2$  respectively. There was no difference in the reduction in mean MRI-PDFF between the aramchol group at  $-1.3\%$  (baseline-MRI-PDFF:15.6% vs end-of-treatment MRI-PDFF:14.4%,  $p=0.24$ ) versus placebo at  $-1.4\%$  (baseline-MRI-PDFF:13.3% vs end-of-treatment MRI-PDFF:11.9%,  $p=0.26$ ), respectively. There was no difference in the relative decline in mean MRI-PDFF between aramchol group and placebo (6.8% versus 1.1%,  $p=0.68$ ). There were no differences in MRE and VCTE derived liver-stiffness, and whole body (fat and muscle) composition analysis by MRI or DXA. Compared to baseline, end-of-treatment aminotransferases were lower in the aramchol group but not in the placebo arm. There were no significant adverse events.

**Conclusion:** Aramchol, over a 12-week period, did not reduce hepatic-fat or change body fat and muscle composition by utilizing novel MRI-based assessment in patients with HIV-associated NAFLD. ([clinicaltrials.gov ID:NCT02684591](https://clinicaltrials.gov/ct2/show/study/NCT02684591))

## Keywords

NASH; steatosis; MRI-PDFF

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent etiologies of chronic liver disease worldwide and an increasingly common cause of cirrhosis and hepatocellular carcinoma (1, 2). NAFLD can be broadly classified into primary NAFLD that is typically seen in the presence of metabolic syndrome and secondary NAFLD that may be associated with other causes such as medications inducing steatosis, viral hepatitis, inborn errors of metabolism, or human immunodeficiency virus (HIV) infection.

Among patients with HIV infection, liver disease is one of the leading causes of death (3). NAFLD affects 20–40% of people with HIV(4) and is associated with increased histologic severity compared to HIV negative controls (5). While many aspects of the pathogenesis of

HIV-associated NAFLD likely overlap with primary NAFLD, unique factors may also exist in patients with HIV-associated NAFLD. Increased disease severity can be seen at lower BMI and may be related to a greater amount of visceral adipose tissue (6), antiviral therapy (7) direct viral effects and increased permeability of the gut epithelium (8). Despite the ongoing development of therapeutic treatments for NAFLD (9–11) patients with HIV-associated NAFLD are largely excluded from those trials and currently have no approved options available for treatment.

Aramchol, also known as arachidyl amido cholanoic acid, is a fatty acid and a bile acid conjugate (FABAC) that was created by conjugating 2 natural components, cholic acid and arachidic acid, through a stable amide bond. Aramchol inhibits stearoyl-coenzyme A desaturase 1 (SCD1), a key enzyme in fatty acid synthesis. SCD1 is an endoplasmic reticulum enzyme that catalyzes the rate-limiting step in the biosynthesis of monounsaturated fatty acids from saturated fatty acids. Inhibiting SCD1 decreases synthesis and increases beta-oxidation of fatty acids, causing decreased storage of fatty acids. In 2003, Gilat and colleagues showed that (12) aramchol significantly reduced hepatic fat content in animals (rats, hamsters, and mice) with a high-fat diet model. In 2014, Safadi and colleagues (13) demonstrated that aramchol significantly reduced hepatic fat content, measured by magnetic resonance spectroscopy (MRS), in a randomized trial of 60 Israeli NAFLD patients (without HIV) after 12 weeks of 300mg aramchol orally daily. A Phase I study of aramchol, found a good safety profile with dosing up to 900 mg daily and an international, multicenter trial is currently being conducted in patients with biopsy-proven NASH, however, aramchol has not yet been studied in HIV-associated NAFLD.

Thus, we hypothesized that aramchol 600 mg orally daily would be superior to placebo in improving hepatic fat on MR imaging in patients with HIV-associated NAFLD. Utilizing a double-blind randomized controlled trial of patients with HIV-associated NAFLD, we tested the efficacy of aramchol versus placebo on improvement in MRI proton density fat fraction (MRI-PDFF) over 12 weeks. Secondary aims were to assess the effect of aramchol on serum aminotransferases and liver stiffness and exploratory aims included longitudinal assessment of body composition using advanced MRI and dual energy X-ray absorptiometry (DXA).

## MATERIAL AND METHODS

### Study Design and Population

This ARRIVE trial was a double-blind, randomized, investigator-initiated, placebo-controlled trial to test the efficacy of 12 weeks of treatment with 600 mg of oral aramchol daily versus placebo in the treatment of HIV-associated NAFLD. The study was designed and conducted according to CONSORT Guidelines and registered at [clinicaltrials.gov](https://clinicaltrials.gov) (registration number [NCT02684591](https://clinicaltrials.gov/ct2/show/study/NCT02684591)) (CONSORT Checklist: Supplemental Materials). Patients were enrolled between March 1, 2016 and January 1, 2018 and the study was conducted at the University of California at San Diego (UCSD) NAFLD Research Center (14–18). The ARRIVE trial patient population was derived from primary care clinics, subspecialty clinics including HIV-treatment and liver disease clinics and through institutional review board approved advertisements. The protocol was Health Insurance Portability and Accountability Act (HIPAA) compliant and was approved by the UCSD

Institutional Review Board. Informed written consent was obtained from each participant before study enrollment.

### **Inclusion and Exclusion Criteria**

Inclusion Criteria: All patients were aged 18 years or older with a history of stable HIV defined by an unchanged antiretroviral therapy (ART) regimen for at least 12 weeks prior to study screening and had the presence of NAFLD defined by  $\geq 5\%$  steatosis by MRI-PDFF. Patients also had to have at least one or more of the following risk factors for more severe fatty liver disease; hypertriglyceridemia ( $>150$  mg/dL), dyslipidemia (low density lipoprotein (LDL)  $> 160$  mg/dL or high density lipoprotein (HDL)  $< 40$  mg/dL), serum ALT above the upper limit of normal ( $>19$  U/L for women and  $>30$  U/L for men), BMI  $>25$  kg/m<sup>2</sup>, hyperuricemia, prediabetes or diabetes defined by American Diabetes Association criteria.

Subjects were excluded if they had evidence of other forms of liver disease including the presence of serum hepatitis B surface antigen, hepatitis C viral RNA, positive autoimmune serologies with biopsy consistent with autoimmune hepatitis, cholestatic liver disease, hemochromatosis by 3+ or 4+ stainable iron on biopsy and homozygosity/heterozygosity on genetic analysis, low ceruloplasmin levels with biopsy suggestive of Wilson's disease, or low alpha-1-antitrypsin levels with biopsy suggestive of alpha-1-antitrypsin disease. Further exclusion criteria included alcohol intake of more than 30 g/day in the previous 10 years or greater than 10 g/day in the previous year, evidence of cirrhosis based on clinical assessment or imaging, active illicit drug use, pregnancy, evidence of hepatocellular carcinoma, ingestion of drugs known to cause hepatic steatosis, ingestion of drugs known to improve NASH such as vitamin E or pioglitazone, or inability to undergo MRI. Patients on medications with known drug-drug interactions with aramchol or hypersensitivity to aramchol or associated medications were excluded. Patients with significant other systemic illness or poorly controlled diabetes defined by a hemoglobin A1c  $> 9\%$  were excluded.

### **Baseline Assessments**

Patients were screened in the UCSF NAFLD research center clinic with history, physical examination, review of outside medical records (including HIV status) and routine blood tests. Alcohol history was assessed with the AUDIT and Skinner lifetime alcohol consumption inventories. All patients were asked to stop any medications being used for their liver disease, including herbal medications. Only those meeting all inclusion criteria and avoiding all exclusion criteria were invited to participate in the study. Those who met all eligibility criteria and had no exclusion criteria underwent more thorough evaluation with laboratory testing including homeostatic model assessment for insulin resistance (HOMA-IR), adipose insulin resistance (Adipo-IR), liver MRI, cardiac MRI, vibration controlled transient elastography (VCTE) with controlled attenuation parameter (CAP), DXA for full body fat assessment, a commercial MRI protocol for body composition analysis (19) and an oral glucose tolerance test (OGTT). CD4 cell count and HIV viral loads were measured at baseline and lipodystrophy was assessed by physical examination for facial, temporal, upper or lower extremity lipodystrophy and visceral or dorsocervical fat accumulation.

### Definition of HIV Associated NAFLD

Subjects were defined to have HIV-associated NAFLD based on MRI-PDFF > 5% after exclusion of other causes of liver disease, excessive alcohol use or use of medications that cause hepatic steatosis (see exclusion criteria above for details).

### Randomization and Allocation Concealment

Subjects were randomized to either an aramchol or a placebo group in blocks of four in a 1:1 ratio by the investigational drug services at the University of California at San Diego using computer-generated numbers. Patients were randomized to receive 600 mg daily of aramchol (including 200 mg tablet and 400 mg tablet) versus *identical* placebo orally for a total of 12 weeks. Medication diaries and a count of residual tablets monitored patient compliance at scheduled visits. Independent investigational drug services pharmacists dispensed either active or placebo treatment pills, which were identical in appearance. Pills were prepackaged in identical bottles, labeled according to the computer-generated randomization numbers, and delivered to the research clinic. The allocation sequence was concealed from the research coordinators and all investigators including hepatologists and radiologists. Radiology investigators were blinded to clinical data. Treatment allocation was unblinded only after the completion of all study procedures in the entire study including all post-treatment MRI studies on all patients. Data analysis were performed by an experienced statistician using a pre-specified analysis plan.

### Study Visits

Patients returned to the research clinic at weeks 4, 8, and 12 after randomization. At these clinic visits, routine blood tests were obtained, body weight and vital signs were recorded, and the number of pills was counted to document compliance. A physical exam and careful history of liver-related symptoms as well as possible side effects of aramchol were also obtained at each visit. At the completion of 12 weeks of therapy, patients underwent MRI-PDFF, MRE, cardiac MRI, VCTE with CAP, OGTT, DXA and MRI for body compositional analysis and repeat blood work.

### Primary and Secondary Endpoints

The primary endpoint was a change in liver fat as measured by MRI-PDFF in co-localized regions of interest (ROI) within each of the nine liver segments as performed previously.(20–22) Secondary endpoints included change in aminotransferases and liver stiffness and exploratory endpoints included body compositional analysis.

### Advanced Liver MRI Phenotyping

MRI was performed at the UCSD MR3T Research Laboratory using the 3T research scanner (GE Signa EXCITE HDxt; GE Healthcare, Waukesha, WI) with all participants in the supine position. MRI-PDFF was used to measure hepatic steatosis and MRE was used to measure hepatic stiffness. The details of the MRI protocol have been previously described.(15, 23)

**MRI-PDFF for Fat Quantification:** MRI-PDFF utilizes a low-flip angle, gradient echo sequence to acquire multiple echoes in which fat and water protons are in and out of phase.

A custom algorithm created by the Liver Imaging Group at UCSD was used to create MR imaging-PDFF maps. MRI-PDFF measurements are reliable and independent of field strength, scanner manufacturer, and patient characteristics including BMI, age and sex.(23–25) Co-localized ROIs were used to assess changes in liver fat over time with one co-localized ROI placed in each of nine liver segments, which allows for increased efficiency and higher precision and accuracy in assessing changes over time.(14, 20, 21)

**MR Elastography:** MRE is the most accurate biomarker for the quantitative assessment of liver stiffness as a surrogate for hepatic fibrosis.(18, 26, 27) A passive driver was fitted around the body over the liver and connected to an acoustic active driver that delivered continuous vibrations at 60 Hz to produce shear waves in the liver, which were processed to generate elastograms depicting liver stiffness. Four slices were assessed, and co-localized ROIs were manually specified.

### Ultrasound-Based Assessment

Transient elastography was performed by an experienced operator using the FibroScan 502 Touch model (M Probe, XL Probe; Echosens, Paris, France) as previously described.(28) The liver stiffness measurement was obtained after at least a three hour fast and included a minimum of 10 measurements. All patients were initially scanned with the M probe and when indicated by the initial assessment rescanned with the XL probe. An unreliable liver stiffness measurement was defined as a ratio of successful to total acquisitions of < 60% and/or < 10 valid measurements and/or IQR/median > 30%.(29) The CAP value was measured simultaneously and quantifies the attenuation of ultrasound waves in dB/m to provide a measure that correlates with liver steatosis.(30)

### Body Composition Analysis with MRI and DXA

A commercially available sequence to estimate abdominal adipose tissue and thigh muscle volume was implemented and obtained in approximately six minutes by imaging the base of the skull to the knees using MRI without contrast was performed (AMRA Medical AB, Linkoping, Sweden). This accurate and repeatable method(19, 31) measured visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (ASAT), and four thigh muscle volumes. Multiple indexes(32) were calculated as follows: visceral fat ratio =  $VAT / (VAT + ASAT) * 100$ , muscle ratio =  $weight / total\ thigh\ volume$ ; fat storage =  $(VAT + ASAT) / height^2$ ; visceral fat index =  $VAT / height^2$ ; fat ratio =  $(VAT + ASAT) / (VAT + ASAT + total\ thigh\ volume) * 100$ . In addition, full body DXA scan was performed at baseline and week 12. Finally, epicardial fat volume was calculated on cardiac MRI as the region of high signal intensity external to the myocardium to the pericardium.

### Rationale for Total Body MRI

Pharmacologic agents in NAFLD targeting lipid metabolism can have off-target effects, and assessment of body composition including visceral adiposity is of particular importance in patients with HIV who are at risk for lipodystrophy. Frequently, therapeutic trials in NAFLD incorporate advanced liver imaging with MRI.(10, 33) Body composition analysis with MRI allows for accurate and reproducible assessment of extrahepatic muscle and fat in three dimensions and without ionizing radiation.



## Sample Size Assessment and Statistical analysis

Our primary endpoint was the absolute difference in change in MRI-PDFF between placebo and aramchol arms. Based upon previous studies we expected a 5% greater improvement in MRI-PDFF in the aramchol group compared to the placebo arm. This requires a sample size of 22 patients in each arm to have a power 90% (or higher) with an  $\alpha$  of 0.05. These estimates were also based upon our recent trial using MRI-PDFF as an accurate and reproducible marker of hepatic steatosis.(34) Therefore, we planned to randomize 25 patients to each arm to have adequate power to detect a difference in placebo and experimental groups accounting for a 10% drop rate, consistent with prior trials using MRI based endpoints.(14, 20). The chi-squared test was used for comparisons between categorical variables and a paired *t test* was used to compare mean differences between continuous variables in the aramchol versus placebo groups. The primary endpoint of this study was improvement in hepatic steatosis by liver MRI and the difference in improvement between aramchol and placebo was tested using a two-sample t-test.

## RESULTS

Between March 1, 2016 and January 1, 2018, 50 patients with HIV-associated NAFLD were randomized to either aramchol or placebo. Of the 25 patients into each arm, 24 patients in the treatment arm and 22 patients in the placebo arm completed 12 weeks of intervention with pre and post treatment MRI-PDFF (CONSORT Diagram: Supplemental Figure 1). The study population included 92% men and was predominantly non-white (66%) with the majority of patients of Hispanic ethnicity (66%). Baseline characteristics of the two groups are shown in Table 1.

### Primary Endpoint: Effect of Aramchol on Liver Fat as Assessed by MRI-PDFF

Aramchol was not significantly better than placebo at reducing liver fat content as measured by mean  $\pm$  standard deviation (SD) MRI-PDFF change in aramchol and placebo arms  $-1.3\% \pm 5.9$  and  $-1.4\% \pm 5.1$ ,  $p=0.93$  (Table 2) (Supplemental Figure 2). The mean percent change in MRI-PDFF did not differ in the aramchol and placebo groups (Figure 1). Similarly, in sub-analysis restricted to patients with MRI-PDFF  $\geq 8\%$  at baseline (N=38) aramchol was not significantly better than placebo at reducing liver fat content measured by mean  $\pm$  SD MRI-PDFF change ( $-1.66\% \pm 5.5$  and  $-3.01\% \pm 5.3$ ,  $p=0.46$ ). Advanced MRI imaging of representative patients are shown in Figures 2 and 3.

### Effect of Aramchol on Liver Stiffness by MRE and VCTE

The mean  $\pm$  SD liver stiffness by MRE in the aramchol and placebo groups at baseline were similar  $2.6 \pm 0.6$  kPa and  $2.4 \pm 0.6$  kPa respectively. At the end of study the liver stiffness by MRE in the aramchol and placebo groups  $2.5 \pm 0.5$  kPa and  $2.5 \pm 0.6$  kPa respectively, and aramchol was not associated with an improvement in liver stiffness by MRE compared to placebo,  $p=0.45$  (Table 2).

Liver stiffness was also assessed by VCTE and was similar between the aramchol and placebo groups at baseline  $6.7 \pm 2.3$  kPa and  $6.3 \pm 5.2$  kPa respectively. Change in liver



stiffness measurement by VCTE was not significantly different between the aramchol and placebo groups (Table 2).

### **Effect of Aramchol on Anthropometric and Biochemical Measurements**

There were no significant differences in change in BMI, aminotransferases, HOMA-IR or CD4 count between the aramchol and placebo groups. Median total cholesterol increased in the aramchol group (18 mg/dL) compared to a decrease in placebo (-2.0 mg/dL), which was statistically significant,  $p=0.02$ . Within the aramchol group, ALT, AST and AST:ALT ratio improved at end of treatment compared to baseline. Within the placebo group, fasting free fatty acids (FFA) and glucose improved from baseline to end of treatment (Table 3).

### **Change in Body Composition by DXA and Novel Whole-Body MRI Measures Including Visceral Adipose and Epicardial Fat Volume**

DXA assessment of total % body fat were similar at baseline and at the end of study. The mean  $\pm$  SD trunk/limb fat mass ratio was higher in the placebo group than the aramchol group at baseline  $1.9 \pm 0.7$  and  $1.6 \pm 0.5$  respectively,  $p=0.04$  (Table 1). Other measures of the distribution of fat including Android/Gynoid ratio, % Fat Trunk/% Fat legs, did not differ between groups at baseline and the differences in change over the study period between aramchol and placebo were not significant (Table 4a).

Epicardial fat volume in  $\text{mm}^3$  was similar in both groups at baseline and increased in both groups over the study period, however, the difference over the study period between aramchol and placebo was not significant.

Further characterization of body composition using MRI confirmed VAT, ASAT and the ratio of visceral to total adiposity were similar between groups at baseline. Assessment of mean  $\pm$  SD muscle mass with thigh volume was higher in the treatment group than placebo at baseline  $12.8 \pm 3.2$  L and  $11.6 \pm 2.6$  L respectively,  $p=0.02$ . There were no significant differences in changes in fat distribution or muscle indices between the aramchol and placebo groups (Table 4b).

### **Sensitivity Analysis in Patients with Obesity**

Fourteen obese patients in the aramchol group and nine obese placebo patients were assessed in subgroup analysis. ALT was higher in the drug group at baseline 76 IU/L vs 43 IU/L,  $p=0.01$  (Supplemental Table 1a). Mean ALT improved in the aramchol group to 61 IU/L at follow up,  $p=0.01$  (Supplemental Table 1b). The difference in improvement in ALT between the aramchol and placebo groups was not statistically significant,  $p=0.47$ . Changes in MRI-PDFF, MRE and CAP were not different between groups, however, liver stiffness measured by VCTE improved more in the placebo (-1.1 kPa) group than the treatment group (+0.3 kPa),  $p=0.03$ .

### **Adverse Events**

No patients in the treatment arm discontinued treatment due to an adverse event. One patient in the placebo arm discontinued treatment due to an asymptomatic increase in creatinine phosphokinase level. There were no serious adverse events or deaths. All adverse events

were mild, Grade 1, and occurred with similar frequency in the placebo 48% and treatment 40% groups (Supplemental Table 2).

## DISCUSSION

In this randomized, double-blind, placebo controlled trial, aramchol was not superior to placebo at reducing hepatic fat measured by MRI-PDFF, improving aminotransferases or liver stiffness by MRE or VCTE. Treatment with aramchol was associated with lower ALT at end of treatment particularly among obese patients with HIV-associated NAFLD. Aramchol was well tolerated with no significant adverse events and a similar safety profile to placebo.

The major innovation applied in the design of this clinical trial was incorporation of advanced MRI based measures of body composition including total visceral adipose tissue volume, total abdominal subcutaneous adipose tissue volume, thigh muscle volume as well as epicardial fat volume assessment before and after treatment in a NAFLD trial. This trial has **three-fold innovation** in longitudinal body composition analysis in NAFLD. First, this trial utilized MRI-PDFF and MRE, to non-invasively assess longitudinal changes in liver fat content and liver stiffness in co-localized regions. These modalities are the most accurate non-invasive tools for the assessment of liver fat(34) and liver stiffness(35) in primary NAFLD and this study is the first to incorporate them in a trial of HIV-associated NAFLD. Second, this trial evaluated body composition changes with whole-body MRI for the first time in a NAFLD trial. The distribution of obesity, which is not captured by BMI, is associated with cardiovascular risk, metabolic disease activity and the development of NAFLD (36, 37). Clinical trials of NAFLD should consider the potential impact of treatment on other body fat compartments. This is of particular importance in HIV where peripheral fat atrophy and visceral fat accumulation occur at lower BMI and might be independent of ART(38). We utilized multiple modalities to assess fat and muscle compartments throughout the body. Specifically, we utilized the well-established DXA, which is widely available and inexpensive but limited by its two-dimensional assessment of volumes. As more treatment trials of NAFLD incorporate MRI based imaging (39, 40), we successfully incorporated the addition of body composition analysis using an MRI based brief commercial protocol. Third, we assessed epicardial fat volume on magnetic resonance imaging, which may be an independent risk factor for endothelial dysfunction(41) and cardiovascular disease(42), which are the leading cause of death in patients with NAFLD. This trial demonstrates the feasibility of incorporating these measurements into a clinical trial of NAFLD and sets the stage for incorporation of these advanced measures and how to assess them before and after treatment in longitudinal studies in NAFLD.

NAFLD is increasingly common among persons with HIV and may be associated with increased disease severity (5, 43). However, unique factors affecting patients with HIV including changes in the intestinal microbiome (44), and metabolic changes associated with chronic infection and antiretroviral therapy (45) may contribute to a differential response to therapy from patients with primary NAFLD. This is the first clinical trial of pharmacologic intervention for HIV-associated NAFLD. While pre-clinical and clinical studies of aramchol in patients with primary NAFLD demonstrated promising results, including improvement in

hepatic steatosis, this study showed no benefit on hepatic fat fraction by MRI-PDFF at 12 weeks.

While we performed in-depth, longitudinal, non-invasive assessments of liver and body composition we acknowledge certain limitations to this study. The study did not include liver histology, which may have limited the ability to detect an improvement in disease activity, particularly, related to the improvement in ALT seen in the aramchol group. However, currently, early phase studies of NAFLD have utilized highly accurate MRI based imaging to accurately characterize changes in hepatic fat content and liver stiffness. Furthermore, MRI-PDFF may be more accurate than liver histology for detecting quantitative changes in hepatic steatosis and the putative mechanism of aramchol suggested a strong effect on liver fat content, which was recently demonstrated to be associated with histologic disease progression (34, 46). Furthermore, the trial period was short, which may have limited detection of changes in liver stiffness, however, changes in the primary endpoint, hepatic fat content, are dynamic and had been demonstrated in a previous trial of aramchol for the treatment of primary NAFLD (13). Finally, while our sample size estimate was reasonable based on a previous phase 2a study of patients with primary NAFLD(13), our negative results could be secondary to a potential type II error, particularly if the effect size of aramchol is more modest than estimated. Importantly, this work will help inform sample size estimates for future studies in HIV-associated NAFLD.

In conclusion, aramchol was not superior to placebo in reducing liver fat in patients with HIV-associated NAFLD. Aramchol was well tolerated and patients in the treatment group had a significant reduction in ALT. There were no off-target effects of aramchol on body composition measured by advanced, whole-body MRI. This trial also demonstrates that noninvasive assessment of body composition is feasible in NAFLD trials.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations:

<b>SCD1</b>	stearoyl-coenzyme-A-desaturase-1
<b>NAFLD</b>	Non-alcoholic fatty liver disease
<b>HIV</b>	human immunodeficiency virus
<b>MRI-PDFF</b>	magnetic resonance imaging-proton density fat fraction

<b>MRE</b>	magnetic resonance elastography
<b>VCTE</b>	vibration controlled transient elastography
<b>MRI</b>	magnetic resonance imaging
<b>DXA</b>	Dual-Energy X-ray Absorptiometry
<b>MRS</b>	magnetic resonance spectroscopy
<b>NASH</b>	nonalcoholic steatohepatitis
<b>OGTT</b>	oral glucose tolerance test
<b>ROI</b>	region of interest
<b>HOMA-IR</b>	homeostatic model assessment for insulin resistance
<b>ADIPO-IR</b>	adipose insulin resistance
<b>FFA</b>	free fatty acids
<b>BMI</b>	body mass index
<b>UCSD</b>	University of San Diego

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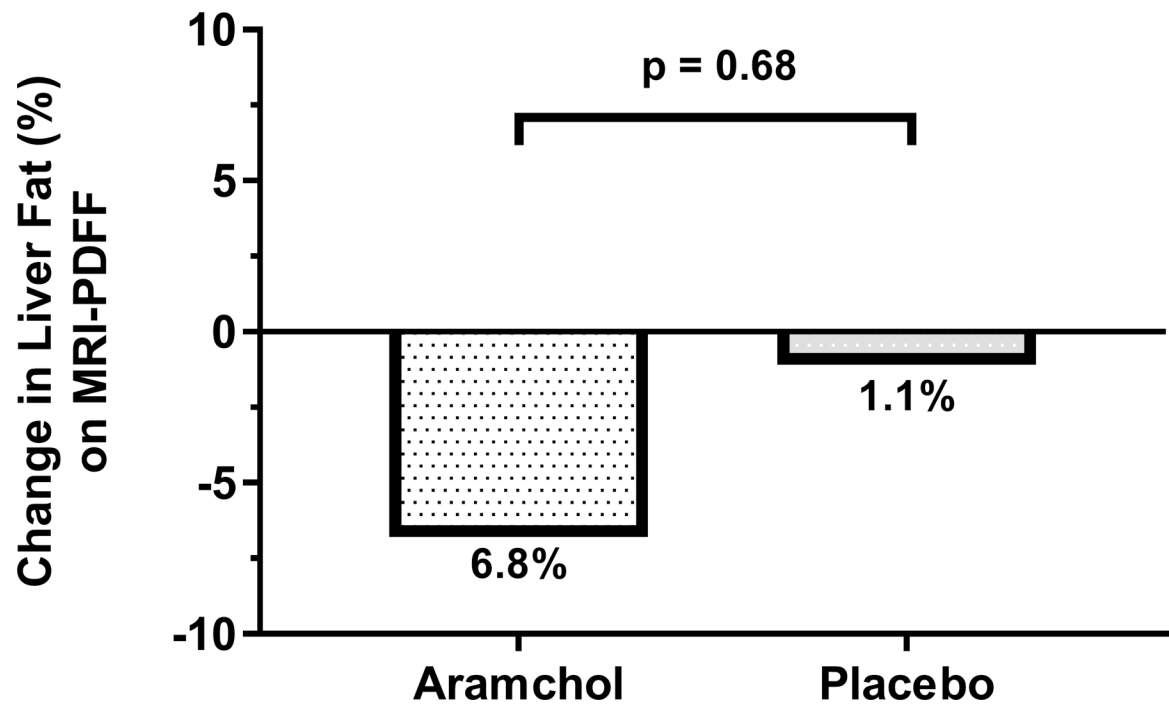
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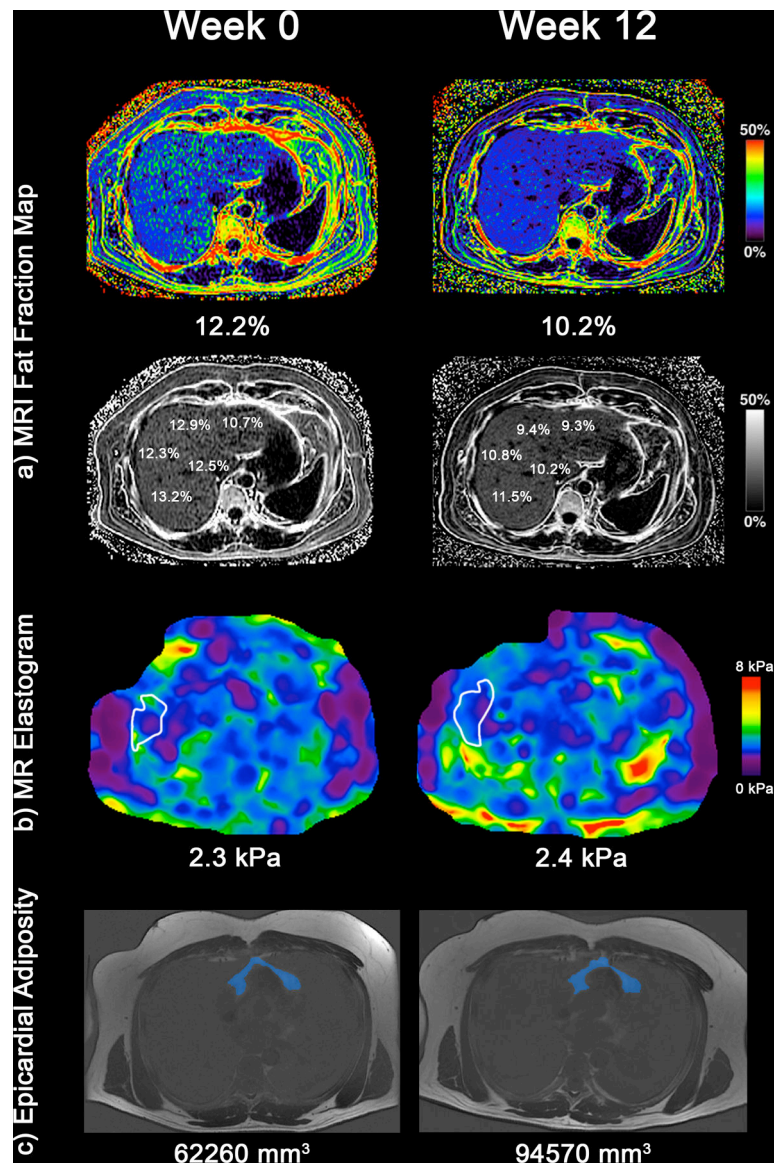
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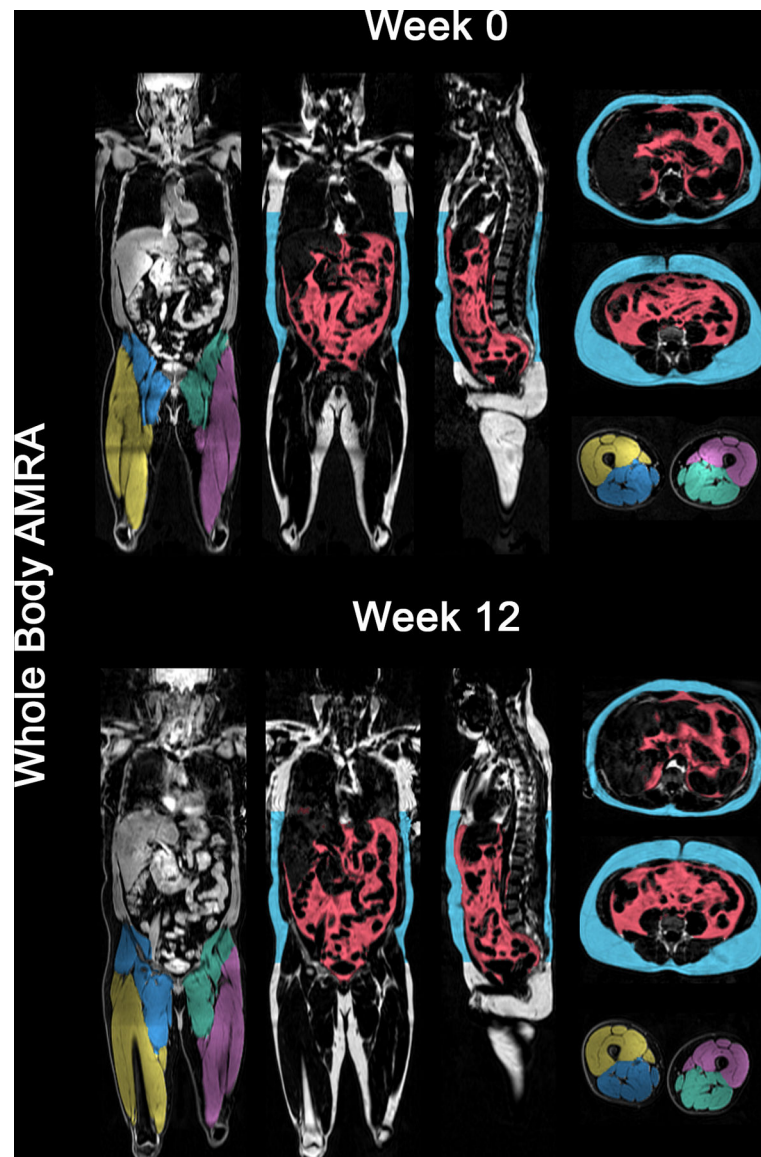


**Figure 1:** Percent mean change in liver fat relative to baseline as assessed by MRI-PDFF by treatment group. The difference between the aramchol group and placebo group was not statistically significant ( $p=0.68$ )



**Figure 2:**

In a representative patient (a) MRI-PDFF fat mapping of the liver. The patient's average liver fat fraction decreased from 12.2% (Week 0) to 10.2% (Week 12) (b) MRE elastograms depicting liver stiffness throughout the entire liver with average liver stiffness increasing from 2.3 kPa to 2.4 kPa (c) Epicardial fat volume on MRI increased from 62,260 mm<sup>3</sup> at week 0 to 94,570 mm<sup>3</sup> at week 12.



**Figure 3:**

Advanced whole-body composition analysis with MRI in a representative patient. Changes from Week 0 to Week 12 in visceral adipose tissue, abdominal subcutaneous adipose tissue, were 4.38 to 4.78 L and 7.67 to 7.88 L respectively. Week 0 to Week 12 thigh muscle volume changes for left posterior, right posterior, left anterior and right anterior were 3.84 to 3.89 L, 3.95 to 3.98 L, 2.23 to 2.32 L and 2.45 to 2.56 L respectively.

**Table 1.**

Baseline Demographic, Biochemical, and Histologic Characteristics of Subjects

	N	Aramchol (n=25)	Placebo (n=25)	P-Value
<b>Demographics</b>				
Age (years)	50	46.6 ± 11.4	49.7 ± 9.0	0.3002
Male patients	50	22 (88%)	24 (96%)	0.6092
White (vs. non-White)	50	11 (44%)	6 (24%)	0.1355
Hispanic (vs. non)	50	14 (56%)	19 (76%)	0.1355
<b>Clinical</b>				
Weight (kg)	50	94.7 ± 16.8	88.9 ± 16.6	0.2196
Height (m)	49	172.4 ± 8.5	171.4 ± 7.0	0.6601
BMI (kg/m <sup>2</sup> )	49	31.4 ± 5.0	30.1 ± 4.2	0.3086
BMI ≥ 30	49	14 (58.3%)	9 (36.0%)	0.1174
Diabetes	50	2 (8%)	8 (32%)	<b>0.0339</b>
<b>Biochemical profile</b>				
ALT (IU/L)	50	58.0 (55.0)	43.0 (26.0)	<b>0.0080</b>
AST (IU/L)	50	42.0 (29.0)	29.0 (23.0)	0.0624
AST:ALT	50	0.6 (0.2)	0.7 (0.2)	<b>0.0397</b>
Alk Phos (U/L)	50	77.0 (32.0)	85.0 (40.0)	0.9536
Total bilirubin (mg/dL)	50	0.6 (0.3)	0.5 (0.3)	0.3222
Direct bilirubin (mg/dL)	47	0.2 (0.0)	0.2 (0.0)	0.2811
Albumin (g/dL)	49	4.5 (0.4)	4.5 (0.3)	0.4240
Protome (s)	50	11.3 (0.9)	11.3 (0.9)	0.6903
Triglycerides (mg/dL)	50	166.0 (67.0)	184.0 (124.0)	0.3986
Total cholesterol (mg/dL)	50	177.0 (58.0)	171.0 (36.0)	0.9690
LDL (mg/dL)	48	109.0 (46.0)	95.0 (31.0)	0.4090
FFA (mmol/L)	49	0.5 (0.3)	0.7 (0.3)	0.3318
Glucose (mg/dL)	50	98.0 (17.0)	108.0 (33.0)	0.2401
Insulin (μU/mL)	50	30.0 (17.0)	23.0 (25.0)	0.9831
HOMA-IR	50	7.2 (4.0)	6.3 (7.3)	0.7859
Hgb A1C (%)	48	5.5 (0.6)	5.6 (0.7)	0.5278
<b>HIV-related</b>				
CD4 count (cells/mm <sup>3</sup> )	48	696.0 (351.0)	680.0 (351.0)	0.9671
Viral load (copies/ml)	46	0 (0)	0 (0)	0.4018
Time since HIV diagnosis (years)	46	14.5 (14)	18.0 (17)	0.4343
<b>Imaging</b>				
MRI PDFF	50	16.2 (10.1)	12.1 (10.3)	0.5094
MRE (kPa)	46	2.5 (0.8)	2.3 (0.7)	0.4221
VCTE LSM (kPa)				

	N	Aramchol (n=25)	Placebo (n=25)	P-Value
Median	49	6.1 (3.0)	5.5 (3.5)	0.4831
IQR	49	1.2 (0.7)	0.7 (1.0)	0.2211
IQR/M	49	15.0 (12.0)	11.0 (8.0)	0.1961
Success rate <60%, n(%)	48	2 (8%)	4 (17.4%)	0.9232
Unreliable liver stiffness, n(%)	50	0	1(4%)	1.0000
Controlled attenuation parameter (dB/m)				
Median	48	334.0 (42.0)	319.0 (105.0)	0.6423
IQR	48	33.0 (21.0)	30.0 (26.0)	0.7883
Probe size, n(%)				0.5712
Medium	23	11 (44%)	12 (52.2%)	
XL	25	14 (56%)	11 (47.8%)	
<b>Adipose Indices</b>				
Total Body % Fat	43	31.7 (9.5)	31.8 (6.8)	0.4952
Fat Mass/Height <sup>2</sup> (kg/m <sup>2</sup> )	43	9.8 (5.6)	9.0 (2.7)	0.1885
Android/Gynoid Ratio	43	1.4 (0.3)	1.4 (0.5)	0.2278
% Fat Trunk/% Fat Legs	43	1.3 (0.3)	1.5 (0.9)	0.0659
Trunk/Limb Fat Mass Ratio	43	1.6 (0.5)	1.9 (0.7)	<b>0.0445</b>
Est. VAT Mass (g)	43	956 (418)	1051 (299)	0.6262
Est. VAT Volume (cm <sup>3</sup> )	43	1033 (452)	1137 (323)	0.6262
Est. VAT Area (cm <sup>2</sup> )	43	198 (87)	218 (62)	0.6348
<b>Lean Indices</b>				
Lean/Height <sup>2</sup> (kg/m <sup>2</sup> )	43	19.7 (3.8)	19.4 (2.4)	0.3805
Appen. Lean/Height <sup>2</sup> (kg/m <sup>2</sup> )	43	8.7 (2.1)	8.1 (1.7)	0.3122
<b>Metabolic Factors, n(%)</b>				
Waist >102cm men, > 88cm women	49	15 (62%)	15 (60%)	0.8575
Triglycerides 150 mg/dL	50	14 (56%)	16 (64%)	0.5637
HDL < 40mg/dL men, < 50 mg/dl women	48	14 (58%)	8 (33%)	0.0822
SBP 130 and/or DBP 85	50	10 (40%)	12 (48%)	0.5688
FPG 100 mg/dL	50	10 (40%)	16 (64%)	0.0894
Metabolic Syndrome	50	12 (48%)	11 (44%)	0.7766
<b>MRI Body Composition Indices</b>				
Pericardial Volume (mm <sup>3</sup> )	32	174700 (117770)	134100 (63059)	0.2582
VAT (l)	44	6.3 (2.5)	6.6 (2.7)	0.8142
ASAT (l)	43	7.9 (4.6)	6.8 (5.2)	0.4963
VAT + ASAT (l)	43	14.3 (4.3)	14.6 (5.1)	0.7246
Visceral fat ratio (%)	43	43.4 (15.6)	46.4 (19.3)	0.5040
Thigh volume (l)	40	12.8 (3.2)	11.6 (2.6)	<b>0.0216</b>
Muscle ratio (kg/l)	40	7.3 (1.0)	7.5 (2.1)	0.3918
Fat storage (l/m <sup>2</sup> )	42	5.0 (1.5)	4.9 (1.5)	0.9699

	N	Aramchol (n=25)	Placebo (n=25)	P-Value
Visceral Fat Index (l/m <sup>2</sup> )	43	2.1 (0.8)	2.3 (0.7)	0.5031
Fat ratio (%)	39	53.3 (8.8)	53.6 (11.2)	0.7890

BMI, Body Mass Index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; Hgb A1C, hemoglobin A1C; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein; FFA, Free Fatty Acids; CRP, C-Reactive Protein; ALK Phos, Alkaline Phosphatase; GGT, Gamma-Glutamyl Transferase; HOMA, homeostatic model assessment; LSM, Liver Stiffness Measurement; VAT, Visceral Adipose Tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; ASAT, abdominal subcutaneous adipose tissue.

Muscle ratio = weight/total thigh volume; Fat storage = (VAT + ASAT)/height<sup>2</sup>; Visceral Fat Index = VAT/height<sup>2</sup>; Fat ratio = (VAT + ASAT) / (VAT + ASAT + total thigh volume) \* 100

Data presented as mean ± sd, median(iqr) or n(%) as appropriate.

T-test performed on continuous variables presented as mean ± sd, Wilcoxon-Mann-Whitney performed on all other continuous/ordinal variables. Chi-square or Fisher's exact test as appropriate on all categorical variables.

P-values in bold denote statistical significance < 0.05.

Aramchol Versus Placebo: Longitudinal Full Liver Fat Mapping Using Magnetic Resonance Imaging Proton Density Fat-Fraction (MRI PDFF)

Table 2.

Liver Segments	Aramchol (n=24)			Placebo (n=22)			Difference	
	Baseline	Posttreatment	P-Value	Baseline	Posttreatment	P-Value	P-Value	P-Value
1	15.4 (6.9)	14.3 (7.9)	0.2989	13.3 (5.8)	11.6 (5.0)	0.1890	0.7589	
2	14.1 (6.3)	13.3 (7.3)	0.4123	11.9 (6.2)	10.2 (5.0)	0.1779	0.5409	
3	14.8 (6.5)	13.7 (7.7)	0.3371	13.2 (6.6)	11.7 (6.1)	0.2744	0.8000	
4a	15.7 (6.8)	14.3 (7.6)	0.1993	13.0 (6.1)	11.5 (5.0)	0.2361	0.9609	
4b	15.8 (6.9)	14.3 (7.8)	0.2118	13.3 (6.4)	11.9 (5.5)	0.3042	0.9486	
5	15.6 (7.1)	14.6 (8.3)	0.3967	13.0 (5.5)	11.9 (6.1)	0.4579	0.9407	
6	15.5 (7.1)	14.3 (8.0)	0.2709	13.5 (6.5)	11.3 (6.3)	0.1236	0.6302	
7	16.5 (7.8)	15.2 (8.5)	0.2360	14.3 (6.3)	13.1 (6.6)	0.4051	0.9281	
8	16.6 (7.7)	15.6 (8.5)	0.3820	14.4 (6.2)	13.3 (6.9)	0.4348	0.9756	
MRI PDFF average	15.6 (6.9)	14.4 (7.8)	0.2400	13.3 (5.9)	11.9 (5.6)	0.2648	0.9308	
MRE average (kPa)	2.6 (0.6)	2.5 (0.5)	0.4777	2.4 (0.5)	2.5 (0.6)	0.7151	0.4459	
VCTE (kPa)								
Median	6.7 (2.3)	7.4 (3.3)	0.2582	6.3 (2.8)	5.2 (1.4)	<b>0.0321</b>	<b>0.0307</b>	
IQR	1.0 (0.5)	1.3 (0.8)	0.1319	0.9 (0.6)	0.8 (0.5)	0.2175	0.0542	
IQR/M	15.6 (8.4)	16.3 (6.2)	0.7102	14.4 (6.8)	13.6 (6.9)	0.7256	0.6068	
Controlled attenuation parameter (dB/m)								
Median	330.3 (41.2)	313.5 (59.4)	0.1912	316.4 (70.9)	312.5 (54.8)	0.8193	0.5287	
IQR	32.8 (14.9)	35.0 (20.5)	0.6577	33.3 (18.8)	35.9 (16.9)	0.6823	0.9615	

Data are expressed as means (sd) or mean difference with p-values in parentheses. Associated p-values are from t test. P-values in bold denote statistical significance < 0.05.

MRI-PDFF, Magnetic Resonance Imaging Proton Density Fat Fraction;

MRI PDFFs measured in all nine liver segments are used to calculate segmental and overall fat fraction averages at baseline and posttreatment between Drug and placebo group.

Only reliable VCTE values used



**Table 3.** Changes in Anthropometric and Biochemical Variable Between the Aramchol- Versus Placebo-Treated Patients

	Aramchol (n=22)			Placebo (n=21)			Difference	
	Baseline	Posttreatment	P-Value	Baseline	Posttreatment	P-Value	P-Value	
Weight (kg)	89.3 (11.9)	90.0 (8.2)	0.6486	87.3 (18.8)	85.4 (20.5)	0.4683	0.3685	
BMI (kg/m <sup>2</sup> )	30.4 (4.3)	30.1 (4.3)	0.2919	29.1 (3.1)	29.3 (4.0)	0.0831	0.0595	
ALT (IU/L)	58.0 (53.0)	51.0 (37.0)	<b>0.0002</b>	43.0 (26.0)	36.0 (44.0)	0.1211	0.1051	
AST (IU/L)	40.5 (29.0)	37.0 (15.0)	<b>0.0189</b>	28.5 (25.0)	25.5 (16.0)	0.1572	0.5180	
AST:ALT	0.6 (0.2)	0.7 (0.3)	<b>0.0002</b>	0.7 (0.2)	0.7 (0.3)	0.4392	0.1625	
Alk Phos (U/L)	77.0 (31.0)	80.5 (32.0)	0.5437	79.5 (42.0)	76.5 (41.0)	0.1478	0.2003	
Total bilirubin (mg/dL)	0.6 (0.3)	0.5 (0.3)	0.4484	0.5 (0.3)	0.4 (0.4)	0.1172	0.5262	
Direct bilirubin (mg/dL)	0.2 (0.0)	0.2 (0.0)	1.0000	0.2 (0.0)	0.2 (0.0)	0.5000	0.3101	
Albumin (g/dL)	4.5 (0.4)	4.5 (0.2)	0.2366	4.5 (0.2)	4.5 (0.3)	0.1928	0.0830	
Protine (s)	11.2 (0.7)	11.3 (0.9)	1.0000	11.4 (0.9)	11.2 (1.1)	0.8138	0.8785	
Triglycerides (mg/dL)	166.0 (79.0)	180.0 (184.0)	0.0797	187.0 (165.0)	159.0 (147.0)	0.2764	0.0907	
Total cholesterol (mg/dL)	177.0 (58.0)	187.0 (181.0)	0.0029	179.0 (54.0)	169.0 (37.0)	0.7683	<b>0.0176</b>	
LDL (mg/dL)	110.0 (45.0)	113.0 (42.0)	<b>0.0450</b>	96.0 (27.0)	102.0 (37.5)	0.8910	0.2352	
FFA (mmol/L)	0.6 (0.2)	0.5 (0.3)	0.4894	0.7 (0.2)	0.5 (0.2)	<b>0.0006</b>	0.0559	
Glucose (mg/dL)	98.0 (17.0)	101.0 (19.0)	0.8017	107.5 (36.0)	98.5 (24.0)	<b>0.0357</b>	0.2218	
Insulin (µU/mL)	29.5 (17.0)	25.5 (19.0)	0.9919	24.5 (25.0)	23.0 (19.0)	0.2798	0.2448	
HOMA-IR	7.2 (4.6)	6.3 (4.7)	0.7756	6.2 (8.2)	5.6 (5.9)	0.1756	0.2177	
Hgb A1C (%)	5.4 (0.4)	5.4 (0.4)	0.7646	5.6 (0.7)	5.6 (0.7)	0.2188	0.1266	
CD4 count (cells/mm <sup>3</sup> )	644.0 (354.0)	640.0 (469.0)	0.5412	680.0 (383.0)	633.0 (371.0)	0.7500	0.4813	

Data are expressed as median (IQR) with p-values from Wilcoxon signed rank test or mean difference with P-value in parentheses. P-values in bold denote statistical significance < 0.05.

BMI, Body Mass Index; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; Hgb A1c, hemoglobin A1c; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein; FFA, Free Fatty Acids; Alk Phos, Alkaline Phosphatase; GGT, Gamma-Glutamyl Transferase; HOMA, homeostatic model assessment; CRP, C-Reactive Protein.

Changes in Adipose and Lean Indices Between the Aramchol- Versus Placebo-Treated Patients on DXA

Table 4a.

	Aramchol (n=20)			Placebo (n=15)			Difference	
	Baseline	Posttreatment	P-Value	Baseline	Posttreatment	P-Value	P-Value	P-Value
<b>Adipose Indices</b>								
Total Body % Fat	31.4 (9.2)	31.8 (9.4)	0.4121	31.8 (7.0)	32.4 (7.7)	0.3029	0.5687	
Fat Mass/Height <sup>2</sup> (kg/m <sup>2</sup> )	9.8 (4.9)	10.1 (4.5)	0.3577	8.4 (3.4)	9.3 (2.7)	0.6097	0.9751	
Android/Gynoid Ratio	1.4 (0.3)	1.3 (0.2)	0.2794	1.4 (0.7)	1.4 (0.8)	0.7609	0.1349	
% Fat Trunk/% Fat Legs	1.3 (0.3)	1.3 (0.2)	0.4918	1.6 (0.9)	1.4 (0.7)	<b>0.0007</b>	0.1948	
Trunk/Limb Fat Mass Ratio	1.6 (0.4)	1.6 (0.4)	0.5706	2.0 (0.8)	1.8 (0.8)	<b>0.0317</b>	0.7873	
Est. VAT Mass (g)	957 (501)	982 (537)	0.8983	971 (408)	996 (543)	0.1070	0.2619	
Est. VAT Volume (cm <sup>3</sup> )	1034 (541)	1061 (583)	0.8983	1050 (441)	1076 (588)	0.1070	0.2637	
Est. VAT Area (cm <sup>2</sup> )	198 (104)	204 (112)	0.8909	201 (85)	207 (112)	0.1037	0.2626	
<b>Lean Indices</b>								
Lean/Height <sup>2</sup> (kg/m <sup>2</sup> )	19.9 (3.3)	19.8 (2.7)	0.9317	19.4 (2.2)	19.0 (3.5)	<b>0.0276</b>	0.2608	
Appen. Lean/Height <sup>2</sup> (kg/m <sup>2</sup> )	8.8 (2.0)	8.7 (1.6)	0.5705	8.1 (1.4)	7.9 (1.9)	<b>0.0145</b>	0.1939	

Data are expressed as median (IQR) with p-values from Wilcoxon signed rank test or mean difference with P-value from t-test in parentheses. P-values in bold denote statistical significance < 0.05.

**Table 4b.** Changes in whole body fat depots and muscle volume by MRI in Aramchol- Versus Placebo-Treated Patients

	Aramchol (n=19)			Placebo (n=20)			Difference	
	Baseline	Posttreatment	P-Value	Baseline	Posttreatment	P-Value	P-Value	P-Value
VAT (l)	6.1 (2.8)	5.6 (3.0)	0.5533	6.7 (2.7)	6.4 (2.3)	0.6280	0.6118	0.6118
ASAT (l)	7.9 (4.7)	8.2 (5.9)	0.9530	8.1 (4.3)	8.1 (3.9)	0.6507	0.4959	0.4959
VAT + ASAT (l)	14.6 (4.3)	14.3 (4.9)	0.9323	14.9 (4.2)	15.3 (4.1)	0.7906	0.4634	0.4634
Visceral fat ratio (%)	42.1 (18.9)	40.9 (13.5)	0.9323	44.1 (15.6)	44.0 (15.5)	0.5678	0.9403	0.9403
Thigh volume (l)	12.6 (1.8)	12.5 (1.5)	0.4037	11.8 (2.9)	11.7 (2.9)	0.8063	0.4447	0.4447
Muscle ratio (kg/l)	7.2 (1.2)	7.1 (1.1)	0.3894	7.7 (2.3)	7.7 (1.9)	0.8176	0.3661	0.3661
Fat storage (l/m <sup>2</sup> )	5.0 (1.3)	5.0 (1.6)	0.8900	5.0 (1.8)	5.0 (1.2)	0.3529	0.8737	0.8737
Visceral Fat Index (l/m <sup>2</sup> )	2.0 (0.7)	1.9 (0.9)	0.6685	2.3 (0.6)	2.1 (0.6)	0.2121	0.7221	0.7221
Fat ratio (%)	53.2 (7.3)	52.9 (8.1)	0.8904	54.4 (11.9)	55.0 (9.5)	0.9661	0.3584	0.3584
Pericardial Volume (mm <sup>3</sup> )	183300 (103100)	202800 (75900)	0.2500	136500 (72019)	171050 (176500)	<b>0.0134</b>	0.1633	0.1633

Data are expressed as median (IQR) with p-values from Wilcoxon signed rank test or mean difference with P-value from t-test in parentheses. N's are "best case". P-values in bold denote statistical significance < 0.05.