Received: 24 April 2021 | Revised: 18 November 2021 | Accepted: 4 December 2021

DOI: 10.1002/hep.32403

ORIGINAL ARTICLE

Randomized placebo-controlled trial of losartan for pediatric NAFLD

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Funding information
NASH CRN, which is supported by the NIDDK (U01DK061713, U01DK061718, U01DK061728, U01DK061731, U01DK061732, U01DK061734,

Abstract

Background and Aims: To date, no pharmacotherapy exists for pediatric NAFLD. Losartan, an angiotensin II receptor blocker, has been proposed as a treatment due to its antifibrotic effects.

Approach and Results: The Nonalcoholic Steatohepatitis Clinical Research Network conducted a multicenter, double-masked, placebo-controlled, randomized clinical trial in children with histologically confirmed NAFLD at 10 sites (September 2018 to April 2020). Inclusion criteria were age 8–17 years,

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; COVID-19, 2019 coronavirus pandemic; CRN, Clinical Research Network; DSMB, Data and Safety Monitoring Board; GGT, gamma-glutamyltransferase; HbA1C, hemoglobin A1C; HOMA-IR, homeostatic model assessment of insulin resistance; NAS, NAFLD activity score; NIDDK, National Institute of Diabetes, Digestive, and Kidney Diseases.

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U01DK061737, U01DK061738, U01DK061730, and U24DK061730); National Center for Advancing Translational Sciences (UL1TR000077, UL1TR000150, UL1TR000424, UL1TR000006, UL1TR000448, UL1TR000040, UL1TR000100, UL1TR000004, UL1TR000423, and UL1TR000454); and the Intramural Research Program of the National Institutes of Health, National Cancer Institute

histologic NAFLD activity score ≥ 3, and serum alanine aminotransferase (ALT) ≥ 50 U/l. Children received 100 mg of losartan or placebo orally once daily for 24 weeks. The primary outcome was change in ALT levels from baseline to 24 weeks, and the preset sample size was n = 110. Treatment effects were assessed using linear regression of change in treatment group adjusted for baseline value. Eighty-three participants (81% male, 80% Hispanic) were randomized to losartan (n = 43) or placebo (n = 40). During an enrollment pause, necessitated by the 2019 coronavirus pandemic, an unplanned interim analysis showed low probability (7%) of significant group difference. The Data and Safety Monitoring Board recommended early study termination.

Baseline characteristics were similar between groups. The 24-week change in ALT did not differ significantly between losartan versus placebo groups (adjusted mean difference: 1.1 U/l; 95% CI = −30.6, 32.7; p = 0.95), although alkaline phosphatase decreased significantly in the losartan group (adjusted mean difference: −23.4 U/l; 95% CI = −41.5, −5.3; p = 0.01). Systolic blood pressure decreased in the losartan group but increased in placebo (adjusted mean difference: −7.5 mm Hg; 95% CI = −12.2, −2.8; p = 0.002). Compliance by pill counts and numbers and types of adverse events did not differ by group.

Conclusions: Losartan did not significantly reduce ALT in children with NAFLD when compared with placebo.

INTRODUCTION

NAFLD is the most common chronic liver disease in children in the developed world. It is commonly diagnosed in the setting of obesity, insulin resistance, and a sedentary lifestyle, and is often considered the liver manifestation of metabolic syndrome.[1] NAFLD can lead to worsening hepatic inflammation and fibrosis during childhood, with progression of disease despite standard-of-care lifestyle counseling regarding healthier dietary intake and exercise.[2,3] Although definitive long-term natural history data for clinical outcomes remain lacking,[1] a heightened risk for future morbidity is likely based on the common occurrence of fibrosis in children,[2,3] the high incidence and prevalence of type 2 diabetes in children with fatty liver,[4,5] and the established natural history data for NAFLD in adults demonstrating a higher risk of type 2 diabetes, cardiovascular disease, and progression to cirrhosis in the setting of fibrosis.[6,7]

The current standard-of-care management approach for children with NAFLD is lifestyle change to achieve healthier metabolic and/or weight status.[1,8] Success of lifestyle changes is limited by the difficulty of improving diet and exercise for many children, and a nonuniform response even when fully implemented. High-dose vitamin E appears to be significantly beneficial in improving histological severity in a subset of patients, but has not been demonstrated to reverse fibrosis in children.[9,10] Thus, treatments that may improve liver injury and insulin resistance seen in pediatric NAFLD are being investigated.

A number of studies suggest the utility of losartan in NAFLD.[11–17] In adults, two meta-analyses have found that angiotensin receptor blockers (ARBs) improve insulin sensitivity and reduce the incidence of type 2 diabetes.[18,19] A large retrospective review of hypertensive patients treated with angiotensin-converting enzyme inhibitors and/or ARBs demonstrated a significant association of renin-angiotensin system (RAS) antagonists with reduced odds of advanced hepatic fibrosis on biopsy.[20] The Fatty Liver Protection Trial by Telmisartan or Losartan Study compared telmisartan to losartan in adult fatty liver patients with type 2 diabetes mellitus, and neither improved alanine aminotransferase (ALT) significantly.[13] However, for this study, there was no placebo group, ALT levels were relatively low at baseline, and a low dose of losartan was used (50 mg once a day). A pilot cross-over study of losartan in 12 normotensive children with NAFLD demonstrated safety and a trend of improvement in ALT, aspartate aminotransferase (AST), and homeostatic model assessment of insulin resistance (HOMA-IR) after 50 mg of daily losartan for 8 weeks versus placebo.[21] A higher proportion of

children achieved reduction in ALT when taking losartan versus placebo (89% vs. 56%) in that study.

The objective of this study was to determine whether a 24-week treatment with losartan improves biomarkers of liver inflammation in children with NAFLD. ALT was selected as the primary outcome, as it is most closely associated with histologic change in fibrosis and in NASH in children with NAFLD.[22,23]

METHODS

Study design

The Losartan for the Treatment of Pediatric NAFLD trial (STOP-NAFLD) was a multicenter, randomized, double-masked, placebo-controlled, parallel treatment group phase 2 trial of 24 weeks of losartan versus placebo in children with biopsy-proven NAFLD. Participants were enrolled at 10 pediatric clinical centers (Appendix S1) from October 2018 through March 2020 as part of the National Institutes of Health–sponsored NASH Clinical Research Network (CRN). The protocol, informed consent and informed assents, and all participant materials were approved by the institutional review boards (IRBs) at each clinical site and the Data Coordinating Center. An independent Data and Safety Monitoring Board (DSMB) was appointed by the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) to monitor the study. All parents or guardians of participants provided written consent, and children provided written assent.

Inclusion criteria were as follows: (1) age 8–17 years at initial screening, (2) histologic evidence of NAFLD with or without fibrosis and a NAFLD activity score (NAS) of ≥3 (without requirement for all three components of the NAS, other than steatosis) on liver biopsy that predated enrollment by no more than 2 years, and (3) serum ALT at screening ≥50 U/l. Key exclusion criteria were (1) weight <70 kg or ≥150 kg at screening (to remain within established pediatric dosing parameters of 0.7–1.4 mg/kg day at the study dose of 100 mg per day); (2) presence of cirrhosis; (3) history of hypotension or stage 2 hypertension (>140 systolic or >90 at screening) or if receiving treatment with any antihypertensive medication; (4) current use of potassium, nonsteroidal anti-inflammatory drugs, or lithium; and (5) ALT ≥ 300 U/l (Appendix S2). There were no exclusions based on sex or race. In the NASH CRN, children with NAFLD are mostly male and Hispanic, reflecting a higher risk of NAFLD among these demographics in childhood in the USA.

Dosing, randomization, and treatment groups

Losartan, an ARB, is approved by the U.S. Food and Drug Administration (FDA) for the treatment of hypertension in children, and dosing has been established in pediatric populations within a range of 0.7–1.4 mg/kg/day, up to maximum dose of 100 mg.[24] In this study, the weight range for eligibility was ≥70 kg to <150 kg at screening to maintain the daily dose of 100 mg within the currently established pediatric dosing range of 0.7–1.4 mg/kg/day. The starting dose was one 50-mg capsule of losartan or matching placebo per day for 1 week, then two capsules of 50 mg of losartan or matching placebo once per day (100 mg total) from weeks 2–24. This titration approach is recommended to minimize any side effects after starting medication.[24] The FDA reviewed the study protocol and recommended this approach and dosing before study initiation.

All children who had had a clinical liver biopsy within the past 2 years were screened for eligibility. Participants meeting eligibility criteria were enrolled by clinic personnel and assigned (1:1) to losartan or placebo using a computer-generated random allocation sequence with permuted blocks, by a centrally administered procedure stratified by clinic. Clinical site investigators, clinical coordinators, staff, and participants were blinded to treatment assignment.

Procedures and follow-up

After screening and randomization, participants returned for study visits at weeks 4, 12, at completion of treatment (24 weeks) and at 36 weeks for posttreatment follow-up. Blood samples were obtained at screening, 4-, 12-, 24-, and 36-week visits for routine biochemical tests to assess renal function and hepatic chemistries. A focused physical exam was performed each visit. This included height, weight, waist and hip circumference measurements, and vital signs. Additional labs at the baseline, 12-, 24-, and 36-week visits included a complete blood count, uric acid, C-reactive protein (CRP), and plasma and serum for banking at a central repository. At 12- and 24- week visits, a fasting lipid profile, glucose, insulin, and hemoglobin A1c (HbA1C) were obtained. A pregnancy test was performed for females of child-bearing age. At each study visit, trained study staff provided standardized evidence-based, written nutrition and exercise recommendations (Appendix S3) to all participants, in accordance with the current standard-of-care lifestyle intervention for pediatric NAFLD.[1,8] Study drug adherence as well as adverse effects were reviewed at weeks 4, 12, and 24.

During randomization, participants and their families were taught how to use an automated blood pressure monitor (Omron 5 Series Upper Arm Blood Pressure Monitor Model BP742N) and were provided with one for home use. Participants were instructed to take their blood pressure each morning for the first 14 days of treatment and to bring the log to their next clinic visit. Participants and parents were instructed to call the

clinic if blood pressure decreased below 90 mm Hg systolic or 60 mm Hg diastolic. During week 2, research staff called participants' families over the phone and reviewed the blood pressure log and asked about any adverse effects their child may have experienced. Blood pressure log was reviewed again at week 4.

At the screening visit, frequency and amount of alcohol intake was obtained using the Alcohol Use Disorders Identification Test, and participants and parents completed the health-related quality-of-life questionnaire (PedsQL) and a beverage intake questionnaire (BEV-Q) to document habitual beverage intake (grams, energy). The PedsQL and Bev-Q were repeated at week 24.

The histologic confirmation of NAFLD and the NAS was initially determined by a NASH CRN pathologist at each clinical center, who reviewed the available slides from the center at which the participant received their care. Liver biopsy slides for all 83 participants were later centrally reviewed by the NASH CRN Pathology Committee in person for 69 and virtually for 14 participants (due to the onset of the 2019 coronavirus pandemic [COVID-19]) and scored according to the criteria described by Kleiner et al.[25]

Compliance was assessed by pill counts at the 24-week visit and calculated as pills dispensed at randomization minus pills returned at the week 24 visit, as a percentage of the pills expected to remain at the time of that visit.

Outcomes

The primary outcome was change in ALT from baseline to 24 weeks in the losartan group compared with placebo. Secondary outcomes included the relative change in AST, gamma-glutamyltransferase (GGT), HOMA-IR, anthropometric measurements, serum lipids, CRP, PedsQL scores, and adverse events.

Study oversight and early termination

Safety oversight of the study was conducted by an independent DSMB appointed by the NIDDK. They approved the study protocol before the start of the study, monitored safety data as the trial progressed, and reviewed the overall progress of the trial in terms of recruitment and data quality. At the end of each scheduled quarterly meeting, the DSMB made a recommendation as to whether the trial should continue unmodified, continue with protocol modifications, or be stopped. Because of the temporary closure of most clinical research sites due to COVID-19, new enrollment paused in March 2020, and some follow-up visits were converted to virtual visits after IRB approval of such changes.

In response to the pandemic-triggered pause in enrollment, the DSMB was shown unplanned interim futility analyses of the primary outcome (ALT) on April 17, 2020. These analyses were performed on data obtained from 33 losartan and 34 placebo patients with 24-week change in ALT data (from date of first patient enrollment on September 11, 2018, through April 15, 2020). The estimated mean losartan–placebo difference in 24-week change in ALT adjusted for ALT at baseline was 2 U/l (95% CI = −31, 35). The conditional power analysis showed that, given the available data and trends, the probability (i.e., the conditional power) of finding a significant difference favoring losartan of the hypothesized magnitude was 7%. Based on the lack of efficacy and low conditional power, the DSMB recommended discontinuation of treatment in all remaining participants. All participants were contacted for per-protocol close-out visits. These were conducted virtually or in person, as permitted by institutional guidelines at each site and per family preference.

Statistical analysis

The trial was powered at 90% to detect a difference in the primary outcome of 24-week change from baseline in ALT of 28 U/l between the losartan versus placebo groups, given 55 patients per group, SD of 24-week change in ALT of 55 U/l and correlation between baseline and 24-week ALT of 0.64 (estimated from a prior randomized control trial of vitamin E or metformin for the treatment of children with biopsy-proven NAFLD[10]), analysis of covariance (ANCOVA) method of analysis, two-sided type 1 error of 5%, and a 10% increase in sample size due to loss of power from missing data. There were no planned interim analyses for efficacy. At the suspension of the treatment phase of the trial, there were 33 losartan and 34 placebo patients with complete 24-week change in ALT data. At this sample size, the trial had 76% power to detect a significant treatment effect, given similar assumptions as described previously. However, a subsequent conditional power analysis during the pandemic-driven pause in enrollment showed that, given the available data and trends, the probability (i.e., the conditional power) of finding a significant difference favoring losartan of the hypothesized magnitude was 7%.

The losartan and placebo groups were compared at baseline using the t-test with unequal variance for continuous variables and Fisher's exact test for categorical variables. Both the primary and secondary continuous outcomes were analyzed using ANCOVA, adjusting for the baseline measure of the outcome. There were no planned compliance analyses or subgroup analyses. Exploratory subgroup analyses were performed for the primary outcome of 24-week change in ALT using the ANCOVA method of analysis and an indicator variable for the treatment by subgroup interaction to test whether the treatment effect differed by subgroup. Subgroups examined included sex, age

(>14 or ≤14 years), race (non-White vs. White), ethnicity (non-Hispanic or Hispanic), weight (>100 or ≤100 kg), HOMA-IR (>8.0 or ≤8.0), NAS (>4 or ≤4), or NASH diagnosis (none/borderline or definite). Number and severity of adverse events by treatment group were compared using Cochran's chi-square test for trend. *p* Values were nominal and not adjusted for multiple comparisons. Analyses were performed using SAS version 9.2 and Stata version 11.1.

Data analyses were performed by the Data Coordinating Center and reviewed by the study investigators and the DSMB. The manuscript was written by a subcommittee and approved by the members of the NASH CRN Steering Committee, who assume responsibility for the conduct of the trial, integrity of the data, and the content of the manuscript. All authors had access to the study data and approved the final manuscript.

Role of funding sources

The NASH CRN is funded by the NIDDK as a U01 cooperative agreement. The STOP-NAFLD protocol was written by a subcommittee and approved by the Steering Committee of the NASH CRN and the Program Officers of the cooperative agreement. The trial was conducted under an Investigational New Drug application held by the NIDDK and was registered with ClinicalTrials.gov (NCT01913470).

RESULTS

Study participants

Of the 132 consented participants, a total of 83 patients with histologically proven NAFLD were randomly assigned to receive either losartan (*n* = 43) or placebo (*n* = 40) (Figure 1). The remaining 39 participants who consented but were not randomized were ineligible, primarily due to exclusion criteria, with ALT < 50 U/l being the most common exclusion. There were no statistically significant differences between treatment groups at baseline (Table 1). Most of the participants were Hispanic (77% losartan vs. 82% placebo) and male (77% losartan vs. 85% placebo), with a mean age of 14 (losartan) and 13 (placebo) years. Mean baseline ALT was 115 U/l in the losartan group and 126 U/l in the placebo group.

Primary outcome

Week-24 follow-up laboratory data were available from 67 patients (*n* = 33 on losartan and *n* = 34 on placebo).

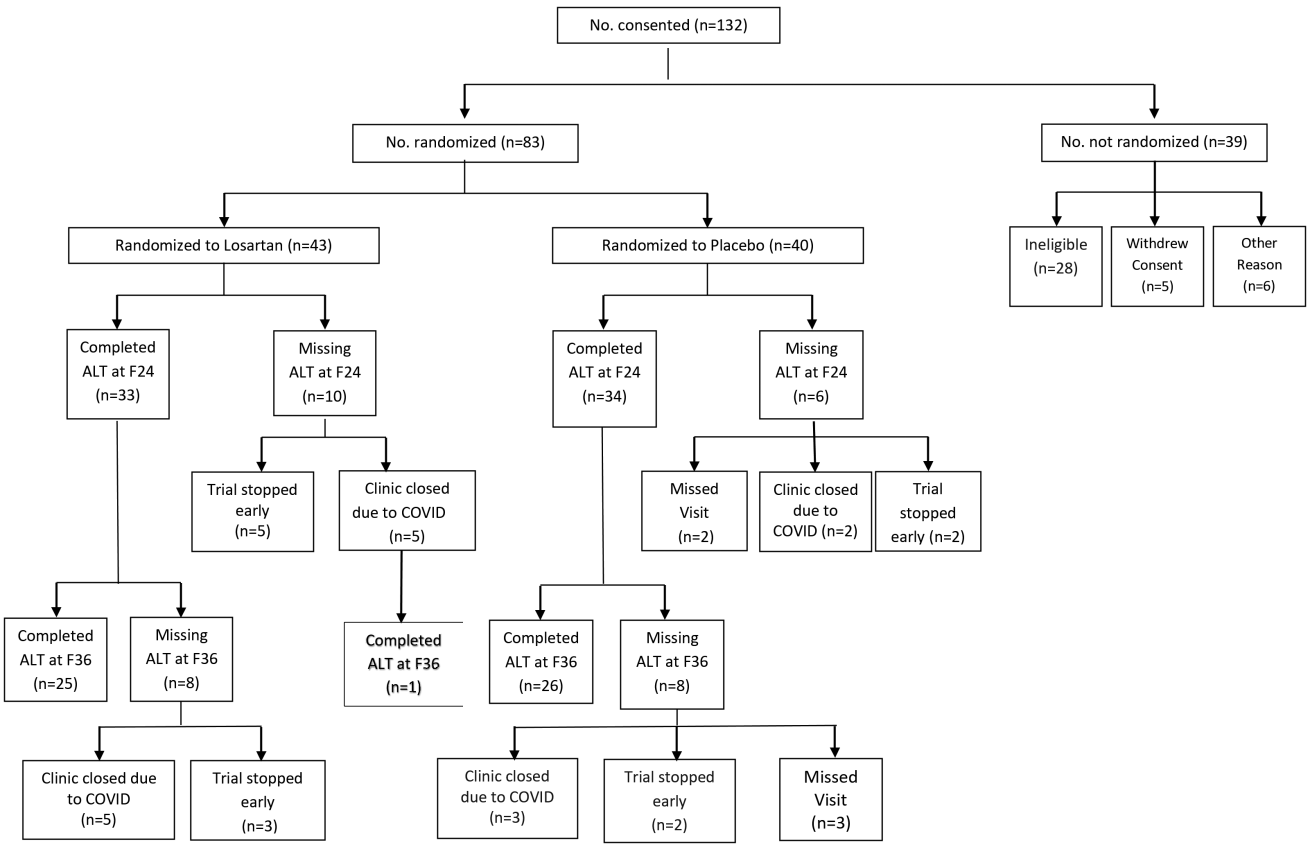

FIGURE 1 Consolidated Standards of Reporting Trials flow diagram. ALT, alanine aminotransferase; and COVID, 2019 coronavirus pandemic

TABLE 1 Baseline characteristics in the losartan and placebo treatment groups

	Losartan (*n* = 43)	Placebo (*n* = 40)	Total (*n* = 83)	
	Mean (SD)/median [IQR]/*n* (%)	Mean (SD)/median [IQR]/*n* (%)	Mean (SD)/median [IQR]/*n* (%)	*p* value
Demographics				
Age [range], years[a]	14 (2) [9–17]	13 (2) [9–17]	13 (2) [9–17]	0.57
Male sex, *n* (%)	33 (77%)	34 (85%)	67 (81%)	0.41
Onset of menarche, *n*/girls (%)	6/10 (60%)	5/6 (83%)	11/16 (69%)	0.59
White race, *n*/nonrefusal (%)	28/34 (82%)	25/32 (78%)	53/66 (80%)	0.76
Hispanic ethnicity, *n* (%)	33 (77%)	33 (82%)	66 (80%)	0.59
Liver enzymes				
ALT, U/l	115 (50)	126 (61)	120 (55)	0.39
AST, U/l	59 (26)	70 (38)	64 (33)	0.13
ALP, U/l	192 (114)	202 (99)	197 (106)	0.68
GGT, U/l	53 (44)	51 (28)	52 (37)	0.82
Total bilirubin, mg/dl	0.6 (0.3)	0.6 (0.3)	0.6 (0.3)	1.00
Direct bilirubin, mg/dl	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.84
Lipids				
Cholesterol				
Total, mg/dl	159 (44)	155 (31)	157 (38)	0.64
HDL, mg/dl	39 (7)	41 (9)	40 (8)	0.35
LDL, mg/dl	91 (40)	92 (22)	91 (32)	0.83
Trigylcerides, mg/dl	158 (105)	128 (58)	143 (87)	0.11
Metabolic factors				
Fasting serum glucose, mg/dl	91 [84, 100]	87 [81, 96]	89 [82, 97]	0.27
Insulin, umol/ml	29 [18, 42]	38 [21, 44]	32 [21, 44]	0.38
HOMA–IR[e], mg/dl × umol/ml/405	6.5 [3.9, 10.7]	8.3 [4.0, 10.2]	7.1 [4.0, 10.2]	0.57
HbA1C, %	5.4 (0.6)	5.4 (0.3)	5.4 (0.5)	0.94
Height, cm	165 (9)	165 (9)	165 (9)	1.00
Weight, kg	95 (17)	96 (19)	95 (18)	0.68
Body mass index, kg/m^2	34 (5)	35 (5)	35 (5)	0.61
Midarm circumference, cm	33 (4)	33 (4)	33 (4)	0.56
Waist circumference, cm	108 (11)	111 (11)	110 (11)	0.38
Hip circumference, cm	110 (11)	112 (11)	111 (11)	0.37
Waist to hip ratio	0.99 (0.06)	0.99 (0.06)	0.99 (0.06)	0.94
Systolic blood pressure, mm Hg	121 (9)	119 (9)	120 (9)	0.54
Diastolic blood pressure, mm Hg	69 (6)	69 (6)	69 (6)	0.93
Pulse, min	79 (13)	80 (13)	79 (13)	0.72
Breath rate, min	18 (3)	19 (4)	19 (3)	0.37
Laboratory results				
Hemoglobin, g/dl	14.5 (1.1)	14.0 (1.2)	14.2 (1.2)	0.06
Hematocrit, %	43.2 (3.3)	42.0 (3.4)	42.6 (3.4)	0.11
MCV, fL	84.9 (4.4)	84.7 (3.4)	84.8 (4.0)	0.77
WBC, I0^3 cells/μl	7.2 (1.5)	8.6 (2.4)	7.9 (2.1)	0.002
RBC, mill cells/μl	509 (39)	496 (37)	503 (39)	0.10
Neutrophils, cells/μl	3541 (1123)	4231 (1867)	3869 (1552)	0.05
Lymphocytes, cells/μl	2656 (727)	3031 (1003)	2835 (884)	0.06
Monocytes, cells/μl	501 (165)	578 (217)	537 (194)	0.08

TABLE 1 (Continued)

	Losartan (*n* = 43)	Placebo (*n* = 40)	Total (*n* = 83)	
	Mean (SD)/median [IQR]/*n* (%)	Mean (SD)/median [IQR]/*n* (%)	Mean (SD)/median [IQR]/*n* (%)	*p* value
Eosinophils, cells/μl	297 (245)	278 (249)	288 (245)	0.72
Basophils, cells/μl	35 (37)	65 (126)	49 (91)	0.16
Platelet, 1000 cells/mm^3	277 (57)	311 (66)	293 (64)	0.01
Sodium, mEq/l	140.0 (2.0)	139.7 (2.0)	139.9 (2.0)	0.41
Potassium, mEq/l	4.1 (0.3)	4.2 (0.2)	4.2 (0.3)	0.43
Chloride, mEq/l	103.9 (2.7)	102.9 (2.5)	103.4 (2.6)	0.10
Bicarbonate, mEq/l	24.3 (2.5)	24.7 (2.0)	24.4 (2.3)	0.36
Calcium, mEq/l	9.8 (0.4)	9.8 (0.3)	9.8 (0.3)	0.91
Blood urea nitrogen, mg/dl	10.9 (3.1)	10.5 (2.5)	10.7 (2.8)	0.56
Creatinine, mg/dl	0.56 (0.14)	0.56 (0.13)	0.56 (0.14)	0.89
eGFR[f], ml/min/1.73 m^2	156 (19)	155 (17)	156 (18)	0.92
Prothrombin time, seconds	12.3 (1.3)	11.9 (1.3)	12.1 (1.3)	0.19
International normalized ratio	1.04 (0.07)	1.04 (0.08)	1.04 (0.07)	0.68
Uric acid, mg/dl	6.6 (1.7)	6.6 (1.5)	6.6 (1.6)	0.81
C-reactive protein, mg/l	3.8 (2.5)	3.8 (3.0)	3.8 (2.7)	1.00
Comorbidities				
Cardiovascular disease, *n* (%)	0 (0%)	0 (0%)	0 (0%)	NC
Diabetes, *n* (%)	2 (4%)	1 (2%)	3 (4%)	1.00
Concomitant medications in the past 6 months				
Anti-lipidemic, *n* (%)	3 (7%)	0 (0%)	3 (4%)	0.24
Anti-diabetic, *n* (%)	0 (0%)	0 (0%)	0 (0%)	NC
Anti-obesity, *n* (%)	0 (0%)	0 (0%)	0 (0%)	NC
Anti-psychotic, *n* (%)	2 (5%)	2 (5%)	4 (5%)	1.00
Liver histology findings				
Time from biopsy, years	0.7 (0.6)	0.8 (0.6)	0.8 (0.6)	0.47
Steatohepatitis				0.49
NAFLD, not NASH, *n* (%)	15 (35%)	9 (22%)	24 (29%)	
Borderline Zone 3, *n* (%)	9 (21%)	7 (18%)	16 (19%)	
Borderline Zone 1, *n* (%)	11 (26%)	12 (30%)	23 (28%)	
Definite, *n* (%)	8 (19%)	12 (30%)	20 (24%)	
Fibrosis stage[b]				0.08
Stage 0 (none), *n* (%)	17 (40%)	7 (18%)	24 (29%)	
Stage 1 (mild), *n* (%)	13 (30%)	20 (50%)	33 (40%)	
Stage 2 (moderate), *n* (%)	8 (19%)	5 (12%)	13 (16%)	
Stage 3 (bridging), *n* (%)	5 (12%)	8 (20%)	13 (16%)	
Mean (SD) stage	1.0 (1.0)	1.4 (1.0)	1.2 (1.0)	0.15
Total NAS[c]	4.6 (1.5)	4.4 (1.2)	4.5 (1.4)	0.66
Hepatocellular ballooning score				0.40
0 (none), *n* (%)	31 (72%)	27 (68%)	58 (70%)	
1 (few), *n* (%)	6 (14%)	10 (25%)	16 (19%)	
2 (many), *n* (%)	6 (14%)	3 (8%)	9 (11%)	
Mean (SD)	0.4 (0.7)	0.4 (0.6)	0.4 (0.7)	0.90
Steatosis score				0.74

(Continues)

TABLE 1 (Continued)

	Losartan (*n* = 43)	Placebo (*n* = 40)	Total (*n* = 83)	
	Mean (SD)/median [IQR]/*n* (%)	Mean (SD)/median [IQR]/*n* (%)	Mean (SD)/median [IQR]/*n* (%)	*p* value
1 (<34%), *n* (%)	4 (9%)	6 (15%)	10 (12%)	
2 (34%–66%), *n* (%)	13 (30%)	11 (28%)	24 (29%)	
3 (≥67%), *n* (%)	26 (60%)	23 (58%)	49 (59%)	
Mean (SD)	2.5 (0.7)	2.4 (0.7)	2.5 (0.7)	0.58
Lobular inflammation score				0.24
1 (<2 foci under ×20 mag), *n* (%)	21 (49%)	17 (42%)	38 (46%)	
2 (2–4 foci under ×20 mag), *n* (%)	16 (37%)	21 (52%)	37 (45%)	
3 (>4 foci under ×20 mag), *n* (%)	6 (14%)	2 (5%)	8 (10%)	
Mean (SD)	1.7 (0.7)	1.6 (0.6)	1.6 (0.7)	0.86
Portal inflammation score[d]				0.84
1 (none), *n* (%)	7 (16%)	5 (12%)	12 (14%)	
2 (mild), *n* (%)	30 (70%)	28 (70%)	58 (70%)	
3 (>mild), *n* (%)	6 (14%)	7 (18%)	13 (16%)	
Mean (SD)	1.0 (0.6)	1.0 (0.6)	1.0 (0.6)	0.55
Pediatric quality of life[g]				
Self-report				
Physical health score	83 (14)	83 (17)	83 (15)	0.99
Psychosocial health score	75 (16)	76 (15)	75 (15)	0.96
Parent-proxy report				
Physical health score	72 (23)	68 (26)	70 (25)	0.52
Psychosocial health score	72 (18)	72 (19)	72 (18)	0.85

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; HbA1C, hemoglobin A1c; IQR, interquartile range; NAS, NAFLD activity score; NC, not calculable.

[a]Median [IQR].

[b]Fibrosis was assessed on a scale of 0 to 4, with higher scores indicating more severe fibrosis.

[c]Total NAFLD activity was assessed on a scale of 0 to 8, with higher scores indicating more severe disease; the components of this measure are steatosis (assessed on a scale of 0 to 3), lobular inflammation (assessed on a scale of 0 to 3), and hepatocellular ballooning (assessed on a scale of 0 to 2).

[d]Portal inflammation was assessed on a scale of 0 to 2 with higher scores indicating more severe inflammation.

[e]Homeostasis model assessment–estimated insulin resistance.

[f]Estimated glomerular filtration rate (eGFR) calculated using CHK-EPI.

[g]Scored from 0 to 100 with higher scores indicating better quality of life.

There was no significant difference in change in ALT from baseline to 24 weeks (adjusted mean difference of change: 1.1 U/l; 95% CI = −30.6, 32.7; $p = 0.95$; Table 2) between the losartan and the placebo group, respectively. As shown in Figure 2, there were no significant differences in mean ALT during each study visit in the losartan group when compared with placebo. There were no significant treatment effects for 24-week change in ALT in any subgroup (Table S1). At the posttreatment follow-up visit (36 weeks), there were no significant differences between treatment groups (Table 3).

Secondary outcomes

There were no significant differences in change in AST and GGT between the two treatment groups (Table 2 and Figure 2). Alkaline phosphatase (ALP) decreased significantly in the losartan group compared with placebo at 24 weeks (adjusted mean difference of change: −23.4 U/l; 95% CI = −41.5, −5.3; $p = 0.01$). There was a statistically nonsignificant increase in HOMA-IR in the losartan group compared with the placebo group (adjusted mean difference of change: 3.4 mg/dl*μU/ml/405; 95% CI = 0.0, 6.8; $p = 0.06$). There were no significant differences in lipid profiles or other biochemical measurements. There was a significant reduction in systolic blood pressure in the losartan group compared with those on placebo (adjusted mean difference of change: −7.4 mm Hg; 95% CI = −12.0, −2.8; $p = 0.002$). At the posttreatment follow-up visit (36 weeks), the changes in blood pressure and ALP were no longer evident (Table 3).

TABLE 2 Changes from baseline to 24 weeks in liver enzymes, lipids, and metabolic features in the losartan and placebo treatment groups

	24-week changes		Losartan–Placebo		
	Lorsartan (*n* = 33)	Placebo (*n* = 34)	Difference of differences		
Outcomes	**Adjusted[a] mean (SD)**	**Adjusted[a] mean (SD)**	**Adjusted[a] mean**	**95% CI**	***p* value**
Primary outcome					
ALT, U/l	−5.3 (51.4)	−6.3 (77.5)	1.1	−30.6, 32.7	0.95
Liver enzymes					
ALT, % relative change[b]	−2.7 (43.8)	5.7 (73.2)	−8.4	−37.5, 20.8	0.57
AST, U/l	0.2 (27.2)	−4.5 (37.0)	4.7	−10.3, 19.8	0.53
ALP, U/l	−32.8 (45.1)	−9.4 (35.8)	−23.4	−41.5, −5.3	0.01
GGT, U/l	−1.9 (13.9)	0.6 (19.7)	−2.5	−11.0, 6.0	0.56
Total bilirubin, mg/dl	−0.01 (0.26)	−0.01 (0.25)	0.00	−0.10, 0.10	1.00
Direct bilirubin, mg/dl	0.01 (0.05)	0.00 (0.07)	0.01	−0.02, 0.04	0.53
Albumin, g/dl	−0.02 (0.25)	−0.11 (0.33)	0.09	−0.04, 0.22	0.19
Protein, g/dl	0.05 (0.38)	−0.05 (0.44)	0.10	−0.08, 0.27	0.28
Lipids					
Cholesterol, mg/dl	−6.7 (28.4)	−4.1 (25.2)	−2.6	−13.9, 8.7	0.64
Triglycerides, mg/dl	13.2 (98.1)	6.2 (41.1)	7.0	−25.6, 39.7	0.67
HDL, mg/dl	−2.1 (6.2)	−1.1 (4.9)	−0.9	−3.4, 1.5	0.45
LDL, mg/dl	−6.7 (29.2)	−6.2 (14.8)	−0.4	−10.3, 9.4	0.93
Metabolic					
Height, cm	2.1 (2.1)	1.9 (2.0)	0.2	−0.7, 1.1	0.65
Weight, kg	4.4 (4.2)	3.9 (4.3)	0.5	−1.5, 2.5	0.64
BMI, kg/m^2	0.8 (1.2)	0.8 (1.3)	0.0	−0.6, 0.6	0.98
Waist circumference, cm	2.6 (5.4)	0.1 (6.4)	2.4	−0.5, 5.3	0.10
Hip circumference, cm	1.2 (4.8)	1.5 (4.5)	−0.3	−2.6, 2.0	0.81
Waist to hip ratio	0.01 (0.05)	−0.01 (0.06)	0.02	0.00, 0.05	0.08
Midarm circumference, cm	0.7 (2.6)	0.4 (2.3)	0.3	−1.1, 1.7	0.65
SBP, mm Hg	−1.6 (9.4)	5.8 (11.3)	−7.4	−12.0, −2.8	0.002
DBP, mm Hg	1.1 (7.2)	2.5 (9.0)	−1.5	−5.2, 2.2	0.43
Pulse, min	−1.2 (9.8)	−0.8 (12.5)	−0.4	−5.1, 4.3	0.87
Breath rate, min	−0.1 (2.6)	−0.6 (3.3)	0.5	−0.7, 1.8	0.40
Glucose, mg/dl	7.5 (22.9)	2.5 (10.8)	4.9	−1.2, 11.0	0.11
Insulin, uU/ml	13.4 (34.0)	3.4 (20.2)	10.0	−3.4, 23.5	0.14
HOMA-IR, mg/dl*uU/ml/405	4.5 (8.1)	1.1 (5.2)	3.4	0.0, 6.8	0.06
HbA1C, %	0.1 (0.3)	0.1 (0.3)	0.0	−0.2, 0.2	0.98
Lab results					
Hemoglobin, g/dl	−0.2 (0.8)	0.0 (0.7)	−0.2	−0.6, 0.2	0.37
Hematocrit, %	−0.3 (2.5)	0.1 (2.2)	−0.5	−1.7, 0.7	0.42
MCV, fl	0.1 (2.8)	−0.4 (2.0)	0.5	−0.5, 1.5	0.29
WBC, l0^3 cells/µl	0.1 (1.4)	−0.3 (1.4)	0.4	−0.3, 1.1	0.23
RBC, mill cells/µl	−4.7 (24.3)	4.0 (24.7)	−8.6	−21.0, 3.4	0.17
Neutrophils, cells/µl	239 (1018)	−146 (1652)	385	−217, 986	0.21
Lymphocytes, cells/µl	−59 (736)	−121 (882)	63	−247, 372	0.69
Monocytes, cells/µl	23 (168)	−1 (282)	24	−80, 128	0.65

(Continues)

TABLE 2 (Continued)

	24-week changes		Losartan–Placebo		
	Lorsartan (*n* = 33)	Placebo (*n* = 34)	Difference of differences		
Outcomes	Adjusted[a] mean (SD)	Adjusted[a] mean (SD)	Adjusted[a] mean	95% CI	*p* value
Eosinophils, cells/μl	−44 (221)	−23 (198)	−21	−90, 49	0.56
Basophils, cells/μl	−15 (33)	0 (135)	−15	−33, 3	0.09
Platelet, 1000 cells/mm^3	8.1 (36.5)	-8.5 (25.0)	16.6	0.3, 33.9	0.05
Sodium, mEq/l	−0.6 (1.8)	0.2 (2.4)	−0.8	−1.6, 0.0	0.04
Potassium, mEq/l	0.0 (0.4)	0.1 (0.3)	−0.1	−0.2, 0.1	0.30
Chloride, mEq/l	0.3 (2.0)	0.9 (2.3)	−0.6	−1.5, 0.4	0.22
Bicarbonate, mEq/l	0.3 (2.7)	0.6 (2.2)	−0.3	−1.4, 0.8	0.62
Calcium, mEq/l	−0.12 (0.33)	−0.06 (0.34)	−0.06	−0.19, 0.07	0.37
Blood urea nitrogen, mg/dl	0.2 (2.8)	0.0 (2.3)	0.2	−1.0, 1.4	0.74
Creatinine, mg/dl	0.02 (0.08)	−0.01 (0.07)	0.03	−0.01, 0.06	0.14
eGFR, ml/min/1.73 m^2	−1.9 (10.2)	1.9 (9.2)	−3.8	−9.3, 0.08	0.10
Prothrombin time, seconds	−0.06 (0.50)	0.20 (1.40)	−0.26	−0.77, 0.26	0.32
International normalized ratio	−0.02 (0.07)	0.01 (0.14)	−0.03	−0.08, 0.03	0.34
Uric acid, mg/dl	−0.6 (2.1)	−0.2 (1.0)	−0.4	−1.0, 0.3	0.29
C-reactive protein, mg/l	−0.2 (2.2)	0.2 (5.0)	−0.4	−2.1, 1.3	0.64
Pediatric quality of life					
Self-report					
Physical health score	0.9 (14.7)	−2.2 (13.3)	3.1	−2.8, 9.0	0.29
Psychosocial health score	2.7 (12.2)	−0.2 (13.9)	2.9	−2.5, 8.3	0.29
Parent-proxy report					
Physical health score	3.3 (24.8)	−5.6 (31.1)	8.8	−2.5, 20.1	0.12
Psychosocial health score	0.8 (17.7)	−3.1 (19.0)	3.9	−4.0, 11.7	0.33

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; MCV, mean corpuscular volume; RBC, red blood count; SBP, systolic blood pressure; and WBC, white blood count.

[a]Adjusted for baseline value of outcome; SD based on unadjusted change.

[b]100*(F24-BL)/BL, where "BL" indicates baseline.

Follow-up and adherence

There were no significant group differences in adherence to assigned treatment, as determined by pill counts (Table 4). In the losartan group, 24 (69%) of those with 24-week data had 80% compliance compared with 27 (73%) in the placebo group (p = 0.80).

Adverse events

There were no severe adverse events during the study, and there were 15 moderate adverse events. Of these, 10 occurred in the placebo group and 5 in the losartan group (Table 5). There was no difference between the number or types of events in the two groups (p = 0.14). Also, there were no treatment group differences in study drug dose reductions (1 losartan vs. 1 placebo) or discontinuation of study drug due to an adverse event (1 losartan vs. 1 placebo).

DISCUSSION

We report the results of a multicenter, randomized controlled clinical trial of losartan as a treatment for NAFLD in children with biopsy-confirmed disease. Losartan is a safe, inexpensive, widely available generic drug that has been reported as potentially beneficial for NAFLD. However, while losartan used for 24 weeks to treat pediatric NAFLD was safe, it did not improve ALT or GGT, two serum biomarkers that are significantly associated with histologic improvements in NAFLD in children, including fibrosis regression.[22] The significant difference in systolic blood pressure between the groups shows that the dose and compliance were sufficient to induce recognized clinical effects.

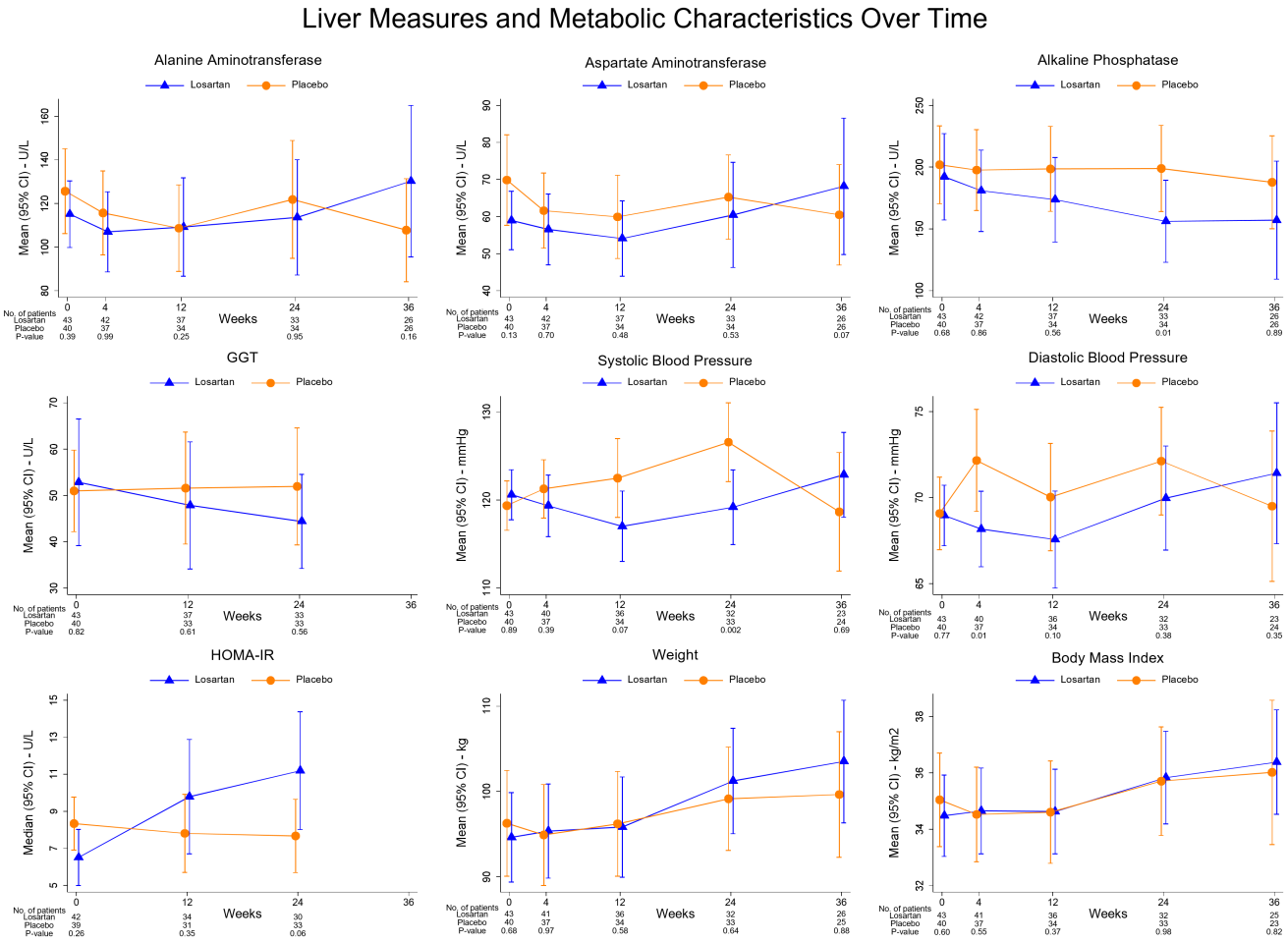

FIGURE 2 Liver measures and metabolic characteristics over time

Despite prior pilot data from a randomized, double-blind, placebo-controlled pediatric clinical trial,[21] supportive data in an animal model,[26] and the theoretical scientific benefit,[16,20,26] this randomized, placebo-controlled trial found no improvement in ALT, AST, or GGT after treatment with losartan in adolescents with NAFLD. We selected ALT as our primary outcome measure, because ALT is the most validated and accurate biomarker of fibrosis change in children with NAFLD. In an analysis of children enrolled in the placebo arms of two earlier NASH CRN clinical trials, for each 10--U/l increase in ALT, there was a 2.4 increased odds of progression in fibrosis (by one stage or more).[5] Furthermore, in a secondary analysis in the cysteamine bitartrate versus placebo trial in children with NAFLD, baseline and change in ALT was the only independent predictor associated with improvement in fibrosis after multivariable modeling (area under the receiver operating characteristic curve [AUROC], 0.80; 95% CI, 0.67–0.93).[22] In addition, GGT, a secondary outcome measure in this trial, has also been shown to predict change in fibrosis in children,[5] as well as change in NASH.[22] By comparison, other serologic biomarkers of fibrosis validated in adults perform poorly in children with NAFLD (e.g., AST-to–platelet ratio index, Fibrosis-4), with AUROC ranging from 0.50–0.67.[27] In contrast, MRI and ultrasound-elastography methods have not been validated as longitudinal noninvasive biomarkers of progression or regression of fibrosis in children.[28,29]

There are several data points that suggest that dosing and compliance with losartan was sufficient to detect an effect, if there were to be one. First, the systolic blood pressure was significantly decreased in the losartan group, as expected, by the recognized action of losartan as an anti-hypertensive. Second, compliance using pill counts found that most children had used >80% of the expected amounts of pills. Compliance can be challenging in all clinical trials, and previous pediatric NAFLD clinical trials have shown lower average levels of adherence.[30] Reasons for higher compliance in this study may have included the use of once-a-day dosing, the relatively short duration of treatment, and the lack of significant side effects.

In this study we found no benefit of losartan on markers of insulin sensitivity, despite an improvement in systolic blood pressure. Rather, there was a small increase in HOMA-IR levels in the losartan-treated group

TABLE 3 Changes from baseline to 36 weeks in liver enzymes and metabolic features in the losartan and placebo treatment groups

	36-week changes		Losartan–Placebo		
	Lorsartan (*n* = 26)	Placebo (*n* = 26)	Difference of differences		
Outcomes	Adjusted[c] mean (SD)	Adjusted[c] mean (SD)	Adjusted[c] mean	95% CI	*p* value
Liver enzymes					
ALT, U/l	18 (76)	−9 (64)	27	−11, 64	0.16
ALT, % relative change[d]	23 (72)	6 (62)	17	−19, 53	0.35
AST, U/l	10 (39)	−8 (33)	18	−2, 37	0.07
ALP, U/l	−22 (55)	−20 (46)	−2	−30, 26	0.89
Total bilirubin, mg/dl	0.03 (0.31)	−0.06 (0.29)	0.09	−0.06, 0.24	0.21
Direct bilirubin, mg/dl	0.03 (0.08)	−0.02 (0.07)	0.05	0.00, 0.09	0.03
Albumin, g/dl	0.0 (0.4)	−0.1 (0.3)	0.1	0.0, 0.3	0.09
Protein, g/dl	−0.2 (0.8)	0.0 (0.4)	−0.2	−0.6, 0.1	0.13
Metabolic					
Height, cm	2.2 (2.6)	2.7 (2.1)	−0.5	−1.9, 0.8	0.44
Weight, kg	5.8 (5.0)	5.9 (4.2)	−0.1	−2.7, 2.5	0.93
BMI, kg/m^2	1.1 (1.5)	1.0 (1.3)	0.1	−0.7, 1.0	0.77
Waist circumference, cm	2.4 (6.0)	2.2 (7.3)	0.1	−3.7, 4.0	0.95
Hip circumference, cm	2.3 (5.5)	3.8 (5.2)	−1.5	−4.4, 1.5	0.31
Waist to hip ratio	0.00 (0.05)	0.01 (0.07)	0.01	−0.02, 0.04	0.48
SBP, mm Hg	1.2 (8.5)	-0.3 (13.6)	1.4	−4.8, 7.7	0.65
DBP, mm Hg	3.0 (8.4)	0.4 (8.7)	2.6	−2.2, 7.4	0.28
Pulse, min	2.8 (14.2)	2.0 (9.3)	0.8	−5.1, 6.7	0.78
Breath rate, min	−0.5 (2.6)	0.2 (3.3)	−0.7	−1.9, 0.6	0.29
Lab results					
Hemoglobin, g/dl	0.0 (0.8)	0.1 (0.6)	−0.1	−0.5, 0.3	0.66
Hematocrit, %	0.4 (2.6)	0.3 (1.8)	0.2	−1.1, 1.5	0.77
MCV, fl	−0.2 (2.9)	0.5 (3.0)	-0.8	−2.3, 0.8	0.33
WBC, I0^3 cells/µl	0.7 (1.6)	−0.2 (1.5)	1.0	0.1, 1.8	0.03
RBC, mill cells/µl	5.6 (26.3)	3.5 (22.3)	2.2	−12.2, 16.6	0.76
Neutrophils, cells/µl	676 (1260)	−18 (1238)	694	−5, 1393	0.05
Lymphocytes, cells/µl	13 (951)	119 (848)	−106	−566, 355	0.65
Monocytes, cells/µl	39 (161)	1 (187)	37	−42, 117	0.35
Eosinophils, cells/µl	−65 (228)	−45 (274)	−20	−93, 53	0.58
Basophils, cells/µl	−3 (47)	1 (183)	−4	−52, 44	0.87
Platelet, 1000 cells/mm^3	−6.7 (32.6)	−2.0 (25.3)	−4.8	−21.1, 11.5	0.56
Sodium, mEq/l	0.2 (2.0)	0.9 (2.1)	−0.7	−1.5, 0.2	0.14
Potassium, mEq/l	−0.03 (0.36)	0.01 (0.35)	−0.04	−0.22, 0.13	0.63
Chloride, mEq/l	0.2 (3.2)	0.7 (2.4)	−0.5	−1.9, 0.8	0.43
Bicarbonate, mEq/l	−0.5 (3.3)	0.7 (2.0)	−1.3	−2.5, 0.0	0.04
Calcium, mEq/l	−0.10 (0.44)	−0.13 (0.48)	0.03	−0.18, 0.24	0.76
Blood urea nitrogen, mg/dl	−0.5 (2.8)	0.2 (2.9)	-0.7	−2.2, 0.9	0.40
Creatinine, mg/dl	0.06 (0.11)	0.04 (0.07)	0.02	−0.03, 0.07	0.45
eGFR, ml/min/1.73 m^2	−6.5 (13.8)	−3.6 (8.7)	−2.9	−8.9, 3.1	0.34
Uric acid, mg/dl	−0.1 (2.2)	−0.2 (1.1)	0.1	−0.7, 0.9	0.80
C-reactive protein, mg/l	0.3 (3.3)	0.0 (3.5)	0.3	−1.2, 1.8	0.67

TABLE 3 (Continued)

Outcomes	36-week changes		Losartan–Placebo		
	Lorsartan (n = 26)	Placebo (n = 26)	Difference of differences		
	Adjusted[c] mean (SD)	Adjusted[c] mean (SD)	Adjusted[c] mean	95% CI	p value
Pediatric quality of life					
Self-report					
Physical health score	3.5 (13.8)	2.1 (10.0)	1.4	−4.6, 7.3	0.65
Psychosocial health score	7.2 (11.4)	6.4 (13.4)	0.8	−5.2, 6.7	0.79
Parent-proxy report					
Physical health score	−1.1 (23.0)	6.6 (23.8)	−7.6	−18.9, 3.7	0.18
Psychosocial health score	−1.1 (14.4)	0.8 (19.5)	−1.9	−9.9, 6.1	0.64

[a]Adjusted for baseline value of outcome; SD based on unadjusted change.
[b]100*(F36-BL)/BL, where "BL" indicates baseline.

TABLE 4 Adherence to treatment

	Losartan (n = 35[e])	Placebo (n = 37[e])	p value
Compliance[f]			
Median [IQR], %	96 [73, 102]	94 [73, 102]	0.43
80% compliance, %	24 (69%)	27 (73%)	0.80

[a]Number with complete 24-week pill history data.
[b](pills dispensed at randomization – pills returned at week 24 visit)/(1 pill/day for 7 days then 2 pills/day until week 24 visit).

relative to placebo, but this did not reach statistical significance (p = 0.06). Previous small studies of losartan have demonstrated either a beneficial effect or lack of impact on glucose homeostasis in both animal and human studies. In an animal model of fructose-treated rats, both acute and chronic exposure to losartan led to improved insulin sensitivity, shown using intravenous glucose tolerance testing (GTT).[31] Similar results have been generated using a rat model of neonatal type 2 diabetes mellitus, in which treatment with losartan led to improved oral GTT.[32] In adults with type 2 diabetic nephropathy, treatment with losartan 100 mg orally daily for 3 months led to reductions in fasting glucose and HbA1C levels, as well as increases in C-peptide levels and the insulin sensitivity index.[33] Likewise, in adults undergoing hemodialysis, treatment with losartan 50 mg orally daily for 12 months led to a reduction in HOMA-IR.[34] Conversely, some studies found no beneficial effect of losartan on glucose homeostasis. In a study of 20 hyperinsulinemic adults, Laakso et al. showed that a daily dose of 50 mg losartan for 12 weeks did not lead to a change in insulin sensitivity, assessed using the euglycemic clamp technique.[35] Similar neutral metabolic effects on HOMA-IR were noted in 21 hypertensive adults with metabolic syndrome who were given 50 mg losartan daily for 8 weeks.[36] Considering the conflicting results, dedicated studies are needed to determine whether losartan can exert a meaningful beneficial effect on glucose homeostasis in children with NAFLD. Notably, both insulin and glucose levels can be influenced by fasting duration and circadian variations (such as timing of blood draw). Although we asked all children to fast for at least 12 hours before blood draws, there is the potential for some variation in the duration of fasting. HbA1C, which is not is not influenced by fasting status, did not differ significantly between the groups (p = 0.98).

Patients treated with losartan in this study were found to have a significant reduction in ALP levels; this was not accompanied by a reduction in serum GGT levels. This potentially suggests an increased contribution from the bone-derived isoenzyme in this reduction. This reduction has been previously reported in other cohorts treated with losartan and other ARBs.[37] Both osteoclasts and osteoblasts express angiotensin II receptors. Angiotensin II leads to osteoclast activation, and through this mechanism, is thought to contribute to osteoporosis in adults.[38–40] Furthermore, it attenuates the differentiation of osteoblasts, preventing osteogenesis.[41] Activation of the RAS has been shown to contribute to osteoporosis in transgenic mice expressing human renin and angiotensinogen genes.[42] In addition, bone mineral density may be reduced with increasing severity of NAFLD.[43] As such, it is possible that losartan was associated with beneficial effects on bone turnover, which may be manifested by a decrease in serum ALP levels. However, ALP interpretation is complicated in the setting of adolescence, due to the combined effect of liver disease and bone growth that occurs in adolescence. To date, however, there have been no studies indicating a negative effect on linear growth in children using losartan prescribed for hypertension.

TABLE 5 Adverse events in the losartan and placebo treatment groups

Adverse event	Category	Losartan	Placebo	Trend *p*-value
Max grade	0 (none)	15	12	0.05
	1 (mild)	23	15	
	2 (moderate)	5	10	
	3 (severe)	0	1	
	4 (life-threatening)	0	2	
	5 (death)	0	0	
	Total patients	43	40	
Number	0 (none)	15	12	0.14
	1	16	11	
	2	8	9	
	3	4	6	
	4	0	2	
	Total events	44	55	
	Total patients	43	40	
Type	Auditory	1	1	0.85[c]
	Cardiovascular	1	2	
	Constitutional	0	1	
	Gastrointestinal	12	11	
	Infection	2	8	
	Liver/pancreatic	0	1	
	Lymphatic	1	0	
	Musculoskeletal	2	3	
	Neurologic	10	11	
	Psychiatric	1	1	
	Pulmonary	6	9	
	Other	5[a]	5[b]	
	Not specified	3	2	
	Total events	44	55	

[a]Not specified (n = 1); general disorder (n = 2); nasal obstruction (n = 1); vascular (n = 1).
[b]General disorder (n = 1); sore throat (n = 2); surgical (n = 1); surgical infection (n = 1).
[c]Derived from chi-square test.

Although this study did not demonstrate anticipated efficacy, it has several important strengths. The participants demonstrated high compliance with study medication by pill counts, further supported by significant difference in the systolic blood pressure between groups. Other strengths include the multicenter placebo-controlled trial design, conducted at 10 geographically dispersed pediatric clinical centers experienced in conducting pediatric NAFLD studies. Accordingly, the histologic severity of disease in our study cohort was very reflective of typical NAFLD disease severity in children,[4,44] with most having fibrosis (71% with ≥ stage 1), nearly one third with significant (32% ≥ stage 2 fibrosis), and 16% having stage 3 fibrosis. Likewise, 71% of the cohort had either borderline or definite NASH. As is typical in pediatric cohorts, ballooning degeneration and lobular inflammation were less common; a portal-predominant pattern of NASH rarely found in adults was found in 28% of this cohort. Whether losartan has effects specifically on ballooning degeneration or lobular inflammation are important questions, but would require dedicated trials in adults, in whom these histologic features are more common.

A limitation of the study was the smaller than planned sample enrolled in the trial. As with numerous clinical trials in 2020, enrollment was interrupted by the onset of COVID-19 after 75% of the planned participants had been enrolled and randomized. Thus, an interim conditional power analysis was conducted, which supported futility of continued enrollment due to a low probability (7%) of finding a significant difference favoring losartan with continuation. The DSMB therefore recommended early termination of the study. Other limitations include the reliance on a surrogate biomarker rather than the use of liver histology as an outcome. However, for early-phase, proof-of-concept trials in children with NAFLD, reduction of elevated serum ALT is an accepted primary outcome that has been shown to significantly correlate with improvement in histology in children, including fibrosis.[5,22,45] Plasma and serum samples were collected in this trial for biobanking and are available for secondary analyses, should novel fibrosis biomarkers in children be identified and validated in the future, such as plasma metabolomic markers. Likewise, we did not have genetic polymorphism data available in this study, but can incorporate this into future studies, recognizing that genetic polymorphisms could potentially influence response to treatment. The predominance of male and Hispanic children aligns with the ethnic and sex distribution of children enrolled in prior NASH CRN cohorts.[10,30] This is reflective of the higher risk of NAFLD among boys and children of Hispanic ethnicity, but limits generalizability to females and patients of non-Hispanic ethnicity. Finally, the mean body mass index (BMI) of 35 kg/m^2 of our study cohort is within the range of severe obesity for children; thus, generalizability to children with overweight or milder degrees of obesity is uncertain. However, even in a prior pediatric NASH CRN clinical trial that did not require any weight limitations,[10] the mean BMI was comparable (mean 34 ± 6 kg/m^2, with BMI z score of 2.34 ± 0.30). Thus, the results of this present study are still likely to be generalizable to a substantial proportion of children with NAFLD.

Treatment for 24 weeks with losartan did not improve ALT in children with NAFLD, compared with placebo. This study underscores the importance of continuing to conduct well-designed clinical trials treating NAFLD in children, given the high prevalence of the disease, the differences often observed from histology found in adults, and recognized risk of progression despite provision of standard-of-care lifestyle counseling.

CONFLICTS OF INTEREST

Dr. Karpen consults for Albireo, Mirum, and Vertex. Dr. Lavine consults for Intercept and Novo Nordisk. Dr. Mohammad advises Albireo. Dr. Molleston received grants from Abbvie, Albireo, and Mirum. Dr. Sanyal consults and received grants from Conatus, Gilead, Mallinckrodt, Immuron, Boehringer Ingelheim, Novartis, Bristol Myers Squibb, Merck, Lilly, Novo Nordisk, Fractyl, Siemens, Madrigal, Inventiva, and Covance. He owns stock in and consults for Genfit and Hemoshear. He consults for Intercept, Pfizer, Salix, Galectin, Sequana, Terns, Albireo, Sanofi, Janssen, Takeda, Northsea, AMRA, Perspectum, Poxel, 89 Bio, AstraZeneca, NGM Bio, Amgen, Regeneron, Genentech, Roche, Albireo, Prosciento, Histoindex, Path AI, and Biocellvia. He received grants from Echosens-Sandhill, OWL, and Second Genome. He received royalties from Elsevier and UptoDate. He owns stock in Sanyal Bio, Exhalenz, Durect, Indalo, Tiziana, and Rivus. Dr. Schwimmer received grants from Intercept and Genfit. Dr. Vos consults for Novo Nordisk, Boehringer Ingelheim, and Eli Lilly. She received grants from Bristol Myers Squibb. Dr. Xanthakos received grants from Target RWE.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Vos MB, Van Natta ML, Blondet NM, Dasarathy S, Fishbein M, Hertel P, et al; NASH Clinical Research Network. Randomized placebo-controlled trial of losartan for pediatric NAFLD. Hepatology. 2022;76:429–444. https://doi.org/10.1002/hep.32403