Supplementary Online Content

Schwimmer JB, Ugalde-Nicalo P, Welsh JA, et al. Effect of a low free sugar diet vs usual diet on nonalcoholic fatty liver disease in adolescent boys: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2018.20579

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

1. Inclusion and Exclusion Criteria:

Participant Inclusion Criteria

- Boys age 11-16
- Liver biopsy for standard of care within 2 years of screening for the study
- Clinical history consistent with NAFLD
- Definite NAFLD based on liver histology
- Hepatic fat by MRI PDFF ≥ 10% on baseline MRI
- Serum ALT ≥ 45 u/L
- Written informed consent from parent or legal guardian
- Written informed assent from the child
- Currently consumes ≥ 3.5 eight ounce sugar drinks (or juice) per week

2. **Participant Exclusion Criteria**
   - Participants with a history of health issues that make it unsafe for them to participate in the opinion of the investigators
   - History of significant alcohol intake (AUDIT questionnaire) or inability to quantify alcohol consumption
   - Chronic use (more than 2 consecutive weeks) of medications known to cause hepatic steatosis or steatohepatitis (systemic glucocorticoids, tetracycline, anabolic steroids, valproic acid, salicylates, tamoxifen) in the past year.
   - The use of other known hepatotoxins within 90 days of liver biopsy or within 120 of baseline
   - Medications with the intent to treat NAFLD/NASH in the past 60 days
   - History of total parenteral nutrition (TPN) use in the year prior to screening
   - History of bariatric surgery or planning to undergo bariatric surgery during the study duration
   - Significant depression
   - Non-compensated liver disease with any one of the following hematologic, biochemical, and serological criteria on entry into protocol:
     a. Hemoglobin < 10 g/dL
     b. White blood cell < 3,500 cells/mm
     c. Neutrophil count < 1,500 cells/mm3 of blood
     d. Platelets < 130,000 cells/mm3 of blood
     e. Direct bilirubin > 1.0 mg/dL
     f. Total bilirubin > 3 mg/dL
     g. Albumin < 3.2 g/dL
     h. International normalized ratio (INR) > 1.4
   - Diabetes

- Evidence of other chronic liver disease
- Children who are currently enrolled in a clinical trial or who received an investigational study drug within the past 60 days
- Participants who are not able or willing to comply with the diet protocol or have any other condition that would impede compliance or hinder completion of the study, in the opinion of the investigator
- Unable to have an MRI due to metal device, claustrophobia or other reason
- Failure to give informed consent
- Families with > 5 individuals
- Recipient of a liver transplant

3. MRI-PDFF Detailed Methods

Key MRI-PDFF scanning parameters were: 3T MRI scanner (GE 3T 750, and Siemens 3T TrioTim)[16], 2D axial spoiled-gradient-echo breath-hold acquisitions, TR > 100 ms, six TE values evenly spaced from 1.15 to 6.9 ms, flip angle 10 degrees, number frequency-encoding steps between 140 and 192, number phase-encoding steps between 128 and 140, no filters, no saturation, slice thickness 6 to 10 mm (contiguous), and rectangular field-of-view to accommodate body habitus. MRI-PDFF values were derived from parametric maps computed pixel-by-pixel from source images on an Osirix platform using a custom MatLab™ non-linear, least-squares fitting algorithm. For each participant, data analysts co-localized the region-of-interest placements for 4- and 8-week exams to locations on screening exams.

4. Adverse Events Monitoring

Adverse Events: The PI at each site monitored adverse events throughout the course of the study. Any adverse events occurring during the study were documented and reported according to applicable IRB policies and procedures. AE's were entered into RedCap after each visit and were assessed as related or unrelated. The following questions were asked at the 4 week and 8 week visit:

a. Are there any severe adverse events to report (hospitalizations, death)?
b. Any significant changes since the last visit?
c. Any hospital or ER visits?
d. Any GI illnesses that lasted more than 24 hours?
e. Any infections that require taking antibiotics?

Serious Adverse Events: Any SAE were planned be reported to the other site, respective IRBs, and sponsor within 10 business days of first knowledge of the event. Standard definitions of SAE was used.

eResults

Adverse Events

1. There were 3 adverse events reported during the study time period including 1 instance of diarrhea for 4 days, persistent cough requiring antibiotics and a sprained ankle. These were assessed as unrelated to participation in the study by the investigators.

eTable 1. Estimated Mean Sweetness and Pleasantness Scores[a,b,c]

	Intervention Group (n=19)		Control Group (n=18)		Week 8 Difference (Control – Intervention)	
Sweetness	**Baseline**	**Week 8**	**Baseline**	**Week 8**	**Difference (95% CI)**	**p-value**
Concentration (mM)						
150	2.4 (1.5, 3.4)	1.5 (0.5, 2.5)	2.4 (1.4, 3.4)	2.5 (1.5, 3.5)	-1.0 (-2.9, 0.9)	0.53
300	3.9 (2.9, 4.8)	4.3 (3.3, 5.2)	4.9 (4.0, 5.9)	4.5 (3.6, 5.5)	-0.3 (-2.2, 1.7)	0.98
500	7.0 (5.8, 8.3)	6.6 (5.4, 7.8)	6.8 (5.5, 8.0)	6.5 (5.2, 7.8)	0.1 (-2.6, 2.8)	1.00
700	8.1 (7.0, 9.1)	7.9 (6.8, 8.9)	7.1 (6.0, 8.2)	8.3 (7.2, 9.4)	-0.5 (-2.5, 1.6)	0.93
1000	8.4 (7.3, 9.5)	8.9 (7.8, 9.9)	7.9 (6.8, 9.0)	8.1 (7.0, 9.2)	0.8 (-1.3, 2.8)	0.73
Pleasantness						
Concentration (mM)						
150	-0.8 (-1.8, 0.1)	-0.8 (-1.8, 0.2)	-1.5 (-2.5, -0.6)	-1.8 (-2.7, -0.8)	0.2 (-1.6, 2.1)	0.98
300	-0.2 (-1.1, 0.8)	0.7 (-0.2, 1.7)	0.1 (-0.8, 1.1)	-0.2 (-1.2, 0.7)	0.4 (-1.4, 2.2)	0.94
500	0.6 (-0.5, 1.7)	0.8 (-0.4, 1.9)	1.0 (-0.1, 2.2)	0.7 (-0.4, 1.9)	0.3 (-1.8, 2.4)	1.00
700	0.9 (-0.1, 1.9)	0.5 (-0.5, 1.6)	0.9 (-0.1, 2.0)	1.0 (0.0, 2.0)	-0.1 (-2.0, 1.9)	1.00
1000	0.1 (-1.0, 1.1)	0.5 (-0.5, 1.5)	0.9 (-0.1, 1.9)	0.5 (-0.6, 1.5)	0.5 (-1.5, 2.4)	0.92

[a] Participants rated the sweetness intensity of model beverages using visual analog scales (range: 0 to 11) and the pleasantness of model beverages using a standard category scale (range: “dislike extremely” (-4) to “neither like nor dislike” (0) to “like extremely” (4)) at sucrose concentrations ranging from barely sweet (100 mM) to very sweet (1000 mM).
[b] Data are presented as least square means (95% CI) from penalized B-splines.
[c] Week 8 difference and p-value between groups adjusted for baseline are presented. Similarly, there were no significant differences in any other comparisons (Intervention Baseline – Control Baseline; Intervention Week8 – Intervention Baseline, Control Week 8 – Control Baseline). Random intercepts used. Tukey’s method was used to account for multiple comparisons.

eTable 2. Model Based Baseline and Comparison of Week 8 Post Hoc Outcomes

Outcome	Time	Mean[a] (95% CI)		Difference in week 8 means[b] adjusted for baseline, (95% CI)	p-value[c]
		Intervention	Control	Intervention-Control	
Weight (kg)	Baseline	88.1 (77.1, 99.1)	88.7 (77.7, 99.7)		
	Week 8	86.7 (75.9, 97.4)	89.3 (78.5, 100.1)	-2.0 (-3.3, -0.79)	.002
BMI (kg/m^2)	Baseline	34 (31.0, 36.4)	32 (29.6, 35.0)		
	Week 8	33 (30.1, 35.6)	32 (29.5, 34.9)	-0.78 (-1.2, -0.35)	.001
BMI z-score	Baseline	2.38 (2.2, 2.6)	2.22 (2.04, 2.40)		
	Week 8	2.32 (2.1, 2.5)	2.21 (2.02, 2.40)	-0.06 (-0.09, -0.03)	<.001
Waist Circumference (cm)	Baseline	110.2 (103.5, 116.9)	107.7 (101.0, 114.4)		
	Week 8	109.2 (102.8, 115.6)	108 (101.7, 114.5)	-1.2 (-3.1, 0.81)	.24
Systolic (mm Hg)	Baseline	116 (111.6, 120.8)	118 (113.0, 122.2)		
	Week 8	112 (107.0, 116.8)	119 (114.5, 124.3)	-6.9 (-13.4, -0.38)	.04
Diastolic (mm Hg)	Baseline	66 (62.4, 69.7)	65 (61.6, 68.9)		
	Week 8	64 (59.8, 69.0)	66 (61.5, 70.7)	-2.1 (-8.3, 4.1)	.50

[a] Data are presented as least squares means (95% CI) from the mixed model, adjusted for study site.
[b] The adjusted mean difference is the difference in means between intervention and control group at week 8 estimated using mixed models conditioned on baseline measurement
[c] P-value from mixed model conditioned on baseline measurement and adjusted for study site.

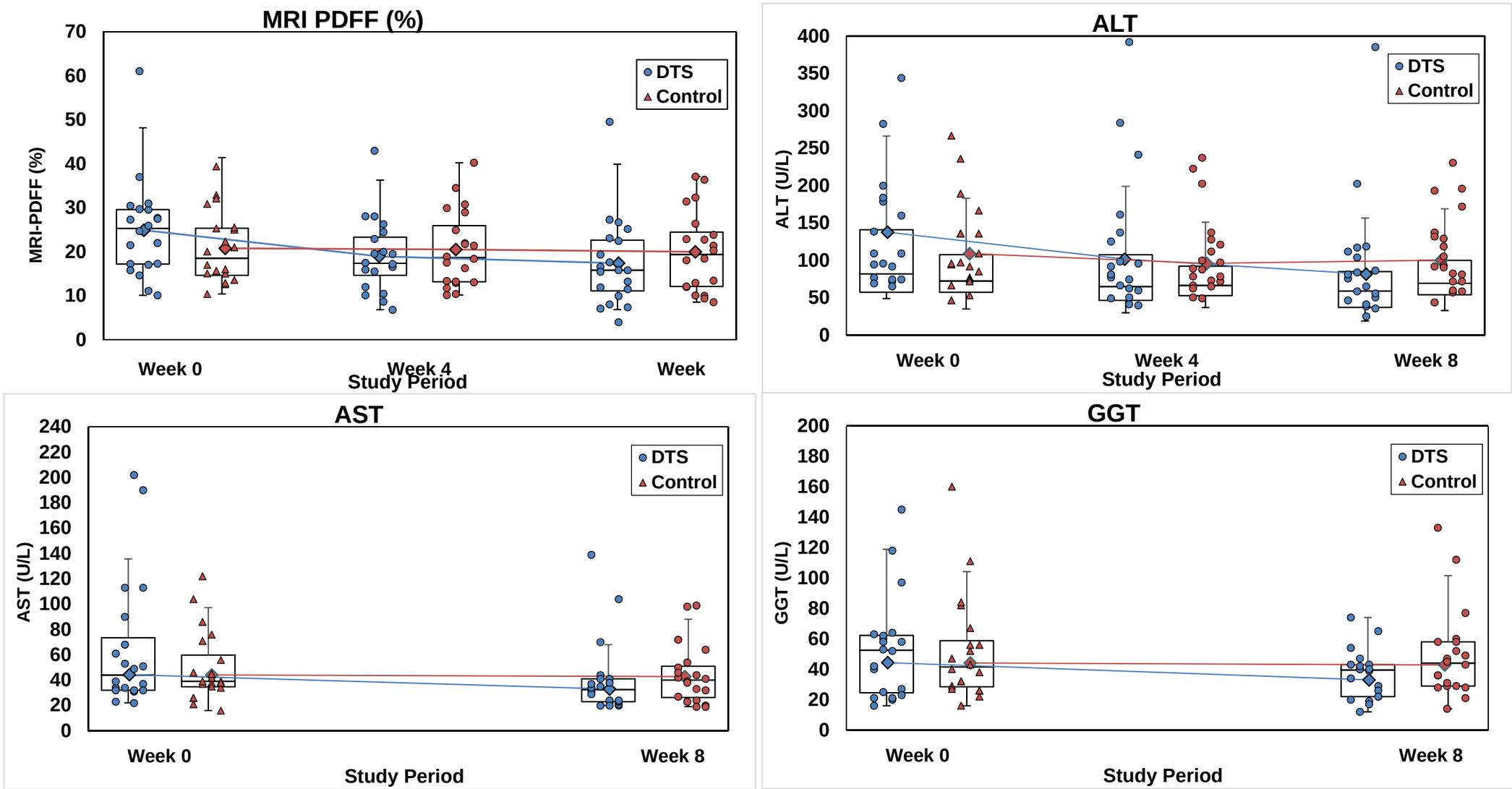
eTable 3. Post Hoc Analysis of Effect of Center in the Model

Handling of Center	Adjusted difference in means at week 8 (95% CI)	p-value
Fixed effect	-6.23 (-9.45, -3.02)	<.001
Random effect	-6.54 (-9.14, -3.96)	<.001

eTable 4. Post Hoc Sensitivity Analysis for Missing Data

Imputation[a]	Adjusted difference in means at week 8 (95% CI)	p-value
No imputation	-6.23 (-9.45, -3.02)	<.001
Imputed data point	-6.12 (-9.34, -2.91)	<.001

[a]Missing week 8 data for one participant was imputed from week 4 results. There was no difference in significant level for the adjusted difference in means at week 8 for the primary outcome when the data point was imputed versus no imputation (missing).



eFigure. Means of Hepatic Steatosis, ALT, AST and GGT at 0, 4 and 8 Weeks for Intervention and Control Groups.

Values are expressed as least squares mean (hepatic steatosis) or geometric mean (ALT, AST and GGT) with error bars spanning the 95% confidence intervals around the estimate. Blue line represents the intervention group; orange line represents the control group. All estimates were obained from a mixed models adjusted for center. The * indicate significant difference between groups, after adjusting for baseline levels and study site. Panel A. Model estimated means of hepatic steatosis by MRI-PDFF at week 0, week 4 and week 8; Panel B. Model estimated geometric means of ALT at week 0 , week 4 and week 8; Panel C. Model estimated geometric means of AST at week 0, week 4 and week 8; Panel D. Model estimated geometric means of GGT at week 0, week 4, and week 8.