View Article Online
View Journal

Food & Function

Linking the chemistry and physics of food with health and nutrition

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: F. T. Jeyaraj, S. S. Vennam, K. L. Nelson, K. Watts, L. R. Goss, B. C. Nance, B. B. Mass and V. S. Voruganti, *Food Funct.*, 2026, DOI: 10.1039/D5FO03248B.

View Article Online
DOI: 10.1039/D5FO03248B

ARTICLE

Received 00th January 20xx,
Accepted 00th January 20xx
DOI: 10.1039/x0xx00000x

NAFLD-related SNPs are linked to changes in liver fat, measured by CAP score, and serum lipids in response to a 3-week sugar sweetened beverage intervention: A pilot study

Faustina Therase Jeyaraj,[1*] Sai Sravani Vennam,[1*] Kendra Nelson,[1] Katherine Watts,[2] Lydia R. Goss,[1] Brea C. Nance, [3]Baba B. Mass,[1] Venkata Saroja Voruganti[1,4]

[1] Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, NC
[2] University of North Carolina at Charlotte, Charlotte, NC
[3] Standard Process Inc., Kannapolis, NC
[4] Department of Nutrition, University of North Carolina at Chapel Hill, Kannapolis, NC
† *Both authors contributed equally to the work

Keywords: Nutrigenetics, Added sugars, Individual variability in responses,

Running title: Genetic variants associated with response to SSB

ABSTRACT

View Article Online
DOI: 10.1039/D5FO03248B

Studies show that increased consumption of sugar sweetened beverage (SSB) is linked to non-alcoholic fatty liver disease (NAFLD), a condition characterized by excess fat accumulation in hepatocytes. Genetic factors also influence NAFLD. We conducted a clinical trial (NCT03783195) to determine if SNPs related to NAFLD are associated with liver fat content and its changes in response to a 3-week SSB intervention in Caucasian adolescents and young adults. Fifteen participants (Males-5, Females–10, mean age 25.5 ± 9 yrs) consumed a beverage, daily for 3 weeks, consisting of fructose:glucose in 60:40 ratio. Liver fat content was measured by transient elastography through controlled attenuation parameter (CAP) score. At baseline, the CAP score was 212.5±10.1dB/m and was not significantly different between sexes. We genotyped ten NAFLD-related SNPs, of which rs1227756 in *COL13A1* (β=-22.4±7.5, $p<0.05$) was associated with baseline CAP score. Individuals carrying AA alleles had significantly higher CAP score than those carrying GG (234±34.7dB/m vs. 188 ± 25.3 dB/m). The CAP score decreased post SSB intervention, and the change was significantly associated with rs2228603 in *NCAN* (β=-20.1± 7.6, $p< 0.05$). The T allele carriers showed a greater reduction in CAP score as compared to CC carriers (Mean ± SE -23.3 ± 5.8 dB/m vs. -18.24 ± 43.2 dB/m). This change was, however, not observed when adjusted for age, sex and body composition. Significant associations were also observed between serum HDL and rs1260326 in *GCKR* and triglycerides and rs58542926 in *TMS6F2*. This pilot study shows a potential role for genetics in liver fat changes in response to SSB intervention that warrants a detailed investigation in a larger sample for a longer duration.

View Article Online
DOI: 10.1039/D5FO03248B

INTRODUCTION

Non-communicable diseases like cancer, obesity, diabetes, cardiovascular diseases (CVD) and non-alcoholic fatty liver disease (NAFLD), have become a significant public health concern(1–7). NAFLD, representing a group of disorders including steatosis(2,8) and non-alcoholic steatohepatitis with fibrosis(9,10), has substantially risen in prevalence over the last two decades with the estimated prevalence being 20% among US adults and 25% in young adults(11–13). Over 64 million individuals are believed to have NAFLD with annual medical costs rising to more than $100 billion(14–16). NAFLD is more commonly observed in individuals who have obesity or diabetes and/or have metabolic syndrome, and has been associated with increased cirrhosis, liver-related mortality and hepatocellular carcinoma(17–19).

Unhealthy diet plays a major role in the development of NAFLD(20–22). Fructose, contained in soft drinks, fruit juices and energy drinks, affects many metabolic processes, foremost being an increase in fat accumulation in the liver(23–25) and contributing to the onset and progression of NAFLD(26–29). Fructose is almost entirely metabolized in liver and is rapidly phosphorylated to fructose 1 phosphate by ketohexokinase (KHK) with ATP depletion in parallel(30,31). The fructose 1-phosphate gets metabolized to dihydroxyacetone-phosphate and glyceraldehyde and finally to triglycerides[3-5] which get deposited in liver and leads to NAFLD(29,30,32–34). Although both glucose and fructose affect fat accumulation in the liver, fructose seems to be the more potent stimulator of *de novo* lipogenesis (DNL) than glucose(23,35,36). In population studies, it has been shown that fructose, and not glucose, is associated with increased visceral adiposity(37–40), insulin resistance(26,37,39,40) and hyperuricemia(41–43). Fructose is unique in its effects that it stimulates KHK and thus potentiates its own metabolism. Second, the rate of phosphorylation of fructose by KHK is 10 times higher than the phosphorylation of glucose by glucokinase(44,45). Third, fructose is directly absorbed into portal vein and delivered to the liver without entering the systemic circulation(30,44). Because of this pass through the liver, it is exposed to a much higher fructose load than other tissues. Fourth, fructose activates the lipogenic transcriptional factors, SREBP1c(46–48) and ChREBP(49–51) in the liver, promoting DNL. Finally, fructose tends to deplete liver ATP levels and one of the outcomes is generation of more AMP which get converted to uric acid(23,30,31). Uric acid has been shown to stimulate fat synthesis in the hepatocyte thus pointing to an additional pathway through which fructose can increase liver fat content(30,31).

In addition to diet, genetic factors contribute to the onset and progression of NAFLD(52,53). NAFLD is a complex and heritable phenotype. Family-based studies have reported heritability estimates for NAFLD to be between 20% and 70%(54–57) and genome-wide and candidate gene studies have identified several genes associated with NAFLD(58,59). Few studies have shown that fructose affects lipogenesis in liver in a genotype-specific manner. Davis and colleagues found that Hispanic children with GG genotypes of *PNPLA3* SNP rs738409 were more inclined to accumulate fat in the liver as compared to children with CC or CG genotypes(58). Similarly, another study investigating the effects of

View Article Online
DOI: 10.1039/D5FO03248B

added sugars on liver fat found that individuals with TT of rs1260326 of *GCKR* increased their *de novo* lipogenesis by 44% during an oral fructose + glucose challenge(59). The aim of our study was to determine the role of key NAFLD-related single nucleotide polymorphisms (SNPs) in liver fat response to a 3-week sugar-sweetened beverage (SSB) intake in adolescents and young adults.

EXPERIMENTAL

Study design

This pilot open label trial was conducted at the University of North Carolina at Chapel Hill Nutrition Research Institute's (UNC-NRI). The review protocol is available at Clinical Trials.gov (# NCT03783195). A total of 72 participants aged 12 to 40 were screened. Inclusion criteria included ages between 12-40 years, no history of alcohol abuse (>7 drinks per week for a year), history of fructose intake of < 14 drinks per week and Caucasian ethnicity. Both ethnicity and race affect the deposition of fat in the liver(60). Studies have shown that the tendency to accumulate fat in the liver is higher in Asians and Hispanics as compared to While or Black Americans(61). To avoid differences in liver fat content changes that may be due to ethnic differences, we focused only on one ethnic group (Caucasians) in this pilot study. The exclusion criteria included ages < 12 and > 40 years, pregnant/lactating, known alcohol abuse or fructose intake > 14 drinks per week, not of Caucasian ethnicity, glucose levels > 100 mg/dl if fasting, > 140mg/dl if within 2 hours post meal and > 200 mg/dl if random sample, taking anti-hypertensive, anti-diabetic, uric acid and/or lipid-lowering medications, known diagnosis of diabetes, fructose intolerance, chronic kidney disease, NAFLD or any liver-related disease, hypertriglyceridemia, polycystic ovary syndrome, hypothyroidism, obstructive sleep apnea, hypopituitarism and hypogonadism, and liver fat fraction >5% as per baseline MRI scan. We excluded 54 participants after screening for being in the exclusion criteria, 2 participants dropped out due to taste issues, and 1 participant was excluded after baseline liver MRI scan due to their liver fat fraction being greater than 5%. The mean ages and BMI for the three participants who dropped out after visit 1 were 30 years and 27.5 kg/m^2, respectively. All participants gave written informed consent to the study and its procedures. The study was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill (IRB # 17-3348).

15 participants completed the 3-week study. The study was divided into two visits. Both visits, spaced three weeks apart, followed the same procedure (**Figure 1**). Visit 1 and 2: Following a 12-hour overnight fast, participants arrived at UNC-NRI. They were given a standardized meal for dinner for the previous night and were asked to refrain from drinking. The participants collected their 24-hr urine the day prior to their visit and returned the samples at their visit. After signing the consent form, the participants had their anthropometrics measured. The weight measures of the subjects were taken standing while wearing shoes and light clothing. A stadiometer against the wall was used to measure height, in an upright standing position, to the closest 0.1cm. Their body mass index (BMI kg/m2) was calculated using the height and weight values. A stretch-resistant tape close to the umbilical region was

View Article Online
DOI: 10.1039/D5FO03248B

measured for waist circumference (WC) to the nearest 0.1 inch. Bioelectric impedance analysis (BIA) using Tanita Dual Frequency Total Body Composition Analyzer (DC-430U, Tokyo, Japan) was used to record every subject's body composition. All anthropometric measurements were taken by the same staff member to minimize measurement variation and margin of error. Omron digital blood pressure monitor (HEM907XL, Omron Healthcare Inc., Lake Forest, IL, USA) was used on the right arm of the subjects to measure blood pressure. Two measurements of BP were taken with an interval of 1 min; the average was calculated and used in the statistical analysis. A detailed questionnaire on medical and dietary data was administered to the participants on the day of the visit. Participants were then provided individually packaged packets of powder containing 0.75g/kg body weight of fructose + 0.45g/kg body weight of glucose which approximates to the 60:40 ratio found in regular sodas(62). Participants were instructed to consume the packets of fructose dissolved in 24 oz of water and consume the drink daily for the three-week intervention period. They were also instructed to not consume any other SSBs during the 3-week period. All participants brought back empty packets for compliance checking.

Table 1: SNPs selected for genotyping

SNP	Gene	Risk allele	SNP position	Allele frequency in general population (ensembl)	Allele frequency in our study sample
rs12137855	*LYPLAL1*: Intergenic variant	C	1: 219275036	0.84	0.79
rs1227756	*COL13A1*: Intron variant	G	10: 69828748	0.61	0.43
rs738409	*PNPLA3*: Missense Variant	G	22: 43928847	0.26	0.27
rs887304	*EFCAB4B* : 3' UTR variant	T	12: 3648382	0.14	0.39
rs1260326	*GCKR* : Missense variant	T	2: 27508073	0.29	0.43
rs2854116	*APOC3* : Regulatory region variant	C	11: 116829453	0.55	0.47
rs2645424	*FDFT1* : Intron Variant	A	8: 11826954	0.52	0.30
rs58542926	*TMS6F2*: Stop gained Variant	C	19: 19268740	0.93	0.77
rs2228603	*NCAN* : Missense Variant	T	19: 19219115	0.04	0.13
rs4240624	*PPP1R3B* :Intron Variant	A	8: 9326721	0.89	0.87

SNP selection for genotyping

Ten single nucleotide polymorphisms (SNPs) were selected for genotyping based on their association with NAFLD or liver fat from previous published studies: rs12137855 in *LYPLAL1*, for its influence on

View Article Online
DOI: 10.1039/D5FO03248B

triglyceride lipase in adipose tissue and depalmitoylating calcium-activated potassium channels(63), rs1227756 in *COL13A1,*for its association with connective tissue proliferation(64) and liver injury (65), missense variant rs738409 in *PNPLA3* for its role in energy usage/ storage in adipocytes(63,64), rs887304 in *EFCAB4B* gene for its role in calcium binding and regulation(65), *GCKR* rs1260326 for its involvement in hepatic fat accumulation along with large very low density lipoprotein (VLDL) and triglyceride levels(63,66), the missense variant in *APOC3* gene rs2854116 for its role in hypertriglyceridemia and impact of dietary fat intake and NAFLD(67,68), rs2645424 in *FDFT1* for its role in cholesterol biosynthesis(69), *TMS6F2* gene variant, rs58542926 for its role in post prandial lipemia(70,71), rs2228603 of the *NCAN* gene for its association with increased risk for liver inflammation and fibrosis(72), and rs4240624 of the *PPP1R3B* gene for its association with glycogen metabolism(73,74). The SNP positions and their frequencies are shown in **Table 1**.

Genotyping

DNA was extracted from saliva via an automated nucleic acid extraction platform (Anaprep 12, Biochain, Institute Inc. Newark, CA, USA) using the Anaprep Forensic DNA extraction kit (Biochain Institute Inc. Newark, CA, USA) Concentration and purity of genomic DNA was measured using NanoDrop Spectrophotometer. Genotyping was performed via Taqman® predesigned SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) on Quant Studio 12k Flex Real-time PCR system (Thermo Fisher Scientific, USA).

Liver fat content and stiffness measurement

Transient elastography using Fibroscan® (Echosens, Netherlands) was used to assess fat content in the liver and liver stiffness(75,76). Fibroscan was done at both visits. This is a non-invasive measure that uses ultrasound technology to measure liver stiffness and adiposity. The CAP score, which is measured in decibels per meter (dB/m) is used to grade liver steatosis. A score below 238 dB/m is considered normal. A score between 238 dB/m to 260 dB/m indicates grade 1 (S1) steatosis. A score between 260 dB/m to 290 dB/m is S2 and 290 dB/m to 400 dB/m is S3. As for Emed values, a score between 2 kPa and 7 kPa (F0 -F1) is normal, a score of 7.5 kPa to 10 kPa (F2) is considered moderate scarring 10 kPa to 14 kPa (F3) is considered severe scarring and 14 kPa or higher (F4) is considered cirrhosis. In this study, CAP score was used to indicate the amount of fat in the liver[82,83].

Sample collection, processing and storage

At both visits, a fasting blood and a 24-hr urine sample were collected. A trained phlebotomist collected blood through venous puncture using 6-mL ethylenediaminetetraacetic acid (EDTA)-coated tubes and serum tubes (BD Vacutainer, Becton, Dickinson & Company, Franklin Lakes, NJ, USA). Immediately after collection, EDTA tubes were placed on wet ice and centrifuged at 3000 RPM for 15 min at 4^0C. Serum, plasma, buffy coat and urine samples were aliquoted and stored at -80^0C for further biochemical measurements.

Biomarker measurements

View Article Online
DOI: 10.1039/D5FO03248B

At each visit, serum concentrations of lipids, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglycerides were measured using fluorometric assays (Abcam, Cambridge, MA, USA). Uric acid concentration in serum was measured using fluorometric assays, as per the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA) on BioTek Synergy 2 Multi-Mode plate reader (BioTek, Winooski, VT, USA). All measurements were conducted in duplicate, and their coefficient of variation was less than 10%.

Statistical Analysis

Statistical analyses were performed using STATA software version 19.5 (College Station, TX, USA). The primary outcomes for this study were CAP and Emed scores and the secondary outcomes were body composition measurements (BMI, waist circumference, waist-height ratio, percent body fat, fat mass and fat free mass), and serum concentrations of lipids (total, HDL, VLDL, LDL cholesterol and triglycerides) and uric acid. As a first step, paired t-tests were conducted to determine whether there were any significant changes in variables pre- and post-intervention. Linear regression analysis was performed to determine the association between the ten selected SNPs and baseline values and changes in body composition, liver fat content and serum concentrations of lipids and uric acid. Baseline or changes in liver fat content and other biomarkers were the outcome or dependent variables and SNPs were the predictor or independent variables. All analyses were adjusted for sex, age and baseline concentrations of the variables and waist-height ratio wherever applicable. All results were considered significant at $p < 0.05$.

RESULTS

The descriptive characteristics of participants are listed in **Table 2**. Fifteen males (n = 5) and females (n = 10) participated and completed the 3-week SSB intervention. Their mean BMI and age were 25.03 ± 1.1 Kg/m^2 and 25.53 ± 2.4 years, respectively. At baseline, significant differences were observed between females and males with respect to fat free mass (104.8 ± 3.8 vs 137.7±8 11.2g, $p < 0.05$), and percent body fat (31.5 ± 2.0 vs, 17.0 ± 3.0%, $p < 0.05$). Serum urate concentrations were significantly higher in males than females (7.35 ± 0.4 vs. 6.1 ± 0.3 mg/dl, $p < 0.05$). Baseline CAP score was significantly associated with fat mass ($\beta = 1.2 \pm 0.5$, $p < 0.05$), waist circumference ($\beta = 2.3 \pm 0.9$, $p < 0.05$), and systolic blood pressure ($\beta = 1.6 \pm 0.4$, $p < 0.005$).

Table 2: Descriptive characteristics of participants at baseline

	Female (N = 10)	Male (N = 5)	Total (N = 15)	
	Mean ± SE	Mean ± SE	Mean ± SE	P-value
BMI (kg/m^2)	25.54(1.24)	24(2.19)	25.03(1.07)	0.52
Age (years)	26.60(2.81)	23.40(4.66)	25.53(2.37)	0.54

View Article Online
DOI: 10.1039/D5FO03248B

Percent body fat (%)	31.5 (2.0)	17.0 (3.0)	27.0 (26.0)	0.003
Fat Mass (lbs)	50.29(5.92)	31(8.31)	43.86(5.24)	0.08
Fat-free mass (lbs)	104.77(3.82)	137.68(11.20)	115.74(5.95)	0.003
Waist Circumference (Cm)	87.02(3.33)	89.36(6.33)	87.92(3.04)	0.73
Waist-height ratio	0.42(0.07)	0.50(0.03)	0.44(0.05)	0.43
SBP (mm Hg)	124.00(5.59)	126.00(8.50)	124.67(4.51)	0.84
DBP (mm Hg)	78.50(4.20)	81.40(5.49)	79.47(3.25)	0.69
Emed (kPa)	4.46(0.34)	4.59(0.38)	4.51(0.25)	0.82
CAP score (dB/m)	222.23(13.07)	192.00(12.66)	212.15(10.15)	0.17
Triglycerides (mg/dl)	65.7(17.18)	56(20.24)	62.47(12.92)	0.74
HDL (mg/dl)	34.19(8.29)	24.58(10.17)	30.98(6.39)	0.50
LDL (mg/dl)	126.06(13.30)	138.64(22.32)	130.25(11.21)	0.62
Total cholesterol (mg/dl)	172.38(15.58)	174.42(21.02)	173.73(12.09)	0.97
UA (mg/dl)	6.07(0.34)	7.35(0.41)	6.49(0.30)	0.04

A paired t-test was conducted to detect differences between pre and post intervention anthropometrics, liver fat content, and serum biomarker values (**Table 3**). Most of the biomarkers showed no changes post intervention. The CAP score, however, showed a decreasing, albeit statistically insignificant, trend ($p = 0.07$), contrary to our hypothesis. When data were analyzed with residuals after adjusting for age, sex and waist-height ratio, there was no difference in CAP score between pre and post intervention ($p = 1.00$). Similarly, we observed a decrease in serum urate concentrations after the 3-week intervention which was mitigated after adjusting for the covariates. The change in CAP score was significantly correlated with baseline waist circumference ($r2 = 0.53$, $p < 0.05$) and not with fat mass or systolic blood pressure as observed with baseline CAP score. Although baseline CAP score was not correlated with baseline waist-height ratio, the change in the two variables were significantly correlated ($r2 = 0.71$, $p < 0.005$). Change in Emed score, waist circumference and waist-height ratio were also associated with their baseline values ($p < 0.05$), respectively.

The ten genotyped SNPs were selected based on their links to NAFLD. Their minor allele frequencies ranged between 13 and 47%. Genotype-specific analysis showed that rs1227756, an intronic variant in *COL13A1* ($\beta = -22.4 \pm 7.5$, $p < 0.05$) was associated with baseline CAP score, with individuals carrying AA alleles having significantly higher CAP score (234 ± 34.7 dB/m) as compared to those with GG alleles (188 ± 25.3 dB/m).

Journal Name ARTICLE

View Article Online
DOI: 10.1039/D5FO03248B

Table 3: Anthropometrics and biomarkers pre and post SSB intervention

Variable	Visit 1 (mean ± SE)	Visit 2 (mean ± SE)	P-value
BMI (kg/m^2)	25.03(1.07)	25.13(1.05)	0.27
PBF (%)	0.27(0.03)	0.27(0.03)	0.16
FM (lbs)	43.86(5.24)	44.71(5.24)	0.15
FFM (lbs)	115.74 (5.95)	115.77(6.15)	0.94
Waist circumference (cm)	87.92(3.04)	88.16(2.82)	0.88
Wc/Ht	0.45(0.05)	0.51(0.02)	0.16
SBP (mmHg)	124.67(4.51)	123.07(3.18)	0.75
DBP (mmHg)	79.47(3.25)	79(2.03)	0.88
E_med (kPa)	4.51(0.25)	5.01(0.49)	0.41
CAP score (dB/m)	212.15(10.15)	192.91(13.57)	0.07
Triglycerides (mg/dl)	62.47(12.92)	73.07(21.47)	0.41
HDL (mg/dl)	30.98(6.39)	29.72(5.66)	0.79
LDL (mg/dl)	130.25(11.21)	128.35(11.08)	0.79
Total cholesterol (mg/dl)	173.73(12.09)	172.68(12.95)	0.92
Uric acid (mg/dl)	6.50(0.30)	6.43(0.28)	0.04

At baseline, systolic blood pressure was associated with rs121137855, an intronic variant in *LYPLAL1* (β ± SE- -11.14 ± 4.3, $p < 0.05$). The same SNP was associated with changes in diastolic blood pressure (-9.91 (± 1.80), $p < 0.005$). The CAP score decreased post SSB intervention, and the change was significantly associated with rs2228603, a missense variant in *NCAN* (β=-20.1± 7.6, $p< 0.05$). The T allele carriers showed a greater reduction in CAP score as compared to CC carriers (Mean ± SE -23.3 ± 5.8 dB/m vs. 18.24 ± 43.2 dB/m). Significant associations were also observed between change in serum HDL and rs1260326, a missense variant in *GCKR,* (β ± SE 11.73 ± 4.6, $p < 0.05$), and triglycerides and rs58542926, a stop gained variant in *TMS6F2* (β ± SE 77.1 ± 26.2, $p < 0.05$).

Table 4: Regression analysis (change in variable as dependent variable and SNP as independent variable adjusted for baseline of the variable value, gender, age and waist circumference)

Primary outcome:

Change in variable	SNP Id	Beta coefficient ± SE	P value*	95% CI

View Article Online
DOI: 10.1039/D5FO03248B

CAP_med dBm	rs58542926	22.26 (± 9.61)	0.05	-0.47	44.98
	rs2228603	-20.10 (± 7.59)	0.03	-38.05	-2.16

Secondary outcomes:

Change in variable	SNP Id	Beta coefficient (SE)	P value*	95% CI	
Systolic blood pressure	rs121137855	-11.14 (4.33)	0.04	-21.39	-0.9
	rs1227756	0.62 (4.60)	0.05	-1.44	0.01
Diastolic blood pressure	rs121137855	-9.91 (1.80)	0.001	-14.06	-5.56
HDL	rs1260326	11.73 (4.61)	0.04	0.82	22.63
TAG	rs58542926	77.07 (26.18)	0.02	15.16	138.97
VLDL	rs58542926	15.41 (5.24)	0.02	3.03	27.79

*results with p values < 0.06 shown here

DISCUSSION

The main aim of this study was to determine whether SNPs previously linked with NAFLD tend to associate with CAP score at baseline and CAP score's response to SSB intake. In this study we utilized transient elastography FibroScan, a non-invasive assessment of liver stiffness/fibrosis (Emed) and steatosis (CAP score). These measures have been increasingly used for NAFLD and NASH evaluation of patients over liver biopsy(77–80). The CAP scores present the total attenuation of sound waves, an indirect measure of steatosis(81–83),(84). However, in this study we used CAP scores as a surrogate measure of liver fat content as reported in some previous studies(85,86). We found that CAP score at baseline was positively associated with body composition and systolic blood pressure. Similar results have been shown by other studies(87,88),(89). In a study on NAFLD patients, CAP score was positively correlated with fat mass, waist circumference and waist-height ratio(87). In another study of patients with overweight and obesity, CAP values were positively associated with fat mass, BMI and homeostasis model assessment of insulin resistance (HOMA-IR) (88). Another study in youths, found that individuals with higher CAP score had higher BMI, waist circumference and other fat distribution measures(85).

A CAP score below 238dB/m is considered normal liver fat content (90). In our study, both males and females had a CAP score lower than 238dB/m. We hypothesized that the three-week SSB intervention will increase the liver fat content. In contrast, the CAP scores decreased slightly after the 3-week period. The non-alcohol related fat deposition in the liver is dependent on an individual's age, sex, dietary intake of simple sugars and saturated fats, physical activity and presence of other metabolic disorders such as obesity, insulin resistance and metabolic syndrome (91–93),(94). In our study, we think that the decrease in CAP score may be due to many reasons: participants' low CAP score at baseline, our stringent inclusion criteria with the exclusion of adverse metabolic conditions, young age of the participants, and the participants' activities and healthy lifestyle. This may also explain as to why there was no change in CAP score when the data were adjusted for age, sex and body composition.

View Article Online
DOI: 10.1039/D5FO03248B

Although we had advised the participants to not alter any of their dietary habits, except reduction in SSBs, it is possible that the participants may have cut down their other sources of sugars. Studies under controlled or domicile conditions may reflect the true effect of simple sugars on liver fat content.

Emed score or the measure of fibrosis / liver stiffness where higher values indicate higher levels of liver stiffness or liver tissue scarring. In our study Emed scores were very similar between males and females, and we observed an increase in the mean scores after the fructose intervention which was negligible and non-significant. Like the liver physiological measures, anthropometrics and body composition measures such as percent body fat and fat mass showed no significant changes. There were significant differences in percent body fat and fat mass measures between males and females at baseline. Studies have shown that the response to fructose intervention is different in males and females (95,96). Males are usually reported to have adverse metabolic effects such as insulin resistance, high blood pressure and hyperlipidemia in response to increased fructose intake(96–98). Females, although higher in fat mass and percent body fat, tend not to have higher metabolic adversities compared to males(96,97). This may be due to the role of hormones such as estrogen in lipid metabolism and storage(99).

Fructose metabolism inherently generates uric acid (41), and hyperuricemia has been linked to the onset and progression of NAFLD(100–102). In a previous fructose response study in our lab, we found that fructose intervention caused serum uric acid level to spike and did not return to the baseline levels until 150 minutes after the intake of fructose(42,43). In this trial we wanted to understand the long-term effects of SSB on adiposity and uric acid. Examination of 3-week fructose exposure on serum urate levels in this study did not show any significant change in post intervention compared to baseline. However, we did find that males had a higher serum uric acid level than females at baseline which is consistent with other studies(42,43). This finding again highlights the difference in uric acid metabolism in males and females highlighting protective effects against hyperuricemia in pre-menopausal females (103,104).

In addition to sex-specific differences, interindividual variability - particularly genetic variation - affects hepatic lipid response to fructose intake(105). In this study we explored and found few potential links between NAFLD-related SNPs and liver fat content and serum lipids. The SNP rs121137855 of the *LYPLAL1* gene was related to systolic blood pressure especially the homozygous T genotype. This gene has been studied for metabolic traits such as fat distribution, obesity, and hypertension(63,106). Other SNPs rs2605100 and rs4846567 in the same gene, *LYPLAL1,* have been found to be associated with the above-mentioned phenotypes and appetite suppression in Japanese population(107,108). Another SNP rs2605100 in *LYPLAL1*was found to be associated with high blood pressure in a cohort of Chinese children(109). These findings suggest that *LYPLAL1* has several variants that could influence blood pressure and thereby cardiovascular function.

View Article Online
DOI: 10.1039/D5FO03248B

SNP rs1227756 of the *COL13A1* gene was associated with increased systolic blood pressure (A/A genotype). This SNP has been studied in relation to lobular inflammation in Caucasian women(110). SNP rs2854116 in *APOC3* gene has been significantly associated with decreased diastolic blood pressure, especially in individuals with homozygous C genotype. Although this gene has been traditionally studied for its role in liver fat and dyslipidaemia(111–113), our results suggest a novel role in blood pressure regulation.

The other interesting link we found was between SNP rs58542926 and CAP values. Individuals with T allele of rs58542926 is in the *TM6SF2* gene has been observed to have lower CAP values. But in a previous study conducted in Hans Chinese population the same allele was associated with increased CAP scores and increased risk for NAFLD(114) . Although in another meta-analysis study of different populations including Chinese, the T allele of rs58542926 has been shown to be associated with lower lipid profile and protective against CVD risk(115). Combined with findings from our study which is 100% Caucasian, the SNP seems to take on a dual and opposite role depending on the ethnicity of the study population.

Individuals with homozygous C allele and homozygous T allele of rs2228603 has been observed to have lower CAP values. This SNP is in the *NCAN* gene and has shown a strong association with CAP score, with T alleles being linked to greater reduction in CAP score. In a previous study that consisted of European descent Caucasian and old order Amish population, the SNP's T allele was associated with increased risk of hepatic fat accumulation(116). In another study involving Chinese population, this same allele was associated with high level of HDL and also increased level of alkaline phosphatase showing opposite dual effects(117) . Interestingly in a study conducted in 80% female bariatric patient cohort, rs2228603 T was associated with increased risk of steatosis(72).

We also found relation between genetic variants and changes in serum HDL and triglycerides. The SNP rs1260326 in *GCKR* was associated with HDL in our study. In a children study, this SNP was associated with liver fat content as measured by MRI(118). Most studies reported T allele carriers to have lower levels of HDL which are in contrast to what we observed in our study(118–120). We found T allele carriers to have higher HDL levels than CC carriers. We found similar differences with respect to our association of rs58542926 in *TM6SF2* with serum triglycerides. Other studies reported T allele to be associated with lower triglycerides(121,122). Although we found higher concentrations in T carriers at baseline, their triglyceride levels decreased after the 3-week SSB intervention while CC carriers increased their triglycerides. This indicates that T allele may have a protective effect on serum triglycerides in our sample. The SNP rs738409 in *PNPLA3* has been linked to liver fat in many studies but we did not find its association either with the baseline levels or its changes.

There are a few limitations to the study which may need to be addressed in future studies. The sample of 15 is small for any type of clinical trial. Secondly, each genotype carriers were limited to 3-5 participants, and third, 3 weeks may be too short of a duration to observe significant changes in lipid

View Article Online
DOI: 10.1039/D5FO03248B

profile such as LDL-C, HDL-C, total cholesterol etc. It is also possible that short-term fructose exposure might cause biochemical changes in hepatocytes without reaching the threshold for measurable fat accumulation. Also, anthropometrics such as weight gain and fat redistribution typically require more than 3 weeks to show significant changes. Underlying hormonal changes such as leptin, adiponectin, cortisol and other metabolic hormones do not significantly shift with the limited duration of SSB intervention. Also, the dosage of fructose might be too low to elicit measurable changes within this timeframe, as our dose is equivalent to two 12-ounce soda cans. The results may not be generalizable across all ethnic groups. And moreover, all participants had low or normal CAP scores at baseline (non-steatotic range) which may limit the sensitivity of CAP score. However, the key strengths of the study are its stringent inclusion criteria and homogenous sample, and that it is one of the very few that studied the association of genetic variants on changes in CAP score in response to a SSB ingestion in an ethnically homogenous population.

CONCLUSIONS

In summary, a 3-week SSB intervention did not affect the liver fat content or the liver fat markers in our young adult population. Genetic heterogeneity is another important puzzle piece in NAFLD to understand individual variability in disease susceptibility and progression. This study unlocks the possible role of SNPs that may influence the NAFLD onset and progression to better understand the role of genetics in this disease. It also provides pilot data for conducting larger studies where genetically susceptible groups could be identified and their response to nutrient intake can be measured. Together this could implement novel treatment, management and prevention strategies for NAFLD.

AUTHOR CONTRIBUTIONS

Conceptualization, VSV; methodology, FTJ, KN, KW, LRG, BCN, BBM; software, SSV, FTJ, VSV; formal analysis, SSV., FTJ, VSV; investigation, FTJ, SSV, VSV; resources, VSV; writing original draft preparation, FTJ, SSV, VSV; writing -review and editing, FTJ, SSV, LRG, VSV; project administration, VSV; funding acquisition, VSV. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

There are no conflicts to declare.

DATA AVAILABILITY

Data supporting the findings of this study are not publicly available due to privacy reasons, but are available from the corresponding author upon reasonable request with the corresponding author.

ACKNOWLEDGEMENTS

We thank all our participants whose contributions and cooperation made this research a reality.
This trial is registered with clinicaltrials.gov (NCT03783195). This research was supported by the UNC Nutrition Obesity Research Center (NORC) NIH/NIDDK/P01DK056350, Trans-NORC complementary pilot grant award, NIH/NIDDK/P30DK056336-16S1 and UNC Nutrition Research Institute funds.

View Article Online
DOI: 10.1039/D5FO03248B

FIGURE LEGEND

Figure 1: Consort diagram of the intervention study

Figure 2: Mechanism of dietary sugars and fat accumulation

ATP- Adenosine triphosphate; AMP- Adenosine monophosphate; *GCKR* – Glucokinase regulatory protein; ChREBP – Carbohydrate response element binding protein; SREBP- Sterol regulatory element binding protein; *PNPLA3* – Patatin-like phospholipase domain -containing protein 3; *TM6SF2* – Transmembrane 6 superfamily member 2; *APOC3* – Apolipoprotein C-III; *FDFT1* – Farnesyl-diphosphate farnesyltransferase 1; *LYPLAL1* - Lysophopholipase - like1 ; *PPP1R3B* – Protein phosphatase 1 regulatory subunit 3B; *EFCAB4B* – *EF* hand calcium binding domain containing protein 4B; *NCAN* – Neurocan; *COLI3A1* – Collagen type III alpha 1 chain

Abbreviations

ACC- acetyl coA carboxylase
ALP- alkaline phosphatase
ALT – alanine transaminase
AMP- Adenosine monophosphate
APOC3 – Apolipoprotein C-III
AST- Aspartate transaminase
ATP- Adenosine triphosphate
BMI – body mass index
CAP – controlled attenuation parameter
ChREBP – Carbohydrate response element binding protein
COLI3A1 – collagen type III alpha 1 chain
CVD – cardiovascular disease
DBP- diastolic blood pressure
DNL- de novo lipogenesis
EFCAB4B – EF hand calcium binding domain containing protein 4B
FAS – fatty acid synthase
FDFT1 – farnesyl-diphosphate farnesyltransferase 1
FLFS – fructose liver fat study
GCKR – Glucokinase regulatory protein
GGT – gamma glutamyl transferase
GLUT 2 – glucose transporter protein 2
GLUT 4 – glucose transporter protein 4
HDL- high-density lipoprotein

Journal Name ARTICLE

View Article Online
DOI: 10.1039/D5FO03248B

LDL- low-density lipoprotein

LYPLAL1 - lysophopholipase - like1

NAFLD – Nonalcoholic fatty liver disease

NASH – non-alcoholic steatosis

NCAN – neurocan

PNPLA3 – patatin-like phospholipase domain -containing protein 3

PPP1R3B – protein phosphatase 1 regulatory subunit 3B

PWV – pulse wave velocity

SBP- systolic blood pressure

SNP- single nucleotide polymorphism

SREBP- Sterol regulatory element binding protein

TAG – triacylglycerols

TC – total cholesterol

TM6SF2 – transmembrane 6 superfamily member 2

UA – uric acid

VLDL – very low- density lipoprotein

WC – waist circumference

MAFLD - metabolic dysfunction-associated fatty liver disease

KHK – ketohexokinase

SSB- sugar sweetened beverage

CITATIONS

View Article Online
DOI: 10.1039/D5FO03248B

1. Lazarus JV, Mark HE, Villota-Rivas M, Palayew A, Carrieri P, Colombo M, et al. The global NAFLD policy review and preparedness index: Are countries ready to address this silent public health challenge? J Hepatol. 2022 Apr;76(4):771–80.

2. Rajan V, Das A, Venkatachalam J, Lohani KK, Lahariya C. Managing Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD) in Primary Care Settings: A Review. Prev Med Res Rev. 2025 July;2(4):183–91.

3. Crane H, Gofton C, Sharma A, George J. MAFLD: an optimal framework for understanding liver cancer phenotypes. J Gastroenterol. 2023 Oct;58(10):947–64.

4. Zhou XD, Targher G, Byrne CD, Somers V, Kim SU, Chahal CAA, et al. An international multidisciplinary consensus statement on MAFLD and the risk of CVD. Hepatol Int. 2023 Aug;17(4):773–91.

5. Guo Z, Wu D, Mao R, Yao Z, Wu Q, Lv W. Global burden of MAFLD, MAFLD related cirrhosis and MASH related liver cancer from 1990 to 2021. Sci Rep [Internet]. 2025 Feb 27 [cited 2025 July 27];15(1). Available from: https://www.nature.com/articles/s41598-025-91312-5

6. Ramírez-Mejía MM, Díaz-Orozco LE, Barranco-Fragoso B, Méndez-Sánchez N. A Review of the Increasing Prevalence of Metabolic-Associated Fatty Liver Disease (MAFLD) in Children and Adolescents Worldwide and in Mexico and the Implications for Public Health. Med Sci Monit [Internet]. 2021 Aug 30 [cited 2025 July 27];27. Available from: https://www.medscimonit.com/abstract/index/idArt/934134

7. Pipitone RM, Ciccioli C, Infantino G, La Mantia C, Parisi S, Tulone A, et al. MAFLD: a multisystem disease. Ther Adv Endocrinol Metab [Internet]. 2023 Jan [cited 2025 July 27];14. Available from: https://journals.sagepub.com/doi/10.1177/20420188221145549

8. Byrne CD, Armandi A, Pellegrinelli V, Vidal-Puig A, Bugianesi E. Metabolic dysfunction-associated steatotic liver disease: a condition of heterogeneous metabolic risk factors, mechanisms and comorbidities requiring holistic treatment. Nat Rev Gastroenterol Hepatol. 2025 May;22(5):314–28.

9. Yamamura S, Eslam M, Kawaguchi T, Tsutsumi T, Nakano D, Yoshinaga S, et al. MAFLD identifies patients with significant hepatic fibrosis better than NAFLD. Liver Int. 2020 Dec;40(12):3018–30.

10. Cheung JTK, Zhang X, Wong GL, Yip TC, Lin H, Li G, et al. MAFLD fibrosis score: Using routine measures to identify advanced fibrosis in metabolic-associated fatty liver disease. Aliment Pharmacol Ther. 2023 Dec;58(11–12):1194–204.

11. Habibullah M, Jemmieh K, Ouda A, Haider MZ, Malki MI, Elzouki AN. Metabolic-associated fatty liver disease: a selective review of pathogenesis, diagnostic approaches, and therapeutic strategies. Front Med. 2024;11:1291501.

12. Xie ZQ, Li HX, Wang BK, Yang ZM, Zhang ZY, Tan WL, et al. Trends in prevalence and all-cause mortality of metabolic dysfunction-associated fatty liver disease among adults in the past three decades: Results from the NHANES study. Eur J Intern Med. 2023 Apr;110:62–70.

13. Li W, Ng CH, Quek J, Chan KE, Tan C, Zeng RW, et al. The growing prevalence of nonalcoholic fatty liver disease (NAFLD), determined by fatty liver index, amongst young adults in the United States. A 20-year experience. Metab Target Organ Damage. 2022;2(4):19.

View Article Online
DOI: 10.1039/D5FO03248B

14. Le P, Tatar M, Dasarathy S, Alkhouri N, Herman WH, Taksler GB, et al. Estimated Burden of Metabolic Dysfunction–Associated Steatotic Liver Disease in US Adults, 2020 to 2050. JAMA Netw Open. 2025 Jan 17;8(1):e2454707.

15. Allen AM, Lazarus JV, Younossi ZM. Healthcare and socioeconomic costs of NAFLD: A global framework to navigate the uncertainties. J Hepatol. 2023 July;79(1):209–17.

16. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018 Jan;15(1):11–20.

17. Kaya E, Yilmaz Y. Metabolic-associated Fatty Liver Disease (MAFLD): A Multi-systemic Disease Beyond the Liver. J Clin Transl Hepatol. 2022 Apr 28;10(2):329–38.

18. Dao AD, Nguyen VH, Ito T, Cheung R, Nguyen MH. Prevalence, characteristics, and mortality outcomes of obese and nonobese MAFLD in the United States. Hepatol Int. 2023 Feb;17(1):225–36.

19. He Y, Yao N, Tian F, Liu L, Lin X, Chen X, et al. Prevalence and risk factors of MAFLD and its metabolic comorbidities in community-based adults in China: A cross-sectional study. Diabetes Metab Syndr Clin Res Rev. 2024 Mar;18(3):102973.

20. Gillespie J. "You Are What You Eat": The Role of Dietary Macronutrients and Micronutrients in MAFLD. Clin Liver Dis. 2021 Aug;18(2):67–71.

21. Yao N, Yang Y, Li X, Wang Y, Guo R, Wang X, et al. Effects of Dietary Nutrients on Fatty Liver Disease Associated With Metabolic Dysfunction (MAFLD): Based on the Intestinal-Hepatic Axis. Front Nutr [Internet]. 2022 June 17 [cited 2025 July 27];9. Available from: https://www.frontiersin.org/articles/10.3389/fnut.2022.906511/full

22. Li T, Zhao J, Cao H, Han X, Lu Y, Jiang F, et al. Dietary patterns in the progression of metabolic dysfunction-associated fatty liver disease to advanced liver disease: a prospective cohort study. Am J Clin Nutr. 2024 Sept;120(3):518–27.

23. Softic S, Cohen DE, Kahn CR. Role of Dietary Fructose and Hepatic De Novo Lipogenesis in Fatty Liver Disease. Dig Dis Sci. 2016 May;61(5):1282–93.

24. Softic S, Gupta MK, Wang GX, Fujisaka S, O'Neill BT, Rao TN, et al. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. J Clin Invest. 2017 Nov 1;127(11):4059–74.

25. Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C, et al. Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. J Hepatol. 2018 May;68(5):1063–75.

26. Dekker MJ, Su Q, Baker C, Rutledge AC, Adeli K. Fructose: a highly lipogenic nutrient implicated in insulin resistance, hepatic steatosis, and the metabolic syndrome. Am J Physiol-Endocrinol Metab. 2010 Nov;299(5):E685–94.

27. Choi Y, Abdelmegeed MA, Song BJ. Diet high in fructose promotes liver steatosis and hepatocyte apoptosis in C57BL/6J female mice: Role of disturbed lipid homeostasis and increased oxidative stress. Food Chem Toxicol. 2017 May;103:111–21.

28. Fan Y, Zhang Y, Chen C, Ying Z, Su Q, Li X, et al. Fasting serum fructose is associated with metabolic dysfunction-associated fatty liver disease: A prospective study. Hepatol Res. 2023 June;53(6):479–88.

View Article Online
DOI: 10.1039/D5FO03248B

29. Drożdż K, Nabrdalik K, Hajzler W, Kwiendacz H, Gumprecht J, Lip GYH. Metabolic-Associated Fatty Liver Disease (MAFLD), Diabetes, and Cardiovascular Disease: Associations with Fructose Metabolism and Gut Microbiota. Nutrients. 2021 Dec 27;14(1):103.

30. Muriel P, López-Sánchez P, Ramos-Tovar E. Fructose and the Liver. Int J Mol Sci. 2021 June 28;22(13):6969.

31. Abdelmalek MF, Lazo M, Horska A, Bonekamp S, Lipkin EW, Balasubramanyam A, et al. Higher dietary fructose is associated with impaired hepatic adenosine triphosphate homeostasis in obese individuals with type 2 diabetes. Hepatology. 2012 Sept;56(3):952–60.

32. Herman MA, Birnbaum MJ. Molecular aspects of fructose metabolism and metabolic disease. Cell Metab. 2021 Dec 7;33(12):2329–54.

33. Gómez-Zorita S, Milton-Laskibar I, Macarulla MT, Biasutto L, Fernández-Quintela A, Miranda J, et al. Pterostilbene modifies triglyceride metabolism in hepatic steatosis induced by high-fat high-fructose feeding: a comparison with its analog resveratrol. Food Funct. 2021;12(7):3266–79.

34. Lodge M, Dykes R, Kennedy A. Regulation of Fructose Metabolism in Nonalcoholic Fatty Liver Disease. Biomolecules. 2024 July 13;14(7):845.

35. Lustig RH. Fructose and Nonalcoholic Fatty Liver Disease. J Calif Dent Assoc. 2016 Oct 1;44(10):613–7.

36. Geidl-Flueck B, Gerber PA. Fructose drives de novo lipogenesis affecting metabolic health. J Endocrinol [Internet]. 2023 Feb 8 [cited 2025 July 27];257(2). Available from: https://joe.bioscientifica.com/view/journals/joe/257/2/JOE-22-0270.xml

37. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest. 2009 May 1;119(5):1322–34.

38. Tappy L, Lê KA. Metabolic Effects of Fructose and the Worldwide Increase in Obesity. Physiol Rev. 2010 Jan;90(1):23–46.

39. Silbernagel G, Machann J, Unmuth S, Schick F, Stefan N, Häring HU, et al. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. Br J Nutr. 2011 July 14;106(1):79–86.

40. Kovačević S, Brkljačić J, Vojnović Milutinović D, Gligorovska L, Bursać B, Elaković I, et al. Fructose Induces Visceral Adipose Tissue Inflammation and Insulin Resistance Even Without Development of Obesity in Adult Female but Not in Male Rats. Front Nutr [Internet]. 2021 Nov 11 [cited 2025 July 27];8. Available from: https://www.frontiersin.org/articles/10.3389/fnut.2021.749328/full

41. A SARL MCMULLEN, 53 rue Blanquerie, 11300 Limoux, France, K. McMullen M. Fructose Increases Uric Acid Contributing to Metabolic Syndrome - Herbal, Nutritional and Dietary Strategies to Reduce Uric Acid. OBM Integr Complement Med. 2018 Sept 28;3(3):1–1.

42. Zhang X, Mass BB, Talevi V, Hou R, North KE, Voruganti VS. Novel Insights into the Effects of Genetic Variants on Serum Urate Response to an Acute Fructose Challenge: A Pilot Study. Nutrients. 2022 Sept 28;14(19):4030.

View Article Online
DOI: 10.1039/D5FO03248B

43. Vennam SS, Talevi V, Venkataraman G, Syed RA, Zhang X, Mass BB, et al. A Pilot Study to Evaluate the Role of Obesity and Genetic Variants in Serum C-Reactive Protein Response to an Acute Fructose Load. Lifestyle Genomics. 2025 Feb 27;18(1):64–75.

44. Patel C, Sugimoto K, Douard V, Shah A, Inui H, Yamanouchi T, et al. Effect of dietary fructose on portal and systemic serum fructose levels in rats and in $KHK^{-/-}$ and $GLUT5^{-/-}$ mice. Am J Physiol-Gastrointest Liver Physiol. 2015 Nov 1;309(9):G779–90.

45. Khitan Z, Kim DH. Fructose: A Key Factor in the Development of Metabolic Syndrome and Hypertension. J Nutr Metab. 2013;2013:1–12.

46. Mastrocola R, Nigro D, Chiazza F, Medana C, Dal Bello F, Boccuzzi G, et al. Fructose-derived advanced glycation end-products drive lipogenesis and skeletal muscle reprogramming via SREBP-1c dysregulation in mice. Free Radic Biol Med. 2016 Feb;91:224–35.

47. Aragno M, Tomasinelli CE, Vercellinatto I, Catalano MG, Collino M, Fantozzi R, et al. SREBP-1c in nonalcoholic fatty liver disease induced by Western-type high-fat diet plus fructose in rats. Free Radic Biol Med. 2009 Oct 1;47(7):1067–74.

48. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002 May;109(9):1125–31.

49. Iizuka K. Recent Progress on Fructose Metabolism-Chrebp, Fructolysis, and Polyol Pathway. Nutrients. 2023 Apr 5;15(7):1778.

50. Lee HJ, Cha JY. Recent insights into the role of ChREBP in intestinal fructose absorption and metabolism. BMB Rep. 2018 Sept;51(9):429–36.

51. Kim MS, Krawczyk SA, Doridot L, Fowler AJ, Wang JX, Trauger SA, et al. ChREBP regulates fructose-induced glucose production independently of insulin signaling. J Clin Invest. 2016 Nov 1;126(11):4372–86.

52. Dong R, Tian T, Ming C, Zhang R, Xue H, Luo Z, et al. Multifaceted environmental factors linked to metabolic dysfunction-associated fatty liver disease: an environment-wide association study. BMC Public Health [Internet]. 2025 Feb 20 [cited 2025 July 27];25(1). Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-025-21930-1

53. Sharma D, Mandal P. NAFLD: genetics and its clinical implications. Clin Res Hepatol Gastroenterol. 2022 Nov;46(9):102003.

54. Gidziela A, Ahmadzadeh YI, Michelini G, Allegrini AG, Agnew-Blais J, Lau LY, et al. A meta-analysis of genetic effects associated with neurodevelopmental disorders and co-occurring conditions. Nat Hum Behav. 2023 Feb 20;7(4):642–56.

55. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011 Aug;34(3):274–85.

56. Garza AL, Lee M, Blangero J, Bauer CX, Czerwinski SA, Choh AC. Genetic correlations between liver fat content, metabolic health, and adiposity distribution in the Fels Longitudinal Study. Nutr Metab Cardiovasc Dis. 2024 July;34(7):1610–8.

View Article Online
DOI: 10.1039/D5FO03248B

57. Kim Y, Lee Y, Lee S, Kim NH, Lim J, Kim YJ, et al. On the Estimation of Heritability with Family-Based and Population-Based Samples. BioMed Res Int. 2015;2015:1–9.

58. Davis JN, Lê KA, Walker RW, Vikman S, Spruijt-Metz D, Weigensberg MJ, et al. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. Am J Clin Nutr. 2010 Dec;92(6):1522–7.

59. Santoro N, Caprio S, Pierpont B, Van Name M, Savoye M, Parks EJ. Hepatic De Novo Lipogenesis in Obese Youth Is Modulated by a Common Variant in the GCKR Gene. J Clin Endocrinol Metab. 2015 Aug 1;100(8):E1125–32.

60. Kubiliun MJ, Cohen JC, Hobbs HH, Kozlitina J. Contribution of a genetic risk score to ethnic differences in fatty liver disease. Liver Int. 2022 Oct;42(10):2227–36.

61. Tota-Maharaj R, Blaha MJ, Zeb I, Katz R, Blankstein R, Blumenthal RS, et al. Ethnic and sex differences in fatty liver on cardiac computed tomography: the multi-ethnic study of atherosclerosis. Mayo Clin Proc. 2014 Apr;89(4):493–503.

62. Walker RW, Dumke KA, Goran MI. Fructose content in popular beverages made with and without high-fructose corn syrup. Nutr Burbank Los Angel Cty Calif. 2014;30(7–8):928–35.

63. Sliz E, Sebert S, Würtz P, Kangas AJ, Soininen P, Lehtimäki T, et al. NAFLD risk alleles in PNPLA3, TM6SF2, GCKR and LYPLAL1 show divergent metabolic effects. Hum Mol Genet. 2018 June 15;27(12):2214–23.

64. Larrieta-Carrasco E, Flores YN, Macías-Kauffer LR, Ramírez-Palacios P, Quiterio M, Ramírez-Salazar EG, et al. Genetic variants in COL13A1, ADIPOQ and SAMM50, in addition to the PNPLA3 gene, confer susceptibility to elevated transaminase levels in an admixed Mexican population. Exp Mol Pathol. 2018 Feb;104(1):50–8.

65. Ye H, Liu W. Transcriptional networks implicated in human nonalcoholic fatty liver disease. Mol Genet Genomics MGG. 2015 Oct;290(5):1793–804.

66. Li J, Zhao Y, Zhang H, Hua W, Jiao W, Du X, et al. Contribution of Rs780094 and Rs1260326 Polymorphisms in GCKR Gene to Non-alcoholic Fatty Liver Disease: A Meta-Analysis Involving 26,552 Participants. Endocr Metab Immune Disord Drug Targets. 2021;21(9):1696–708.

67. Wang J, Ye C, Fei S. Association between APOC3 polymorphisms and non-alcoholic fatty liver disease risk: a meta-analysis. Afr Health Sci. 2020 Dec;20(4):1800–8.

68. Tong M, Wang F. APOC3rs2854116, PNPLA3rs738409, and TM6SF2rs58542926 polymorphisms might influence predisposition of NAFLD: A meta-analysis. IUBMB Life. 2020 Aug;72(8):1757–64.

69. Colak Y, Coskunpinar EM, Senates E, Oltulu YM, Yaylim I, Gomleksiz OK, et al. Assessment of the rs2645424 C/T single nucleotide polymorphisms in the FDFT1 gene, hepatic expression, and serum concentration of the FDFT in patients with nonalcoholic fatty liver disease. Meta Gene. 2018 Dec;18:46–52.

70. Liao S, An K, Liu Z, He H, An Z, Su Q, et al. Genetic variants associated with metabolic dysfunction-associated fatty liver disease in western China. J Clin Lab Anal [Internet]. 2022 Sept [cited 2025 July 27];36(9). Available from: https://onlinelibrary.wiley.com/doi/10.1002/jcla.24626

71. Parry SA, Hodson L. Influence of Dietary Macronutrients on Liver Fat Accumulation and Metabolism. J Investig Med. 2017 Dec;65(8):1102–15.

View Article Online
DOI: 10.1039/D5FO03248B

72. Gorden A, Yang R, Yerges-Armstrong LM, Ryan KA, Speliotes E, Borecki IB, et al. Genetic Variation at NCAN Locus Is Associated with Inflammation and Fibrosis in Non-Alcoholic Fatty Liver Disease in Morbid Obesity. Hum Hered. 2013;75(1):34–43.

73. Stender S, Smagris E, Lauridsen BK, Kofoed KF, Nordestgaard BG, Tybjærg-Hansen A, et al. Relationship between genetic variation at PPP1R3B and levels of liver glycogen and triglyceride. Hepatology. 2018 June;67(6):2182–95.

74. Seidelin AS, Nordestgaard BG, Tybjærg-Hansen A, Stender S. Genetic Variation at*PPP1R3B*Increases Hepatic CT Attenuation and Interacts With Prandial Status on Plasma Glucose. J Clin Endocrinol Metab. 2020 June 1;105(6):1963–72.

75. Cournane S, Browne JE, Fagan AJ. The effects of fatty deposits on the accuracy of the Fibroscan® liver transient elastography ultrasound system. Phys Med Biol. 2012 June 21;57(12):3901–14.

76. Lv S, Jiang S, Liu S, Dong Q, Xin Y, Xuan S. Noninvasive Quantitative Detection Methods of Liver Fat Content in Nonalcoholic Fatty Liver Disease. J Clin Transl Hepatol. 2018 June 28;6(2):217–21.

77. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. Nat Rev Gastroenterol Hepatol. 2013 Nov;10(11):666–75.

78. Bedossa P, Patel K. Biopsy and Noninvasive Methods to Assess Progression of Nonalcoholic Fatty Liver Disease. Gastroenterology. 2016 June;150(8):1811-1822.e4.

79. Ajmera V, Loomba R. Imaging biomarkers of NAFLD, NASH, and fibrosis. Mol Metab. 2021 Aug;50:101167.

80. Troelstra MA, Witjes JJ, Van Dijk A, Mak AL, Gurney-Champion O, Runge JH, et al. Assessment of Imaging Modalities Against Liver Biopsy in Nonalcoholic Fatty Liver Disease: The Amsterdam NAFLD-NASH Cohort. J Magn Reson Imaging. 2021 Dec;54(6):1937–49.

81. Siddiqui MS, Vuppalanchi R, Van Natta ML, Hallinan E, Kowdley KV, Abdelmalek M, et al. Vibration-Controlled Transient Elastography to Assess Fibrosis and Steatosis in Patients With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol. 2019 Jan;17(1):156-163.e2.

82. Tapper EB, Sengupta N, Hunink MMG, Afdhal NH, Lai M. Cost-Effective Evaluation of Nonalcoholic Fatty Liver Disease With NAFLD Fibrosis Score and Vibration Controlled Transient Elastography. Am J Gastroenterol. 2015 Sept;110(9):1298–304.

83. Yoneda M, Honda Y, Nogami A, Imajo K, Nakajima A. Advances in ultrasound elastography for nonalcoholic fatty liver disease. J Med Ultrason. 2020 Oct;47(4):521–33.

84. Berzigotti A. Non-invasive assessment of non-alcoholic fatty liver disease: ultrasound and transient elastography. Rev Recent Clin Trials. 2014;9(3):170–7.

85. Ramírez-Vélez R, Izquierdo M, Correa-Bautista JE, Correa-Rodríguez M, Schmidt-RioValle J, González-Jiménez E, et al. Liver Fat Content and Body Fat Distribution in Youths with Excess Adiposity. J Clin Med. 2018 Dec 7;7(12):528.

86. Isis Trujillo-Gonzalez*, David A. Horita, , Julie Stegall, , Rachel Coble, , Evan M. Paules , Anju A. Lulla, , Emmanuel Baah, et al. Choline and betaine concentrations in plasma predict dietary choline intake in healthy humans: a double-blind randomized control feeding study. [Internet]. Available from: https://www.medrxiv.org/content/10.1101/2025.07.15.25331567v1.full.pdf

View Article Online
DOI: 10.1039/D5FO03248B

87. Arslanow A, Lammert F, Stokes C. Controlled attenuation parameter (CAP) for the quantification of hepatic steatosis: Relation to body composition in patients with nonalcoholic fatty liver disease. Z Für Gastroenterol. 2014 Aug 11;52(08):s-0034-1386146.

88. Li Z, Liu R, Gao X, Hou D, Leng M, Zhang Y, et al. The correlation between hepatic controlled attenuation parameter (CAP) value and insulin resistance (IR) was stronger than that between body mass index, visceral fat area and IR. Diabetol Metab Syndr. 2024 July 9;16(1):153.

89. Douard V, Ferraris RP. Am J Physiol Endocrinol Metab. 2008;295:227–37.

90. Price JC, Dodge JL, Ma Y, Scherzer R, Korn N, Tillinghast K, et al. Controlled attenuation parameter and magnetic resonance spectroscopy-measured liver steatosis are discordant in obese HIV-infected adults. AIDS. 2017 Sept 24;31(15):2119–25.

91. Bertolotti M. Nonalcoholic fatty liver disease and aging: Epidemiology to management. World J Gastroenterol. 2014;20(39):14185.

92. Vancells Lujan P, Viñas Esmel E, Sacanella Meseguer E. Overview of Non-Alcoholic Fatty Liver Disease (NAFLD) and the Role of Sugary Food Consumption and Other Dietary Components in Its Development. Nutrients. 2021 Apr 24;13(5):1442.

93. Bawiskar N, Acharya S, Kumar S. Fatty liver disease - non alcoholic to metabolic - A transition of concepts!! J Fam Med Prim Care. 2024 Aug;13(8):2857–62.

94. Sliz E, Sebert S, Würtz P, Kangas AJ, Soininen P, Lehtimäki T, et al. Hum Mol Genet. 2018;27:2214–23.

95. Tran C, Jacot-Descombes D, Lecoultre V, Fielding BA, Carrel G, Lê KA, et al. Sex differences in lipid and glucose kinetics after ingestion of an acute oral fructose load. Br J Nutr. 2010 Oct 28;104(8):1139–47.

96. Rattanavichit Y, Chukijrungroat N, Saengsirisuwan V. Sex differences in the metabolic dysfunction and insulin resistance of skeletal muscle glucose transport following high fructose ingestion. Am J Physiol-Regul Integr Comp Physiol. 2016 Dec 1;311(6):R1200–12.

97. Couchepin C, Lê KA, Bortolotti M, Da Encarnaçao JA, Oboni JB, Tran C, et al. Markedly Blunted Metabolic Effects of Fructose in Healthy Young Female Subjects Compared With Male Subjects. Diabetes Care. 2008 June 1;31(6):1254–6.

98. Brown CM, Dulloo AG, Yepuri G, Montani JP. Fructose ingestion acutely elevates blood pressure in healthy young humans. Am J Physiol-Regul Integr Comp Physiol. 2008 Mar;294(3):R730–7.

99. Sharma N, Li L, Ecelbarger CM. Sex differences in renal and metabolic responses to a high-fructose diet in mice. Am J Physiol-Ren Physiol. 2015 Mar 1;308(5):F400–10.

100. Gong S, Song J, Wang L, Zhang S, Wang Y. Hyperuricemia and risk of nonalcoholic fatty liver disease: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol. 2016 Feb;28(2):132–8.

101. Fan J, Wang D. Serum uric acid and nonalcoholic fatty liver disease. Front Endocrinol. 2024 Nov 28;15:1455132.

102. Lanaspa MA, Sanchez-Lozada LG, Choi YJ, Cicerchi C, Kanbay M, Roncal-Jimenez CA, et al. Uric acid induces hepatic steatosis by generation of mitochondrial oxidative stress: potential role in fructose-dependent and -independent fatty liver. J Biol Chem. 2012 Nov 23;287(48):40732–44.

View Article Online
DOI: 10.1039/D5FO03248B

103. Liu C, Ruan J, Ruan F, Ding X, Han C, Huang C, et al. Estradiol protects female mice from hyperuricemia induced by PCB138 exposure. Ecotoxicol Environ Saf. 2023 Aug;261:115093.

104. Li G yun, Qian X dong, Ma C ming, Yin F zai. The dose-response relationship between sex hormones and hyperuricemia in different gender: NHANES 2013-2016. Front Endocrinol. 2022 Nov 1;13:1035114.

105. Hou R, Panda C, Voruganti VS. Heterogeneity in Metabolic Responses to Dietary Fructose. Front Genet. 2019;10:945.

106. Bille DS, Banasik K, Justesen JM, Sandholt CH, Sandbæk A, Lauritzen T, et al. Implications of Central Obesity-Related Variants in LYPLAL1, NRXN3, MSRA, and TFAP2B on Quantitative Metabolic Traits in Adult Danes. Mailund T, editor. PLoS ONE. 2011 June 2;6(6):e20640.

107. Hotta K, Kitamoto A, Kitamoto T, Mizusawa S, Teranishi H, So R, et al. Replication study of 15 recently published Loci for body fat distribution in the Japanese population. J Atheroscler Thromb. 2013;20(4):336–50.

108. Bandstein M, Mwinyi J, Ernst B, Thurnheer M, Schultes B, Schiöth HB. A genetic variant in proximity to the gene *LYPLAL1* is associated with lower hunger feelings and increased weight loss following Roux-en-Y gastric bypass surgery. Scand J Gastroenterol. 2016 Sept 1;51(9):1050–5.

109. Gu Z, Hu D, Cui W, Liu H, Zhang C. A clinical study on the factors associated with nasopharyngeal carcinoma among the Chinese population. Exp Ther Med. 2021 Apr;21(4):375.

110. Chalasani N, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, et al. I. Chen J Rotter Gastroenterol. 2010;139:1567-1576 6.

111. Ginsberg HN, Reyes-Soffer G. Is APOC3 the driver of cardiovascular disease in people with type I diabetes mellitus? J Clin Invest. 2019 Oct 1;129(10):4074–6.

112. Hieronimus B, Stanhope KL. Dietary fructose and dyslipidemia: new mechanisms involving apolipoprotein CIII. Curr Opin Lipidol. 2020 Feb;31(1):20–6.

113. Jo G, Kwak SY, Kim JY, Lim H, Shin MJ. Association between Genetic Variant of Apolipoprotein C3 and Incident Hypertension Stratified by Obesity and Physical Activity in Korea. Nutrients. 2018 Oct 30;10(11):1595.

114. Li Y, Liu S, Gao Y, Ma H, Zhan S, Yang Y, et al. Association of TM6SF2 rs58542926 gene polymorphism with the risk of non-alcoholic fatty liver disease and colorectal adenoma in Chinese Han population. BMC Biochem. 2019 Dec;20(1):3.

115. Pirola CJ, Sookoian S. The dual and opposite role of the TM6SF2-rs58542926 variant in protecting against cardiovascular disease and conferring risk for nonalcoholic fatty liver: A meta-analysis. Hepatology. 2015 Dec;62(6):1742–56.

116. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-Wide Association Analysis Identifies Variants Associated with Nonalcoholic Fatty Liver Disease That Have Distinct Effects on Metabolic Traits. McCarthy MI, editor. PLoS Genet. 2011 Mar 10;7(3):e1001324.

117. Wu MJ, Yuan C, Lu LL, An BQ, Xuan SY, Xin YN. Role of NCAN rs2228603 polymorphism in the incidence of nonalcoholic fatty liver disease: a case-control study. Lipids Health Dis. 2016 Dec;15(1):207.

View Article Online
DOI: 10.1039/D5FO03248B

118. Santoro N, Zhang CK, Zhao H, Pakstis AJ, Kim G, Kursawe R, et al. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. Hepatol Baltim Md. 2012 Mar;55(3):781–9.

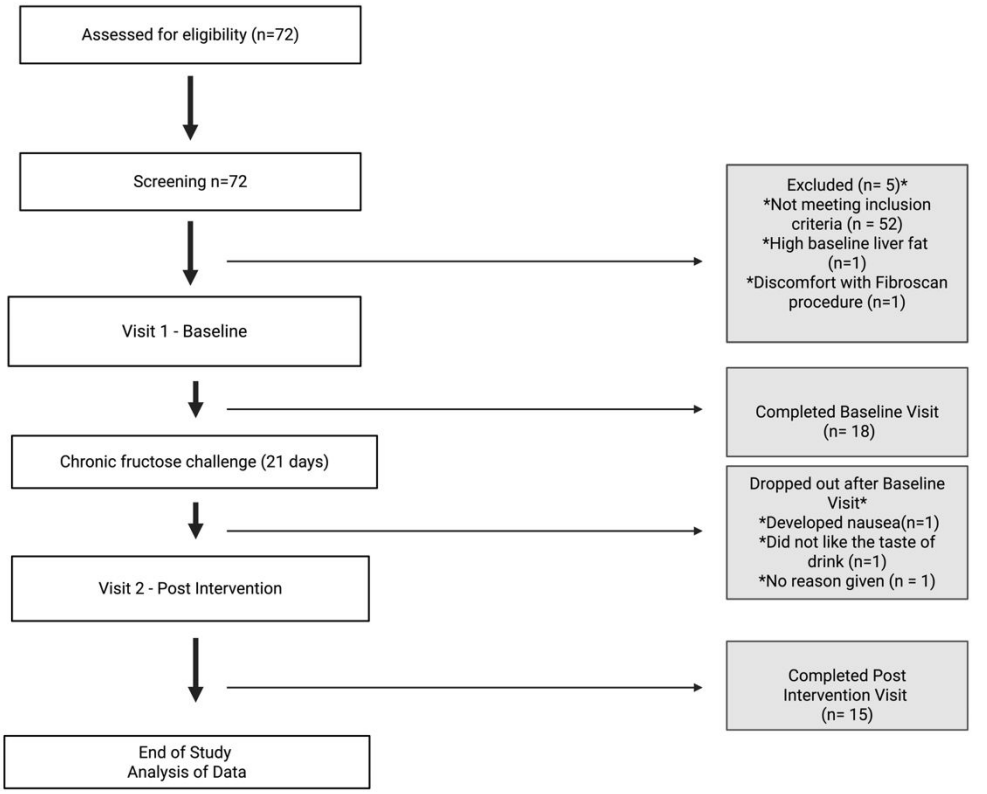
119. Zhou YJ, Hong SC, Yin RX, Yang Q, Cao XL, Chen WX. Polymorphisms in the GCKR are associated with serum lipid traits, the risk of coronary artery disease and ischemic stroke. Int J Clin Exp Med. 2015;8(7):10678–86.

120. Yuan F, Gu Z, Bi Y, Yuan R, Niu W, Ren D, et al. The association between rs1260326 with the risk of NAFLD and the mediation effect of triglyceride on NAFLD in the elderly Chinese Han population. Aging. 2022 Mar 25;14(6):2736–47.

121. Kalafati IP, Dimitriou M, Revenas K, Kokkinos A, Deloukas P, Dedoussis GV. TM6SF2-rs58542926 Genetic Variant Modifies the Protective Effect of a "Prudent" Dietary Pattern on Serum Triglyceride Levels. Nutrients. 2023 Feb 23;15(5):1112.

122. Petta S, Maida M, Grimaudo S, Pipitone RM, Macaluso FS, Cabibi D, et al. TM6SF2 rs58542926 is not associated with steatosis and fibrosis in large cohort of patients with genotype 1 chronic hepatitis C. Liver Int Off J Int Assoc Study Liver. 2016 Feb;36(2):198–204.

Figure 1: Consort diagram of the intervention study

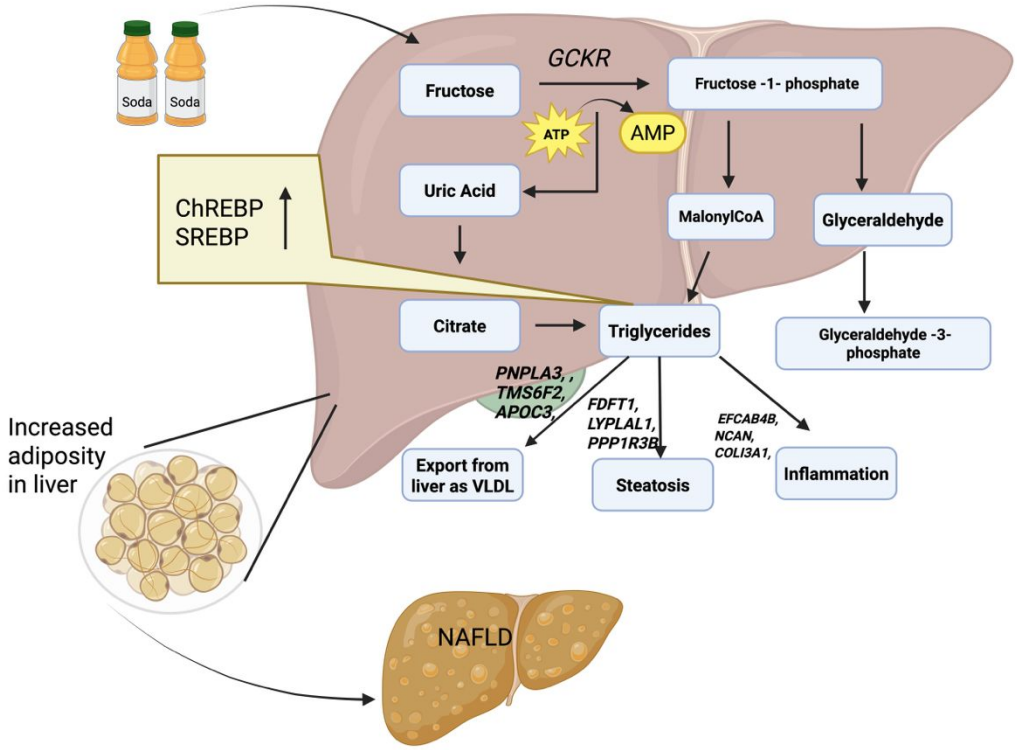


Created with Bio-Render

Journal Name ARTICLE

View Article Online
DOI: 10.1039/D5FO03248B

Figure 2: Mechanism of dietary sugars and fat accumulation



Created with Bio-Render

View Article Online
DOI: 10.1039/D5FO03248B

DATA AVAILABILITY

Data supporting the findings of this study are not publicly available due to privacy reasons, but are available from the corresponding author upon reasonable request with the corresponding author.