Effects of fructose restriction on liver steatosis (FRUITLESS); a double-blind randomized controlled trial

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ABSTRACT

Background: There is an ongoing debate on whether fructose plays a role in the development of nonalcoholic fatty liver disease.

Objectives: The aim of this study was to investigate the effects of fructose restriction on intrahepatic lipid (IHL) content in a double-blind randomized controlled trial using an isocaloric comparator.

Methods: Between March 2017 and October 2019, 44 adult overweight individuals with a fatty liver index $\geq$ 60 consumed a 6-wk fructose-restricted diet (<7.5 g/meal and <10 g/d) and were randomly assigned to supplementation with sachets of glucose (= intervention group) or fructose (= control group) 3 times daily. Participants and assessors were blinded to the allocation. IHL content, assessed by proton magnetic resonance spectroscopy, was the primary outcome and glucose tolerance and serum lipids were the secondary outcomes. All measurements were conducted in Maastricht University Medical Center.

Results: Thirty-seven participants completed the study protocol. After 6 wk of fructose restriction, dietary fructose intake and urinary fructose excretion were significantly lower in the intervention group (difference: -57.0 g/d; 95% CI: -77.9, -39.5 g/d; and -38.8 μmol/d; 95% CI: -91.2, -10.7 μmol/d, respectively). Although IHL content decreased in both the intervention and control groups ($P < 0.001$ and $P = 0.003$, respectively), the change in IHL content was more pronounced in the intervention group (difference: -0.7% point, 95% CI: -2.0, -0.03% point). The changes in glucose tolerance and serum lipids were not significantly different between groups.

Conclusions: Six weeks of fructose restriction per se led to a small, but statistically significant, decrease in IHL content in comparison with an isocaloric control group. This trial was registered at clinical trials.gov as NCT03067428.   *Am J Clin Nutr* 2021;113:391–400.

Keywords: nonalcoholic fatty liver disease, hepatic steatosis, intrahepatic lipid, fructose, nutrition, dietary intervention, randomized controlled trial, glucose metabolism

Introduction

The drastic increase in fructose consumption since the Industrial Revolution has paralleled the current epidemic of obesity and its cardiometabolic complications, such as dyslipidemia, type 2 diabetes, nonalcoholic fatty liver disease (NAFLD), and cardiovascular disease (1–4). Furthermore, fructose overfeeding trials have convincingly been shown to increase the accumulation of fat in the liver (5), the principal organ involved in the metabolism of fructose (6).

There is, however, an ongoing debate on whether fructose is more detrimental for liver health than other simple sugars,

such as glucose ([6], [7]). Whereas there is abundant evidence from animal studies on the prominent role of fructose in, and the putative mechanism by which fructose causes, IHL accumulation ([8–12]), a previous meta-analysis of controlled trials in humans did not show any effects of isocaloric fructose exchange for other carbohydrates on intrahepatic lipid (IHL) content or serum alanine aminotransferase concentrations ([5]). On the other hand, Schwarz et al. ([13]) more recently demonstrated that a 9-d isocaloric fructose-restricted diet successfully reduced IHL content in obese children with high habitual sugar intake, which was mainly attributed to a decrease in *de novo* lipogenesis.

The discrepancies between these studies may be attributed to the amount of fructose that was consumed, i.e., either moderate to high ([5]) or very low ([13]). Fructose can serve as a substrate for de novo lipogenesis *and* stimulate de novo lipogenesis via upregulation of transcription factors that enable the expression of genes involved in *de novo* lipogenesis, such as sterol regulatory element binding protein 1 (SREBP1), carbohydrate response element binding protein (ChREBP), and liver X receptor (LXR) ([14], [15]). On the other hand, even at small ("catalytic") amounts, fructose can also increase hepatic glucose disposal ([16], [17]).

Because the interpretation of the intervention study by Schwarz et al. was limited by its single-arm design and, hence, the lack of a control group, the aim of the current study ("eFfects of fRUctose restrIcTion on LivEr SteatosiS"—FRUITLESS) was to investigate the effects of fructose restriction on IHL content in a double-blind randomized controlled trial, using an isocaloric comparator.

Methods

Study population

Between March 2017 and October 2019, individuals were recruited via advertisements or contacted directly if they had participated in scientific research before and agreed to be contacted for future studies. Inclusion criteria for participation were age $\geq$ 18 y, a high prior chance of having an increased IHL content [i.e., a BMI (in kg/m^2) $\geq$ 28 and a fatty liver index (FLI) $\geq$ 60 ([18], [19])], and a daily fructose intake above the Dutch average [i.e., $\geq$ 45 g/d ([20]) according to a 3-d food journal]. If an individual did not meet the inclusion criterion of BMI $\geq$ 28, but had an elevated FLI, he or she was still considered eligible to participate. Owing to a slow recruitment rate, the inclusion criterion of fructose intake $\geq$ 45 g/d was abandoned (protocol amendment 18 July, 2018). Individuals were excluded from participation in case of (history of) liver disease, (history of) excessive alcohol consumption (i.e., > 3 and > 2 units/d for men and women, respectively), major change in weight (i.e., > 5%) and/or physical activity pattern in the 3 mo before the study, use of glucose-lowering drugs, recent illness, pregnancy and/or lactation, contraindications for MRI, and inability to give informed consent. Eligibility assessment was performed by the clinical researcher of the study (NS).

All participants gave written informed consent before inclusion in the study. The study was performed according to the Declaration of Helsinki ([21]) and approved by the medical ethical committee of Maastricht University Medical Center. The full trial protocol can be accessed via the corresponding author or clinicaltrials.gov (NCT03067428).

Dietary intervention

Because restriction of 1 nutrient (in this case fructose) without affecting other components of the diet is practically impossible, both groups were asked to follow a 6-wk fructose-restricted diet. In addition to this diet, the control group was supplemented with fructose powder aimed at achieving a fructose intake similar to baseline, whereas the intervention group remained fructose-restricted and received glucose powder to allow an isocaloric comparison.

At screening, baseline, and completion of the study, dietary intake was assessed with a 3-d food journal along with a personal interview by the clinical researcher (NS) who was blinded to the intervention assignment. Weight of the food products was provided by the participant or estimated using average quantities per portion. Macro- and micronutrient composition and caloric content of the diet were calculated using the Dutch food composition table ([22]). Average fructose intake was calculated with an extensive database of food products from Wageningen University ([20]). This database was also used for the composition of the fructose-restricted diet throughout the study. If the fructose content of a specific product was not available in the database, a comparable food product or the sucrose content (= 50% fructose) of the specific product was used.

All participants received extensive counseling on the fructose-restricted diet by the clinical researcher of the study (NS) under the supervision of an experienced metabolic dietitian (EMCvdP), both of whom were blinded to the intervention assignment. Participants were allowed to have an ad libitum food intake, as long as the fructose intake per meal and per day was < 7.5 g and < 10 g, respectively. For this, participants were provided a list of permitted and prohibited food products as well as examples of fructose-restricted meals. Furthermore, participants received dietary counseling and diets were adjusted on a weekly basis according to the fructose intake that was assessed by personal interviews. Substantial weight loss/gain was checked every week and (if necessary) corrected with dietary advice. Random assignment to either glucose or fructose supplementation was computer-generated using block sizes of 4 and performed by an independent researcher (MDGVdE). The allocation sequence was concealed in an opaque, sealed envelope and remained blinded to the participants and assessors (NS, PIHGS, MCGJB, CDAS, NCS, PV, and VBS-H) upon completion of all analyses. Supplementation of either glucose or fructose equaled the amount of fructose that was restricted from the diet. In case of a baseline fructose intake below the Dutch average (i.e., 45 g/d), glucose or fructose supplementation was set at 45 g/d. The glucose and fructose powders (indistinguishable in terms of color and odor) were prepacked in identical sachets by an independent researcher (MDGVdE) and distributed to the participants on a weekly basis. Participants were instructed to dissolve the glucose or fructose supplementation in water or food (e.g., yogurt or cottage cheese) for consumption during the 3 main meals. If participants preferred to dissolve the glucose or fructose powder in water, they were urged to consume the supplementation solely during or directly after finishing the meal. Because the 6-wk fructose-restricted diet is

devoid of fruits and vegetables, all participants received vitamin C supplementation (70 mg/d) throughout the entire study to prevent deficiency. No additional requirements were imposed in terms of food/liquid intake or medication use. Participants were asked not to alter their physical activity throughout the study.

Primary outcome measure

Participants were asked to visit the research ward of Maastricht University Medical Center after an 8-h fast and to refrain from alcohol for 3 d before the measurements. Proton magnetic resonance spectroscopy (^{1}H-MRS) was performed to determine IHL content at baseline and completion. All magnetic resonance (MR) measurements were performed on a 3T MR system (Achieva 3T-X, Philips Healthcare) using a 32-channel sense cardiac/torso coil (Philips Healthcare). Owing to morbid (abdominal) obesity, 5 participants were scanned on a wide-bore 1.5 T MR system (Ingenia, Philips Healthcare) at both time points (i.e., baseline and follow-up measurement). A T_2-weighted turbo spin echo MR image was acquired in 3 (axial, coronal, and transversal) orientations and a $20 \times 20 \times 20$-mm voxel was placed in the right hepatic lobe, avoiding vascular structures and edges of the liver, diaphragm, and biliary structures. Special care was taken to ensure that the placement of the voxel was in the same position at both time points. Hepatic lipid spectra were acquired using a PRESS sequence with water suppression (frequency-selective prepulses) using the following MR parameters: repetition time (TR)/echo time (TE) = 4000/32.5 ms, 32 acquisitions with 2 signal averages in each, bandwidth = 2000 Hz, and data points = 2048. In addition, spectra without water suppression were acquired (8 acquisitions with 2 signal averages in each) as an internal reference. The long TR was chosen to let the participant breathe in the rhythm of the measurement, and MR acquisition was performed at the end of expiration. A pressure-sensitive probe was placed on the abdomen of the participant to monitor the breathing pattern throughout the measurement. All MR spectra obtained were individually frequency-aligned and -phased, and lipid-CH_2 and water peaks were fitted to the respective spectrum as described before (23), using a custom-built MATLAB script (MATLAB R2017b, Mathworks). The signal intensities obtained were corrected for T_2 decay using the literature T_2 values of 3T (26.3 ms, 59.1 ms) (24) and 1.5 T (50 ms, 60 ms) (25) for water and lipid, respectively. Finally, the IHL percentage was represented as the area ratio of the CH_2:H_2O peak using the T_2-corrected signal intensities of lipid-CH_2 and water. Because of technical reasons, MRS was not available in 1 participant. The IHL content at both time points was subsequently assessed using mDIXON imaging, which was validated against ^{1}H-MRS in a former study [intraclass correlation coefficient $(ICC)_{agreement} = 0.82$; $P < 0.001$ (26)].

Secondary outcome measures

On the same day as the ^{1}H-MRS measurements, blood was drawn for the determination of serum insulin and lipids (i.e., total, HDL, and LDL cholesterol and triglycerides) and a 2-h 75-g oral-glucose-tolerance test (OGTT) was performed, all exactly as described previously (27). The AUC was calculated as a measure of glucose tolerance. Insulin resistance was estimated with the homeostasis model assessment of insulin resistance (HOMA2-IR) calculator (www.dtu.ox.ac.uk).

Other measurements

All participants filled in a health questionnaire concerning smoking, habitual alcohol consumption, and medical history. Weight was measured in solely underwear, height was assessed with a stadiometer, and waist and hip circumference were determined with a measuring tape at the level of the umbilicus and trochanter major, respectively. Twenty-four-hour urine was collected in preacidified plastic containers at $T = 0$, 2, 4, and 6 wk for measurement of urinary fructose, an objective biomarker of fructose consumption (28), with ultra-performance LC–tandem MS [variation coefficient (VC%) 6.6].

Statistical analysis

For this double-blind randomized controlled trial, sample size calculations showed that 19 individuals/group were required to detect a mean difference of 3% ($\sigma = 3.2$%) in IHL content ($\alpha = 0.05$, $\beta = 0.20$, allocation ratio $n_{\text{intervention group}}$:$n_{\text{control group}} = 1$:1). To account for dropout (15%), the number of individuals was increased to 22/group.

Dichotomous data are expressed as frequencies. Continuous data are presented as median [IQR]. Changes from baseline within and between groups were analyzed with Wilcoxon's Signed Rank test and a Mann–Whitney U test, respectively. The 95% CIs for these changes were calculated according to the Hodges–Lehmann method.

Sensitivity analyses were conducted *1*) to assess the influence of unbalanced randomization, if present, on the primary outcome measure — for this, a 1-way ANCOVA was conducted with the unbalanced variable as a covariate; *2*) to determine the effect of the protocol amendment (i.e., the omission of the fructose intake $\geq$ 45 g/d inclusion criterion) on the primary outcome measure; *3*) to assess the effect of the degree of IHL content at baseline [i.e., above or below the 5.56% cutoff value (25)] on the primary outcome measure — for both analyses, interaction terms were tested in a 1-way ANCOVA; and *4*) to explore a dose–response relation by testing the association between the change in fructose intake and the change in IHL content and by testing for an interaction between the intervention and the change in fructose intake on the change in IHL content (1-way ANCOVA).

A P value < 0.05 was considered statistically significant. All analyses were carried out with IBM SPSS version 25 for Windows (SPSS Inc.).

Results

Randomization and follow-up

Forty-four participants were randomly assigned to the intervention group (fructose-restricted diet plus glucose supplementation) or control group (fructose-restricted diet plus fructose supplementation) (see **Figure 1** for a flowchart). Six participants discontinued participation because of various reasons [i.e., claustrophobia during MR measurements ($n = 2$), nonadherence to the diet (as indicated by the participant) ($n = 1$), immobility
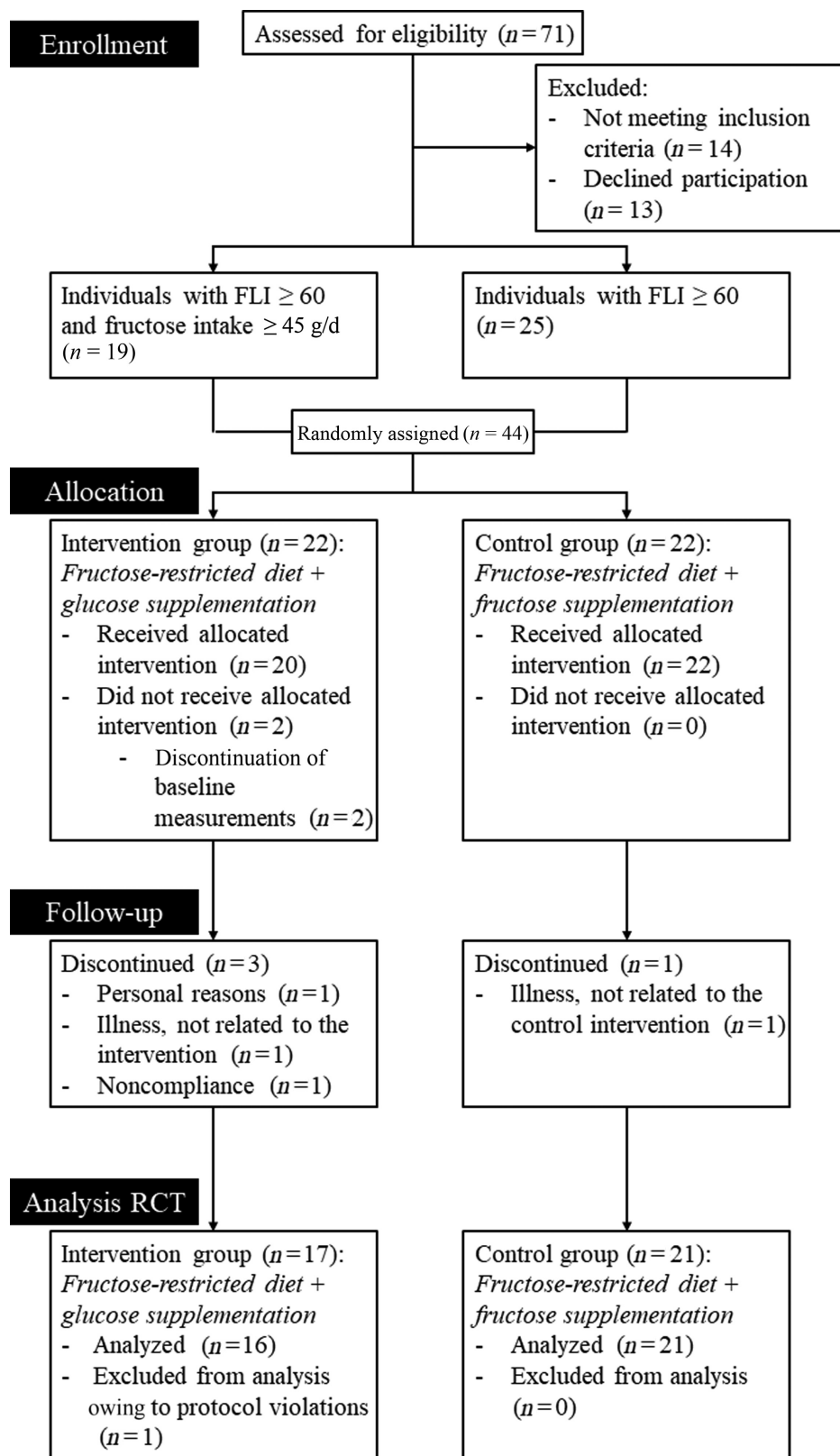
FIGURE 1 Flowchart of study. Seventy-one individuals were assessed for eligibility. Forty-four participants were randomly assigned to the intervention group (fructose-restricted diet plus glucose supplementation) or control group (fructose-restricted diet plus fructose supplementation), of whom 19 were assigned before and 25 after the protocol amendment (i.e., abandonment of the fructose intake $\geq$ 45 g/d inclusion criterion). Six participants discontinued participation because of various reasons. At completion of the study, 1 participant did not appear to meet the inclusion criteria (i.e., FLI $\geq$ 60) and was therefore excluded. Per-protocol analyses were carried out with 16 and 21 participants in the intervention and the control group, respectively. FLI, fatty liver index; RCT, randomized controlled trial.

TABLE 1 General baseline characteristics of the study population[1]

Characteristic	Intervention group ($n = 16$)	Control group ($n = 21$)
Age, y	55 [35–62]	52 [38–62]
Sex, n (M/F)	6/10	6/15
Smoking, % yes	12.5	14.3
Alcohol intake, units/wk	2 [1–5]	3 [0–5]
BMI, kg/m^2	34.1 [28.8–37.3]	31.1 [30.2–35.6]
Waist circumference, cm	117.9 [106.5–128.4]	110.0 [104.3–113.6]
HOMA2-IR	0.84 [0.50–1.37]	0.86 [0.73–1.14]
Serum total cholesterol, mmol/L	5.2 [4.3–5.6]	5.2 [4.2–6.0]
Serum HDL cholesterol, mmol/L	1.2 [1.1–1.3]	1.3 [1.0–1.5]
Serum LDL cholesterol, mmol/L	3.1 [2.3–3.8]	2.7 [2.3–3.8]
Serum triglycerides, mmol/L	1.6 [1.2–1.9]	1.3 [0.9–1.7]
Dietary fructose intake, g/d	42.1 [20.3–73.4]	36.9 [27.1–54.6]
Fatty liver index	94 [74–96]	84 [64–91]
Intrahepatic lipid content, %	4.9 [2.3–10.3]	2.1 [0.9–7.7]

[1] Values are medians [IQRs]. HOMA2-IR, homeostasis model assessment of insulin resistance.

due to ankle distortion ($n = 1$), symptoms related to irritable bowel syndrome (before start of the diet) ($n = 1$), and personal reasons ($n = 1$)]. Upon completion of the study, 1 participant did not appear to meet the inclusion criteria (i.e., FLI $\geq$ 60) and was therefore excluded from the analyses. The individualized supplementation was successfully supplied to and ingested by all participants. Because follow-up of the primary outcome could not be acquired for the 6 individuals who discontinued participation, the intention-to-treat analyses did not differ from the per-protocol analyses, which were carried out with 16 and 21 participants in the intervention group and the control group, respectively. **Table 1** displays baseline characteristics.

Adherence and tolerability of the intervention

Compared with baseline, the habitual dietary intake of fructose decreased drastically in both groups (**Figure 2**A). The median dietary fructose intake after 6 wk of fructose restriction was 1.4 g/d in the intervention group and 2.2 g/d in the control group (change from baseline: −45.7 g/d; 95% CI: −61.4, −29.8 g/d; and −37.9 g/d, 95% CI: −48.3, −28.6 g/d, respectively). The change from baseline was not significantly different between the groups (difference between change from baseline: −6.7 g/d; 95% CI: −24.0, 13.0 g/d). When the supplementation was taken into account, the fructose intake in the control group increased to 49.1 g/d (change from baseline: +12.4 g/d; 95%
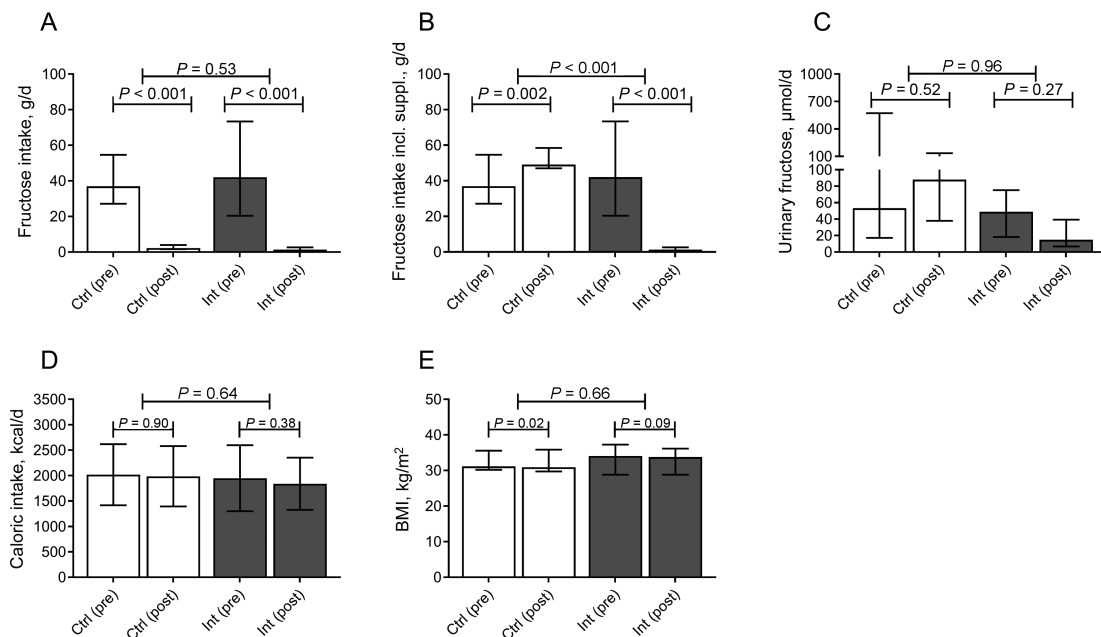

FIGURE 2 Adherence to the intervention. (A) Daily fructose intake, (B) daily fructose intake including supplementation, (C) 24-h urinary fructose concentration, (D) daily caloric intake, and (E) BMI in Ctrl (white bars, $n = 21$) and Int (grey bars, $n = 16$) at baseline (pre) and after completion of the study (post). Data are expressed as median ± IQR. Differences within groups are analyzed with Wilcoxon's Signed Rank test. Differences between groups are analyzed with a Mann–Whitney U test. Ctrl, control group; IHL, intrahepatic lipid; Int, intervention group.

CI: 6.2, 20.8 g/d). This was higher than baseline, because supplementation of fructose (and glucose) was set at 45 g/d in all participants with a baseline fructose intake below the Dutch average (see the Methods section). This change was significantly different from the intervention group (difference between change from baseline: −57.0 g/d; 95% CI: −77.9, −39.5 g/d) (Figure 2B; **Supplemental Figure 1**). The cumulative difference in fructose intake during the full study period — calculated as the individual difference from baseline times the number of days each participant was included in the study — was 1894 g (95% CI: 1727, 2052 g). This difference was also reflected by a significantly different urinary fructose excretion between the intervention and the control group at completion of the study (−38.8 μmol/d; 95% CI: −91.2, −10.7 μmol/d). The change from baseline between the groups was not significantly different, which was mainly attributed to the large variation in urinary fructose concentration at baseline in the control group (Figure 2C).

Caloric intake remained stable throughout the intervention as a consequence of the isocaloric supplementation and did not differ between the 2 groups (Figure 2D). Nevertheless, BMI decreased in both the intervention and control groups (change from baseline: −0.2; 95% CI: −0.5, 0.0; and −0.4; 95% CI: −0.6, −0.1, respectively), a decrease which was not significantly different between both groups (difference between change from baseline: −0.1; 95% CI: −0.3, 0.5) (Figure 2E). No statistically significant effect was observed for macronutrient composition (i.e., carbohydrate, protein, total fat, and saturated fat intake) (**Supplemental Figure 2**A–D). When macronutrient composition was expressed as percentage of total daily energy intake, a significant difference between change from baseline was observed for carbohydrate intake (difference between change from baseline: 7.1%, 95% CI: 0.7%, 14.3%) (**Supplemental Figure 3**A), which appeared to be in exchange for protein intake (difference between change from baseline: −5.9%; 95% CI: −13.3%, 0.4%) (Supplemental Figure 2B).

The intervention was generally well tolerated. Diarrhea (grade 1–2) was reported in 3 and 5 participants in the intervention and the control group, respectively. Only 1 participant in the control group experienced a serious adverse event, which occurred between the screening visit and baseline measurements.

Effect of fructose restriction on IHL content

IHL content decreased in both treatment arms (**Figure 3**A). The reduction in IHL content was significantly greater in the intervention group, albeit small (difference between change from baseline: −0.7%, 95% CI: −2.0%, −0.03%) (Figure 3A). Because baseline BMI and IHL content tended to be higher in the intervention group (Table 1), we subsequently conducted a sensitivity analysis to assess the impact thereof on the primary outcome measure. Addition of baseline BMI and IHL content as covariates to a 1-way ANCOVA did not have substantial effects on the (log-transformed) primary outcome measure (β: −0.06; 95% CI: −0.116, 0.007; $P = 0.08$).

Subgroup analyses did not show significantly different effect sizes between participants with either high or low habitual fructose intake, or high or low baseline IHL content (P-interaction: 0.63 and 0.75, respectively) (**Supplemental Figure 4**). Finally, the change in fructose intake was not associated with the change in IHL content ($P = 0.34$), nor was there an interaction between the intervention and the change in fructose intake on the change in IHL content ($P = 0.72$).

Effect of fructose restriction on glucose tolerance and serum lipid concentrations

Plasma glucose excursions during a 2-h 75-g OGTT were not significantly different between the 2 groups (difference between change from baseline: 32.3; 95% CI: −62.3, 119.0) (Figure 3B). HOMA2-IR was affected neither in the intervention nor in the control group (change from baseline: 0.12; 95% CI: −0.17, 0.51; and 0.06; 95% CI: −0.13, 0.19, respectively), nor was there a difference between the groups (difference between change from baseline: 0.10; 95% CI: −0.21, 0.42). In addition, no effects were observed on serum lipids (Figure 3C–F).

Discussion

In this double-blind randomized controlled trial, we have shown that a 6-wk fructose-restricted diet resulted in a small decrease in IHL content in adult overweight individuals with a high FLI in comparison with an isocaloric comparator. No effects were observed on glucose tolerance or serum lipid concentrations.

Although glucose and fructose are both simple sugars with identical molecular formulas, their metabolic fate differs greatly. In contrast to glucose, which is widely metabolized throughout the body, a proportion of ingested fructose is already cleared in the small intestine, as shown by animal studies (29). After absorption, fructose reaches the portal blood where it is almost entirely taken up by the liver to be converted to glucose, lactate, glycogen, or triglycerides (30). Furthermore, at a low dose, fructose facilitates hepatic glucose disposal by dissociation of the glucokinase (GCK)-glucokinase regulatory protein (GKRP) complex (GCK-GKRP) (16, 31). Dissociation of this complex appears clinically relevant, because carriers of a missense variant in *GCKR* (glucokinase regulatory protein gene) — the gene that encodes a GKRP protein that binds GCK less effectively — are prone to develop NAFLD (32). Thus, although clear metabolic differences between fructose and glucose metabolism exist, such differences cannot be easily extrapolated to net effects on metabolic health, including IHL content, and experiments such as the current trial may provide important insights.

Dose-dependent metabolic pathways such as those aforementioned may also explain the conflicting results of previous clinical trials (5, 13). To date, fructose intervention studies have mainly focused on the role of fructose added to the diet and shown contrasting effects on IHL content (5, 33–36). Recently, Schwarz et al. (13) showed that restriction of fructose from the diet reduced IHL content in obese children and adolescents. The lack of a control group, however, makes it difficult to conclude whether this change in IHL content can be attributed to fructose restriction per se or, alternatively, to the change in the diet in general or other behaviors that are associated with participation in a clinical trial [i.e., the Hawthorne effect (37)].

The current study is the first that we know of to have used a randomized controlled study design in which both the intervention and control groups followed the same
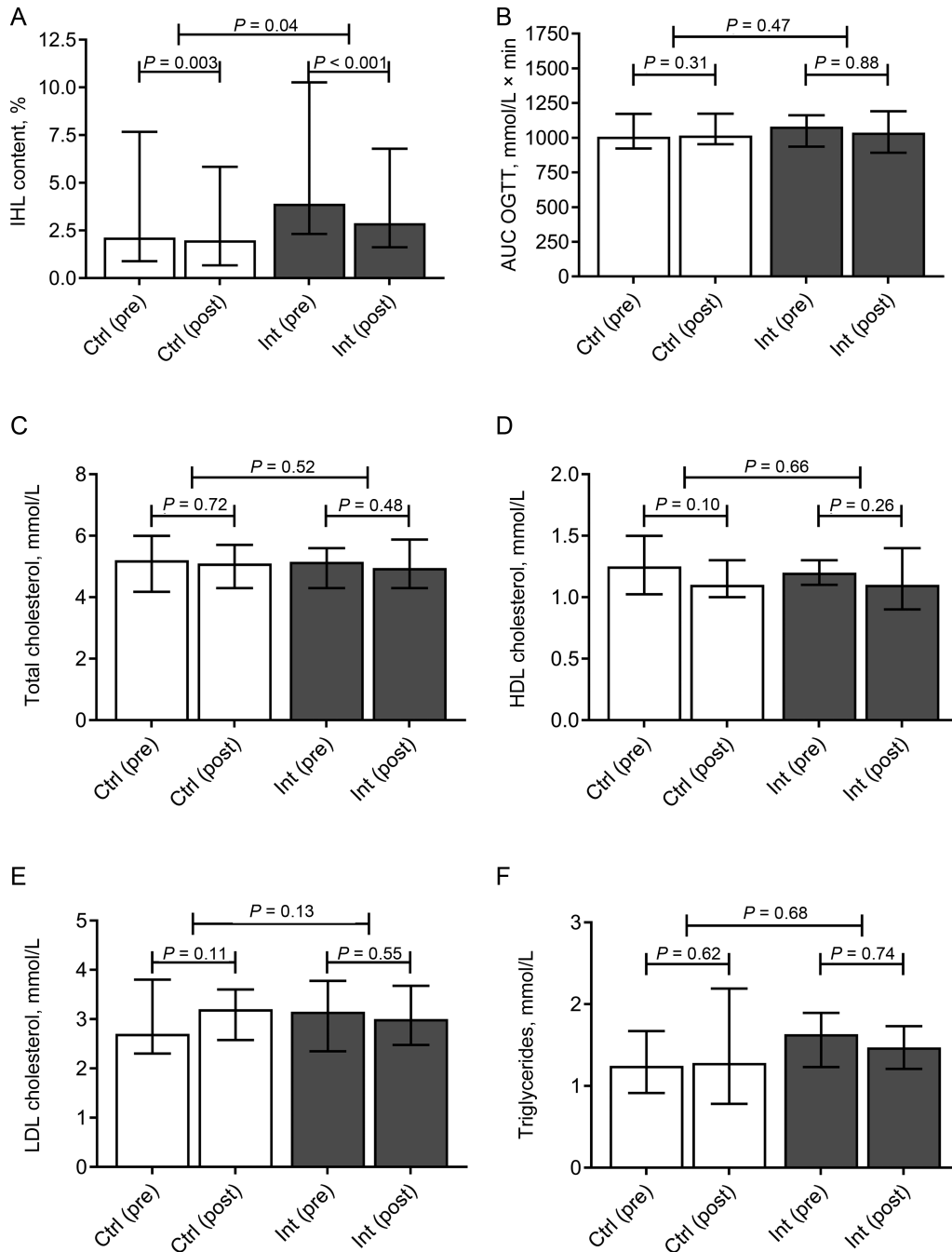
FIGURE 3 IHL content, glucose tolerance, and serum lipid concentrations. (A) IHL content, (B) glucose AUC during an OGTT, (C) serum total cholesterol, (D) serum HDL cholesterol, (E) serum LDL cholesterol, and (F) serum triglycerides in Ctrl (white bars, $n = 21$) and Int (grey bars, $n = 16$) at baseline (pre) and after completion of the study (post). Data are expressed as median ± IQR. Differences within groups are analyzed with Wilcoxon's Signed Rank test. Differences between groups are analyzed with a Mann–Whitney U test. Ctrl, control group; IHL, intrahepatic lipid; Int, intervention group; OGTT, oral-glucose-tolerance test.

fructose-restricted diet. Together with a similar caloric intake in both treatment arms (as a consequence of the isocaloric glucose supplementation in the intervention group), we were able to study the effect of fructose restriction per se. Indeed, the results in the control group show that the diet by itself was already associated with a decrease in IHL content, independently of a change in fructose intake (which was supplemented). More importantly, by comparing both treatment arms we are able to

show that fructose restriction per se reduces IHL content, albeit with a small effect size (0.7% point).

In the present study, we did not observe any effect of fructose restriction on serum lipid concentrations, glucose tolerance, or HOMA2-IR, which was somewhat surprising because these variables have been associated with IHL content (38, 39). A possible explanation might be that the effect of fructose restriction on IHL content was too small to result in a detectable

change in serum lipid concentrations and/or glucose tolerance. In addition, we did not assess insulin sensitivity with the gold-standard method, i.e., a hyperinsulinemic-euglycemic clamp. Another possible explanation might be that the duration of the intervention was too short. Yet, this period is comparable with most other fructose feeding trials, which varied between 3 and 10 wk in duration (40). Moreover, it has been shown that IHL accumulation can resolve within days (13, 41). We, therefore, believe that a 6-wk intervention period — in which the cumulative difference in fructose consumption was almost 2 kg — is sufficient to reach a steady state in these metabolic outcomes.

Our study has strengths and limitations. First, the sample size was relatively small, but should be viewed in perspective of the major dietary restrictions that were imposed. More importantly, our study was adequately powered with an expected dropout rate. Second, we used the FLI as a screening tool for fatty liver (42). Despite a high positive likelihood ratio to rule in fatty liver with an FLI ≥ 60 (43–45), the median IHL content of the study participants was only 3.4%. This was not attributable to regression to the mean, because FLI was high at both the screening and baseline visits (91 and 87, respectively). Nevertheless, sensitivity analysis with stratification for high and low liver fat showed that similar effect sizes were observed in individuals with a high IHL content at baseline. Furthermore, we did not observe a dose–response relation between the change in fructose intake and the change in IHL content. It should, however, be noted that this study was not designed and, hence, not powered to assess a dose–response relation. Third, owing to a slow inclusion rate we decided to abandon the inclusion criterion of fructose intake ≥ 45 g/d. To safeguard a detectable effect on IHL content, the supplementation of either glucose or fructose was increased to 45 g/d in participants with a low habitual fructose intake. Again, a sensitivity analysis did not show any significant difference when we stratified for fructose intake at baseline. Fourth, supplemented fructose may not have the same metabolic effects as fructose-containing food products. The food matrix may play a role in the timing of fructose absorption — in the current study the supplemented fructose was dissolved in water or food — and may cause an interaction between different nutrients. For instance, it has been postulated that that the presence of vitamin C in fruit and vegetables abolishes the toxic effects of fructose (46–48). Fifth, despite major efforts to achieve an isocaloric diet in comparison with the habitual baseline diet of participants, BMI decreased similarly in both the intervention and control groups, which could be explained by the Hawthorne effect.

A major strength of this study is the double-blind randomized controlled design, using an isocaloric comparator. As previously mentioned, both groups followed the same fructose-restricted diet, which is of importance given the differential impact of macronutrients on IHL content (49, 50). Despite this design, we did find some small, probably accidental, differences in carbohydrate intake expressed as percentage of total energy intake (i.e., relatively higher in the intervention group), which tended to be in exchange for protein intake (i.e., relatively lower in the intervention group). Given the previously described effects of carbohydrates and proteins on IHL content (i.e., predisposing and protective, respectively) (49, 50), the effects of these subtle differences in macronutrient composition on IHL content, if any,

have probably mitigated the observed difference in the current study. Finally, not only did we use a 3-d food journal combined with personal interviews to assess fructose intake throughout the study, we were also able to objectively confirm adherence to the study protocol by measuring urinary fructose concentrations, a biomarker of fructose intake (28).

In conclusion, in this double-blind randomized controlled trial, we showed that a 6-wk fructose restriction per se results in a small but statistically significant decrease in IHL content in overweight adult individuals with a high FLI in comparison with an isocaloric control group. No effects were observed on glucose tolerance or serum lipid concentrations.

Data availability

The data sets generated and/or analyzed during the current study are available from the corresponding author on request.

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