Abating abdominal adiposity: Modifiable lifestyle risk factors for visceral and liver fat deposition

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Consumption of Alcoholic and Sugar-Sweetened Beverages is Associated with Increased Liver Fat Content in Middle-Aged Men and Women

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ABSTRACT

Background
Fatty liver is the leading cause of chronic liver diseases and increases the risk of cardiovascular disease. Besides alcohol consumption, energy-containing non-alcoholic beverages may contribute to liver fat accumulation.

Objective
We aimed to study the consumption of alcoholic and non-alcoholic beverages and their mutual replacement in relation to hepatic triglyceride content (HTGC) in middle-aged men and women.

Methods
In this cross-sectional analysis, HTGC was assessed by ^{1}H-MRS. Habitual consumption of alcoholic and non-alcoholic beverages was assessed using a validated food frequency questionnaire. All beverages were converted to standard servings and to percent of total energy intake (En%). We performed linear regression to examine the association of alcoholic and non-alcoholic beverages with HTGC, adjusted for age, sex, smoking, education, ethnicity, physical activity, total energy intake and total body fat. We studied replacement of alcoholic beverages with non-alcoholic beverages per serving/d and per 5 En%/d.

Results
After exclusion of individuals with missing values, 1,966 participants (47% men) were analyzed, with a mean ± SD age of 55 ± 6 years, BMI of 26 ± 4 kg/m^2, and HTGC of 5.7 ± 7.9 %. Each extra alcoholic serving per day was associated with more liver fat (1.09 times, 95% CI: 1.05, 1.12). Replacing 5 En% of alcoholic beverages with milk was associated with less liver fat (0.89 times, 95% CI: 0.81, 0.98), whereas replacement with 5 En% of sugar sweetened beverages was associated with liver fat to a similar extent as the alcoholic beverages (1.00 times, 95% CI: 0.91, 1.09).

Conclusion
In a population-based cohort, consumption of each extra daily alcoholic beverage was associated with more liver fat. In isocaloric replacement, milk was associated with less liver fat, whereas sugar sweetened beverages were equally associated with liver fat. This suggests that intake of alcohol and sugars may contribute to liver fat accumulation.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is often defined as a hepatic triglyceride content of more than 5.56% not due to excessive alcohol consumption [1]. NAFLD covers a broad clinical spectrum, ranging from the most common feature, hepatic steatosis, to non-alcoholic steatohepatitis (NASH), and liver cirrhosis [2], and increases the risk of end-stage liver disease and liver-related and all-cause mortality [3-6]. Although the incidence of NAFLD is underreported and varies widely [7], the prevalence has risen considerably over the last two decades [8] to 14 to 34% of the general population in Europe [9, 10], Asia [11] and the United States of America [7, 11]. The prevalence of NAFLD in obesity might even be as high as 90% [12], possibly due to excessive calorie intake [13]. It is the leading cause of chronic liver diseases worldwide [14], and is also strongly associated with the metabolic syndrome [15] and cardiovascular diseases [16]

Excessive alcohol consumption[17] is a well-established risk factor of both hepatic steatosis (liver fattening) and liver disease. Current guidelines to prevent or reduce liver fat accumulation therefore recommend that heavy alcohol consumption should be discouraged [18]. However, there is much controversy whether moderate alcohol consumption should also be discouraged, as there are studies indicating that light to moderate alcohol consumption might be protective in relation to fatty liver and (extra) hepatic complications [18-23], whereas in a mendelian randomization study it has been suggested there is no beneficial effect of moderate alcohol consumption on the severity of nonalcoholic fatty liver disease [24]. Moreover, it has been shown that liquid food leads to less satiety and more postprandial hunger [25]. Particularly alcohol is very inefficient in activating the satiety mechanism, and consuming alcohol during meals might lead to higher food consumption [26].

In addition, sugar sweetened beverages (SSB), but not diet sodas have been associated with fatty liver [27]. This suggests that energy-containing drinks in general, or specifically dietary sugars may increase liver fat as well [28, 29]. As the relative contributions of different types of non-alcoholic and alcoholic beverages consumption to liver fat accumulation remain unclear, we aimed to directly compare the associations of consumption of alcoholic beverages and non-alcoholic energy-containing and non-energy containing beverages with hepatic triglyceride content in a large sample of the general population. Insight in these associations may contribute to lifestyle guidelines, especially with regard to beverages, for both primary and secondary prevention aims.

METHODS

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study in 6671 individuals aged 45 to 65 years, with an oversampling of persons with a BMI of 27 kg/m^2 or higher. Men and women aged between 45 and 65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI. Detailed information about the study design and data collection has been described elsewhere [30].

The present study is a cross-sectional analysis of the baseline measurements of the participants with a measurement of hepatic triglyceride content (HTGC). For our analyses we excluded participants with an implausibly high or low total energy intake (<600kcal or >5000kcal) and missing data on beverage consumption or potential confounding factors. The study was approved by the medical ethics committee of the LUMC and all participants gave written informed consent.

Beverage consumption

Habitual consumption of beverages of all participants was estimated using a semi-quantitative food frequency questionnaire, which was originally designed to study dietary fat intake [31, 32]. Consumption of alcoholic and non-alcoholic beverages was assessed in absolute frequency (times per day, week, month). Participants were asked about consumption of different alcoholic beverages (beer, wine, liquor and mixed drinks (such as cocktails). For each alcoholic beverage we used a standard serving as based on the Dutch Food Composition Database (NEVO-2011): 200 grams for beer, 110 for wine, 50 for liquor and 258 for mixed long drinks so that each consumption contained 10 grams of alcohol. Non-alcoholic beverages were also converted to standard servings: 200 grams for non-alcoholic beers, 125 grams for coffee and tea, 150 grams for milk and 150 grams for sugar-sweetened beverages (NEVO-2011). Non-alcoholic beverages were divided into energy-containing (non-alcoholic beers, milk and sugar sweetened beverages) or non-energy containing (tea and coffee without milk) beverages. No information on water consumption or diet sodas was collected using the FFQ. After the conversion to standard servings, all non-alcoholic beverages were also summed up into one variable. The same was done for all alcoholic beverages. Total alcoholic beverage consumption was divided into subcategories: 0 to 0.5 grams of alcohol per day (g/d) (including abstainers), 0.5 to 5 g/d, 5 to 15 g/d for women and 5-30 g/day for men and lastly >15 g/d for women and >30 g/d for men.

We assessed the reproducibility of the habitual consumption of different beverages in a random subgroup of 100 participants who completed the FFQ for a second time approximately three months after the baseline measurement. The individual measurement intraclass correlation coefficients of the different beverages were 0.63 for sugar sweetened beverages, 0.81 for milk, 0.82 for coffee, 0.91 for tea, 0.79 for beer, 0.82 for wine, 0.67 for mixed drinks and 0.89 for liquor, which can be considered good to excellent[33].

^{1}H-MR spectroscopy of liver fat content

^{1}H-MR spectroscopy of the liver was performed on a 1.5 Tesla whole-body MR scanner (Philips Medical Systems, Best, the Netherlands), and spectra were obtained as described previously [34]. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium) [35]. Hepatic triglyceride content relative to water was calculated as (signal amplitude of triglyceride) / (signal amplitude of water) x 100.

Data collection of covariates

In the baseline questionnaire, participants reported smoking behaviour in three categories: current, former or never smoking (reference group). Ethnicity was reported by self-identification in eight categories which we grouped into white (reference group) and other. Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high (including higher vocational school, university, and post-graduate education) versus low education (reference group). Physical activity during leisure time was reported using the Short Questionnaire to Assess Health-enhancing physical activity and was expressed in MET-hours per week (36). Data collection on other covariates has been described previously [30].

Statistical analyses

In the NEO study there is an oversampling of persons with a BMI of 27 kg/m^2 or higher. To correctly represent associations in the general population [37], adjustments for this oversampling were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality [38], whose BMI distribution was similar to the BMI distribution of the general Dutch population [39] (**Supplemental table 1**). All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2. As a result of the weighting, only percentages and proportions can be given instead of numbers of participants. Baseline characteristics are displayed in percentages or means (standard deviations) for the total population, and stratified by sex and categories of alcohol consumption.

We performed linear regression analyses to examine the association between alcohol consumption and liver fat. We performed three different models and also stratified each model by sex, due to the known differences in both alcohol consumption and liver fat content between men and women. Because of the skewed distribution of HTGC, we used the natural logarithm of this variable in the analyses. For an easier interpretation of these results, we back transformed the regression coefficients towards a ratio (using exp(beta)) with 95% confidence interval. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%.We first performed linear regression analysis to examine the association of alcohol consumption as a categorical variable (0 to 0.5 g/d (reference),≥0.5 to 5 g/d, ≥5 to 15 g/d for women and ≥5 to 30 g/d for men, and ≥15 g/d for women and≥30 g/d for men) with HTGC. We tested for a linear trend (p=0.01) and also added a quadratic term (p=0.49) to the model to check for non-linearity.

Then, we studied alcohol consumption as a continuous outcome in three different ways. Firstly, we studied the association between one serving of alcohol (total alcohol, beer, wine, mixed drinks and liquor) and one serving of non-alcoholic beverages (sugar sweetened beverages, milk, coffee, tea and non-alcoholic beer) per day and liver fat content. This was done in both a crude model and a multivariable linear regression model, which was adjusted for age, sex, smoking, education, ethnicity, physical activity and total energy intake. Models studying separate alcoholic beverages were additionally adjusted for all other alcoholic beverages, and models on non-alcoholic beverages were additionally adjusted for all other non-alcoholic beverages.

Secondly, we studied the effect of substituting one serving of an alcoholic beverage with one serving of a non-alcoholic beverage. In these substitution models we included a sum variable of all beverages, in addition to each beverage separately, except for the beverage to be substituted, in this case alcoholic beverages. Instead of total energy intake, these substitution models were adjusted for caloric intake from food only, to adjust for possible confounding when substituting different beverages. Accordingly, the regression coefficients can be interpreted as the relative change in HTGC if one serving/d of an alcoholic beverage was substituted by one serving/d of a non-alcoholic beverage.

Third, in addition to the substitution analyses based on servings, we also performed an isocaloric substitution model of alcoholic beverages with energy-containing non-alcoholic beverages. This model was adjusted for both caloric intake from beverages and caloric intake from food. In these analyses 5 En% of alcoholic beverages is replaced with 5 En% of energy containing non-alcoholic beverages (sugar-sweetened beverages, milk, and non-alcoholic beer) in relation to HTGC, to examine to what extent the caloric content contributes to liver fat content. To study whether the associations are specific for liver fat, we additionally adjusted all three models for total body fat.

To examine to what extent consumption of alcoholic beverages was associated with liver fat content in participants without a fatty liver, we stratified the analyses by the arbitrary cut-off point of 5.56% which indicates a fatty liver. Next to that, we stratified by the rs738409 single nucleotide polymorphism (SNP) in the *PNPLA3* gene that is associated with diffuse fat deposition in the liver and may promote NASH, fibrosis and cirrhosis throughout the liver [40], to investigate whether the associations differ between carriers and non-carriers of the SNP.

As a means of sensitivity analysis, we repeated the substitution analysis based on servings after taking into account the milk and sugar potentially added to coffee and tea. In the analyses with categories of alcohol consumption, we repeated the analyses after excluding alcohol abstainers (0 g/d) from the reference group. Additionally, we performed the models after exclusion of participants with diabetes type 2 or cardiovascular disease, as they might have changed their drinking habits after being diagnosed, or might potentially react differently to sugars.

All above mentioned analyses were pre-defined, and analyses not pre-specified are considered exploratory. We performed all analyses using STATA statistical Software (Statacorp, College Station, Texas, USA), version 14.

RESULTS

In total, 6,671 participants were included in the NEO study between September 2008 and October 2012, of whom 2,580 underwent a liver fat measurement by ^{1}H-MRS. However, due to the limited time slot that was available per participants did not allow time for a repeat examination when technical failures were present (n=497), leaving 2,083 participants with a successful liver fat measurement. After exclusion of participants with extreme energy intake (n=18), missing dietary data (n=26), missing data on potential confounding factors (n=1 for smoking, n=16 for education, n=2 for ethnicity, n=44 for physical activity in leisure time, n=3 for total body fat and n=6 for visceral adipose tissue) and one participant for whom the log transformation of the liver fat could not be calculated, 1,966 participants were included in the analyses. Baseline characteristics of these participants are presented in **table 1**. Participants with higher

Table 1. Characteristics of participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS [1]

				Alcohol consumption			
	Total population	**Men (47%)**	**Women (53%)**	**0-0.5 g/d (16%)**	**>0.5-5 g/d (21%)**	**>5-15 g/d women >5-30 g/d men (38%)**	**>15 g/d women >30 g/d men (24%)**
Age (year)	55 ± 6	56 ± 6	55 ± 6	55 ± 7	55 ± 6	56 ± 6	56 ± 6
Sex (% men)	47			30	34	60	44
Ethnicity (% white)	96	96	96	92	93	97	99
Education (% high)	46	51	42	32	41	50	55
Smoking (% current)	14	16	13	13	7	13	23
Physical activity in leisure time (MET h/wk)	37.8 ± 31.9	39.1 ± 37.1	36.7 ± 27.3	38.6 ± 38.4	35.3 ± 28.0	38.4 ± 31.9	38.9 ± 31.4
Sugar sweetened beverages (serving/d)[2]	0.8 ± 1.0	0.9 ± 1.1	0.8 ± 0.9	1.1 ± 1.4	0.9 ± 1.0	0.8 ± 0.8	0.8 ± 0.9
Milk (serving/d)[2]	1.1 ± 1.0	1.2 ± 1.2	0.9 ± 0.9	1.1 ± 1.2	1.1 ± 1.0	1.0 ± 0.9	1.0 ± 1.1
Coffee (serving/d)[2]	3.7 ± 2.1	4.3 ± 2.4	3.2 ± 1.8	3.3 ± 2.4	3.1 ± 2.0	4.0 ± 2.1	4.0 ± 2.1
Tea (serving/d)[2]	2.0 ± 1.9	1.4 ± 1.7	2.4 ± 2.0	2.1 ± 2.2	2.3 ± 1.9	1.8 ± 1.7	1.7 ± 2.1
Non-alcoholic beer (serving/d)[2]	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1
CVD (%)	5	5	4	8	4	3	5
Diabetes (%)	3	4	2	7	2	2	3
BMI (kg/m^2)	25.9 ± 3.9	26.6 ± 3.5	25.2 ± 4.0	26.5 ± 5.0	25.6 ± 4.1	25.8 ± 3.3	25.9 ± 4.1
Waist circumference (cm)	91.0 ± 12.6	97.5 ± 10.7	85.4 ± 11.3	91.0 ± 15.1	89.6 ± 11.8	91.4 ± 11.6	91.5 ± 13.7
Total body fat (%)	30.7 ± 8.2	24.6 ± 5.7	36.1 ± 6.0	34.3 ± 8.8	31.9 ± 8.6	28.6 ± 7.6	31.3 ± 7.7
VAT (cm^2)	88.6 ± 55.1	114.5 ± 60.0	65.9 ± 39.8	85.1 ± 60.0	81.5 ± 49.5	89.9 ± 52.2	95.0 ± 61.3
HTGC (%)	5.7 (7.9)	7.0 (8.3)	4.6 (7.3)	5.4 (8.2)	5.0 (7.4)	5.5 (6.9)	6.8 (9.5)
HTGC>5.56% (%)	29	39	21	25	24	29	35
Fasting serum triglycerides (mmol/L)	1.2 (0.8)	1.4 (1.0)	1.1 (0.7)	1.2 (0.8)	1.2 (0.8)	1.3 (0.8)	1.3 (1.0)

[1]Values are means ± SDs or percentage. Results are based on analyses weighted toward the BMI distribution of the general population (*n*=1,966). [2]Servings equal 150 grams for sugar-sweetened beverages, 150 grams for milk, 125 grams for coffee and tea and 200 grams for non-alcoholic beers. BMI, body mass index; CVD, cardiovascular disease, HTGC; hepatic triglyceride content, MET, metabolic equivalent of task; VAT, visceral adipose tissue.

alcohol consumption were more often smokers and had on average a higher education. Hepatic triglyceride content and the prevalence of a fatty liver was also higher in the categories with higher alcohol consumption. Whereas men on average have a higher coffee and beer consumption, women have a higher tea consumption.

Table 2 displays the association between different categories of alcohol consumption and liver fat content. Despite a linear trend (P for trend 0.01), light and moderate consumption were not significantly associated with liver fat (Table 2). Compared with no alcohol consumption (0-0.5 g/d), high alcohol consumption (>15 g/d for women and >30 g/d for men) was associated with more liver fat, for total alcohol consumption (1.28 times, 95% CI: 1.06, 1.55), beer consumption (1.39 times, 95% CI: 1.08, 1.80) and wine consumption (1.28 times, 95% CI: 1.04, 1.58) (Table 2). Results were similar when excluding alcohol abstainers (0g/d) from the reference group (data not shown).

Table 3 shows the associations between consumption of different alcoholic beverages as continuous variables and liver fat content. Each extra alcoholic serving was associated with more liver fat (1.09 times, 95% CI: 1.06; 1.13). When additionally adjusted for total body fat to examine whether the associations were specific for liver fat, associations attenuated for liquor and mixed drinks, although total alcoholic beverages remained associated with more liver fat (1.09 times, 95% CI: 1.05, 1.12).

The associations of non-alcoholic beverages are shown in **table 4.** In the total population, each extra serving of non-alcoholic beverages was associated with less liver fat (0.97 times, 95% CI: 0.95, 0.99). Consumption of coffee (0.96 times for each extra serving, 95% CI: 0.93, 0.99), tea (0.97 times, 95% CI: 0.94; 1.00) and milk (0.95 times, 95% CI: 0.89; 1.00) was also associated with less liver fat. Results did not differ after exclusion of participants with diabetes type 2 or cardiovascular disease or when taking the milk and sugar added to coffee and tea into account (data not shown).

Table 5 shows that substituting one alcoholic serving with one non-alcoholic serving was associated with less liver fat (0.90 times, 95% CI: 0.86, 0.94) in the total population after adjustment for potential confounding factors and total body fat. Of the different non-alcoholic beverages, replacement with milk (0.88 times, 95% CI: 0.82, 0.95), tea (0.89 times; 95% CI: 0.85; 0.94) and coffee (0.88 times, 95% CI: 0.84; 0.92) was associated with less liver fat. Results were similar when taking the milk and sugar added to coffee and tea into account (data not shown).

Isocaloric substitution of 5 En% of alcoholic beverages with 5 En% of non-alcoholic beverages (**table 6**) showed that substitution of alcohol with milk was associated with less HTGC (0.89 times, 95% CI: 0.81, 0.98) in the total population. Replacing 5 En% of alcohol with 5 En% of sugar sweetened beverage was associated with liver fat equally strong as with alcohol (1.00 times, 95% CI: 0.91, 1.09).

Table 2. Relative change in HTGC and 95% confidence intervals for different categories of alcohol consumption in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS

	0 to 0.5 g/d	≥0.5 to 5 g/d	≥5 to 15 g/d women ≥5 to 30 g/d men	≥15 g/d women ≥30 g/d men	*P*-trend
Alcohol (total)					
Multivariable-adjusted[1]relative change (95% CI)	1 (ref)	1.05 (0.87, 1.25)	1.07 (0.90, 1.28)	1.28 (1.06, 1.55)	**0.01**
Proportion of population, %	13.7	22.2	41.0	23.1	
Beer[2]					
Multivariable-adjusted relative change (95% CI)	1 (ref)	0.94 (0.83, 1.08)	1.10 (0.93, 1.29)	1.39 (1.08, 1.80)	**0.03**
%	48.1	27.5	18.5	6.0	
Wine[2]					
Multivariable-adjusted relative change (95% CI)	1 (ref)	1.01 (0.88, 1.16)	1.02 (0.89, 1.18)	1.28 (1.04, 1.58)	**0.16**
%	23.3	32.7	34.8	9.3	

[1]Adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time, total energy intake and total body fat. Results are based on analyses weighted towards the body mass index distribution of the general population (*n*=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change compared with the reference category. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%.CI, confidence interval; HTGC, hepatic triglyceride content.

[2]Additionally adjusted for other alcoholic beverages. Servings equal 200 grams for beer and 110 grams for wine.

Table 3. Relative change in HTGC and 95% confidence intervals per 1 serving/d higher consumption of alcoholic beverage in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Alcohol (total)			
Total	1.15 (1.11; 1.19)	1.09 (1.06; 1.13)	1.09 (1.05; 1.12)
Men	1.11 (1.07; 1.15)	1.11 (1.07; 1.15)	1.09 (1.05; 1.13)
Women	1.05 (1.07; 1.15)	1.09 (1.00; 1.18)	1.10 (1.02; 1.19)
Beer[2]			
Total	1.14 (1.09; 1.19)	1.07 (1.02; 1.11)	1.08 (1.03; 1.13)
Men	1.08 (1.04; 1.13)	1.08 (1.04; 1.13)	1.09 (1.04; 1.15)
Women	0.95 (0.84; 1.07)	1.02 (0.89; 1.17)	1.06 (0.96; 1.17)
Wine[2]			
Total	1.13 (1.06; 1.20)	1.13 (1.06; 1.21)	1.11 (1.05; 1.18)
Men	1.13 (1.06; 1.21)	1.13 (1.06; 1.21)	1.08 (1.02; 1.15)
Women	1.12 (1.02; 1.24)	1.13 (1.02; 1.26)	1.15 (1.04; 1.28)
Liquor[2]			
Total	1.64 (1.45; 1.86)	1.22 (1.08; 1.38)	1.06 (0.93; 1.21)
Men	1.33 (1.17; 1.50)	1.24 (1.10; 1.40)	1.10 (0.97; 1.26)
Women	1.28 (0.66; 2.48)	0.95 (0.52; 1.73)	0.62 (0.37; 1.04)
Mixed drinks[2]			
Total	1.56 (1.34; 1.83)	1.18 (1.00; 1.40)	0.97 (0.83; 1.15)
Men	1.26 (1.09; 1.47)	1.18 (1.00; 1.40)	0.98 (0.83; 1.16)
Women	1.74 (0.95; 3.20)	1.53 (0.86; 2.71)	1.12 (0.63; 1.97)

After stratifying the analyses by the cut-off point of fatty liver (HTGC>5.56%), associations between alcohol consumption and liver fat were similar in both groups (**Supplemental table 2**). Regarding the *PNPLA3* polymorphism, the association between each alcoholic beverage and HTGC was similar in both groups (1.14 times for each alcoholic serving extra; 95% CI: 1.07, 1.21 for GC and GG carriers and 1.09 times; 95% CI: 1.04, 1.15 for CC carriers).

Table 4. Relative change in HTGC and 95% confidence intervals per 1 serving/d higher consumption of non-alcoholic beverages in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by [1]H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Non-alcoholic beverages (total)			
Total	0.98 (0.95; 1.00)	0.97 (0.94; 0.99)	0.97 (0.95; 0.99)
Men	0.98 (0.95; 1.01)	0.99 (0.95; 1.02)	0.98 (0.95; 1.01)
Women	0.95 (0.91; 0.98)	0.94 (0.91; 0.97)	0.95 (0.92; 0.98)
SSB[2]			
Total	1.07 (1.01; 1.14)	1.05 (0.99; 1.11)	1.03 (0.98; 1.08)
Men	1.04 (0.97; 1.12)	1.07 (0.99; 1.15)	1.02 (0.96; 1.09)
Women	1.05 (0.96; 1.14)	1.02 (0.93; 1.11)	1.03 (0.95; 1.11)
Milk[2]			
Total	1.00 (0.94; 1.07)	0.94 (0.88; 1.00)	0.95 (0.89; 1.00)
Men	0.92 (0.86; 1.00)	0.91 (0.84; 0.99)	0.92 (0.86; 0.99)
Women	1.02 (0.91; 1.13)	0.96 (0.87; 1.07)	0.97 (0.89; 1.06)
Coffee (without sugar or milk)[2]			
Total	1.01 (0.98; 1.04)	0.96 (0.93; 0.99)	0.96 (0.93; 0.99)
Men	1.00 (0.96; 1.04)	0.99 (0.95; 1.03)	0.99 (0.95; 1.02)
Women	0.95 (0.91; 0.99)	0.92 (0.88; 0.96)	0.92 (0.88; 0.96)
Tea (without sugar or milk)[2]			
Total	0.92 (0.89; 0.95)	0.96 (0.92; 0.99)	0.97 (0.94; 1.00)
Men	0.96 (0.92; 1.02)	0.98 (0.93; 1.03)	1.00 (0.94; 1.05)
Women	0.95 (0.92; 0.99)	0.94 (0.90; 0.98)	0.95 (0.91; 0.99)
Non-alcoholic beer[2]			
Total	1.35 (0.99; 1.84)	1.13 (0.88; 1.45)	1.09 (0.86; 0.99)
Men	1.22 0.90; 1.65)	1.18 (0.89; 1.57)	1.17 (0.90; 1.52)
Women	0.82 (0.40; 1.70)	0.88 (0.49; 1.59)	0.73 (0.49; 1.10)

[1]Multivariable: adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time and total energy intake. Results are based on analyses weighted towards the body mass index distribution of the general population (*n*=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%. CI, confidence interval; HTGC, hepatic triglyceride content; TBF, total body fat.

[2]Additionally adjusted for all other non-alcoholic beverages. Servings equal 150 grams for SSB, 150 grams for milk, 125 grams for tea and coffee, and 200 grams for non-alcoholic beer.

Table 5. Relative change in HTGC and 95% confidence intervals per 1 serving/d of alcoholic beverage substitution by non-alcoholic beverage in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by ^{1}H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Non-alcoholic beverages (total)			
Total	0.85 (0.81; 0.88)	0.89 (0.85; 0.93)	0.90 (0.86; 0.94)
Men	0.89 (0.85; 0.93)	0.90 (0.86; 0.95)	0.91 (0.87; 0.96)
Women	0.91 (0.83; 0.99)	0.87 (0.79; 0.95)	0.86 (0.80; 0.94)
Tea (without sugar or milk)[1]			
Total	0.81 (0.78; 0.85)	0.87 (0.83; 0.92)	0.89 (0.85; 0.94)
Men	0.88 (0.82; 0.94)	0.90 (0.84; 0.96)	0.92 (0.86; 0.99)
Women	0.88 (0.79; 0.97)	0.86 (0.78; 0.94)	0.86 (0.79; 0.94)
Coffee (without sugar or milk)[1]			
Total	0.85 (0.81; 0.89)	0.88 (0.84; 0.92)	0.88 (0.84; 0.92)
Men	0.89 (0.84; 0.94)	0.90 (0.85; 0.95)	0.91 (0.86; 0.96)
Women	0.88 (0.79; 0.97)	0.84 (0.76; 0.92)	0.83 (0.77; 0.91)
Milk[1]			
Total	0.86 (0.80; 0.92)	0.87 (0.81; 0.93)	0.88 (0.83; 0.94)
Men	0.83 (0.77; 0.91)	0.84 (0.77; 0.91)	0.86 (0.80; 0.92)
Women	0.94 (0.82; 1.09)	0.90 (0.78; 1.03)	0.89 (0.79; 1.00)
SSB[1]			
Total	0.93 (0.87; 1.00)	0.98 (0.92; 1.04)	0.96 (0.91; 1.02)
Men	0.96 (0.89; 1.03)	1.00 (0.92; 1.08)	0.96 (0.90; 1.03)
Women	0.99 (0.88; 1.11)	0.95 (0.84; 1.06)	0.94 (0.85; 1.04)

[1]Multivariable: adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time, total energy intake of food, a sum variable of all beverages and all beverages except for alcohol and itself. Results are based on analyses weighted towards the body mass index distribution of the general population (*n*=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%.CI, confidence interval; HTGC, hepatic triglyceride content; SSB, sugar sweetened beverages; TBF, total body fat.

[1]Servings equal 125 grams for tea and coffee, 150 grams for milk and 150 grams for SSB.

Table 6. Relative change in HTGC and 95% confidence intervals per 5 En% of alcoholic beverage substitution by non-alcoholic beverages in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by ^{1}H-MRS[1]

	Crude	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)	Relative change (95% CI)
Non-alcoholic beverages (total)			
Total	0.91 (0.83; 0.98)	0.94 (0.87; 1.02)	0.94 (0.87; 1.01)
Men	0.90 (0.81; 1.00)	0.90 (0.81; 1.01)	0.88 (0.81; 0.97)
Women	0.99 (0.86; 1.14)	0.96 (0.84; 1.10)	0.96 (0.85; 1.09)
Milk			
Total	0.86 (0.77; 0.97)	0.88 (0.79; 0.98)	0.89 (0.81; 0.98)
Men	0.82 (0.71; 0.95)	0.80 (0.69; 0.92)	0.82 (0.72; 0.93)
Women	0.98 (0.82; 1.17)	0.94 (0.79; 1.13)	0.94 (0.81; 1.10)
SSB			
Total	0.95 (0.85; 1.06)	1.01 (0.91; 1.12)	1.00 (0.91; 1.09)
Men	1.00 (0.86; 1.17)	1.04 (0.89; 1.22)	0.97 (0.85; 1.10)
Women	1.01 (0.87; 1.19)	0.98 (0.85; 1.14)	0.99 (0.86; 1.13)

[1]Multivariable: adjusted for age, sex, smoking, education, ethnicity, physical activity in leisure time, total energy intake of beverages, total energy intake from food, and all beverages except for alcohol and itself. Results are based on analyses weighted towards the body mass index distribution of the general population (***n***=1,966), and derived from beta coefficients with 95% confidence intervals from linear regression analyses and expressed as a relative change. Such ratio, for example 1.2, can be interpreted as 1.2 times HTGC for each extra serving per day, which would reflect an increase in HTGC from, for example, 5% to 6%. CI, confidence interval; HTGC, hepatic triglyceride content; SSB, sugar sweetened beverage; TBF, total body fat.

DISCUSSION

In this population-based cohort of 1,966 middle aged men and women with directly assessed liver fat content, consumption of each extra alcoholic serving per day was associated with more liver fat, with larger increases in liver fat with excessive alcohol consumption. Replacing one alcoholic beverage by one non-alcoholic beverage was associated with less liver fat. Whereas isocaloric replacement of alcohol with milk was associated with less liver fat, isocaloric replacement with sugar sweetened beverages was equally associated with liver fat.

This study was conducted within a large cohort study, in which hepatic triglyceride content has been directly assessed by ^{1}H-MRS. We used substitution analysis to directly compare different types of beverages and their association with liver fat to each other. The comparative nature of our study can contribute to translation to recommendations in clinical practice, as we have shown that consumption of both alcohol and sugar sweetened beverages is associated with more liver fat, whereas milk, tea and coffee are associated with less liver fat. More importantly, replacing alcohol with sugar sweetened beverages is therefore equally associated with liver fat, and replacing it with milk, tea

or coffee is associated with less liver fat. This can be translated into clear advice for patients diagnosed with fatty liver and who are advised to stop consuming alcohol. Lastly, extensive phenotype measurements have been performed, allowing adjustment for many potential confounding factors. However, inherent to the observational, cross-sectional design we cannot exclude residual confounding by lifestyle factors.

Due to the cross-sectional design, a limitation of this substitution analysis is that it is modelled on a group level rather than on an individual level. All participants completed a semi-quantitative FFQ, based on which we estimated the habitual beverage consumption. Although alcohol consumption might have been misreported, intra-class correlations of the beverages showed good to excellent reproducibility. Moreover, by adjusting our analyses for total energy intake we partly corrected for potential misreporting. A limitation of the FFQ is that it did not take drinking habits into account, so we cannot make any statements on the potential role of drinking patterns. Also no information on diet sodas or water was available, so no statements on these beverages can be made. Nevertheless, this will not have influenced the isocaloric substitution models, as only energy-containing beverages were taken into account in this analysis. Results from these isocaloric substitution models suggest that it is not energy per se, but possibly sugars that contribute to liver fat accumulation.

Alcohol is mainly metabolized in the liver [41], and can induce fatty liver by increasing the fatty acid synthesis in the liver. Together with the impaired oxidation of these compounds caused by an increased accumulation of the reduced form of nicotinamide adenine dinucleotice (NADH), alcohol consumption may lead to increased triglyceride synthesis, which is the main form of stored fat stored in the liver [42]. Although many studies investigated the association between light to moderate alcohol consumption and liver fat, results have been inconsistent and inconclusive, and the exact mechanism remains unidentified. A prospective randomized study concluded that moderate red wine consumption during three months increased HTGC in subjects without steatosis at baseline [43], whereas red wine consumption during four weeks in another randomized controlled trial did not significantly increase liver fat compared to de-alcoholized red wine [44]. Additionally, Ekstedt et al. concluded from their long-term follow-up study that moderate alcohol consumption was associated with fibrosis progression in patients with NAFLD and that they should be advised to refrain from heavy episodic drinking [45]. Modest wine consumption has been associated with reduced prevalence of suspected (NA)FLD in other studies [19, 21, 46, 47]. In another study, light to moderate alcohol consumption had a potentially protective effect against insulin resistance in severely obese patients, but not on the severity of activity and stage of liver disease [48]. Although in a recent review an association between moderate alcohol consumption and decreased NASH and fibrosis was shown, it was also observed that heavy episodic drinking may accelerate fibrosis progression [49]. Most of the studies on alcohol consumption, however, including ours, did not take drinking habits into account, only habitual total amount of alcohol consumed. However, even though certain drinking patterns such as drinking outside mealtimes and drinking multiple different alcoholic beverages lead to an increased risk of developing alcohol related liver damage [50], it seems to be the cumulative consumption that is most strongly associated with the progression of alcoholic fatty liver disease [42]. Although current literature is in disagreement about the role of moderate alcohol consumption, none of these studies performed substitution analysis to take into account that a person does not simply stop drinking alcohol but may replace the alcoholic beverages with other drinks. Moreover, results from a recent mendelian randomization suggest that there is no beneficial effect of moderate alcohol consumption on the severity of non-alcoholic fatty liver disease [24]. In our study, light and moderate alcohol consumption were not associated with less liver fat, which is in line with these findings.

Additionally, isocaloric replacement of alcohol with milk was associated with less liver fat in our study. This indicates that it is not caloric intake per se that leads to liver fat accumulation. The exact mechanism behind the seemingly negative association between dairy and liver fat remains unknown, although it is in agreement with current literature. Established biomarkers of dietary dairy fat intake have been associated with higher hepatic and systemic insulin sensitivity, lower fasting glucose concentrations and less liver fat [51]. Moreover, higher low-fat fermented dairy product consumption has also been associated with a decreased risk of developing type 2 diabetes in a prospective study [52].

Importantly, isocaloric replacement of alcohol with sugar sweetened beverage consumption was equally associated with liver fat. Taken together with our results on substitution with milk, this suggest a role for sugars in liver fat accumulation. Our results are in line with recent findings from the Framingham Heart Study that showed a significant dose-response relationship between sugar sweetened beverages and fatty liver disease, but not for diet soda intake [27]. However, replacement of sugar sweetened beverages with other beverages was not investigated in this study.

Multiple underlying mechanisms have been proposed through which sugar sweetened beverages might contribute to the development of diabetes and cardiometabolic diseases not only via overall weight gain, but also independently through the metabolic

effects of constituent sugars [53]. It has also been suggested that liquid foods lead to less satiety and more postprandial hunger [25]. Consumption of sugar sweetened beverages have been shown to induce peaks in blood glucose and insulin levels, contributing to a high glycaemic state, in turn associated with insulin resistance, diabetes and coronary heart disease [53, 54]. In the Netherlands, soft drinks are one of the main sources of fructose [55], which is mostly metabolized to lipids in the liver and might therefore lead to an increase in hepatic de novo lipogenesis [56, 57]. In a recent trial, moderate fructose consumption for 12 weeks increased liver fat despite only a small increase in weight and waist circumference [58]. Moreover, chronic fructose consumption has been shown the decrease resting energy expenditure in a 10-week trial [59]. Our results support the current literature and suggest that both alcoholic beverages and sugar sweetened beverages may contribute to liver fat accumulation. However, in clinical practice, patients with NAFLD are often advised not to consume alcoholic beverages [18] there are no clear guidelines about what they should replace these beverages with.

In conclusion, consumption of alcoholic beverages was associated with a higher liver fat content in a population-based cohort. Replacing a serving of alcoholic beverages with non-alcoholic beverages was associated with less liver fat. Importantly, in isocaloric replacement of alcoholic beverages, sugar sweetened beverages were equally associated with liver fat as alcoholic beverages, suggesting that both alcohol and sugars may contribute to liver fat accumulation. Although intervention studies should confirm to what extent hepatic triglyceride content can actually be changed by altering drinking habits, it is advised to specify with what beverages alcoholic beverages should be replaced in clinical practice, such as non-energy containing beverages or milk, but not with sugar sweetened beverages.

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SUPPLEMENTARY FILES

Supplemental table 1. Body Mass Index distribution of participants of the Netherlands Epidemiology of Obesity study from the Leiderdorp municipality and the resulting weighting factors for the different BMI categories as used in the statistical analyses

Body Mass Index	*n* (%)	Weighting factor
≥30 kg/m²	268 (16.0)	1
≥29-30 kg/m²	83 (5.0)	1.304461
≥28-29 kg/m²	103 (6.2)	1.472934
≥27-28 kg/m²	151 (9.0)	2.458912
≥26-27 kg/m²	172 (10.3)	4.445434
≥25-26 kg/m²	190 (11.4)	8.668198
<25 kg/m²	704 (42.0)	10.26279
Total	**1,671**	

Supplemental table 2. Relative change in HTGC and 95% confidence intervals in stratifications on HTGC, alcohol consumption and PNPLA3 gene in participants of the Netherlands Epidemiology of Obesity study, men and women between 45 and 65 years of age with direct assessment of hepatic triglyceride content by ^{1}H-MRS[1]

	Multivariable	Multivariable + TBF
	Relative change (95% CI)	Relative change (95% CI)
Alcohol (total) - per 1 unit/day higher consumption		
HTGC<5.56%	1.05 (1.01; 1.09)	1.04 (1.00; 1.08)
HTGC≥5.56%	1.01 (0.99; 1.04)	1.02 (1.00; 1.05)
PNPLA3 II	1.09 (1.04; 1.15)	1.06 (1.02; 1.11)
PNPLA3 IM+MM	1.14 (1.07; 1.21)	1.12 (1.06; 1.19)
Non-alcoholic (total) - per 1 unit/day higher consumption		
HTGC<5.56%	0.97 (0.95; 0.99)	0.97 (0.95; 0.99)
HTGC≥5.56%	1.00 (0.98; 1.02)	1.00 (0.98; 1.02)
PNPLA3 II	0.96 (0.92; 0.99)	0.95 (0.92; 0.98)
PNPLA3 IM+MM	0.99 (0.95; 1.03)	0.99 (0.96; 1.03)
Non-alcoholic (total) - per 1 unit/day of alcoholic beverage substitution		
HTGC<5.56%	0.93 (0.89; 0.97)	0.94 (0.90; 0.97)
HTGC≥5.56%	0.99 (0.95; 1.02)	0.98 (0.95; 1.01)
PNPLA3 II	0.89 (0.84; 0.95)	0.91 (0.87; 0.96)
PNPLA3 IM+MM	0.87 (0.81; 0.93)	0.89 (0.83; 0.95)
Non-alcoholic (total) - per 5 En% of alcoholic beverage substitution		
HTGC<5.56%	0.94 (0.88; 1.01)	0.95 (0.90; 1.02)
HTGC≥5.56%	0.98 (0.91; 1.06)	0.97 (0.90; 1.04)
PNPLA3 II	0.98 (0.88; 1.08)	0.99 (0.90; 1.08)
PNPLA3 IM+MM	0.87 (0.74; 1.01)	0.87; 0.77; 0.99)