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Differential effects of low or high-fat dairy and fat derived from dairy products on MASLD

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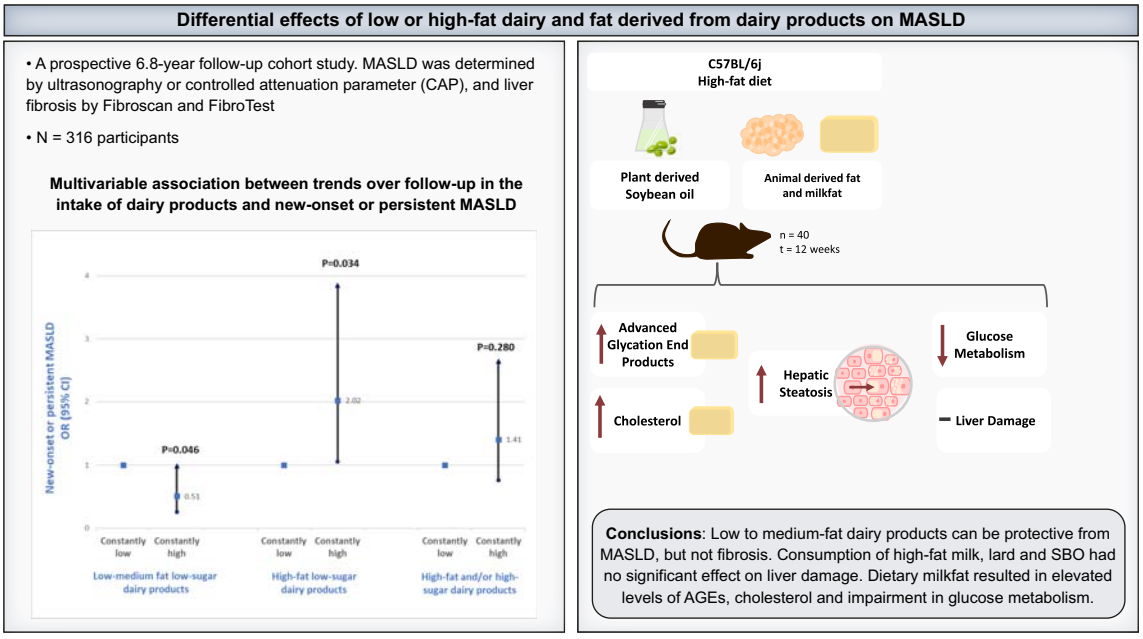
Graphical abstract



Highlights:

- The association between dairy products and MASLD has not been fully established.
- Low-medium fat dairy product consumption was associated with lower risk for MASLD.
- Neither low-medium nor high-fat dairy consumption was related to fibrosis.
- In mice, high-fat diets, including milk fat, induced a similar increase in steatosis.

Impact and implications:

MASLD is related to nutrition, but evidence of an association between high-fat and low-fat dairy products is lacking, therefore, we evaluated this association by performing experimental studies in mice and an observational human study. For MASLD prevention, a differential effect based on the type of dairy products should be considered: low-medium fat low-sugar dairy products were found to be protective, in contrast high-fat dairy and generally high-fat diets may be harmful. It would be advisable to prefer low-fat low-sugar dairy products and minimise intake of high-fat dairy products; however, additional evidence is needed to allow generalisability of our findings.

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Research article

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Differential effects of low or high-fat dairy and fat derived from dairy products on MASLD

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Background & Aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is strongly related to nutrition. However, only a few human and animal studies have tested the association between MASLD and dairy consumption and the effect of milk fat on liver damage. Therefore, we aimed at testing the association between consumption of dairy product and the incidence of MASLD and fibrosis markers in humans, and the effect of milk fat *vs.* other fats on MASLD in animal studies.

Methods: A prospective 7-year follow-up cohort study was performed including baseline and follow-up fasting blood tests, liver evaluation and a face-to-face interview on health status and behaviour using structured questionnaires. MASLD was determined by ultrasonography or by controlled attenuation parameter (CAP), and liver fibrosis by FibroTest™ or FibroScan®. An animal study was performed in which 6-week-old C57BL/6j male mice were fed a high-fat diet (HFD) consisting of lard, soybean oil, and milk fat for 12 weeks. Metabolic impairment was assessed during the animal experiment, and serum advanced glycation end-products (AGEs) and liver damage were evaluated.

Results: A total of 316 patients were included in the prospective cohort. In multivariable analysis, high consumption of low-medium fat low-sugar dairy products (g/day above the baseline sex-specific median) was associated with a lower risk for MASLD incidence (OR 0.42, 95% CI 0.18–0.95, $p = 0.037$) or incidence/persistence at follow-up (OR 0.58, 0.34–0.97, $p = 0.039$). Constantly high consumption of high-fat low-sugar dairy products was associated with greater odds for new onset/persistence of MASLD. Neither low-medium nor high-fat dairy consumption was related to fibrosis markers. In mice, all HFDs induced similar weight gain and steatosis and did not affect liver enzymes. Milk fat increases serum cholesterol and AGEs levels more than lard or soybean oil.

Conclusions: Low-medium fat low-sugar dairy products may be protective and should be preferred over high-fat dairy to prevent MASLD. HFDs from different fat sources with a wide spectrum of fatty acid saturation content are equally deleterious.

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD; formerly known as non-alcoholic fatty liver disease) is the most common chronic liver disease worldwide, with an estimated prevalence of 38% (95% CI 33.7–42.5) among the adult population.[1] MASLD can deteriorate to lobular inflammation, known as metabolic dysfunction-associated steatohepatitis (MASH; formerly called non-alcoholic steatohepatitis), fibrosis, and cirrhosis. It is well established that MASLD is associated with a diet rich in fructose and saturated fat,[2] while the Mediterranean diet and other similar healthy dietary patterns can reduce liver fat accumulation and may be related to lower disease progression.[3] Overfeeding with polyunsaturated (PUFA) and saturated fatty acids (SFA) has distinct effects on liver and visceral fat accumulation, as shown repeatedly in several short-term randomised controlled trials (RCTs).[2,4,5]

Dairy products have high nutritional quality, being a good nutritional source of protein, and contain all essential amino acids, as well as calcium, vitamin D (especially if fortified), vitamin B12, and vitamin A. Dairy products have repeatedly been shown to have benefits in the prevention of several chronic diseases and metabolic alterations.[6,7] Two meta-analyses of observational prospective cohort studies have demonstrated low-fat dairy products to be related to a reduced risk of metabolic syndrome, cardiovascular disease (CVD), type-2 diabetes,[7,8] and a meta-analysis of clinical trials, demonstrated they have a lowering effect on insulin resistance.[6] In an umbrella review of prospective studies, cheese consumption was inversely associated with all-cause mortality, cardiovascular morbidity and mortality, and other health outcomes.[9]

However, few studies have tested the direct association between dairy products and dairy fat and MASLD, with inconsistent results. In a Korean study, higher dairy protein intake

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was inversely associated with the risk of MASLD in men and women aged over 50 years, but MASLD was defined only based on a calculated score.[10] Similarly, another cross-sectional study, using transient elastography controlled attenuation parameter (CAP) to diagnose MASLD, found full-fat dairy products to be inversely related to MASLD.[11] However, two meta-analyses of observational studies (mostly cross-sectional or case-control studies) showed conflicting results:[12,13] one study found no significant association between dairy product consumption and MASLD,[12] whereas the other found a significant inverse association between dairy intake and MASLD. In addition, high-fat dairy has also been reported to be protective, but this finding was based on two studies defined as having low to moderate quality.[13] In two large prospective cohort studies, high-fat dairy products and butter, but not low-fat dairy, were associated with a higher risk of incident hepatocellular carcinoma, implying a role for cholesterol and saturated fat in liver damage, but not for dairy in general.[14]

The dairy fat composition is characterised by a high content of SFA and prevalent triacylglycerol (TAG) structures, and studies have indicated that the latter and even the food matrix may influence absorption and lipid metabolism.[15,16] The limited animal-based mechanistic studies comparing the effects of a high-fat milk-based diet with a vegetable oil-based diet and an animal fat-based diet on MASLD in mice have yielded confusing results. For example, a deleterious effect on insulin resistance and liver steatosis was linked to a high saturated-fat lard-based high-fat diet (HFD) compared with a corresponding soybean oil (SBO)-based diet.[17] However, lard contains a significant component of monounsaturated fatty acid. Functional milk fat HFD enriched in conjugated linoleic acid has been suggested to prevent hepatic steatosis compared with HFD based on milk or SBO in Wistar rats,[18] however, this may be related to essential fatty acid deficiency. Some beneficial effects of milk derivative phospholipids have also been reported, unrelated to the fatty acid profile.[19] Feeding with lard-based HFD resulted in elevated oxidative stress compared with feeding with vegetable oils,[20] which is difficult to explain based on the high oxidisability of PUFA.

Therefore, this study aimed at evaluating the effects of different dietary fat sources on MASLD in a translational study consisting of a human epidemiological prospective study and animal studies. First, we tested the association between dietary intake of dairy products (categorised by fat and sugar content) and MASLD and presumed significant fibrosis biomarkers in humans. Second, we aimed to compare a HFD based on milk fat to other fat sources, varying in saturation levels, in an animal model of MASLD/MASH. We hypothesised that low-fat dairy consumption is neutral and may even be beneficial for MASLD as it is a good source of protein and vitamin D with low fat and sugar content, whereas high-fat dairy may confer higher risk given the high saturated fat content.

Materials and methods

Human study design and population

A prospective cohort study was conducted, including participants from two metabolic and hepatic screening surveys at least 5 years apart. The first survey was conducted between 2010 and 2015, and the second was conducted between 2017 and 2020. Exclusion criteria at both time points included the presence of HBsAg or anti-HCV antibodies; fatty liver suspected to be secondary to hepatotoxic drugs; inflammatory bowel disease; celiac disease or excessive alcohol consumption (≥30 g/day in men or ≥20 g/day in women). In addition, participants who reported an unreasonable caloric intake below or above the accepted range (in one or both of the surveys): 800-4,000 KCal/day for men and 500-3,500 KCal/day for women were excluded, in accordance with a previous report[21] (Supplementary Materials and methods and Fig. S1). The Tel-Aviv Medical Center IRB committee approved the study, and all patients signed informed consent forms.

Data collection and definitions of variables

At the two time points of the screening survey, participants underwent fasting blood tests, liver ultrasound (US), and/or FibroScan® and a face-to-face interview on health status and behaviour using structured questionnaires, all of which were assembled by the Israeli Ministry of Health and had been used in national surveys. The semi-quantitative Food Frequency Questionnaire (FFQ)[21,22] was assembled by the Food and Nutrition Administration Ministry of Health and was tailored to the Israeli population. It consisted of 117/183 food items (for baseline and follow-up evaluations, respectively). The questionnaire included questions about many foods and food groups including detailed questions on dairy products (this section of the FFQ is described in the Supplementary Materials and methods and Table S1). The nutrient components of each food item were taken from the Israeli National Nutrient Database (BINAT), Food and Nutrition Administration, Ministry of Health. Physical activity was assessed by a structured questionnaire, which included detailed information on the type (branch) of physical activity, duration, and frequency from which physical activity is calculated as hour/week. The alcohol questionnaire was a detailed structured questionnaire asking about the type of alcohol, frequency, and amount of each intake, summed to determine the total daily consumption.

The participants were informed of their US or FibroScan® and blood test results only after completing the questionnaires to avoid reporting bias.

MASLD and liver fibrosis evaluation

MASLD was evaluated at baseline by liver US in all patients, whereas at follow-up, liver US was available only to a sub-sample. FibroScan® analysis was available only at the follow-up survey. At the follow-up survey, one of two methods was used to detect MASLD: 1) liver US using standardised uniform criteria performed by the same operator[21] at both the baseline and follow-up evaluation; or 2) CAP performed by the same operator and the same equipment (FibroScan® 502 Touch; Echosens, Paris, France) with a cut-off of ≥294 dB/m indicating fatty liver.[23] Persistent MASLD was defined as a diagnosis at both time points by either modality.

A non-invasive assessment of liver fibrosis was conducted using two methods. The first method involved FibroMax testing (BioPredictive, Paris, France), which was performed at the two time points. It included the FibroTest™, a fibrosis marker that has been extensively validated, and the presence of significant fibrosis ≥F2 was defined by a value of ≥0.48.[24] In addition, using a sub-sample, only at the follow-up evaluation, we performed liver stiffness measurements (LSM) using vibration-

controlled transient elastography (VCTE). The median of 10 measurements represented the LSM score, and it was considered reliable only if at least 10 successful acquisitions were obtained, and the IQR-to-median ratio was ≤0.3. Significant fibrosis was defined as a value ≥8.2 kPa.[25]

Nutritional variables evaluation and definitions

We calculated dairy product consumption in portions per day for each subject at the two time points. Dairy products were categorised into 1) low-medium fat low-sugar, 2) high-fat low-sugar, and 3) high-fat and/or high-sugar; a detailed list of dairy product variables is provided in Supplemental Materials and methods and Table S2. High dairy product consumption was considered above baseline and follow-up sex-specific medians (Table S2). Change in dairy product consumption was calculated as four categories: consumption under sex-specific medians in both time points (constantly low), consumption above the sex-specific median at baseline and under sex-specific median at follow-up (decreased), consumption under sex-specific median at the baseline and above sex-specific median at follow-up (increased), and consumption above the sex-specific median in both time-points (constantly high). In addition, the Alternate Healthy Eating Index-2010 (AHEI-2010) was calculated, a widely used tool for assessing dietary quality, incorporating evidence-based recommendations. This score has repeatedly been demonstrated to be inversely related to chronic diseases,[26,27] including MASLD.[28] The 11-item scoring system highlights the consumption of vegetables, fruits, whole grains, nuts, and legumes *vs.* low consumption of sodium, sugar-sweetened beverages, alcohol, and red/processed meat. Moreover, it takes into consideration the variation in different fat sources, including trans-fat, and different types of PUFA.

Animal study design and diet composition

Six-week-old C57BL/6J mice (Harlan Laboratories, IL, USA) were randomly divided into four dietary groups (n = 10): normal/control diet (ND/Control, 16% calories from fat, maintenance AIN-93G diet); high-fat lard-based diet (HFL); high-fat soybean-oil based diet (HFSBO), and high-fat milk-fat based diet (HFM). All HFD groups consisted of 60% calories from fat. The diet composition is detailed in Supplemental Materials & Methods and Tables S3 and S4. Animals were fed *ad libitum* with free access to water and were housed in cages within a controlled environment with the temperature set at 22 ± 2 °C under a 12/12 h light/dark cycle. Food consumption was calculated as the difference in consumption between the beginning and the end of the week. Ensuing 12 weeks mice were put in an overnight fast, and body weight and blood glucose measurements were recorded (oral glucose tolerance test [OGTT] at week 7 and fasting glucose at the end of the experiment). The mice were then euthanised in a random order. Blood samples were collected from the inferior vena cava into serum separation tubes and centrifuged at 5,000 × *g* for 10 min at 4 °C. Serum samples were then stored at -80 °C. Epididymal adipose tissue was removed and weighed. Liver tissue was collected and weighed, a sample of the right liver lobe was placed in a 4% formaldehyde solution, and the remaining tissue was frozen in liquid nitrogen and stored at -80 °C. The research complied with the Hebrew University of Jerusalem's Animal Care Guidelines and approval—Ethics Committee research number: AG-21-16610-4.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed as previously described[29] Briefly, following 7 weeks of a control diet or HFD, mice were fasted overnight, marked on the tail, and weighed. Blood glucose levels were measured using FreeStyle Optium Neo glucometer (Abbott Diagnostic Cared Ltd., Maidenhead, Berkshire, UK) according to the manufacturer's instructions. Mice were given a D-glucose solution (2 g/kg body weight) by gavage, and blood glucose levels were recorded at 0, 30, 60, 90, and 120 min post gavage using blood samples obtained from the tail tip.

Serum biochemical analysis

Sample analyses of serum total cholesterol, triglycerides (TG), glutamic pyruvic transaminase (GPT), and aspartate aminotransferase (AST) were performed by American Medical Laboratories Ltd. (Herzliya, Israel) on the chemical analyser Cobas 6000 (Roche Diagnostics, Basel, Switzerland).

Determination of hepatic steatosis grade

Preparation of histological slides was performed by ABBM (Authority of biological and biomedical models of The Hebrew University, Jerusalem, Israel). Briefly, liver tissue was embedded in paraffin, and the blocks were then sectioned into 3–5 μm sections and placed on glass slides. The slides were then stained with haematoxylin and eosin (H&E). Histopathological changes were scored using the MASLD Scoring System as previously described.[30] In brief, macro- and microvesicular steatosis were separately scored, and their severity was graded based on the percentage of the total area affected into one of four categories: 0 (<5%), 1 (5-33%), 2 (34–66%), and 3 (>66%). The score is presented as an average of the combined score.

Gene expression

Total RNA isolation from liver tissue was performed using a Tri-reagent solution (Sigma–Aldrich, Jerusalem, Israel) according to the manufacturer's protocol. Subsequently, complementary DNA was prepared with the high-capacity cDNA reverse transcription kit (Quanta BioSciences, Beverly Hills, CA, USA). Real-time PCR was performed with QuantStudio 1 (ThermoFisher Scientific, Waltham, MA, USA) with specific primers, listed in Table 1. The quantitative changes in gene expression of serum amyloid A1 (SAA1), serum amyloid A2 (SAA2), and tumour necrosis factor-alpha (TNFα) were thereafter determined by normalisation against 18S mRNA. Statistical significance was calculated using the 2–ΔΔCt method.

Quantification of serum AGEs

Serum advanced glycation end-products (AGEs) levels were calculated using the OxiSelect™ Advanced Glycation End Product Competitive ELISA Kit (Cell Biolabs, USA) following the manufacturer's instructions. In brief, samples were added to a pre-coated plate and incubated for 10 min. The first and second antibodies were added separately and incubated for 1 h. A substrate solution was added until sufficient colour development

Table 1. Primer sequences used for quantitative real-time PCR.

Name	Reverse	Forward
18S	5′-CCTCAGTTCCGAAAACCAAC-3′	5′-ACCGCAGCTAGGAATAATGG-3′
SAA1	5′-GATGAAGCTACTCACCAGCCT-3′	5′-GGTCAGCAATGGTGTCCTCA-3′
SAA2	5′-TTTTCTCAGCAGCCCAGACT-3′	5′-AATACTTCCCATGCTCGGGGG-3′
TNFα	5'-CCACAAGCAGGAATGAGAAGA-3'	5'-ACGTGGAACTGGCAGAAGAG-3'

SAA1, serum amyloid A1; SAA2, serum amyloid A2; TNFα, tumor necrosis factor-alpha.

was achieved and the reaction was stopped by the addition of a stop solution. Results were measured using the Infinite M Plex (Tecan Trading AG, Männedorf, Switzerland) at the wavelength 450 nm. The same methods were applied to human serum. Extreme values, more than 4 SD from the mean, were excluded from the analysis (in the human study, n = 4 were excluded with extreme values, and the four others did not have frozen serum samples).

Statistical analysis

Statistical analyses were performed using SPSS version 27 (IBM-SPSS Armonk, NY, USA). Continuous variables are presented as means ± SD. The independent samples *t* test was performed to test differences in continuous variables between the two groups. Associations between nominal variables were performed with the Pearson Chi-Square test, and *p* for trend was calculated when appropriate. A multivariable logistic regression analysis was performed to test the adjusted association between dairy product intake and MASLD, adjusting for potential confounders (MASLD-related variables, which differed between the dairy intake categories at baseline). Model A of the multivariable analysis included the major potential confounders demonstrated to be strongly related to MASLD, whereas model B was adjusted further for nutritional factors that potentially explained the association between dairy product consumption and MASLD. For the outcome, incidence of MASLD, only subjects without these outcomes at the baseline survey were included. For the combined outcome, either new onset or persistence of MASLD, the entire sample was included in the analysis. In this analysis, the comparison was made with subjects who never presented these outcomes or had a remission of the outcome at the follow-up evaluation. The fully adjusted model included potential confounders and mediators (*i.e.* age, sex, energy intake, BMI, and current smoking). The odds ratio (OR) and 95% CI are presented. A calculated sample size of 304 people was found to be sufficient for an expected OR of 0.5, with a significance level of 5% and power of 80%. The data for the animal study are presented as mean ± SEM. Data analysis was carried out by JMP version16 PRO software (SAS Institute, Cary, NC, USA) via unpaired two-tailed Student *t* test or by analysis of variance followed by the Tukey-Kramer honestly significant difference post hoc test. A *p*-value of ≤0.05 was considered statistically significant for all analyses. The statistical software is detailed in the CTAT table.

Results

Epidemiological prospective study

Study population and comparison of subjects with high and low dairy intake consumption

A total of 970 subjects participated in the baseline FFQ survey.[22] Of these, 402 subjects attended the follow-up survey. Eighty-two subjects were excluded because of the exclusion criteria. Of the 320 subjects remaining, 316 were assessed for MASLD in the follow-up survey and were included for analysis (101 subjects underwent liver US, 236 underwent CAP, and 21 subjects underwent both) (Supplemental Materials and methods and Fig. S1). In the final sample, 179 subjects were male (56.6%), the mean age at baseline was 58.65 ± 6.44 years, and the mean baseline BMI was 28.12 ± 5.48 kg/m^2. The mean follow-up time was 6.79 ± 0.67 years.

New-onset or persistence of MASLD was identified in 34.5% (n = 109/316) of the sample. Of participants without MASLD at baseline, 18.2% (n = 36/198) were diagnosed with new-onset MASLD. Remission of MASLD occurred in 38.1% (n = 45/118) of those with a MASLD diagnosis at baseline. New-onset or persistence of presumed significant fibrosis evaluated by the FibroTest was detected in 16.7% (n = 51/305) of the sample. The prevalence of presumed significant fibrosis in the follow-up survey, evaluated by LSM, was 10.2% (n = 24/236) of the sub-sample with LSM measurement.

At baseline, subjects with high consumption of low-medium fat low-sugar dairy products (categorised by sex-specific median) had higher BMI, higher caloric intake, SFA (as a percent of total energy intake [%TEI]), and cholesterol (mg/day), higher calcium and vitamin D intake, but lower carbohydrates (%TEI) intake, and consisted of a lower proportion of current smokers. In contrast, subjects with high consumption of high-fat and/or high-sugar dairy products had a higher homeostasis model assessment for insulin resistance (HOMA-IR) score. Participants who consumed high-fat dairy products also had higher calorie intake, total fat, SFA (%TEI), and calcium, but lower fibre intake (g/1,000 KCal/day) and lower AHEI-2010. There were no differences in alcohol or red and processed meat intake, calculated dietary AGEs at baseline and serum AGEs levels at follow-up across dairy intake groups (Table 2).

Multivariable association of dairy products consumption at baseline with MASLD and significant fibrosis evaluated by FibroTest or LSM

High consumption of low-medium fat low-sugar dairy products was associated with significantly lower odds for new-onset/persistence (OR 0.58, 95% CI 0.34–0.97, $p = 0.039$) or incidence of MASLD (OR 0.42, 95% CI 0.18–0.95, $p = 0.037$), adjusting for potential confounders and mediators: baseline age, sex, BMI, energy and fibre intake (g/1,000 KCal/day), and current smoking (Table 3, Model A). No significant association was observed between high consumption of high-fat low-sugar dairy products with new-onset/persistent MASLD (OR 1.60, 95% CI 0.95–2.69, $p = 0.077$). There was no association between high consumption of high-fat and/or high-sugar dairy

Table 2. Baseline characteristics of participants according to dairy product consumption.

Variable	Low-medium fat low-sugar fat dairy products			High-fat and/or high-sugar dairy products		
	Low consumption* (n = 154)	High consumption* (n = 162)	*p* value	Low consumption† (n = 156)	High consumption† (n = 160)	*p* value
Age (years)	58.49 ± 6.43	58.80 ± 6.46	0.670	58.81 ± 6.35	58.48 ± 6.54	0.647
Sex (%)	59.10	54.30	0.392	57.10	56.30	0.886
BMI (kg/m^2)	27.43 ± 4.52	28.77 ± 6.21	**0.030**	27.63 ± 5.10	28.60 ± 5.81	0.117
Weight change (%)‡	-0.23 ± 12.02	-1.13 ± 7.53	0.422	-0.90 ± 6.81	-0.50 ± 12.29	0.720
HOMA-IR (score)	2.58 ± 1.62	2.91 ± 2.24	0.133	2.37 ± 1.41	3.13 ± 2.33	**0.001**
Triglycerides (mg/dl)	114.12 ± 62.53	110.85 ± 59.80	0.635	106.76 ± 63.48	118.00 ± 58.27	0.103
ALT (U/L)	27.42 ± 18.60	26.78 ± 13.65	0.724	26.39 ± 15.40	27.78 ± 17.00	0.448
Total cholesterol (mg/dl)	180.14 ± 35.66	182.75 ± 34.13	0.506	182.19 ± 35.81	180.79 ± 33.98	0.723
LDL-cholesterol (mg/dl)	105.30 ± 30.78	103.50 ± 27.22	0.586	105.47 ± 28.45	103.32 ± 29.54	0.514
HDL-cholesterol (mg/dl)	52.52 ± 14.73	56.27 ± 17.58	0.042	55.00 ± 16.44	53.89 ± 16.25	0.549
AST (U/L)	25.26 ± 6.35	25.75 ± 8.87	0.616	25.55 ± 8.43	25.48 ± 8.81	0.945
HbA1C (%) (n = 309)	5.84 ± 0.75	5.76 ± 0.60	0.335	5.76 ± 0.49	5.83 ± 0.82	0.396
Serum AGEs at follow-up (μg/ml)§ (n = 308)	12.33 ± 11.13	12.80 ± 12.27	0.723	12.29 ± 12.33	12.84 ± 11.09	0.681
Nutritional and lifestyle habits						
AHEI-2010	59.56 ± 10.40	59.37 ± 10.60	0.869	61.04 ± 10.50	57.93 ± 10.27	0.008
Alcohol consumption (portions/week)	1.80 ± 2.93	1.84 ± 2.66	0.886	1.85 ± 2.68	1.79 ± 2.90	0.862
Energy (KCal/day)	1,848.74 ± 622.85	2,144.96 ± 681.72	**<0.001**	1,859.97 ± 630.71	2,137.72 ± 679.23	**<0.001**
Protein (%TEI)	17.67 ± 4.78	20.18 ± 4.46	<0.001	18.80 ± 5.08	19.11 ± 4.48	0.568
Carbohydrates (%TEI)	42.81 ± 9.51	39.52 ± 7.69	**0.001**	42.01 ± 9.00	40.27 ± 8.47	0.077
Fat (%TEI)	35.97 ± 6.88	36.77 ± 5.58	0.258	35.29 ± 6.07	37.45 ± 6.25	**0.002**
Saturated fatty acids (%TEI)	11.42 ± 3.59	13.57 ± 3.15	**<0.001**	11.37 ± 2.94	13.64 ± 3.71	**<0.001**
Cholesterol (mg/1,000 KCal/day)	165.45 ± 74.27	174.64 ± 66.03	0.245	166.84 ± 76.22	173.40 ± 63.88	0.408
Fibers (g/1,000 KCal/day)	12.37 ± 4.92	11.51 ± 4.13	0.095	13.11 ± 4.90	10.78 ± 3.86	**<0.001**
SSBs (cups/day)	2.07 ± 3.49	1.63 ± 3.06	0.235	1.77 ± 3.08	1.92 ± 3.46	0.671
Calcium mg/1,000 KCal/d	464.49 ± 158.89	632.66 ± 178.97	<0.001	522.61 ± 185.58	578.09 ± 188.85	0.009
Vitamin D μg/1,000 KCal/d	7.84 ± 14.33	19.65 ± 34.41	<0.001	12.70 ± 27.79	15.06 ± 26.66	0.443
Red and processed meat (portions/day)	0.61 ± 0.91	0.72 ± 1.20	0.354	0.57 ± 0.89	0.77 ± 1.21	0.105
Current smokers (%)	16.20	7.40	**0.015**	13.50	10.00	0.339
Physical activity (hour/week)	2.33 ± 2.98	2.83 ± 3.46	0.168	2.88 ± 3.28	2.30 ± 3.18	0.109
Calculated dietary AGEs (Ku/1,000 KCal/day)	5,415.21 ± 2,564.80	5,484.11 ± 1,987.43	0.790	5,423.49 ± 2,373.57	5,476.90 ± 2,199.63	0.836

The independent samples t-test was performed to test differences in continuous variables between the two groups. Associations between nominal variables were performed with the Pearson Chi-Square test. AGEs, advanced glycation end products; AHEI-2010, Alternate Healthy Eating Index-2010; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HbA1C, hemoglobin A1C (Glycated hemoglobin); HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein; SSBs, sugared sweetened beverages; %TEI, percent of total energy intake. In bold *p*-value <0.05.

*High consumption - defined above the baseline sex-specific median ≥1.71 or 2.00 per day for men and women.

†High consumption - defined above the baseline sex-specific median ≥0.36 or 0.21 per day for men and women.

‡Weight change calculated as the percent of change from baseline: [follow-up weight (kg) - baseline weight (kg)]/baseline weight (Kg) * 100.

§Serum AGEs were measured only at the follow-up survey.

products and MASLD**.** Low-medium fat low-sugar or high-fat low-sugar dairy products were not associated with fibrosis evaluated by the FibroTest. High consumption of high-fat and/or high-sugar dairy products tended to be associated with new-onset/persistent fibrosis evaluated by FibroTest, but the association did not reach statistical significance (OR 1.97, 95%CI 0.97–3.99, p = 0.059) (Table 3, Model A). For all the above-mentioned analyses, further adjustments for additional nutritional variables; baseline calcium and vitamin D consumption and AHEI-2010, yielded similar results (Table 3, Model B). There was no significant association between dairy product intake of any type and presumed liver fibrosis evaluated by LSM (Supplemental Materials and methods and Table S5).

Multivariable association of trends over follow-up in dairy product consumption and new-onset/persistence of MASLD

In a multivariable analysis adjusting for the same potential confounders (as in Table 3, Model A), constant high consumption of low-medium fat low-sugar dairy products was associated with lower odds for new-onset/persistence of MASLD (OR 0.51, 95% CI 0.26–0.99, p = 0.046) (Table 4, Model A). Constant high consumption of high-fat low-sugar dairy products was associated with 2.02-fold greater odds of developing new onset/persistence of MASLD (95% CI 1.06–3.85, p = 0.034) (Table 4, Model A). However, a constant high consumption of high-fat and/or high-sugar dairy products was not related to MASLD**.** For all the above-mentioned analyses, further adjustments for additional nutritional variables; baseline calcium and vitamin D consumption and AHEI-2010, yielded similar results (Table 4, Model B).

Animal study supplemented with HFDs

Effect of fat source on weight, glucose tolerance, liver, and epididymal fat weight

No significant differences in body weight or caloric intake (Fig. S2) were observed between HFD groups, whereas the control group gained less weight when compared with all HFD groups (Fig. 1A). HFM exhibited significantly poorer glucose tolerance when compared with the control diet but not when compared with HFL or HFSBO. The HFL group demonstrated better glucose tolerance than HFSBO but not HFM (Fig. 1B). There were no differences between HFD groups in mean liver weight, albeit this was significantly higher than control (Fig. 1C). Epididymal fat tissue weight was significantly higher in the HFM group when compared with HFSBO and controls, but not in comparison with HFL (Fig. 1D).

Dietary source of fat and serum biochemical markers

Serum cholesterol levels were markedly lower in HFL and HFSBO compared with HFM (Table 5). No differences were found between HFD groups in means serum TG values, which were lower compared with the control group (Table 5). Different fat sources had no significant effect on serum alanine aminotransferase (ALT) (Table 5). Similarly, no differences were observed in AST between diet groups (Table 5).

Effect of diet on steatosis grade

Liver sections stained in H&E are shown (Fig. 2). Abnormal liver morphology is evident with higher levels of hepatocellular vesicular steatosis in the HFD groups as compared with the control (Fig. 2A). HFM had the highest steatosis grade when compared with the control group, but there were no significant differences between HFD groups (Fig. 2B).

Effect on gene expression and serum AGEs levels

No significant differences were observed when comparing groups in gene expression of SAA1, SAA2, or TNFα in the liver tissue (Table 6). Although not significant, the HFM group had higher expression of SAA1 and SAA2 (Table 6). Likewise, HFM and HFL groups had higher mRNA expression of TNFα (Table 6). Significant elevation of AGEs level was found in the HFM group when compared with the HFSBO group and controls, but not with the HFL group (Table 6).

Discussion

This study showed that high consumption of low-medium fat low-sugar dairy products in patients with MASLD at baseline and their prolonged high consumption were independently associated with a lower risk of MASLD incidence or persistence over time. In contrast, high-fat, low-sugar dairy consumption showed an inconsistent association with MASLD, with baseline consumption having a non-significant association with MASLD, whereas constantly high consumption was associated with two-fold greater odds for new onset/persistent MASLD. However, there was no significant association between intake of all types of dairy products and presumed liver fibrosis as evaluated by the FibroTest or FibroScan. Our findings are in line with our study hypothesis and partially confirm those of previous studies, especially those of a meta-analyses showing either a neutral association between dairy consumption and MASLD[12] or an inverse association between dairy intake and MASLD.[13]

While low-fat dairy seems safe and even beneficial to consume, the debate remains regarding high-fat dairy products, which are usually not tested in clinical studies, and when these products are evaluated, the definition of the high fat percentage is unclear and varies between different studies. A cross-sectional study found that high consumption of full-fat dairy products (without specifying fat percentage) was inversely related to MASLD and insulin resistance.[11] Moreover, milk consumption was negatively associated with MASLD in cross-sectional studies among Chinese[31] and Iranian[32] populations, but low-fat and high-fat dairy products were not tested separately. Noteworthy, the Dietary Approach to Stop Hypertension (DASH) diet, characterised by high consumption of low-fat dairy products, together with fruits, vegetables, whole grains, and legumes or nuts but low in saturated fat, sodium, and added sugars, has been reported to be beneficial in patients with MASLD.[33] In contrast to epidemiological human feeding studies, it is clear that high saturated fat diets are deleterious. For example, in a double-blind RCT, supplementation for 8 weeks with SFA from palm oil *vs.* PUFA from sunflower oil led to a similar body weight gain of about 2 kg, but only the SFA diet markedly increased liver fat content, liver

Table 3. Multivariable analysis for the association between dairy product consumption (above sex-specific median) at baseline and incidence or persistence of MASLD and presumed significant fibrosis at follow-up.

		New-onset or persistence (vs. never or remission)	Incidence (new onset among those without the outcome at baseline)
		OR (95% CI), *p* value	
MASLD		**n/N (109/316)**	**n/N (36/198)**
Low-medium fat low-sugar dairy products			
	Low <median	1 (ref)	1 (ref)
Modal A	High ≥median (1.71 or 2.00 per day	0.58 (0.34-0.97), **0.039**	0.42 (0.18-0.95), **0.037**
Modal B	for men and women)	0.49 (0.27-0.88), **0.017**	0.37 (0.14-0.95), **0.039**
High-fat low-sugar dairy products			
	Low <median	1 (ref)	1 (ref)
Modal A	High ≥median (0.18 or 0.14 per day	1.60 (0.95-2.69), 0.077	1.67 (0.75-3.71), 0.205
Modal B	for men and women)	1.59 (0.94-2.69), 0.086	1.72 (0.76-3.85), 0.191
High-fat and/or high-sugar dairy products			
	Low <median	1 (ref)	1 (ref)
Modal A	High ≥median (≥0.36 or 0.21 per day	1.15 (0.68-1.92), 0.604	0.94 (0.43-2.06), 0.867
Modal B	for men and women)	1.15 (0.68-1.94), 0.602	0.97 (0.44-2.15), 0.936
Significant fibrosis* (≥F2)		**n/N (51/305)**	**n/N (30/261)**
Low-medium fat, low-sugar dairy products			
	Low <median	1 (ref)	1 (ref)
Modal A	High ≥median (≥1.71 or 2.00 per day	1.25 (0.63-2.46), 0.525	1.71 (0.73-4.00), 0.213
Modal B	for men and women)	1.42 (0.64-3.14), 0.384	1.83 (0.68-4.88), 0.229
High-fat low-sugar dairy products			
	Low <median	1 (ref)	1 (ref)
Modal A	High ≥median (≥0.18 or 0.14 per day	1.51 (0.75-3.02), 0.248	1.48 (0.63-3.48), 0.374
Modal B	for men and women)	1.55 (0.76-3.15), 0.225	1.41 (0.58-3.41), 0.445
High-fat and/or high-sugar dairy products			
	Low <median	1 (ref)	1 (ref)
Modal A	High ≥median (≥0.36 or 0.21 per day	1.97 (0.97-3.99), 0.059	1.59 (0.67-3.78), 0.292
Modal B	for men and women)	1.96 (0.96-3.99), 0.065	1.50 (0.62-3.62), 0.371

A multivariable logistic regression analysis was performed to test the adjusted association between dairy product intake and MASLD outcomes. Model A adjusted for baseline age (years), sex (% male), BMI (Kg/m^2), energy intake (KCal/day), fibres (g/1,000 KCal/day), and current smoking. Model B adjusted for all variables in model A and for baseline calcium consumption (mg/1,000 KCal/day), vitamin D consumption (μg/1,000 KCal/day), and AHEI-2010 tertiles (1st; AHEI score<55, 2nd; AHEI score 55-65, 3rd; AEHI score≥65). AHEI-2010, Alternate Healthy Eating Index-2010; BMI, body mass index; 95% CI, 95% confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease; OR, odds ratio. In bold *p*-value <0.05.

*Significant fibrosis evaluated by FibroTest.

enzymes, and atherogenic serum lipids.[4] In another RCT, a daily intake of 1,000 extra KCal of SFA from a mixture of coconut oil, butter, and blue cheese, compared with PUFA or simple sugars over 3 weeks, revealed that SFA led to the greatest increase in liver fat and insulin resistance.[5]

In the present study, we further elaborated on these epidemiological associations in human studies using an animal model. C57Bl/6j mice were used to compare high milk-fat and lard/soybean-based HFDs for MASLD-related parameters. Higher fat consumption in mice led to an alteration of body composition and metabolism independently of fat sources. Although the dietary SFA content corresponded to the weight of the epididymal fat, a favourable trend in glucose tolerance and liver weight was observed in mice fed HFD based on lard compared with SBO and milk fat. A study conducted on Swiss mice fed with a high-fat, high-sugar diet observed that animals fed a butter-based diet had higher body and liver weight than lard and SBO.[34] In another study, replacing half the fat from lard with SBO in a high-fat, high-fructose diet mitigated weight gain in mice.[35] Our current study observed that the relatively high fraction of saturated fat in milk contributes to metabolic impairment in isocaloric HFDs. Cow/bovine milk contains a higher amount of SFA (≥60%) than chicken fat, lard, and beef tallow (35%, 40%, and 55%, respectively).[36]

Contradictory to plant-based oils, animal-sourced fats inherently contain cholesterol. In murine models, HFD and high-cholesterol diets induced steatosis with inflammation and fibrosis.[37] Furthermore, the addition of cholesterol to HFD contributes to the development of MASLD-associated hepatocellular carcinoma.[38] Serum cholesterol levels can be influenced by variables other than dietary cholesterol intake. In a meta-analysis of 55 randomised trials involving 2,065 participants, the reduction in serum cholesterol was achieved by replacement of butter by lard or SBO, or lard by SBO, and the beneficial effect was attributed to the unsaturated fatty acids content and n-3 and n-6 fatty acids specifically.[39] In an animal study, SBO, though not containing cholesterol alone, might aggravate cholesterol-induced hepatic injury.[40] Our animal study results are in agreement with the above, whereby higher caloric intake from fat resulted in elevated cholesterol, most significant in the milk-fat group.

Cholesterol is usually consumed with SFA, which, according to our results when associated with milk fat, may have a deleterious effect on blood cholesterol levels.[41] Mice fed HFD had lower serum TG when compared with controls receiving a high carbohydrate-supplement. Reducing carbohydrates overall lowers serum TG, and replacing carbohydrates with SFA yields a similar effect, albeit elevating LDL-

Table 4. Multivariable association between trends over follow-up in the intake of low-medium or high-fat dairy products and new-onset or persistent MASLD.

	New-onset or persistent MASLD n/N (109/316)	
	OR (95% CI), *p* value	
	Model A	Model B
Low-medium fat low-sugar dairy products (n/N)		
Constantly low (38/96)	1 (ref)	1 (ref)
Decrease (19/66)	0.53 (0.26-1.09), 0.083	0.45 (0.21-0.96), **0.040**
Increase (20/58)	0.75 (0.37-1.53), 0.427	0.71 (0.34-1.46), 0.351
Constantly high (32/96)	0.51 (0.26-0.99), **0.046**	0.40 (0.19-0.85), **0.018**
High-fat low-sugar dairy products (n/N)		
Constantly low (28/108)	1 (ref)	1 (ref)
Decrease (20/50)	1.65 (0.77-3.56), 0.202	1.65 (0.76-3.58), 0.206
Increase (19/59)	1.55 (0.74-3.23), 0.246	1.58 (0.75-3.31), 0.226
Constantly high (42/99)	2.02 (1.06-3.85), **0.034**	2.01 (1.05-3.87), **0.036**
High-fat and/or high-sugar dairy products (n/N)		
Constantly low (31/103)	1 (ref)	1 (ref)
Decrease (16/52)	0.88 (0.40-1.94), 0.756	0.90 (0.41-1.98), 0.790
Increase (18/53)	1.19 (0.56-2.54), 0.657	1.19 (0.56-2.55), 0.652
Constantly high (44/108)	1.41 (0.76-2.64), 0.280	1.41 (0.75-2.65), 0.288

A multivariable logistic regression analysis was performed to test the adjusted association between dairy product intake and MASLD outcomes. Model A adjusted for baseline age (years), sex (% male), BMI (Kg/m^2), energy intake (KCal/day), fibers (g/1,000 KCal/day), and current smoking. Model B adjusted for all variables in model A and for baseline calcium consumption (mg/1,000 KCal/day), vitamin D consumption (mcg/1,000 KCal/day), and AHEI-2010 tertiles (1st; AHEI score <55, 2nd; AHEI score 55-65, 3rd; AEHI score ≥65). AHEI-2010, Alternate Healthy Eating Index-2010; BMI, body mass index; 95% CI, 95% confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease; OR, odds ratio.[*,†,‡,§] In bold *p*-value <0.05.

*Consistent low consumption: consumption under medians in both evaluations.

†Decreased: consumption above the median at the baseline survey and under the median at the follow-up evaluation.

‡Increased: consumption under median at the baseline survey and above median at the follow-up evaluation.

§Consistent high consumption*:* consumption above the median in both evaluations.

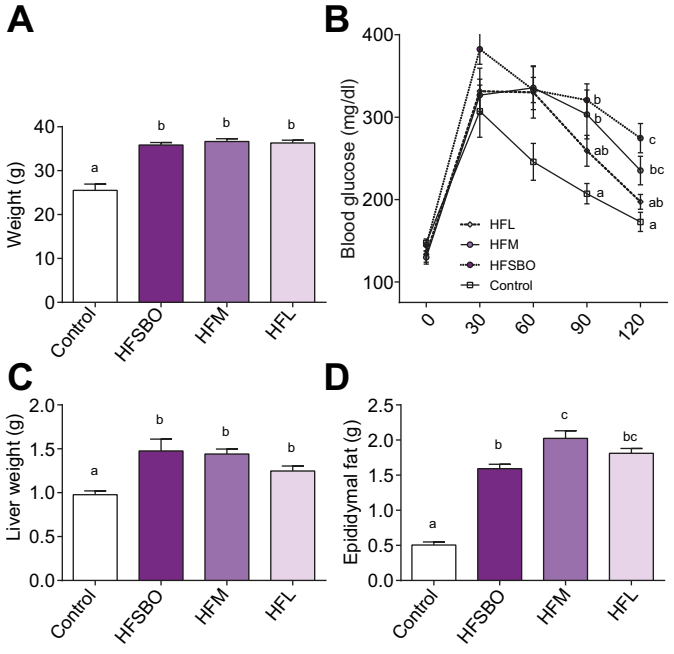

Fig. 1. Effect of dietary fat source on weight gain, glucose tolerance, liver, and epididymal tissue weight. Male C57BL/6J mice fed normal/control diets (ND/control) or high-fat diets (HFD) based on soybean oil (HFSBO), milk fat (HFM), or lard (HFL). (A) Body weight before sacrifice. (B) Oral glucose tolerance test (OGTT) performed at week 7. (C) Liver tissue weight at sacrifice. (D) Epididymal tissue weight at sacrifice. All values are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA Tukey–Kramer HSD for differences between pairs. Means without a common letter are statistically different, p <0.05.

cholesterol levels.[42] These results indicate that the fat composition and fatty acid profile have minimal effect on TG blood level. Another animal study involving high-fat high-fructose diets exhibited lower TG in groups fed diets based on lard alone and lard and SBO mix compared with the control group.[35]

The role of oxidative stress in the pathogenesis of MASLD and MASH has been studied previously and has been shown to be correlated with both dietary patterns and components.[43] Excessive lipid accumulation and impaired metabolism can lead to elevation in reactive oxygen species levels, ensuing oxidative stress and inflammation, factors linked to MASLD progression.[44]

During the processing and storage of food items, oxidative processes can occur in food products and lead to a reduction in quality and the formation of reactive carbonyls and glycation products such as AGEs.[45,46] The levels of AGEs can be evaluated because of their stability and have been proposed as a possible biomarker for MASLD.[47] While it is possible for endogenous production of AGEs, recent studies suggest that dietary AGEs consumption had an adverse contribution to fibrosis and liver injury in mice, and serum AGEs have even been proposed as a possible biomarker for MASLD.[48] In our study, different dietary sources of fat had a significant effect on serum AGEs in mice, probably stemming from the AGEs content of the diet itself. In contrast, there was no association between dairy intake and serum AGEs in the human cohort study, perhaps because of the low level of consumption of high-fat and/or high-sugar dairy products. Dairy products have several factors contributing to higher AGEs content, including

Table 5. Effect of diet on serum cholesterol, triglycerides, and liver enzymes.

	Control diet	High-fat soybean oil	High-fat milk	High-fat lard
Serum cholesterol (mg/dL)	102.8 ± 5.3	147.9 ± 8.9*	184.1 ± 3.8†	155.4 ± 8.2*
Serum triglycerides (mg/dL)	113.8 ± 10.2	81.6 ± 4.7*	87.2 ± 3.4*	86.3 ± 6.0*
Serum ALT (IU/L)	88.1 ± 19.9	100.5 ± 18.0	90.6 ± 14.6	90.1 ± 19.0
Serum AST (IU/L)	87.0 ± 23.0	75.5 ± 17.7	76.9 ± 13.6	62.7 ± 9.1

ALT, alanine aminotransferase; AST, aspartate aminotransferase. All values are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA Tukey–Kramer HSD for differences between pairs, significant difference set as p <0.05.
*Significant difference compared with the control diet.
†Significant difference compared with high-fat lard and high-fat soybean oil.

Table 6. Effect of diet on hepatic inflammation markers and serum AGEs levels.

	Control diet	High-fat soybean oil	High-fat milk	High-fat lard
Relative mRNA expression				
Serum amyloid A1	1.00 ± 0.10	1.07 ± 0.71	1.57 ± 0.41	0.92 ± 0.20
Serum amyloid A2	1.00 ± 0.10	1.23 ± 0.66	1.98 ± 0.52	1.36 ± 0.24
TNFα	1.00 ± 0.10	0.89 ± 0.12	1.63 ± 0.21	1.86 ± 0.47
Serum AGEs (μg/ml)	2.47 ± 0.27	3.05 ± 0.26	4.50 ± 0.42*	2.90 ± 0.31

AGEs, advanced glycation end-products; TNFα, Tumor necrosis factor α. Statistical analysis was performed using one-way ANOVA Tukey–Kramer HSD for differences between pairs, significant difference set as p <0.05.
*Significant difference compared with all groups.

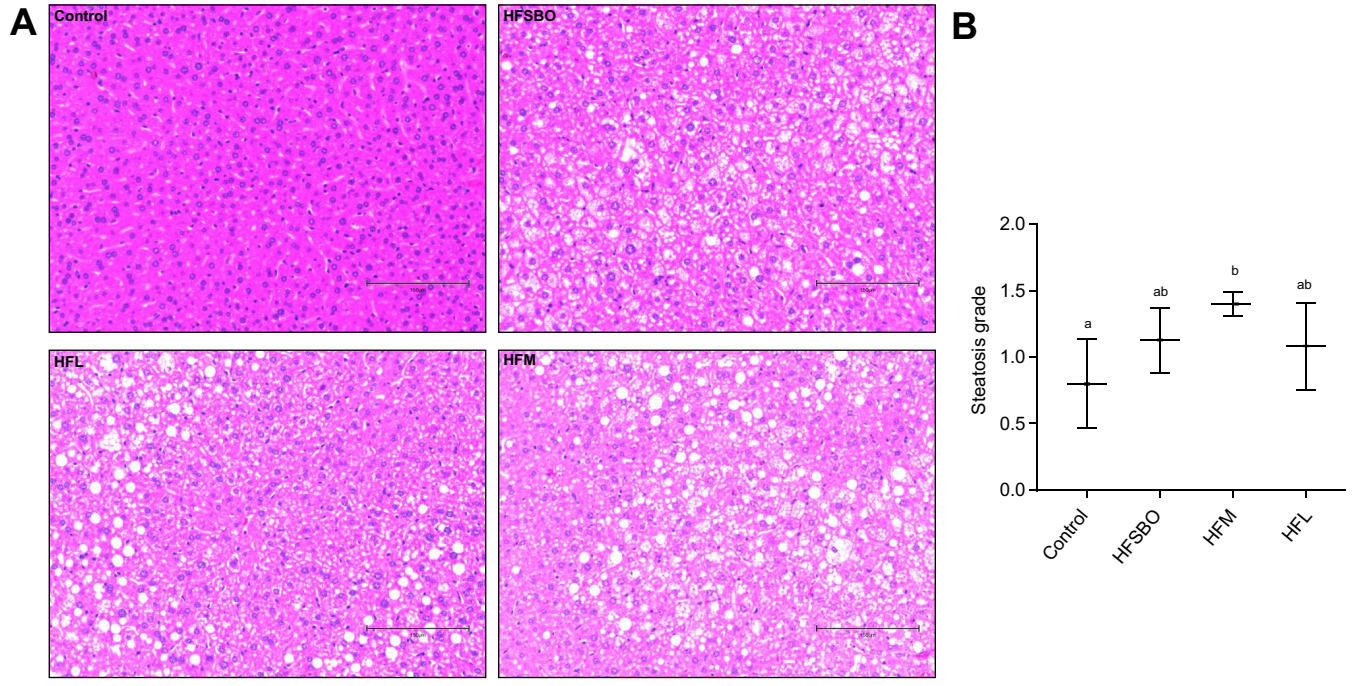

Fig. 2. Effect of different fat sources on liver steatosis and inflammatory markers. (A) Haematoxylin and eosin staining of liver sections, representative sections for diets with scale bars to represent 150 μm. (B) Steatosis grade scored using the general MASLD scoring system for rodent models using semiquantitative grading of 4 grades (0, <5%; 1, 5-33%; 2, 34-66%, and 3, >66%). Statistical analysis was performed using one-way ANOVA Tukey–Kramer HSD for differences between pairs. Means without a common letter are statistically different, p <0.05.

the composition of sugars, fats, and proteins, as well as the process of pasteurisation, sterilisation, and, in some cases, lengthy storage.[49] Higher fat content correlated with higher AGEs content, promoted by lipid oxidation, and aggravated furthermore by the high processing temperatures.[50]

No indication of liver damage was identified regardless of lipid saturation, but the higher SFA content did exacerbate fat accumulation in the tissue. C57BL/6j mice fed HFD typically gain weight and present with impaired metabolism and hepatic steatosis.[51] Dietary saturated fat further induces the accumulation of hepatic fat and is associated with the development and progression of MASLD.[52,53] Our results showed that HFM attributed the highest steatosis grade between test groups, but steatosis remained mild in all groups, and tissue lacked signs of inflammation or fibrosis, suggesting a relatively early stage of the disease. The lack of inflammatory gene expression in the HFD groups further indicates minimal tissue damage. Serum amyloid A (SAA) expression, both in men and mice, marks an acute phase response in reaction to tissue injury or infection and has a role in lipid metabolism and a regulatory role in inflammation.[54] SAA is often associated with MASLD and is used as an indicator of chronic inflammation related to the

progression of the disease.[55,56] TNFα, like SAA, is a regulatory cytokine implicated in MASLD pathogenesis and has even been linked to the severity of disease with higher levels in more progressive states.[57]

The strengths of our study include its prospective study design, two-time points detailed assessments of dairy intake enabling evaluation of changes in intake during follow-up, and separation between low and high-fat or sugary dairy products. Finally, we evaluated liver fibrosis markers. The human study provides practical information since the median consumption of low-medium fat low-sugar dairy products is 1.71 or 2.00 portions per day for men and women respectively in our study, a threshold that aligns with the accepted recommendations for dairy intake by different national food-based dietary guidelines. The recommendation in Europe is mostly in a range of two to four portions of dairy products per day, and in North America two to three portions per day, without sex-specific recommendations.[58]

However, this study has several limitations. First, dietary habits were self-reported, which may lead to a reporting bias. Nevertheless, since the participants and the research team were blinded to the liver and blood test results at baseline and given the prospective nature of the study, it is a non-differential bias and may have only led to underestimation of the observed associations. Second, the diagnosis of MASLD was determined by liver US or CAP *vs.* liver histology, which is impossible to obtain in a study among the general population. In addition, the diagnostic method of MASLD changed during the follow-up study, although both methods are well-validated. Lastly, since this study was conducted in a single country, the generalisability of our findings may be limited to other populations with different types and amounts of dairy product consumption. Dairy consumption characteristics in Israel may differ from those in other countries; for example, a high proportion of dairy products is low fat, whereas the consumption of high-fat products is relatively low in Israel. Thus, the lack of significance in the association of MASLD with high-fat dairy product consumption may be attributed to the limited variability in consumption in Israel, remaining at a relatively low level that may be less harmful: a 0.3 portion per day (roughly the study median) translates for example to one small piece of hard/yellow cheese (20 g) or one-third cup of sour cream (70 g) per day. Therefore, this association should be further tested in populations with higher consumption of high-fat products.

As for the animal models, these models are designed to mimic the morphologic key features of metabolic impairment associated with liver damage, cardiovascular events, and progressive liver damage. Despite metabolic similarities, most models have limitations in developing the features of the disease. However, these models contribute significantly to our understanding of the pathogenic aspects of the disease, despite the obvious differences.

Conclusion

Based on the findings of this study, low-medium fat low-sugar dairy products can be protective for MASLD but not for fibrosis. It may be advised to minimise high-fat dairy products, but more evidence is needed. This is supported by animal model showing that consumption of a HFM diet is not much different from lard and SBO in terms of causing metabolic impairment and liver damage, and it can result in higher levels of AGEs and cholesterol, and minor deleterious effects on glucose metabolism. Additional research is needed to confirm our findings, particularly regarding the specific effects of milk fat on liver pathogenesis.

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Abbreviations

AGEs, advanced glycation end-products; AHEI-2010, Alternate Healthy Eating Index-2010; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CVD, cardiovascular disease; DASH, dietary approaches to stop hypertension; FFQ, food frequency questionnaire; GPT, glutamic pyruvic transaminase; HFD, high-fat diet; HFL, high-fat lard-based diet; HFM, high-fat milk-fat based diet; HFSBO, high-fat soybean-oil based diet; HOMA-IR, homeostasis model assessment for insulin resistance; LSM, liver stiffness measurements; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, Metabolic dysfunction-associated steatotic liver disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ND, normal/control diet; OGTT, oral glucose tolerance test; OR, odds ratio; PUFA, polyunsaturated fatty acids; RCTs, randomised control trials; SAA, serum amyloid A; SAA1, serum amyloid A1; SAA2, serum amyloid A2; SBO, soybean-oil; SFA, saturated fatty acids; TAG, triacylglycerol; %TEI, percent of total energy intake; TG, triglycerides; TNFa, tumour necrosis factor-alpha; US, ultrasound; VCTE, vibration-controlled transient elastography.

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Conflicts of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conceived the research question: OT, SZS Conducted all animal experiments: OT, MV. Critically reviewed the manuscript: MV, LSG, NFI, MW, RK, OS. Developed the methods: SZS, OT, RK. Drafted the manuscript: OT, SZS. Performed the data analysis: MV, SZS, DIV. Performed the data collection: LSG, SZS, DIV, NFI, RK, MV, OT. Performed the ultrasonography evaluation: MW. Provided input on data analysis, interpretation: OT, SZS, MV, DIV, LSG. Approval of the final version of the manuscript: all authors.

Data availability statement

Human study: Given the sensitive nature of data collected in a medical institute, the data are confidential and cannot be shared.

Animal study: Data are available upon request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2024.101194.

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Author names in bold designate shared co-first authorship

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