Short report

Dietary n-6 to n-3 fatty acid ratio is related to liver fat content independent of genetic effects: evidence from the monozygotic co-twin control design

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Words: 1454 including the abstract, excluding references

Keywords: identical twins; twin study; liver fat; nutrition; omega-6: omega-3 ratio

Abstract

Background & aim: Lifestyle changes focusing on diet and exercise remain the cornerstone of the treatment of non-alcoholic fatty liver disease (NAFLD). The present co-twin control study of monozygotic (MZ) twin pairs was designed to identify nutritional factors potentially involved in the pathogenesis of NAFLD.

Methods: Cross-sectional study of 50 MZ twin pairs (age range: 23-36 years), of which ten pairs were discordant for liver fat (liver fat percentage of one twin ≤ 5% and his/her co-twin > 5% and a difference between co-twins of > 5%) as determined by magnetic resonance spectroscopy. Nutrient intake was calculated from 3-day food records.

Results: Among the ten liver fat-discordant twin pairs, the n-6: n-3 ratio was significantly higher in the twins with higher liver as compared to their co-twins with lower liver fat (6.6: 1 vs. 3.2: 1, *p*-value = 0.005). In multiple regression analysis of within-pair differences including all 50 twin pairs, a higher n-6: n-3 ratio was significantly associated with a higher liver fat percentage within MZ twin pairs after adjustment for body mass index, energy intake and other covariates (standardized beta= 0.43, *p*-value = 0.001).

Conclusions: Our findings suggest that the n-6: n-3 ratio is a promising dietary agent for the prevention and treatment of NAFLD. Clinical trials are required to better understand causal relationships and required doses.

Introduction

Non-alcoholic fatty liver disease (NAFLD), characterized by fat accumulation in more than 5% of hepatocytes in the absence of excessive alcohol consumption, is the most common chronic liver disease in the world. It affects up to a quarter of the general population, depending on the country studied[1]. The disease spectrum includes simple steatosis, steatohepatitis, advanced fibrosis and cirrhosis which can progress to liver failure[1].

The heritability of NAFLD is low, underscoring the importance of environmental factors in its etiology[2]. Currently, there are no approved pharmacotherapies for patients with NAFLD. Therefore, weight reduction and lifestyle changes through diet and exercise form the first-line therapy for treatment[3]. Diets high in saturated fatty acids and refined carbohydrates could exacerbate NAFLD[4], while Mediterranean dietary patterns might reduce liver fat even in the absence of weight loss[5]. Randomized controlled trials suggest that supplementation with omega-3 (n-3) polyunsaturated fatty acids (PUFAs) has therapeutic potential for treating patients with NAFLD [6].

Another powerful study design that allows stronger inference about causality than observational studies of unrelated individuals is the co-twin-control design of disease-discordant monozygotic (MZ) twin pairs because MZ twins are genetically identical at the DNA sequence level and discordance within pairs must thus be of environmental origin. Our previous twin study suggests that metabolic abnormalities in acquired obesity are tightly linked to liver fat content[7]. The present study of MZ twins was designed to identify nutritional factors potentially involved in the pathogenesis of NAFLD.

Research design and methods

TwinFat study participants were enrolled from two Finnish population-based longitudinal studies of five consecutive birth cohorts of young adult twins[8]. Participants were invited to the TwinFat study based on their reported body mass index (BMI) during these studies (at the age of 23 to 27 years) with the aim to represent a wide range of intra-pair differences in BMI. Participants were then weighed and their height was measured while barefoot and in light clothing by trained research nurses. Whole-body fat was measured by dual energy x-ray absorptiometry, abdominal subcutaneous and intra-abdominal fat by magnetic resonance imaging and liver fat percentage by magnetic resonance spectroscopy as described earlier[9]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated based on 2-hour oral glucose tolerance tests. Fasting plasma cholesterol and triglyceride concentrations were determined with enzymatic methods. Supine blood pressure was measured using an automatic digital sphygmomanometer (mean of three measurements).

Nutrient intake was calculated from 3-day food records by the Diet32 program (Aivo), based on a national Finnish database (Fineli, www.fineli.fi, National Institute for Health and Welfare, Nutrition Unit, Helsinki, Finland). The sports activity index was calculated from the Baecke questionnaire[10]. Out of 107 twin individuals with data on liver fat and food records, one twin individual was excluded because of missing information on physical activity, two twin individuals because of implausible energy intakes (one woman reported < 714 kcal/day, and one men > 4200 kcal/day) and another four twin individuals because of missing co-twin information. Thus, the final analytic sample consisted of 50 MZ twin pairs, of which 10 twin pairs were discordant for liver fat. Liver fat discordance was defined as the liver fat percentage of one twin ≤ 5% and his/her co-twin > 5% and a difference between co-twins of > 5% resulting in identification of ten such pairs. The study protocol was in accordance with the Helsinki Declaration and approved by local Ethical Committees. Written informed consent was given by all twins.

Data were reported as median and interquartile ranges. Differences between discordant twin pairs were analysed using Wilcoxon signed rank test. In all twin pairs, multiple linear regression analysis was used to relate within-pair differences in log transformed liver fat to within-pair differences in the omega-6 (n-6) to n-3 ratio adjusted for covariates. Statistical analyses were performed using Stata version 13 (Stata Corp, College Station, TX).

Results

The descriptive characteristics (clinical, behavioral, nutritional and liver fat) of the 100 twin individuals (mean age 29.9 years (range: 23-36 years) and of the subset of ten liver fat-discordant twin pairs are shown in **Table 1**. Among these ten pairs, the twins with higher liver fat had more body fat and a worse metabolic profile than their co-twins with lower liver fat. The twins with higher and lower liver fat did not differ in physical activity indices and energy intake, or the intake of protein, fat and carbohydrates. In every single discordant pair, the twin with the higher liver fat percentage had consistently a higher n-6: n-3 ratio than his or her co-twin with less liver fat (**Supplementary Figure)**. The twins with the lower liver fat percentage had a mean n-6: n-3 ratio of 3.2: 1, while the ratio in the co-twins with higher liver fat percentage was 6.6: 1 (p-value = 0.005 for the difference between pairs).

The results from the within-pair linear regression on all 50 MZ pairs are presented in **Table 2**. The n-6: n-3 ratio and BMI were significant predictors and of about equal importance in predicting within-pair differences in liver fat. The gender interaction (male vs. female pairs) was not significant. Additional adjustment for intra-abdominal fat (standardized beta= 0.37, p=0.08) did not change the effect estimate for the n-6: n-3 ratio (standardized beta= 0.39, p-value = 0.002), but removed the influence of BMI (standardized beta= 0.13, p-value = 0.52). In a model that additionally adjusted for sucrose, the effect size for sucrose was not significant (standardized beta= -0.12, p-value = 0.28), and the effect estimate for the n-6: n-3 ratio remained unchanged (standardized beta= 0.40, p-value = 0.001).

Discussion

To our knowledge, this is the first study that evaluated nutrient intake differences in MZ twin pairs discordant for liver fat. Among the 10 twin pairs discordant for liver fat, the co-twins with higher liver fat reported a higher dietary n-6: n-3 ratio than his or her co-twin in every single pair. We employed the MZ co-twin control design, which is analogue to a case-control study, where the twin with the higher liver fat is the case and the co-twin with the lower liver fat the control, with the additional advantage that cases and controls are naturally matched for several factors including age, sex and genotype.

Due to modern agriculture and the popularity of fast foods, Western diets contain excessive amounts of n-6 PUFAs and low amounts of n-3 PUFAs. An overconsumption of n-6 PUFAs coupled with under-consumption of n-3 PUFAs may lead to inflammation and pro-inflammatory cytokine production involved in many disease processes[11]. In addition, n-3 PUFAs may influence the intestinal microbiota[12] and thereby modulate NAFLD susceptibility. Recent meta-analysis of randomized controlled trials suggest that n-3 PUFA supplementation can reduce liver fat and glucose control in patients with NAFLD, at amounts that can be easily obtained through the habitual diet[6].

In the present study of liver-fat discordant MZ twin pairs, a mean n-6: n-3 ratio of 3.2: 1 was associated with normal liver fat content, while a mean ratio of 6.6: 1 was associated with increased liver fat content independent of genetic and familial confounding factors. Dietary advice for preventing and treating NAFLD should focus on shifting consumption away from processed foods

(such as refined vegetable oils rich in n-6 fatty acid used for deep frying) towards fresh foods (such as vegetable oils used in salads), and by increasing fish intake while decreasing meat intake[11]. Our findings could have important implications for the dietary management of NAFLD and encourage clinical trials to better understand causal relationships and required doses.

Competing interest statement

The authors declare no competing interests.

Acknowledgements

KHP was supported by the Academy of Finland (272376, 266286, 314383, and 315035), Finnish Medical Foundation, Finnish Diabetes Research Foundation, Novo Nordisk Foundation, Gyllenberg Foundation, Sigrid Juselius Foundation, Helsinki University Hospital Research Funds, Government Research Funds and University of Helsinki. LHB received a working grant from the Juho Vainio Foundation.

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Table 1. Clinical characteristics, physical activity and macronutrient intakes of the monozygotic twins

		Twin pairs discordant for liver fat (within-pair difference in liver fat > 5%)[1]		
	All twins	Co-twins with liver fat ≤ 5%	Co-twins with liver fat > 5%	p-value[2]
Demographic factors				
Number (n) of individuals[3]	100	10	10	
Males (n)	46	6	6	
Liver fat >5% (n)	22	0	10	
Liver fat (%)[4]	1.0 (0.5, 3.9)	1.1 (0.8, 3.2)	9.4 (7.3, 9.4)	
Clinical parameters[4]				
BMI (kg/m^2)	26.6 (23.7, 29.8)	25.7 (22.9, 27.8)	31.8 (27.6, 35.0)	**0.005**
Body fat (%)	32.4 (26.9, 40.7)	27.7 (21.2, 38.8)	38.3 (31.9, 43.4)	**0.007**
Intra-abdominal fat (dm^3)	807 (422, 1355)	604 (439, 11165)	1709 (1416, 2417)	**0.007**
Subcutaneous fat (dm^3)	3727 (2449, 5513)	3263 (2150, 5498)	5768 (3195, 6965)	**0.007**
LDL-cholesterol (mmol/l)	2.7 (2.3, 3.2)	2.7 (2.5, 2.9)	3.3 (2.7, 3.8)	0.07
HDL-cholesterol (mmol/l)	1.4 (1.2, 1.7)	1.5 (1.3, 1.7)	1.2 (1.1, 1.4)	**0.02**
Triglycerides (mmol/l)	1.0 (0.7, 1.3)	0.7 (0.5, 1.1)	1.3 (1.0, 2.2)	**0.01**
HOMA-IR index	1.4 (0.8, 2.0)	1.1 (0.5, 1.8)	2.4 (1.8, 2.8)	**0.01**
Systolic blood pressure (mmHg)	124 (118, 138)	131 (122, 140)	139 (124, 148)	0.36
Diastolic blood pressure (mmHg)	70 (65, 80)	70 (70, 78)	79 (70, 80)	0.18
Physical activity[4]				
Sports activity index	2.8 (2.3, 3.5)	3.3 (2.3, 4)	2.4 (1.8, 3.5)	0.33
Leisure time activity index	2.8 (2.5, 3.3)	2.8 (2.5, 3.0)	2.9 (2.5, 3.3)	>0.99
Work activity index	2.5 (2.0, 3.3)	2.3 (2.1, 3.0)	2.6 (1.9, 2.9)	0.14
Total energy intake (kcal) [4]	2075 (1774, 2551)	2169 (1809, 2382)	2611 (2319, 2800)	0.07
Macronutrient intake (% energy intake)[4]				
Carbohydrates	43.1 (38.3, 48.1)	43.5 (37.9, 50.0)	37.4 (33.4, 44.0)	0.09
Protein	16.5 (14.8, 18.8)	18.0 (14.2, 22.9)	16.0 (14.1, 18.9)	0.24
Total fat	35.2 (30.6, 39.0)	32.4 (26.3, 38.9)	39.0 (31.6, 42.8)	0.11
Sucrose	9.7 (6.0, 13.0)	4.8 (3.8, 7.0)	8.8 (7.9, 11.2)	**0.04**
Saturated fatty acids	13.5 (11.1, 16.2)	11.3 (10.9, 13.8)	13.1 (10.7, 16.3)	0.58
Monounsaturated fatty acids	10.6 (9.0, 12.3)	8.8 (7.4, 12.8)	12.0 (9.6, 13.4)	0.24
Polyunsaturated fatty acids	4.5 (3.8, 5.8)	4.1 (3.5 5.5)	5.6 (4.8, 5.8)	0.09
Omega-6/omega-3 ratio[4]	4.8 (3.0, 6.1)	3.2 (2.3, 5.4)	6.6 (3.8, 7.4)	**0.005**

Abbreviations: BMI, body mass index; LDL, low-density lipoprotein; HDL, high -density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance

[1]Discordance was defined as the liver fat percentage of one twin ≤ 5% and the liver fat percentage of his/her co-twin > 5% and a within-pair difference in liver fat of > 5%. The difference in liver fat percentage between discordant co-twins ranged from 5 to 20%.

[2]*P*-values are from Wilcoxon signed rank test comparing co-twins discordant for liver fat.

[3]The 100 monozygotic twin individuals are from 50 complete twin pairs of which 10 twin pairs are discordant for liver fat.

[4]Data are median and interquartile ranges.

Table 2. Multiple linear regression analysis predicting (log transformed) liver fat within 50 pairs of monozygotic twins

Dependent variable: Within-pair difference (Δ) in log liver fat	Unstandardized beta coefficient	Standard error	Standardized beta coefficient	p-value
Sex (Female twin pairs)	-0.57	0.23	-0.31	**0.017**
Age of the twin pairs (years)	-0.02	0.02	-0.09	0.42
Δ Omega-6/omega-3 ratio	0.24	0.06	0.43	**0.001**
Δ Total energy intake (per 100 kcal)	0.00	0.02	-0.02	0.83
Δ Body mass index (kg/m^2)	0.10	0.03	0.42	**0.003**
Δ Alcohol intake (% energy intake)	-0.02	0.02	-0.12	0.30
Δ Sports activity index	-0.14	0.10	-0.17	0.17
R^2=0.49				

Supplementary Figure. Omega-6/omega-3 ratio in ten monozygotic twin pairs discordant for liver fat