Dairy consumption is associated with lower plasma dihydroceramides in women from the D.E.S.I.R. study

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ABSTRACT

Aim: In the D.E.S.I.R. cohort, a higher consumption of dairy products is associated with a lower incidence of hyperglycemia, and dihydroceramide concentrations are higher in people who progress to diabetes. Our aim here is to study relationships between dairy consumption and concentrations of dihydroceramides and ceramides.

Methods: In the D.E.S.I.R. cohort, men and women aged 30–65 years, volunteers from western-central France, were included for a 9-year follow-up, with examinations every 3 years, including food frequency questionnaires. Two items concerned dairy products (cheese, other dairy products). At each examination, dihydroceramides and ceramides were determined by mass spectrometry in a subset of the cohort; we analyzed the 105 people who did not progress to diabetes, because disease per se might be a confounding factor.

Results: A higher consumption of dairy products (except cheese) was associated with total plasma dihydroceramides during follow-up, but only in women (sex interaction P=0.01): dihydroceramide levels were lower in women with a high consumption as compared to the low consumers (P=0.03), and significantly increased during the follow-up (P=0.01) in low consumers only. There was also a trend for lower ceramides in women with a high intake of dairy (except cheese) (P=0.08). Cheese was associated with dihydroceramides and ceramides changes during follow-up (P=0.04 for both), but no clear trend could be seen in either the low or high consumers.

Conclusion: These results show, in women, an inverse association between fresh dairy product consumption and predictive markers of type 2 diabetes, the dihydroceramides.

Keywords: dairy product intake; dihydroceramides; ceramides; general population; longitudinal study

Introduction

Sphingolipids, in particular ceramides and dihydroceramides, have been recognized as playing a large role in lipotoxicity, that is known to be associated with impaired glucose metabolism and type 2 diabetes [1]. In the French prospective D.E.S.I.R. (Data from the Epidemiological Study on the Insulin-Resistance syndrome) cohort, we have shown that high levels of plasma ceramides and dihydroceramides are associated with type 2 diabetes, and that dihydroceramides are higher in people who progressed to type 2 diabetes, even dihydroceramides measured 9 years before the onset of the disease [2]. We replicated these results in another cohort, the CoLaus study [2]. Other recent cohort studies yielded similar results with ceramides, but did not report plasma dihydroceramide concentrations [3, 4].

Type 2 diabetes is a multifactorial disease, resulting from interactions between genetic susceptibility and environmental factors, including diet. The influence of dairy product intake in type 2 diabetes risk is controversial, but meta-analyses including large cohorts seem to show a beneficial effect of dairy intake [5-8]. In D.E.S.I.R., we have shown an inverse association between dairy consumption at baseline and onset of impaired fasting glycaemia/type 2 diabetes and of the metabolic syndrome, over a 9-year follow-up [9, 10].

Although ceramides are considered to be an important cardiometabolic risk factor [11], there are few published studies reporting their relation with diet in humans, and to our knowledge, none concerned dairy products. Our aim in this report is to study relationships between dairy consumption and concentrations of dihydroceramides and ceramides in the D.E.S.I.R. cohort.

Subjects and Methods

Population

The D.E.S.I.R. study is a prospective cohort of men and women, aged 30–65 years, recruited from volunteers who were offered periodic health examinations free of charge by the French Social Security system, in 10 health examination centers from the western part of France. They were clinically and biologically evaluated at visits every 3 years, and the final examination was 9 years after inclusion [**12**]. For our previous studies on ceramides, all incident cases of type 2 diabetes and a random sample from the entire pool of people as controls were selected (n=295). For the present study, we include only the 105 controls without diabetes **because disease per se might be a confounding factor. Supporting this concept, we observed no association between dairy products and dihydroceramides and ceramides in people with diabetes (data not shown).**

The D.E.S.I.R. study was approved by the ethics committee of the Kremlin Bicêtre Hospital, and all participants signed an informed consent.

Measurements

Weight, height, and waist circumferences were measured by trained personnel. Venous blood samples were collected in the morning after participants had fasted for 12 h. Systolic and diastolic arterial pressures were measured after 5 min of rest in a supine position, with a mercury sphygmomanometer adapted for arm size. Two measures of blood pressure were taken, and means were used for the analysis. A detailed description of laboratory measurements including fasting triglycerides, and glucose is provided elsewhere [**12**]. Total adiponectin was assayed in fasting plasma EDTA samples from 3249 people **from the D.E.S.I.R. cohort** selected at random, collected at baseline and kept frozen at -80°C. An ELISA kit from Alpco®, Eurobio, Courtaboeuf, France was used. According to the manufacturer, intra- and inter-assay coefficients of variation for adiponectin assay were less than 6.0%.At baseline, then at years 3, 6 and 9, dihydroceramides and ceramides were determined by mass spectrometry, on thawed samples kept at -80°C, in a subset of the

cohort [2]. Lipid extraction, mass spectrometry (MS) based lipid detection and data processing for human samples was performed by Zora Biosciences Oy (Espoo, Finland) [**13**] as described previously [2]. In the present study, we analyzed the 105 people who did not progress to diabetes~~**, because disease per se might be a confounding factor.**~~. Characteristics of participants are shown in table 1.

Type 2 diabetes was defined as fasting plasma glucose ≥ 7 mmol/l or treatment by glucose lowering agents.

A 23-item questionnaire was completed by each participant, to determine the frequency and level of consumption of different foods. This questionnaire has been validated by comparison with the dietary history method, with 30 minute interviews by trained dieticians [**14**]. This validation study enabled the determination of multivariable linear regression equations to estimate the main nutrient intakes **as well as total calories**, from the questions on the consumption of different foods. Two items concerned dairy products: cheese, milk and other dairy products (except cheese). One portion was defined as 30 g for cheese, and 125 mL for milk or dairy products. There were three **possible** responses for cheese intake (0-1 portion/day; 2-3 /day; > 3/day), and four for the intake of dairy products (except cheese) (never; < 1 portion/day; 1-2 /day; > 2 /day) [9, 10]. In the present study, these responses were dichotomized as low/high: <2 portions/day / ≥2 portions/day for cheese and < 1 portion/day / ≥ 1 portion/day for dairy products **(except cheese), in order to optimize group sizes for comparisons**.

Statistics

General characteristics of the studied sample are described by means **±** SD, medians (interquartile range) or %. The associations between plasma dihydroceramide and ceramide concentrations (natural log values) at four follow-up times with baseline dairy intake, sex and

their interactions, were tested by analysis of covariance (ANCOVA) for repeated measures, adjusted for age, BMI, alcohol intake, total energy intake, physical activity, and fasting plasma cholesterol, triglycerides and glucose. ANCOVA tested the average effects of dairy intake and the interactions with sex and time. Results are shown as least squares adjusted geometric means with 95% confidence intervals.

All statistical analyses used SYSTAT 13®software for Windows.

Results

Total dihydroceramide and ceramide concentrations were similar in men and women (**Table 1**), but men had higher triglyceride concentrations, as well as higher alcohol and energy intakes; adiponectin concentrations were higher in women. Dairy intake (except cheese) was similar in men and women, but women ate less cheese.

A higher consumption of dairy products (except cheese) was associated with total plasma dihydroceramides during follow-up, in interaction with sex (P=0.004): dihydroceramide concentrations at follow-up were lower in women with high consumption as compared to the low consumers (P=0.03) but in men, no significant association was found **(Table 2)**. In women, plasma dihydroceramides significantly increased during the follow-up in low consumers (P=0.01) but not in high consumers. No differences in total plasma ceramides according to dairy (except cheese) consumption was found in the whole population **(Table 3)**. Nevertheless, there was a trend for lower total plasma ceramides in women with a high intake of dairy (except cheese) (P=0.08) **(Table 3)**.

When looking at the different dihydroceramide species, on average, all were higher at follow-up in women with low dairy (except cheese) consumption **(Figure 1)**. Two specific ceramide

species (out of nine) were also significantly associated with dairy consumption in women: Cer(d18:1/26:0) and Cer(d18:1/26:1) **(Figure 1)**.

Low and high cheese intake was associated with different changes in plasma dihydroceramides and ceramides during follow-up (P=0.04 for time X cheese consumption interactions for both dihydroceramides and ceramides) **(Table 4)**, however, when looking at changes in high and low consumers separately, no significant change over the follow-up was observed in any group.

Discussion

In this study, we have shown that plasma dihydroceramides, predictive markers of type 2 diabetes, were lower in women from the D.E.S.I.R. study with a higher consumption of dairy products except cheese, i.e. fresh dairy products. This result is consistent with our previous data indicating that dairy intake is associated with a lower risk of impaired fasting glucose/type 2 diabetes [9].

A high-fat diet in mice increased ceramide concentrations concomitantly with insulin resistance [2], but despite the fact that ceramides and related molecules are now recognized as important cardiometabolic risk factors, few dietary influences on plasma ceramides and dihydroceramides have been described in humans [**11**]. Diet induced weight loss has been shown to be associated with a decrease in intramyocellular dihydroceramide levels, but not ceramide levels [15]. A healthy Nordic diet lowered some plasma ceramide species in people with the metabolic syndrome [16]. A diet rich in saturated fat increased ceramides and dihydroceramides when compared to diets rich in unsaturated fat or carbohydrates [17]. In PREDIMED, a score summing concentrations of four ceramides was associated with

cardiovascular risk and modified the effects of a Mediterranean diet on cardiovascular outcomes, nevertheless this score was not modified by a Mediterranean diet [18].

The relationship between dairy and (dihydro)ceramides might explain at least in part, the negative association of dairy with impaired fasting glucose/ type 2 diabetes in the D.E.S.I.R. study [9]. Actually, it has been shown that ceramides play a deleterious role in insulin resistance [1], but this is less clear for dihydroceramides, which are ceramide precursors. Dihydroceramides were previously considered as inert sphingolipid precursors but they have now been proposed as biomarkers of metabolic dysfunction [19]. Dihydroceramides are markers of the ceramide *de novo* synthesis pathway. This pathway might be especially at risk for metabolic disease when compared to the other pathways. In patients with NAFLD, liver dihydroceramides and ceramides were increased in those with high as compared to low insulin resistance, while other markers of ceramide synthesis, by sphingomyelin hydrolysis or salvage pathway, were unchanged [20]. In the same study, serum adiponectin was lower in the high insulin resistance group. Although there is no clear mechanism for the results found in the present study, adiponectin might play a role in the observed association between (dihydro)ceramides and dairy intake. Adiponectin activates ceramidase by its two receptors, which can contribute to lower concentrations of ceramides via increased degradation [21]. Low fat dairy product intake [22], and dietary patterns characterized by low fat dairy and whole grain [23] or by dairy and bread [24] have been positively associated with adiponectin concentrations. As mentioned in our previous papers, it can be assumed that in France, the majority of cheese consumed is high-fat, while other dairy products such as milk and yogurts are low-fat [9, 10]. In women in the D.E.S.I.R. cohort, a higher dairy (but not cheese) consumption was associated with higher adiponectin concentrations (high consumption: 6.84mg/L (adjusted mean) ± 1.02 (SEM) vs. low consumption: 6.35mg/L ± 1.03; P=0.04 by ANCOVA). **We also observed negative but non-significant correlations of adiponectin**

with dihydroceramides and ceramides in women, likely because of the small sample size of people with both measurements. This is consistent with the hypothesis that it is mainly the low fat fresh dairy intake that is associated with high adiponectin, which is associated with lower ceramides, leading to a lower risk of type 2 diabetes. In the D.E.S.I.R. study, cheese consumption (high fat) was not significantly associated with (dihydro)ceramides nor with impaired fasting glycemia/type 2 diabetes [9, 10].

Nevertheless, the association between dihydroceramides and dairy intake was statistically significant only in women. This indicates that it can only be a partial explanation for the lower hyperglycemia risk associated with dairy consumption, since there was no evidence of interaction between sex and dairy consumption on the risk of hyperglycemia in D.E.S.I.R. [9, 10].

This work has some strengths and several limitations. Among the strengths are the prospective nature of the data. The size of our study may seem limited, however repeated measurements of ceramides were performed, which is not the case in larger cohort studies with only one single measurement [3, 4]. We were also able to adjust for confounders such as lifestyle factors. The relationships we observed are modest, but they were obtained after adjustment for lifestyle variables, including physical activity, smoking, alcohol consumption, and energy intake. One of the main limitations is that our questionnaire does not allow the separation of low-fat and high-fat products, or to estimate the effects of different dairy products. However, we could separate cheese from other dairy products. As already stated, it can be assumed that in France, cheese is high-fat, but that the milk included in other dairy products is low fat.

In conclusion, our data show that in women from the general population, higher fresh dairy product consumption is associated with lower concentrations of plasma dihydroceramides, a

protective marker for the onset of type 2 diabetes. Further studies will be needed to clear up the potential mechanism of this association.

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Disclosure of interest

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Table 1. Baseline characteristics of the people without diabetes selected for ceramide analysis. The D.E.S.I.R. Study

	Men	**Women**	**P[a]**
N	54	51	
Age (years)	47.5 ± 9.9	47.9 ± 9.9	0.85
BMI (kg/m²)	25.0 ± 2.7	23.9 ± 4.0	0.09
Fasting glucose (mmol/L)	5.35 ± 0.47	5.17 ± 0.55	0.06
Total cholesterol (mmol/L)	5.63 ± 0.86	5.73 ± 1.06	0.60
Triglyceride (mmol/L)	1.15 (0.79-1.47)	0.84 (0.64-1.26)	0.02
Total dihydroceramides Cer(d18:0) (µmol/L)	0.134 (0.103-0.157)	0.129 (0.105-0.162)	0.91
Total ceramides Cer(d18:1) (µmol/L)	13.1 (11.0-15.3)	12.3 (10.9-14.9)	0.53
Physical activity (%): none or low/moderate/intense	22.2/64.8/13.0	21.6/66.7/11.8	0.98
Alcohol intake (g/d)	24.6 (9.7-35.5)	2.0 (0.0-13.6)	<0.001
Total energy intake (kcal/d)	2458 ± 369	1676 ± 215	<0.001
Dairy (except cheese) intake (%): low/high:	38.9/61.1	29.4/70.6	0.31
Cheese intake (%): low/high	37.0/63.0	56.9/43.1	0.04

Data are means ± SD, medians (interquartile range), or %, when appropriate.

[a]by t-test on continuous variables (on Ln values for those given as medians) or chi square on grouped variables (given as %).

Table 2

Plasma total dihydroceramide concentration (µmol/L) in 105 people without diabetes at baseline or at follow-up, according to intake of dairy products (except cheese). The D.E.S.I.R. Study.

		Baseline	3 years	6 years	9 years	P intake	P intake X sex
Total population	**Low intake** (n=36)	0.139 [0.122-0.158]	0.139 [0.121-0.160]	0.155 [0.135-0.178]	0.149 [0.128-0.173]	0.29[a]	0.004[a]
	High intake (n=69)	0.133 [0.123-0.145]	0.134 [0.23-0.146]	0.140 [0.127-0.153]	0.136 [0.124-0.150]		
Men	**Low intake** (n=21)	0.135 [0.115-0.159]	0.124 [0.105-0.145]	0.127 [0.106-0.150]	0.125 [0.105-0.148]	0.18[b]	
	High intake (n=33)	0.132 [0.118-0.147]	0.145 [0.129-0.164]	0.135 [0.125-0.159]	0.148 [0.131-0.167]		
Women	**Low intake** (n=15)	0.145 [0.119-0.177]	0.156 [0.125-0.194]	0.194 [0.153-0.247]	0.172 [0.133-0.223]	0.03[b]	
	High intake (n=36)	0.133 [0.118-0.150]	0.124 [0.110-0.139]	0.137 [0.119-0.157]	0.126 [0.108-0.145]		

Data are adjusted geometric means [95% CI].

[a]by two-way (sex, intake) ANCOVA (on Ln values) for repeated measures, adjusted for age, BMI, alcohol intake, total energy intake, physical activity, and plasma cholesterol, triglycerides and glucose. No statistically significant interaction effect for intake X time was observed.

[b]by one-way (intake) ANCOVA for repeated measures, same adjustments. No statistically significant interaction effect for intake X time was observed, but in women, in low intake group: P(time)=0.01, in high intake group: P(time)= 0.95.

Table 3

Plasma total ceramide concentration (µmol/L) in 105 people without diabetes at baseline or at follow-up, according to intake of dairy products (except cheese). The D.E.S.I.R. Study.

		Baseline	3 years	6 years	9 years	P intake	P intake X sex
Total population	**Low intake** (n=36)	13.9 [12.8-15.0]	12.6 [11.6-13.6]	13.5 [12.2-14.9]	14.3 [129-15.8]	0.11[a]	0.13[a]
	High intake (n=69)	13.1 [12.6-13.6]	12.6 [12.1-13.1]	12.7 [11.9-13.5]	13.2 [12.4-14.0]		
Men	**Low intake** (n=21)	13.8 [12.4-15.5]	12.8 [11.6-14.2]	12.6 [11.4-14.2]	14.2 [12.7-16.1]	0.71[b]	
	High intake (n=33)	13.1 [12.1-14.2]	13.4 [12.6-14.4]	12.6 [11.6-13.6]	13.4 [12.3-14.49]		
Women	**Low intake** (n=15)	13.7 [12.4-15.2]	12.4 [10.9-14.2]	14.4 [12.3-16.4]	14.6 [12.6-17.0]	0.08[b]	
	High intake (n=36)	13.1 [12.3-13.9]	11.6 [10.8-12.4]	12.7 [11.6-13.9]	12.9 [11.9-14.0]		

Data are adjusted geometric means [95% CI].

[a]by two-way (sex, intake) ANCOVA (on Ln values) for repeated measures, adjusted for age, BMI, alcohol intake, total energy intake, physical activity, and plasma cholesterol, triglycerides, and glucose. No statistically significant interaction effect forintake X time was observed.

[b]by one-way (intake) ANCOVA for repeated measures, same adjustments. No statistically significant interaction effect for intake X time was observed.

Table 4

Plasma total dihydroceramide and ceramide concentrations (µmol/L) in 105 people without diabetes at baseline or at follow-up, according to intake of cheese. The D.E.S.I.R. Study.

		Baseline	3 years	6 years	9 years	P intake	P intake X time
Total dihydroceramides	**Low intake** (n=49)	0.124 [0.112-0.137]	0.130 [0.115-0.147]	0.141 [0.125-0.159]	0.145 [0.129-0.164]	0.66[a]	0.037[a]
	High intake (n=56)	0.142 [0.129-0.157]	0.138 [0.125-0.153]	0.144 [0.130-0.159]	0.129 [0.114-0.145]		
Total ceramides	**Low intake** (n=49)	12.8 [12.1-13.6]	12.6 [11.8-13.3]	13.2 [12.2-14.3]	14.3 [13.2-15.5]	0.44[b]	0.043[b]
	High intake (n=56)	13.6 [12.8-14.4]	12.4 [11.7-13.2]	12.6 [11.6-13.6]	12.8 [12.1-13.6]		

Data are adjusted geometric means [95% CI]. No significant interaction effect intake X sex was observed.

[a]By two-way ANCOVA (on Ln values) for repeated measures, adjusted for age, BMI, alcohol intake, total energy intake, physical activity, and plasma cholesterol, triglycerides, and glucose. One way ANCOVA, in low intake group: P (time)=0.14, in high intake group: P (time)= 0.11.

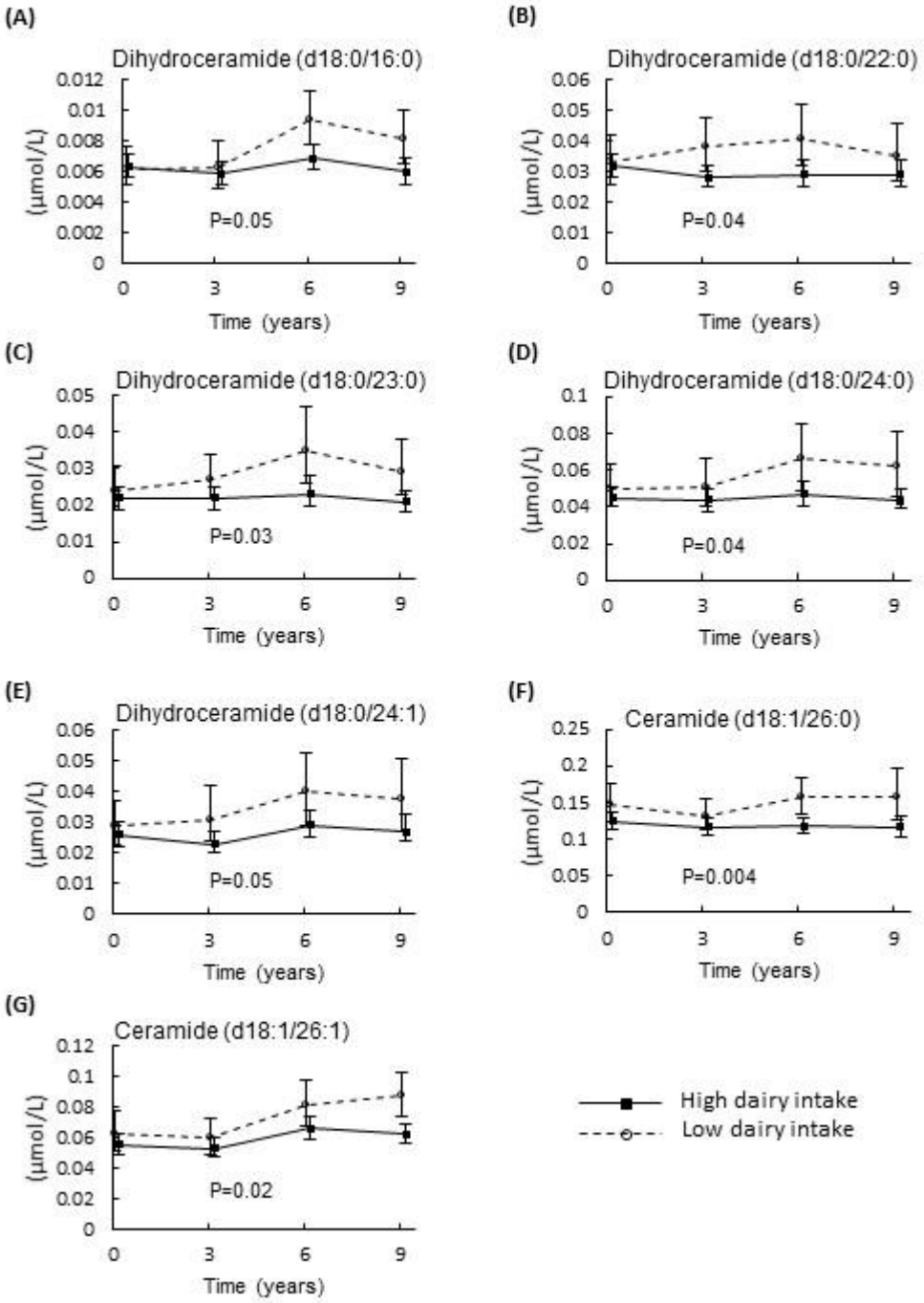
[b]By two-way ANCOVA (on Ln values) for repeated measures, same adjustments as above. One way ANCOVA, in low intake: P time=0.27, in high intake: P time= 0.63

Figure caption

Figure 1

Plasma specific dihydroceramide and ceramide concentrations in women without diabetes at baseline or at follow-up, according to their intake of dairy products (except cheese). The D.E.S.I.R. Study.

P-values are for the average intake effects during the follow-up by ANCOVA for repeated measures, adjusted for age, BMI, alcohol intake, total energy intake, physical activity, and fasting plasma cholesterol, triglycerides, and glucose. No statistically significant interaction between intake and time was observed. Data are adjusted geometric means with 95% CI. (A) Dihydroceramide (d18:0/16:0) (B) Dihydroceramide (d18:0/22:0) (C) Dihydroceramide (d18:0/23:0) (D) Dihydroceramide (d18:0/24:0) (E) Dihydroceramide (d18:0/24:1) (F) Ceramide (d18:1/26:0) (G) Ceramide (d18:1/26:1)


(A)
Dihydroceramide (d18:0/16:0)
(µmol/L)
P=0.05
Time (years)
(B)
Dihydroceramide (d18:0/22:0)
(µmol/L)
P=0.04
Time (years)
(C)
Dihydroceramide (d18:0/23:0)
(µmol/L)
P=0.03
Time (years)
(D)
Dihydroceramide (d18:0/24:0)
(µmol/L)
P=0.04
Time (years)
(E)
Dihydroceramide (d18:0/24:1)
(µmol/L)
P=0.05
Time (years)
(F)
Ceramide (d18:1/26:0)
(µmol/L)
P=0.004
Time (years)
(G)
Ceramide (d18:1/26:1)
(µmol/L)
P=0.02
Time (years)
High dairy intake
Low dairy intake