

Intake of high-fat yogurt, but not of low-fat yogurt or prebiotics, is related to lower risk of depression in women of the SUN cohort study

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Abstract

Background: Yogurt and prebiotic consumption have been related to better health. However no longitudinal study has assessed the association of yogurt and prebiotic consumption with the incidence of depression.

Aim: We longitudinally evaluated the association of yogurt and prebiotic consumption with the incidence of depression in a Mediterranean cohort.

Methods: The SUN Project is a dynamic, prospective cohort of Spanish university graduates. A total of 14,539 men and women (mean age: 37y) initially free of depression were assessed during a median follow-up period of 9.3 years. Validated food frequency questionnaires (FFQ) at baseline and after 10-year follow-up were used to assess prebiotic [fructans and galacto-oligosaccharides (GOSs)] intake and yogurt consumption (0-0.5 servings/week, >0.5 to <3 servings/week, ≥ 3 to <7 servings/week, and ≥ 7 servings/week). Participants were classified as incident cases of depression when they reported a new clinical diagnosis of depression by a physician (previously validated). Multivariable Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: We identified 727 incident cases of depression during follow-up. Whole-fat yogurt intake was associated with reduced depression risk [HR for the highest (>7 servings (1 serving=125 g)/week) versus lowest (0-0.5 servings/week) consumption=0.78 [95% CI:0.63-0.98]; P -trend=0.020]. When we stratified by sex, this association was only significant in women (HR=0.66 [95% CI:0.50-0.85]; P -trend=0.004). On the contrary, low-fat yogurt consumption was associated with higher incidence of depression (HR=1.32 [95% CI:1.06-1.65]; P -trend=0.001), although this association lost significance after excluding early incident cases, suggesting possible reverse causation bias. Prebiotic consumption was not significantly associated with depression risk.

Conclusion: Our study suggests that high consumption of whole-fat yogurt was related to lower risk of depression in women of the SUN cohort. No association was observed for prebiotics. Further studies are needed to clarify why the yogurt-depression association may differ by fat content of the yogurt.

Keywords: Depression, Yogurt, Prebiotics, Fibre, Probiotics.

29 **Introduction**

30 Unipolar depression has reached epidemic proportions worldwide and it is expected to be the leading
31 cause of disability in developing countries by the year 2030 (1). Although depression seems to be a
32 multifactorial disease (2), diet has recently emerged as a determinant factor for the prevention and
33 treatment of this mental disorder (3). Recent studies have shown that diet influences gut microbiota
34 composition and activity (4), and that this in turn may influence brain function, including depressive
35 illness (5, 6). Some of the mechanisms that may link them are immune, neural, and metabolic pathways
36 (5). Therefore, the fast-emerging field of the gut microbiota-brain axis has been proposed as an
37 underlying link between diet quality and depression (6).

38 Both probiotics and prebiotics are able to enhance and maintain a healthy gut microbiota in humans (7).
39 Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a
40 health benefit on the host” (8). Probiotics must survive the gastrointestinal transit to exert a health-
41 promoting effect (9) and they can be consumed in various forms, but mainly as either a functional food,
42 such as in yogurt/fermented milk formulas with live probiotic bacteria, or as encapsulated supplements.
43 Probiotic bacteria such as *Bifidobacterium bifidum* and/or *Lactobacillus acidophilus* can be added to
44 yogurts and fermented milks (8), and nowadays, a wide range of yogurts and fermented milks in the
45 market contain probiotic bacteria (10). Commercial yogurt fulfils the current concept of probiotics if it
46 contains viable, live and abundant beneficial bacteria (namely *Streptococcus thermophilus* and
47 *Lactobacillus bulgaricus*) at a minimum concentration of 10^7 colony forming units (CFUs)/g (9). Above
48 this concentration several health benefits linked to the presence of live bacteria in yogurt have been
49 observed (11). Commercial yogurt consumption has been suggested to favourably alter the gut microbiota
50 and gut function (12), decrease the risk of overweight/obesity and metabolic syndrome (MetS) (13, 14),
51 improve the immune activity (15), and lead to a better lipid profile (16). To date, the association between
52 yogurt/fermented milks and depression has not been investigated; however, probiotic consumption has
53 been previously reported to be associated with reduced anxiety- and depressive-like behaviors in some
54 small human studies (17).

55 A prebiotic is defined as “a selectively fermented ingredient that results in specific changes in the
56 composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health”
57 (18). Fructans (fructo-oligosaccharides (FOSs) and inulin) and galacto-oligosaccharides (GOSs) are the
58 most important prebiotic sources, and they are mostly present in fruits, vegetables and whole grains (19).

Among the beneficial effects attributed to prebiotics are the intestinal growth of beneficial bacteria (e.g., *Bifidobacterium* and/or *Lactobacillus* strains), the improvement of the gut barrier function and host immunity, a lower risk of overweight/obesity and mitigation of inflammatory responses (20-22). Moreover, prebiotic administration may have antidepressant effects by modulating the hypothalamic-pituitary-adrenal (HPA) axis, the immune system and metabolite-mediated pathways (23).

Considering that the suggested beneficial effects of probiotics and prebiotics on brain development and behavior through the gut microbiota-brain axis are mainly based on animal studies and small short-term human studies (5, 6), it is of great interest to determine whether the consumption of prebiotics and yogurt/fermented milks is associated with a reduced risk of depression in the long-term in a large human study. Therefore, our aim was to prospectively assess the association of yogurt (total, whole-fat and low-fat) and prebiotic consumption with the incidence of depression among university graduates enrolled in a longitudinal study with an average follow-up period longer than 9 years, the “Seguimiento Universidad de Navarra” (SUN) Project.

Methods

Study population

The SUN study is a prospective cohort study with continually open recruitment (i.e. a dynamic design), started in December 1999 with alumni of the University of Navarra, registered professionals from some Spanish provinces, and other university graduates. Detailed information on this cohort has been described elsewhere (24). Briefly, at enrolment and every 2 years, self-reported questionnaires are administered to collect and update medical and lifestyle information, although dietary information was only collected twice, at baseline and after 10 years of follow-up.

For the present analysis, we included 21,291 participants who had answered the baseline questionnaire before March 2012 in order to ensure that all participants had the opportunity to answer the 2-year follow-up questionnaire. Participants who reported total energy intake at baseline out of recommended limits (total energy intakes > 4000 kcal/day and > 3500 kcal/day in men and women, respectively and < 800 kcal/d in men and < 500 kcal/d in women) ($n = 2,011$) were not included in the analysis (25). Subjects with cancer, diabetes or cardiovascular disease at the beginning of the study were also excluded ($n = 1,198$). We excluded 2,085 participants who reported antidepressant medication at baseline or had a history of physician diagnosed depression throughout their life. Of the 16,000 remaining participants,

1,427 subjects were lost to follow-up (retention rate: 91%), and 31 participants had missing data on some of the variables of interest. Finally, data from 14,539 participants were included in our main longitudinal analysis.

This study was conducted according to the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of the University of Navarra. The completion of the self-administered questionnaire was considered to imply informed consent (24).

Assessment of prebiotic and yogurt consumption

Dietary intake was measured by a self-administered 136-item semi-quantitative food-frequency questionnaire (FFQ) administered twice, at baseline and after 10 years of follow-up, which has been previously validated in Spain (26). The validity of the FFQ used in this study was not focused on each individual food but on food groups. The intra-class correlation coefficient for dairy products from the FFQ and from four 3-day food records was 0.84 (27). In order to determine usual dietary intake over the previous year frequencies of intake were measured in nine categories, ranging from “never or almost never” to “six or more times per day”.

As previously detailed (22), we used dietary intakes from the FFQ to calculate the total fructans (FOSs and inulin) and GOSs consumption, and these values were updated in 29% of participants who completed the 10-year follow-up FFQ. This percentage is not high largely because of the late entry in the cohort and its dynamic design. Total prebiotic consumption was estimated by summing total fructans and GOSs consumptions. The main food contributors to fructans and GOSs intakes in our cohort were vegetables (the main contributor was asparagus) and cereals (the main contributors were both white bread and whole-grain bread) (22).

Participants reported frequency of whole-fat and low-fat yogurt consumption in both FFQs (at baseline and after 10 years of follow-up), while total yogurt consumption was calculated by summing the previous two food items. Participants were allocated into four categories according to their servings (125 g) of yogurt (total, whole-fat (~3% fat) and low-fat yogurt (~0.1% fat)) per week: 0-0.5 servings/week (0-63 g/week), >0.5 to <3 servings/week (>63 to <250 g/week), ≥ 3 to <7 servings/week (>250 to <875 g/week), and ≥ 7 servings/week (≥ 875 g/week).

Outcome assessment

Information on physician-diagnosed depression is updated biennially (Q_2-Q_14). Thus, we defined as an incident case of depression any participant who responded affirmatively to the question: “Have you ever been diagnosed with depression by a medical doctor?” and was free of depression at baseline. Self-reported medical diagnosis of depression has been previously validated in a subsample of this cohort using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders fourth edition as gold standard (28). The percentage of confirmed depression cases was 74.2 %; (95 % confidence interval [CI] = 63.3–85.1) and the percentage of confirmed non-depression subjects was 81.1 % (95 % CI = 69.1–92.9).

Assessment of covariates

Demographic (e.g. age, sex, marital and employment status), lifestyle behavior [e.g. smoking status, leisure-time physical activity, total energy intake, adherence to the Mediterranean diet (MedDiet) using the MedDiet Score proposed by Trichopoulou (29) and fiber consumption], weight gain (> 3 kg) in the 5 years previous to entering the cohort, body mass index (BMI), and comorbidity information (such as prevalence/history of cancer, diabetes or cardiovascular disease) were collected in the baseline questionnaire. Participants also answered questions about personality and behavior features, such as the level of competitiveness, level of anxiety and level of dependence using Likert scales with values in the range 0–10. Finally, physical activity was assessed with a validated questionnaire (30).

Statistical analysis

Chi squared tests for trend (categorical variables) and ordinary least-squares linear regression analyses (continuous variables) across categories or prebiotic and yogurt consumption were used for the comparisons of baseline characteristics. We categorized our exposure variables in quartiles, because quartiles keep a sufficiently high (and equally sized) sample size in each category and allow to assess dose-response trends.

Cox regression models were fitted to assess the relationship of yogurt and prebiotic consumption at baseline with the risk of developing depression during follow-up, where hazard Ratios (HRs) and their 95% CIs were calculated. Test for linear trend across quartiles of yogurt or prebiotic consumption was performed by assigning the median value of intake within each category and treating these as continuous variables in the respective multivariable-adjusted Cox regression models. Prebiotic and yogurt

consumption data were updated after 10 y of follow-up and Cox regression models with time-dependent exposures were also fitted. Age was used as the underlying time variable. Age at baseline was used as the entry time variable. Birth date was taken as the origin of the time scale. Exit time was defined as depression diagnosis or age at censoring due to death or loss to follow-up. Analyses were stratified by date of recruitment (2-year periods) and deciles of age. The proportional hazard assumption was assessed using the Schoenfeld residuals method.

In the multiple-adjusted models the following potential confounders were included as covariates: age (underlying time variable), sex, smoking (never, current, former, missing), physical activity (quartiles), total energy intake (quartiles), baseline BMI (quartiles), living alone (yes/no), unemployment (yes/no), marital status (married or not), and for the 3 personality traits: competitiveness (higher scores, more competitive), relaxation (lower scores, more relaxed) and dependency/locus of control (higher scores, more dependent). Two a priori-defined tests for interaction by sex and age (≤ 40 years or > 40 years) were conducted by introducing interaction terms in the model, and then comparing the models with and without the interaction term using likelihood ratio tests.

We conducted stratified analyses according to the fat content of yogurt (i.e., low-fat yogurt versus whole-fat yogurt). The rationale for this stratification was that this is a newer area of research, where there is some controversy on the differential effects of dairy products according to their fat content (31).

Finally, we performed sensitivity analyses in order to test the robustness of our estimates. We rerun our analyses using the following alternative assumptions: 1) additionally adjusting for adherence to the MedDiet; 2) additionally adjusting for other fiber consumption apart from prebiotics (only in the prebiotic analysis); 3) additionally adjusting for omega 3 polyunsaturated fatty acids (PUFAs); 4) excluding early incident cases of depression (until 2 years of follow-up); 5) including prevalent cancer, diabetes or cardiovascular disease; 6) Including only those participants with prevalent obesity ($\text{BMI} > 30 \text{ kg/m}^2$); 7) Including only those participants who had gained 3 or more kg in the last 5 years; 8) changing the energy limits: percentiles 5–95; 8) only women and excluding early incident cases of depression (until 2 years of follow-up) and 10) only men and excluding early incident cases of depression (until 2 years of follow-up). The rationale for the exclusion of these early cases was that cases diagnosed early during the follow-up period might be more likely to have been present as subclinical cases at baseline. All analyses were

two-tailed, and $P < 0.05$ was considered significant. Statistical analyses were performed using STATA version 12.0 (StataCorp, College Station, Tex).

Results

The characteristics of participants subdivided by extreme categories of total prebiotic and yogurt consumption are shown in **Table 1**. The highest category of total prebiotic consumption included a higher proportion of women, non-smokers, unemployed subjects, as well as participants with better adherence to the MedDiet, more physically active and with lower BMI. There were higher proportions of women, non-smokers, younger people, unemployed subjects, and participants living alone among participants in the highest category of total yogurt consumption. Moreover, participants with a higher yogurt consumption were physically more active, and had better adherence to the MedDiet.

After 9.3 years of follow-up, a total of 727 incident cases of depression were identified. The exposure variables were all defined using repeated measurements of diet (baseline and after 10 years of follow-up). No interaction was observed between age or sex and any of the dietary exposures (P -interaction=0.12-0.90). However, there was a significant sex x low-fat yogurt intake interaction (P -interaction=0.017) so these data are also presented for each sex separately.

The association between prebiotic consumption and the risk of depression is shown in **Table 2**. In all participants, the multiple-adjusted model showed that although a higher consumption of fructans, GOSs and total prebiotics seemed to be associated with lower risk of depression [HR in the highest versus the lowest quartile of intake = 0.94 (95% CI: 0.74-1.20) for fructans, HR = 0.87 (95% CI: 0.69-1.08) for GOSs, and HR = 0.92 (95% CI: 0.73-1.17) for total prebiotics], this association was not statistically significant. When analyses were divided by sex no association between prebiotic intake (fructans, GOSs and total prebiotics) and depression risk was observed.

Table 3 shows HRs for depression in relation to yogurt consumption. Considering all participants, whole-fat yogurt intake was associated with a decreased risk of depression (HR for the highest (> 7 servings/week) versus the lowest (≤ 0.5 servings/week) consumption was 0.78, 95% CI: 0.63-0.98); P -trend = 0.020). Conversely, a higher consumption of low-fat yogurt was related to a higher risk of depression (HR for the highest (> 7 servings/week) versus the lowest (0-0.5 servings/week) consumption was 1.32, 95% CI: 1.06-1.65); P -trend = 0.001), but there was no evidence of an association with total yogurt intake. Stratified analyses showed that the previous associations between yogurt consumption and

incident depression were only significant in women (HR for the highest (>7 servings/week) versus the lowest (0-0.5 servings/week) consumption = 0.66, 95% CI: 0.50-0.87); *P*-trend=0.004 for whole-fat yogurt, and HR = 1.37, 95% CI: 1.07-1.76; *P*-trend < 0.001 for low-fat yogurt).

The association between fructans, GOSs and total prebiotic consumption and the incidence of depression remained non-significant in all our sensitivity analyses (**Supplemental table 1**), and no association between total yogurt consumption and the risk of depression was observed (**Supplemental table 2**). When non-obese participants and those with a stable weight in the last 5 years were excluded the inverse association between whole-fat yogurt consumption and the risk of depression was attenuated (**Supplemental table 2**). However, excluding early incident cases of depression (until 2 year of follow-up), additionally adjusting for adherence to the MedDiet or omega-3 PUFAs, including participants with prevalent cancer, diabetes or cardiovascular disease, with energy limits between the fifth and 95th percentiles, did not alter the results. In analyses stratified by sex we observed that the inverse association between whole-fat yogurt and depression risk in women remained significant even after excluding early incident cases. Importantly, the positive association of low-fat yogurt consumption with incidence of depression was no longer significant after excluding early cases of depression (**Supplemental table 2**). This was observed in all participants and also in women.

Discussion

In this prospective study, we found that a higher consumption of whole-fat yogurt was related to lower risk of depression in women. The consumption of prebiotics or total yogurt was not significantly associated with depression risk. These longitudinal results are novel, and this is, to our knowledge, the first prospective study that has analyzed the association between yogurt and prebiotic consumption and depression risk.

Unhealthy diets have shown to have a detrimental effect on depression due to their harmful effects on hormones, the immune system, neurodegenerative factors, and the expression of potentially damaging genes (32-36). Conversely, healthy dietary patterns, such as the MedDiet, the Alternative Healthy Eating Index (aHEI), or the plant-based (“pro-vegetarian”) Dietary Pattern, might have a protective effect against the incidence of depression (37).

Recent evidence suggests that prebiotics, including fructans and GOSs, modulate brain function by modifying the gut microbiota, decreasing low-grade inflammation, and/or influencing the production of

neurochemicals (6, 23). Prebiotics are fermented by beneficial bacteria, including *Bifidobacterium* and *Lactobacillus*, to produce short-chain fatty acids (SCFAs), which may suppress pro-inflammatory cytokines (38). A recent study in healthy volunteers showed that those supplemented with Bimuno–GOSs (B-GOSs) had lower cortisol awakening reactivity (a reliable marker of HPA axis activity), while FOSs administration had no effect (39). An animal study showed that prebiotic feeding increases brain derived neurotrophic factor (BDNF) expression probably through the involvement of gut hormones (40). The same authors also showed that the ingestion of B-GOSs attenuated post-inflammatory anxiety in mice (41). In the current study we did not find any significant association between prebiotic consumption and the risk of depression. However, the point estimates suggest that prebiotic consumption, especially GOSs, might be associated with lower risk of depression; therefore it would be of great interest to replicate these analyses in a cohort with a larger number of incident cases of depression to increase the statistical power.

A surprising finding of our study was a direct association between low-fat yogurt consumption and depression risk among women. However, this association was no longer significant after excluding those cases of depression that occurred within the first 2 years of follow-up. Therefore, this association might be due to reverse causality, so that subclinical (i.e., “hidden”) cases of depression at baseline might be responsible for the higher low-fat yogurt consumption among participants who will be diagnosed of depression early in the follow-up of the cohort. In fact, 1,725 out of the 2,085 participants with prevalent depression consumed yogurt in our cohort.

Our finding of an inverse association between whole-fat yogurt consumption and depression is consistent with previous studies (42, 43). A cross-sectional study in 1,745 pregnant Japanese women showed an association between higher intake of yogurt and lower prevalence of depressive symptoms during pregnancy (42). A fermented milk product with probiotic positively might affected the activity of brain regions that control central processing of emotion and sensation in healthy women (43). Furthermore, yogurt consumption has been related to lower risk of overweight/obesity and MetS (13, 14), diseases that have been previously related to depression (44). Moreover, preclinical research in rodents suggested that probiotics produce anti-depressant and anxiolytic effects by beneficially affecting neural systems (noradrenaline), and normalizing corticosterone release and levels of inflammatory biomarkers (45, 46).

An association between the gut microbiota and depression has been found in humans (47), and an increase in beneficial bacteria and a reduction in potentially pathogenic bacteria has been observed after the consumption of commercial yogurt supplemented with probiotics in healthy adults (48). A human

study showed that the status of the gut microbiota of the mother may have important repercussions for the mental and neurodevelopmental health of their children (49). Several studies have reported that above 1×10^7 CFUs/g per day (9) or 1×10^9 CFUs per serving/day (50) are enough to produce health benefits. Therefore, if a determined yogurt contains at least 1×10^7 CFUs/g, consuming 1 serving of yogurt (125 g) per day would be necessary to produce the health benefit. We acknowledge that probiotics have a transient effect on the gut microbiota. As stated by the International Scientific Association for Probiotics and Prebiotics, most probiotics should be consumed daily for obtaining their expected health benefit. In addition, further research is needed in the field of yogurt consumption and changes in gut microbiota as it is still necessary to clarify which bacterial populations are involved in depression (51), although studies with *Bifidobacterium infantis* are showing promising results (52).

The current study showed opposite results for the association of whole- and low-fat yogurt consumption with the incidence of depression. This was the reason to explain why total yogurt was not related to depression risk. Since whole- and low-fat yogurts seem to not differ on bacterial concentration (53), the probiotic hypothesis does not appear to fully explain the opposite associations found between whole- and low-fat yogurt consumption and depression risk in our results, although it may support the inverse association observed between whole-fat yogurt and depression. Together with beneficial bacteria, yogurt also contains zinc, vitamins (riboflavin, vitamin A, vitamin E, thiamine, vitamin B-6 and folate), protein, carbohydrates (lactose, glucose and galactose), fat (including conjugated linoleic acid (CLA)), and minerals (calcium, magnesium, and potassium). However, the nutritional composition of yogurt varies depending on the species and strains of bacteria used in the fermentation, the type of milk (whole, semi or skimmed), fortification methods, store conditions, etc. (15, 54). CLA has been proposed to have gut anti-inflammatory properties (55), which may in turn improve immune activity (15). Depression has been related to low-grade inflammation, and also with low folate consumption (35, 36). Whole-fat yogurt contains higher amount of fat (that includes CLA) and folate than fat-free yogurt (54). This hypothesis may contribute at least partially to explain why we only observed an inverse association for whole-fat yogurt consumption but not for low-fat yogurt.

The inverse association between whole-fat yogurt consumption and depression risk was statistically significant only in women, although no significant interaction between whole-fat yogurt and sex was found. We may hypothesize that these differences are because in general women are more conscious of

their diet (56) and this may lead to a lower measurement error in this group. Moreover, women are more likely to suffer from depression than men (57).

The current study has some strengths and limitations. Strengths of this study include its prospective longitudinal design, the use of previously validated methods (26), the large sample size, and the repeated measurements of diet. Participants were highly educated subjects, which increases the quality of self-reported information and reduces the potential for misclassification bias. In addition, the restriction to a fairly homogenous subgroup of subjects regarding educational levels minimizes the potential for residual confounding and it is an excellent technique to improve the internal validity of our results. Also, the long-term follow-up may reduce the potential for reverse causation bias.

A limitation of this study was that dietary intake and clinical diagnosis of depression were self-reported; however, both methods have been previously validated in our cohort (27, 28). Although our FFQ collects the consumption of low- or whole-fat yogurt, it does not differentiate among other yogurt varieties (e.g. bio- or probiotic-yogurt). Together with fructans and GOSs, other prebiotic sources should be acknowledged, but they were not included in the analysis because nowadays there is not enough data available in the literature (22). Moreover, our FFQ is composed of 136-items and information of some possible foods containing prebiotics was not collected. We assumed a relatively long induction period (from 0 to 10 years) for the association diet-depression. This may potentially be a strong assumption. However, dietary habits tend to be correlated within subjects along years of follow-up. Albeit 10 years was the longest possible follow-up, most cases occurred after a follow-up period considerably shorter than 10 years. Although the follow-up questionnaires were mainly focused on outcomes (disease incidence, including depression incidence) and were completed every 2 years, the full-length FFQ was administered twice, at baseline and after 10 years of follow-up allowing the use of updated information on diet after 10 years. Another potential limitation is that our participants are university graduates and research in other population groups is needed before our findings can be extrapolated to the general population. Finally, it would have been of interest to determine the gut microbiota composition of the participants, but stool samples were not available in this study.

Conclusion

In summary, a high consumption of whole-fat yogurt was related to lower risk of depression in women of the SUN cohort. The consumption of prebiotics was not significantly associated with depression risk. The

different effect observed depending on the fat content of the yogurt encourages to carry out further prospective studies to clarify these matters.

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Table 1. Baseline characteristics of participants according to their total prebiotic and yogurt consumption. The SUN cohort, 1999-2012.¹

	Prebiotic consumption ³				<i>P</i> -trend ²	Total yogurt consumption				<i>P</i> -trend ²
	Q1	Q2	Q3	Q4		(0-0.5 servings ⁵ /week)	(>0.5 to <3 servings ⁵ /week)	(≥3 to <7 servings ⁵ /week)	(≥7 servings ⁵ /week)	
Median	1.1	1.8	2.4	3.4		0	1	3	7	
<i>n</i>	3,635	3,635	3,635	3,634		3,133	1,871	5,489	4,046	
Women, %	55.3	59.5	59.4	62.4	< 0.001	51.2	56.5	59.6	65.8	< 0.001
Age ⁴ , years	37.8 ± 11.7	37.3 ± 11.4	37.4 ± 11.4	37.8 ± 11.9	0.99	41.2 ± 12.6	37.5 ± 11.3	36.2 ± 10.9	36.7 ± 11.26	< 0.001
Baseline BMI ⁴ , kg/m ²	23.7 ± 3.6	23.4 ± 3.4	23.4 ± 3.4	23.3 ± 3.3	< 0.001	23.9 ± 3.6	23.6 ± 3.5	23.4 ± 3.4	23.2 ± 3.3	< 0.001
Marital status, % married	50.7	50.8	50.9	50.3	0.80	58.2	49.8	48.4	48.3	< 0.001
Unemployed, %	4.0	4.5	3.9	3.8	< 0.001	3.3	3.7	4.4	4.3	< 0.001
Smoking status, %					< 0.001					< 0.001
Current smoker	26.5	21.7	21.0	18.4		27.9	25.9	21.2	16.4	
Former smoker	29.0	28.0	28.8	28.2		32.1	30.2	26.1	28.3	
Living alone, %	7.2	6.7	5.7	6.3	0.046	5.5	6.2	6.4	7.3	0.002
Physical activity, MET-h/week	19.0 ± 20.6	21.0 ± 21.9	22.5 ± 22.3	24.6 ± 26.3	< 0.001	19.6 ± 22.0	20.1 ± 21.1	21.8 ± 22.5	24.2 ± 24.8)	0.000
Weight gain >3 kg in the 5 years previous to entering the cohort, %	32.2	30.5	28.9	28.9	0.001	31.1	30.6	31.0	28.0	0.012
Total energy intake ⁴ , kcal/day	1,890 ± 526	2,250 ± 505	2,490 ± 516	2,800 ± 522	< 0.001	2,220 ± 650	2,300 ± 593	2,360 ± 599	2,480 ± 596	< 0.001
Adherence to the Mediterranean dietary pattern ⁴ , 0-9 score	3.1 ± 1.6	3.9 ± 1.6	4.6 ± 1.7	5.2 ± 1.6	< 0.001	4.3 ± 1.8	4.3 ± 1.8	4.1 ± 1.8	4.3 ± 1.8	< 0.001
Omega 3 PUFAs, g/day	2.3 ± 1.2	2.6 ± 1.2	2.7 ± 1.2	2.9 ± 1.3	< 0.001	2.5 ± 1.3	2.6 ± 1.1	2.6 ± 1.2	2.7 ± 1.3	< 0.001
Total dietary fiber intake ⁴ , g/day	18.5 ± 7.3	25.0 ± 7.8	28.9 ± 9.2	38.0 ± 13.2	< 0.001	26.4 ± 12.8	25.9 ± 10.8	26.9 ± 11.1	30.2 ± 12.6	< 0.001
Total prebiotic consumption ^{3, 4} , g/day	1.1 ± 0.3	1.7 ± 0.2	2.4 ± 0.2	3.7 ± 1.0	< 0.001	2.1 ± 1.2	2.1 ± 1.0	2.2 ± 1.1	2.4 ± 1.2	< 0.001
Total yogurt intake ⁴ , g/week	493 ± 619	579 ± 632	592 ± 623	657 ± 707	< 0.001	26 ± 31	147 ± 44	487 ± 151	1,330 ± 756	< 0.001

BMI: body mass index; CVD: cardiovascular disease; MET, metabolic equivalent of task; PUFAs, polyunsaturated fatty acids.

¹ Values are means ± SDs, or %.

² $P < 0.05$ for chi-square test for trend (categorical variables) and linear regression models (continuous variables) across categories of prebiotic and yogurt consumption.

MET-h, metabolic equivalent task hours; SUN, Seguimiento Universidad de Navarra.

³ Sum of galacto-oligosaccharides and fructans.

⁴ Mean \pm SD (all such values).

⁵ serving = 125g.

Table 2. HR and 95% CI for incident depression (diagnosis of depression) according to intake of prebiotics in the SUN cohort (1999-2012) stratified by sex.¹

		Quartile				P-trend
		1	2	3	4	
Overall sample	<i>n</i>	3,635	3,635	3,635	3,634	
	Fructans					
	Median	0.9	1.5	2.0	2.9	
	Fructans ² , g/d	1.0 ± 0.2	1.5 ± 0.1	2.0 ± 0.2	3.1 ± 0.9	
	<i>n</i> cases/person-years	180/29454	184/29494	192/29358	171/29358	
	Multiple-adjusted model	1.00 (ref.)	1.04 (0.84, 1.29)	1.07 (0.86, 1.33)	0.94 (0.74, 1.20)	0.70
	Galacto-oligosaccharides					
	Median	0.1	0.2	0.4	0.5	
	Galacto-oligosaccharides ² , g/d	0.1 ± 0.1	0.25 ± 0.1	0.4 ± 0.0	0.6 ± 0.2	
	<i>n</i> cases/person-years	187/29220	187/29425	172/29415	181/29856	
	Multiple-adjusted model	1.00 (ref.)	0.97 (0.79, 1.19)	0.91 (0.74, 1.12)	0.87 (0.69, 1.08)	0.16
	Total prebiotics					
	Median	1.1	1.8	2.4	3.4	
	Total prebiotics ^{2,3} , g/d	1.1 ± 0.3	1.8 ± 0.2	2.4 ± 0.2	3.6 ± 0.9	
	<i>n</i> cases/person-years	182/29537	193/29443	173/29523	179/29412	
	Multiple-adjusted model	1.00 (ref.)	1.06 (0.86, 1.30)	0.94 (0.76, 1.18)	0.92 (0.73, 1.17)	0.34
Females	<i>n</i>	2,150	2,150	2,150	2,149	
	Fructans					
	Median	1.0	1.5	2.0	3.0	
	Fructans ² , g/d	0.9 ± 0.3	1.5 ± 0.1	2.1 ± 0.2	3.3 ± 1.0	
	<i>n</i> cases/person-years	128/17298	135/17253	123/17261	128/17155	
	Multiple-adjusted model	1.00 (ref.)	1.12 (0.88, 1.44)	1.04 (0.80, 1.35)	1.09 (0.82, 1.44)	0.72
	Galacto-oligosaccharides					
	Median	0.1	0.2	0.4	0.5	
	Galacto-oligosaccharides ² , g/d	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	
	<i>n</i> cases/person-years	131/17174	133/17204	125/17155	125/17435	
	Multiple-adjusted model	1.00 (ref.)	1.01 (0.80, 1.29)	0.97 (0.76, 1.24)	0.90 (0.69, 1.17)	0.41
	Total prebiotics					
	Median	1.1	1.8	2.4	3.4	
	Total prebiotics ^{2,3} , g/d	1.1 ± 0.3	1.8 ± 0.2	2.4 ± 0.2	3.7 ± 1.0	
	<i>n</i> cases/person-years	125/16213	132/17307	121/17255	136/18192	
	Multiple-adjusted model	1.00 (ref.)	1.04 (0.81, 1.34)	0.99 (0.76, 1.29)	1.03 (0.78, 1.36)	0.93
Males	<i>n</i>	1,485	1,485	1,485	1,485	
	Fructans					

Median	0.9	1.4	1.9	2.8	
Fructans ² , g/d	0.9 ± 0.2	1.4 ± 0.1	1.9 ± 0.2	3.1 ± 0.9	
<i>n</i> cases/person-years	52/12156	49/12241	69/12348	43/12203	
Multiple-adjusted model	1.00 (ref.)	0.83 (0.56, 1.25)	1.16 (0.79, 1.71)	0.66 (0.42, 1.05)	0.24
Galacto-oligosaccharides					
Median	0.1	0.2	0.4	0.5	
Galacto-oligosaccharides ² , g/d	0.1 ± 0.1	0.2 ± 0.1	0.38 ± 0.1	0.62 ± 0.2	
<i>n</i> cases/person-years	56/12046	54/12221	47/12260	56/12421	
Multiple-adjusted model	1.00 (ref.)	0.86 (0.59, 1.25)	0.74 (0.49, 1.12)	0.79 (0.52, 1.20)	0.24
Total prebiotics					
Median	1.1	1.8	2.4	3.4	
Total prebiotics ^{2,3} , g/d	1.1 ± 0.3	1.8 ± 0.2	2.4 ± 0.2	3.7 ± 1.0	
<i>n</i> cases/person-years	57/13324	61/12136	52/12268	43/11220	
Multiple-adjusted model	1.00 (ref.)	1.07 (0.73, 1.56)	0.88 (0.59, 1.30)	0.71 (0.45, 1.11)	0.08

¹ Cox regression analysis. Repeated measurements of diet with baseline intake and updated dietary values from the FFQ after 10 years of follow-up (10-y follow-up questionnaire). Multiple-adjusted model adjusted for age (underlying time variable), sex, smoking (never, current, former), physical activity (quartiles), total energy intake (quartiles), baseline BMI (quartiles), living alone, unemployment, marital status, and for the personality traits competitive, relaxed and dependant.

Models are stratified by date of recruitment (2-year periods) and deciles of age.

The SUN (Seguimiento Universidad de Navarra, University of Navarra Follow-up) Project 1999–2012.

² Values are means ± SDs.

³ Total prebiotic consumption was the sum of galacto-oligosaccharides and fructans.

Table 3. HR and 95% CI for incident depression (diagnosis of depression) according to the consumption of yogurt in the SUN cohort (1999-2012) stratified by sex.¹

		(0-0.5 servings ² /week) 0-63 g/week	(>0.5 to <3 servings ² /week) >63 to <250 g/week	(≥3 to <7 servings ² /week) >250 to <875 g/week	(≥7 servings ² /week) ≥875 g/week	P-trend
Overall sample	Total yogurt					
	Median	0	1	3	7	
	<i>n</i>	3,133	1,871	5,489	4,046	
	<i>n</i> cases per person-year	147/24973	93/15300	287/44556	200/33086	
	Multiple-adjusted model	1.00 (ref.)	1.02 (0.79, 1.31)	1.09 (0.90, 1.33)	1.00 (0.81, 1.25)	0.83
	Whole-fat yogurt					
	Median	0	1	3	7	
	<i>n</i>	6,445	1,586	4,091	2,417	
	<i>n</i> cases per person-year	347/52103	76/12951	193/33100	111/19760	
	Multiple-adjusted model	1.00 (ref.)	0.86 (0.67, 1.10)	0.87 (0.73, 1.03)	0.78 (0.63, 0.98)	0.020
	Low-fat yogurt					
	Median	0	1	3	7	
	<i>n</i>	9,376	1,034	2,413	1,716	
	<i>n</i> cases per person-year	422/75959	62/8320	144/19690	99/13946	
	Multiple-adjusted model	1.00 (ref.)	1.39 (1.08, 1.80)	1.32 (1.09, 1.58)	1.32 (1.06, 1.65)	0.001
Females	Total yogurt					
	Median	0	1	3	7	
	<i>n</i>	1,605	1,057	3,274	2,663	
	<i>n</i> cases per person-year	93/12697	69/8363	210/26218	142/21690	
	Multiple-adjusted model	1.00 (ref.)	1.14 (0.84, 1.54)	1.15 (0.91, 1.46)	0.96 (0.74, 1.25)	0.75
	Whole-fat yogurt					
	Median	0	1	3	7	
	<i>n</i>	3,919	904	2,300	1,476	
	<i>n</i> cases per person-year	254/31212	53/7219	137/18524	70/12012	
	Multiple-adjusted model	1.00 (ref.)	0.86 (0.64, 1.15)	0.87 (0.71, 1.07)	0.66 (0.50, 0.87)	0.004
	Low-fat yogurt					
	Median	0	1	3	7	
	<i>n</i>	4,982	682	1,659	1,276	
	<i>n</i> cases per person-year	263/40011	53/5306	119/13296	79/10354	
	Multiple-adjusted model	1.00 (ref.)	1.72 (1.29, 2.29)	1.49 (1.20, 1.84)	1.37 (1.07, 1.76)	< 0.001
Males	Total yogurt					
	Median	0	1	3	7	
	<i>n</i>	1,528	814	2,215	1,383	

<i>n</i> cases per person-year	54/12276	24/6937	77/18338	58/11396	
Multiple-adjusted model	1.00 (ref.)	0.80 (0.49, 1.29)	0.98 (0.68, 1.40)	1.22 (0.84, 1.78)	0.28
Whole-fat yogurt					
Median	0	1	3	7	
<i>n</i>	2,526	682	1,791	941	
<i>n</i> cases per person-year	93/20892	23/5732	56/14576	41/7748	
Multiple-adjusted model	1.00 (ref.)	0.87 (0.55, 1.37)	0.85 (0.60, 1.19)	1.15 (0.79, 1.66)	0.86
Low-fat yogurt					
Median	0	1	3	7	
<i>n</i>	4,394	352	754	440	
<i>n</i> cases per person-year	159/35948	9/3014	25/6394	20/3592	
Multiple-adjusted model	1.00 (ref.)	0.70 (0.36, 1.36)	0.90 (0.59, 1.38)	1.42 (0.89, 2.27)	0.53

¹ Cox regression analysis. Repeated measurements of diet with baseline intake and updated dietary values from the FFQ after 10 years of follow-up (10-y follow-up

questionnaire). Multiple-adjusted model adjusted for age (underlying time variable), sex, smoking (never, current, former), physical activity (quartiles), total energy intake (quartiles), baseline BMI (quartiles), living alone, unemployment, marital status, and for the personality traits competitive, relaxed and dependant.

Models are stratified by date of recruitment (2-year periods) and deciles of age.

The SUN (Seguimiento Universidad de Navarra, University of Navarra Follow-up) Project 1999–2012.

² serving = 125g

Supplemental table 1. Sensitivity analyses. HRs (95% CIs) for incident depression according to quartiles of prebiotic intake in the SUN cohort 1999-2012.¹

	Cases/person -years	Fructans			Galacto-oligosaccharides			Total Prebiotics ²		
		Q1	Q4	P-trend	Q1	Q4	P-trend	Q1	Q4	P-trend
Median		0.9	2.9		0.1	0.5		1.1	3.4	
Overall	727/117,916	1.00 (ref.)	0.94 (0.74, 1.20)	0.70	1.00 (ref.)	0.87 (0.69, 1.08)	0.16	1.00 (ref.)	0.92 (0.73, 1.17)	0.34
Additionally adjusted for adherence to the Mediterranean diet	727/117,916	1.00 (ref.)	1.03 (0.80, 1.33)	0.72	1.00 (ref.)	0.93 (0.73, 1.18)	0.48	1.00 (ref.)	1.00 (0.78, 1.29)	0.84
Additionally adjusted for other fiber consumption	727/117,916	1.00 (ref.)	0.87 (0.94, 0.73)	0.70	1.00 (ref.)	0.86 (0.86, 0.68)	0.15	1.00 (ref.)	0.91 (0.91, 0.71)	0.31
Additionally adjusted for omega-3 PUFAs	727/117,916	1.00 (ref.)	0.95 (0.75, 1.21)	0.76	1.00 (ref.)	0.87 (0.69, 1.08)	0.17	1.00 (ref.)	0.93 (0.73, 1.18)	0.38
Excluding early incident cases of depression (those in the first 2-year follow-up)	529/116,223	1.00 (ref.)	0.98 (0.74, 1.31)	0.90	1.00 (ref.)	0.90 (0.70, 1.17)	0.37	1.00 (ref.)	0.97 (0.73, 1.28)	0.75
Including prevalent cancer, diabetes or cardiovascular disease	779/124,879	1.00 (ref.)	0.95 (0.75, 1.20)	0.77	1.00 (ref.)	0.87 (0.70, 1.07)	0.17	1.00 (ref.)	0.92 (0.73, 1.16)	0.36
Only those with prevalent obesity (BMI ≥ 30 kg/m ²)	32/4,940	1.00 (ref.)	1.48 (0.48, 4.59)	0.50	1.00 (ref.)	0.51 (0.15, 1.77)	0.19	1.00 (ref.)	1.52 (0.50, 4.61)	0.71
Only those who have gained 3 or more kg in the last 5 years	248/35,945	1.00 (ref.)	1.20 (0.79, 1.82)	0.31	1.00 (ref.)	1.07 (0.73, 1.56)	0.88	1.00 (ref.)	1.09 (0.73, 1.65)	0.77
Energy limits: percentiles 5–95	659/106,173	1.00 (ref.)	0.97 (0.76, 1.24)	0.77	1.00 (ref.)	0.96 (0.76, 1.20)	0.53	1.00 (ref.)	0.95 (0.74, 1.22)	0.50
Only women excluding incident cases of depression (until 2 years of follow-up)	369/67,723	1.00 (ref.)	1.16 (0.84, 1.61)	0.43	1.00 (ref.)	0.96 (0.71, 1.31)	0.65	1.00 (ref.)	1.10 (0.80, 1.53)	0.50
Only men excluding incident cases of depression (until 2 years of follow-up)	160/48,499	1.00 (ref.)	0.65 (0.37, 1.13)	0.33	1.00 (ref.)	0.77 (0.48, 1.25)	0.39	1.00 (ref.)	0.70 (0.41, 1.22)	0.14

Abbreviation: BMI, body mass index; PUFAs, polyunsaturated fatty acids.

¹ Cox regression analysis. Repeated measurements of diet with baseline intake and updated dietary values from the FFQ after 10 years of follow-up (10-y follow-up questionnaire). Multiple-adjusted model adjusted for age (underlying time variable), sex, smoking (never, current, former), physical activity (quartiles), total energy intake (quartiles), baseline BMI (quartiles), living alone, unemployment, marital status, and for the personality traits competitive, relaxed and dependent.

Models are stratified by date of recruitment (2-year periods) and deciles of age.

The SUN (Seguimiento Universidad de Navarra, University of Navarra Follow-up) Project 1999–2012.

² Total prebiotic consumption was the sum of galacto-oligosaccharides and fructans.

Supplemental table 2. Sensitivity analyses. HRs (95% CIs) for incident depression according to the consumption of yogurt in the SUN cohort 1999-2012.¹

	Cases/person-years	Total yogurt			Whole-fat yogurt			Low-fat yogurt		
		(0-0.5 servings ² /week)	(≥7 servings ² /week)	P-trend	(0-0.5 servings ² /week)	(≥7 servings ² /week)	P-trend	(0-0.5 servings ² /week)	(≥7 servings ² /week)	P-trend
Median		0	7		0	7		0	7	
Overall	727/117,916	1.00 (ref.)	1.00 (0.81, 1.25)	0.83	1.00 (ref.)	0.78 (0.63, 0.98)	0.02	1.00 (ref.)	1.32 (1.06, 1.65)	0.001
Additionally adjusted for adherence to the Mediterranean diet	727/117,916	1.00 (ref.)	1.00 (0.81, 1.25)	0.85	1.00 (ref.)	0.76 (0.60, 0.95)	0.007	1.00 (ref.)	1.39 (1.11, 1.74)	< 0.001
Additionally adjusted for omega-3 PUFAs	727/117,916	1.00 (ref.)	0.99 (0.78, 1.30)	0.90	1.00 (ref.)	0.78 (0.63, 0.97)	0.018	1.00 (ref.)	1.32 (1.06, 1.64)	0.001
Excluding early incident cases of depression (until 2 years of follow-up)	529/116,223	1.00 (ref.)	1.00 (0.78, 1.28)	0.77	1.00 (ref.)	0.77 (0.60, 0.99)	0.047	1.00 (ref.)	1.23 (0.94, 1.61)	0.010
Including prevalent cancer, diabetes or cardiovascular disease	779/124,879	1.00 (ref.)	1.00 (0.81, 1.24)	0.74	1.00 (ref.)	0.81 (0.65, 1.00)	0.034	1.00 (ref.)	1.27 (1.02, 1.57)	0.001
Only those with prevalent obesity (BMI ≥ 30 kg/m ²)	32/4,940	1.00 (ref.)	1.76 (0.58, 5.29)	0.71	1.00 (ref.)	2.47 (0.79, 7.67)	0.39	1.00 (ref.)	2.51 (1.01, 6.24)	0.14
Only those who have gained 3 or more kg in the last 5 years	248/35,945	1.00 (ref.)	0.92 (0.64, 1.31)	0.68	1.00 (ref.)	0.77 (0.52, 1.14)	0.25	1.00 (ref.)	1.44 (0.97, 2.12)	0.15
Energy limits: percentiles 5–95	659/106,173	1.00 (ref.)	1.00 (0.80, 1.26)	0.91	1.00 (ref.)	0.79 (0.63, 1.00)	0.032	1.00 (ref.)	1.35 (1.07, 1.69)	0.001
Only women excluding incident cases of depression (until 2 years of follow-up)	369/67,723	1.00 (ref.)	1.00 (0.74, 1.37)	0.93	1.00 (ref.)	0.67 (0.48, 0.92)	0.035	1.00 (ref.)	1.29 (0.95, 1.76)	0.008
Only men excluding incident cases of depression (until 2 years of follow-up)	160/48,499	1.00 (ref.)	1.15 (0.75, 1.77)	0.39	1.00 (ref.)	1.08 (0.70, 1.65)	0.81	1.00 (ref.)	1.33 (0.77, 2.32)	0.24

Abbreviation: BMI, body mass index; PUFAs, polyunsaturated fatty acids.

¹ Cox regression analysis. Repeated measurements of diet with baseline intake and updated dietary values from the FFQ after 10 years of follow-up (10-y follow-up questionnaire). Multiple-adjusted model adjusted for age (underlying time variable), sex, smoking (never, current, former), physical activity (quartiles), total energy intake (quartiles), baseline BMI (quartiles), living alone, unemployment, marital status, and for the personality traits competitive, relaxed and dependant.

Models are stratified by date of recruitment (2-year periods) and deciles of age.

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² serving = 125g