Effect of Presymptomatic Body Mass Index and Consumption of Fat and Alcohol on Amyotrophic Lateral Sclerosis

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IMPORTANCE Because dietary intake may influence pathophysiologic mechanisms in sporadic amyotrophic lateral sclerosis (ALS), the association between premorbid dietary intake and the risk of sporadic ALS will provide insight into which mechanisms are possibly involved in ALS pathogenesis.

OBJECTIVE To systematically determine the association between premorbid dietary intake and the risk of sporadic ALS.

DESIGN, SETTING, AND PARTICIPANTS A population-based case-control study was conducted in a general community setting in the Netherlands from January 1, 2006, to September 30, 2011. Analysis was conducted April 1, 2013, to November 15, 2014. All patients with a new diagnosis of possible, probable (laboratory supported), or definite ALS according to the revised El Escorial criteria were included and multiple sources were used to ensure complete case ascertainment. Of 986 eligible patients, 674 gave informed consent and returned a complete questionnaire; 2093 controls randomly selected from the general practitioners’ registers and frequency matched to the patients for sex and age were included.

MAIN OUTCOMES AND MEASURES We studied the premorbid intake of nutrients in association with the risk of ALS by using a 199-item food frequency questionnaire adjusted for confounding factors and corrected for multiple comparisons while minimizing recall bias.

RESULTS Presymptomatic total daily energy intake in patients, reported as mean (SD), was significantly higher compared with controls (2258 [730] vs 2119 [619] kcal/day; P < .01), and premortem body mass index (calculated as weight in kilograms divided by height in meters squared) was significantly lower in patients (25.7 [4.0] vs 26.0 [3.7]; P = .02). With values reported as odds ratio (95% CI), higher premorbid intake of total fat (1.14; 1.07-1.23; P < .001), saturated fat (1.43; 1.25-1.64; P < .001), trans-fatty acids (1.03; 1.01-1.05; P < .001), and cholesterol (1.08; 1.05-1.12; P < .001) was associated with an increased risk of ALS; higher intake of alcohol (0.91; 0.84-0.99; P = .03) was associated with a decreased risk of ALS. These associations were independent of total energy intake, age, sex, body mass index, educational level, smoking, and lifetime physical activity. No significant associations between dietary intake and survival were found.

CONCLUSIONS AND RELEVANCE The combination of independent positive associations of a low premorbid body mass index and a high fat intake together with prior evidence from ALS mouse models transgenic for SOD1 and earlier reports on premorbid body mass index support a role for increased resting energy expenditure before clinical onset of ALS.

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The cause of amyotrophic lateral sclerosis (ALS) is poorly understood. Oxidative stress, mitochondrial dysfunction, excitotoxicity, cortical hyperexcitability, disrupted axonal transport, and inflammation are mechanisms that are potentially involved in ALS pathogenesis. Some of these mechanisms may be influenced by dietary nutrients. Intake of dietary antioxidants, for example, may reduce oxidative stress.

Previous studies did not identify a consistent nutrient that modifies susceptibility to ALS. Most associations have not been replicated, and contradictory results exist for the association with fat intake. A decreased risk of ALS with higher levels of vitamin E intake, a potent cellular antioxidant, has been reported more than once. Furthermore, a recent study replicated the observation that higher intake of polyunsaturated fatty acids (PUFAs) is associated with a decreased risk of ALS. These studies suggest that nutrients might influence pathways involved in ALS pathogenesis.

Diet is highly modifiable. Therefore, in a large, population-based case-control study, we set out to test the association between premorbid intake of many nutrients and the risk of ALS as well as the progression of the disease, adjusted for confounding factors and corrected for multiple comparisons.

Methods

Study Participants
A population-based case-control study was performed in the Netherlands from January 1, 2006, until September 30, 2011. Data analysis was conducted from April 1, 2013, to November 15, 2014.

During the study period, all patients with a new diagnosis of possible, probable, probable laboratory-supported, or definite ALS according to the revised El Escorial criteria were included. Multiple sources were used to ensure complete case ascertainment: neurologists, rehabilitation physicians, the Dutch Neuromuscular Patient Association, and our ALS website (http://www.alsonderzoek.nl/contact). Medical records were scrutinized for eligibility of the patients, excluding patients with an ALS-mimic syndrome or those with a first-, second-, or third-degree family member with ALS, defined as familial ALS. Patients with a C9orf72 (RefGene 203228) repeat expansion, assessed by performing a repeat-primed polymerase chain reaction as described previously, were excluded from our analysis.

Population-based controls were selected from the register of the general practitioner (GP) providing care for the participating patients with ALS. In the Netherlands, the health care system ensures that every inhabitant is registered with a GP, which makes this record representative of the population. The GPs were asked to select individuals from their registers in alphabetical order, starting with the surname of the patient. Controls were matched to patients for sex and age (±5 years). Blood relatives or spouses of the patients were not eligible to be controls to prevent overmatching.

This study was approved by the institutional review board of the University Medical Centre Utrecht. Written informed consent was obtained from all participants; no financial compensation was provided.

Exposure Assessment
Patients and controls were asked to complete a 199-item food frequency questionnaire (FFQ) that covered food consumption, including nutritional supplements, during the previous month (Methods in the Supplement). However, if dietary habits had changed since the onset of symptoms, patients were asked to recall those habits for the 1-month period prior to the onset of muscle weakness or bulbar signs to avoid a possible influence of disease on their dietary intake. If necessary, participants were contacted to clarify inconsistencies or missing data in the questionnaire. The FFQs of 5 control participants were not included in the analyses because of implausibly low or high reported energy intake. To determine whether energy intake was implausible, theoretical physical activity levels were calculated, dividing reported energy intake by the basal metabolic rate using the Schofield formulae and compared with the lower and upper cutoff limits for these physical activity levels. All questionnaires remained anonymous during analyses, and all data were entered in a blinded fashion.

A second self-administered questionnaire was completed by the participants to obtain data on age, sex, educational level, smoking, anthropometric characteristics, and a lifetime history of occupations, sporting activities, and hobbies.

Survival of patients was monitored using the municipal population register.

Statistical Analysis
Baseline characteristics were evaluated for differences using the Pearson χ² test and the Mann-Whitney test. To determine odds ratios (ORs) for the association between intake of a specific nutrient and ALS, we performed a binary logistic regression with 3 adjustment levels: (1) adjusted for age (at ALS onset for patients; at the time of completion of the questionnaire for controls), sex, and educational level; (2) additionally adjusted for body mass index (BMI) (premorbid in patients), smoking (current or nonsmoking), and lifetime physical activity; and (3) additionally adjusted for total energy intake. Lifetime physical activity was calculated from the lifetime history of occupations, sporting activities, and hobbies, as described elsewhere. We also determined the association between nutrient intake and the risk of ALS using the multivariate nutrient density model designed by Willett et al., which is another frequently used model, to account for total energy intake:

\[
\text{Disease Risk} = \beta_1 \frac{\text{Energy Provided by Nutrient}}{\text{Total Energy}} + \beta_2 \text{Total Energy}.
\]

The meaning of coefficient \( \beta_1 \), for nutrient density (ie, energy provided by nutrient divided by total energy), is the difference in disease risk associated with a difference in 1% of energy from the nutrient; total energy intake is kept constant. For nutrients that do not yield energy, nutrient density was expressed as nutrient intake in milligrams per 1000 kcal energy intake. This analysis was also adjusted for age, sex, educational level, BMI, smoking, and physical activity.
An additional logistic regression analysis, with the same covariates, was performed in which nutrient intake was categorized into quintiles based on nutrient intake in controls. The lowest quintile served as the reference group, and the 5-level variables were also entered into the model as continuous variables to determine whether there was a linear trend.

Nutrients that were significantly associated with ALS, either in the analysis with absolute values of nutrient intake or in the analysis with quintiles of intake, were analyzed together in a multivariate binary logistic regression to determine which of these nutrients were independently associated with ALS. This analysis was performed with the maximal level of adjustment.

Sensitivity analysis excluding patients with bulbar onset of ALS was performed because bulbar symptoms may have affected dietary habits and, subsequently, how patients answered the questionnaire despite the fact that patients were asked to recall their dietary habits during the period before the onset of bulbar signs.

Cox proportional hazards regression analysis was performed to determine the association between survival from ALS onset and nutrient intake. Survival was defined as the time from symptom onset to death or the censoring date (February 14, 2012). Analyses were adjusted for sex, age at onset, site of onset, premorbid BMI, energy intake, educational level, current smoking, and lifetime physical activity.24 The same method was used to determine the effect of nutrient intake on age at ALS onset. To adjust appropriately for age, an interaction term of diagnosis and nutrient or dietary pattern was introduced to the Cox regression analysis, using age at the time of completing the questionnaire for the control participants.

All tests were 2-sided, and Bonferroni correction was applied to the α level to adjust for multiple comparisons. Bonferroni-adjusted P values are reported in the tables.

Results

Informed consent was given by 885 of 986 eligible patients (89.8%) identified between January 1, 2006, and September 30, 2011. Of the questionnaires sent to these 885 patients, 747 were returned (response rate, 84.4%). Of the returned questionnaires, 674 (90.2%) were completed without omissions and were included in the analyses. A total of 2480 population-based controls were selected from the GP registers, and 2385 of these (response rate, 96.2%) returned their questionnaire. Of these 2385 controls, 2093 individuals (87.8%) had completed the questionnaires without missing values and were included in the analyses. Table 1 reports the characteristics of the 674 patients and 2093 controls. Sex, mean age at onset, and frequency of bulbar onset did not differ significantly between the responders and nonresponders. Cases and controls were similar for the matching variables, sex, and age.

Presymptomatic BMI was significantly lower in patients than controls (P = .02) (Table 1). In contrast, presymptomatic daily energy intake as calculated from the FFQ was significantly higher in patients compared with controls (P < .01). Median lifetime physical activity did not differ significantly (P = .22).

Table 2 presents adjusted ORs for the association between premorbid intake of individual nutrients and risk of ALS. Higher intake of total fat, saturated fat, trans-fatty acids, and cholesterol was independently associated with an increased risk of ALS irrespective of the level of adjustment and the use of absolute intake or nutrient density in the analysis. In the maximal adjusted model, higher intake of vegetable protein, polysaccharides, fibers, and flavonoids was associated with a decreased risk of ALS. The association with quintiles of intake of these nutrients and P values for trends across quintiles are illustrated in the Figure (significant associations) and
increased risk of ALS. Saturated fat was independently associated with a decreased risk of ALS. The multivariate analysis (Table 2 and Figure) showed that only a higher intake of saturated fat in the multivariate model or in the analysis excluding patients with bulbar onset did not essentially change results. No significant associations between nutrient intake and survival were found with multivariate Cox regression analysis (eFigure in the Supplement). A total of 482 of 674 patients (71.5%) had died by the censoring date of February 14, 2012. Several significant associations between nutrients and age at onset were identified. However, an interaction term of case-control status and the nutrient introduced into the model was not significant for any of these associations; furthermore, the same associations were found when Cox regression was performed in controls using questionnaire completion as the event. Both findings indicate that the association between nutrients and age at onset was an age-related effect and thus not disease specific.

### Table 2. Adjusted Odds Ratios (AORs) for the Association Between Amyotrophic Lateral Sclerosis and Nutrient Intake

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Binary Logistic Regression With Absolute Nutrient Intake</th>
<th>Nutrient Density Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOR (95% CI)a</td>
<td>P Valueb</td>
</tr>
<tr>
<td>Protein, total</td>
<td>1.18 (1.06-1.31)</td>
<td>.002</td>
</tr>
<tr>
<td>Vegetable</td>
<td>1.18 (1.06-1.31)</td>
<td>.82</td>
</tr>
<tr>
<td>Animal</td>
<td>1.18 (1.06-1.31)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Fat, total</td>
<td>1.09 (1.05-1.12)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Saturated</td>
<td>1.27 (1.18-1.36)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>1.23 (1.13-1.33)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>1.19 (1.05-1.35)</td>
<td>.007</td>
</tr>
<tr>
<td>α-Lipoic acid</td>
<td>1.02 (1.01-1.04)</td>
<td>.007</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>1.06 (0.92-1.22)</td>
<td>.42</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>1.06 (0.95-1.18)</td>
<td>.29</td>
</tr>
<tr>
<td>ω-3 Fatty acids, total</td>
<td>1.02 (1.01-1.04)</td>
<td>.006</td>
</tr>
<tr>
<td>trans-Fatty acids</td>
<td>1.03 (1.02-1.04)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.45 (1.31-1.61)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Carbohydrates, total</td>
<td>1.05 (1.02-1.08)</td>
<td>.004</td>
</tr>
<tr>
<td>Monosaccharides and disaccharides</td>
<td>1.11 (1.05-1.17)</td>
<td>.001</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>1.03 (0.98-1.09)</td>
<td>.27</td>
</tr>
<tr>
<td>Fibers</td>
<td>0.81 (0.49-1.34)</td>
<td>.40</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.96 (0.89-1.04)</td>
<td>.36</td>
</tr>
</tbody>
</table>

- Adjusted for age (at onset in patients; at time of questionnaire in controls), sex, and educational level.
- Bonferroni-adjusted α value: .05/25 = .002.
- Adjusted for age (at onset in patients; at time of questionnaire in controls), sex, educational level, body mass index, current smoking, lifetime physical activity, and total energy intake.
- Adjusted for age (at onset in patients; at time of questionnaire in controls), sex, educational level, body mass index, current smoking, lifetime physical activity, and total energy intake.

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Discussion

In the present population-based case-control study, we found an increased risk of sporadic ALS with higher premorbid intake of total fat, saturated fat, trans-fatty acids, and cholesterol and a low intake of alcohol. Furthermore, presymptomatic daily energy intake in patients was significantly higher compared with that in controls, and presymptomatic BMI was significantly lower in patients. The combination of a positive association of high total energy intake, low premorbid BMI, and high fat intake, corrected for lifetime physical activity, supports a role for an altered energy metabolism before clinical onset of ALS.

The finding that higher intake of fat is associated with an increased risk of ALS corroborates observations in a population-based case-control study on ALS in western Washington. Another case-control study found a contrary result: a decreased risk of ALS with a higher intake of fat. Differences in study design may explain this discrepancy. Inclusion of only clinic-based patients may have caused referral bias in the latter study. Furthermore, in the western Washington study and the present study, only incident cases were included, and it is well known that there are many differences in characteristics between an incident and prevalent cohort of patients with ALS.

Multiple studies have shown that after symptom onset, patients with ALS have an increased resting energy expenditure. Our finding that presymptomatic daily energy intake in patients was higher and presymptomatic BMI was lower, which has also been demonstrated in large cohort and case-control studies, supports the hypothesis that energy expenditure is increased in patients with presymptomatic ALS. In general, the intake of saturated fats is not associated with an increased risk for cardiovascular disease. Previous observations, therefore, that the presence of coronary heart disease and the use of angiotensin-converting enzyme inhibitors are less frequent before the onset of ALS further support an altered metabolism with less atherosclerotic deposition in patients with presymptomatic ALS. This is in contrast to our observation of increased fat intake.

Table 3. Adjusted Odds Ratios (AORs) for the Association Between Amyotrophic Lateral Sclerosis and Nutrient Intake in a Multivariate Model

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>AOR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable protein</td>
<td>0.997 (0.991-1.004)</td>
<td>.43</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>1.002 (1.000-1.004)</td>
<td>.04</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>0.999 (0.998-1.000)</td>
<td>.12</td>
</tr>
<tr>
<td>Fibers</td>
<td>0.998 (0.985-1.010)</td>
<td>.69</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.999 (0.998-1.000)</td>
<td>.03</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.996 (0.987-1.004)</td>
<td>.29</td>
</tr>
<tr>
<td>Total energy intake</td>
<td>1.000 (1.000-1.001)</td>
<td>.09</td>
</tr>
<tr>
<td>Premorbid BMI</td>
<td>0.967 (0.944-0.992)</td>
<td>.01</td>
</tr>
<tr>
<td>Age</td>
<td>0.998 (0.988-1.008)</td>
<td>.68</td>
</tr>
<tr>
<td>Sex</td>
<td>0.874 (0.692-1.104)</td>
<td>.26</td>
</tr>
<tr>
<td>Current smoking</td>
<td>1.392 (1.088-1.781)</td>
<td>.01</td>
</tr>
<tr>
<td>Lifetime physical activity</td>
<td>1.017 (0.986-1.049)</td>
<td>.30</td>
</tr>
</tbody>
</table>

Abbreviation: BMI, body mass index.

There is a growing body of evidence that the ALS mouse model transgenic for human SOD1 (NCBI Entrez Gene: 6647) shows metabolic alterations. Resting and total energy expenditure of G86R and G93A mice, when compared with wild-type littermates, were shown to be markedly increased. This finding was also apparent in presymptomatic mice.

Because fat has a high caloric density, the higher premorbid intake of fat in patients with ALS in the present study may be a compensatory mechanism for this increased energy expenditure to prevent weight and muscle loss. This may also explain the positive effect of hypercaloric enteral nutrition on survival in patients with ALS in a recent phase 2 trial. A previous study, however, has shown that a high-fat diet increases resting energy expenditure, which may support a hypothesis that a high intake of fat in patients with presymptomatic ALS is not a compensation for increased energy expenditure but may have partially caused the increased energy expenditure. It remains uncertain whether...
these findings are part of a disease-causing chain of events in ALS or whether they represent premorbid secondary phenomena. This chain of events is testable in a group of carriers of frequently occurring genetic mutations related to ALS (ie, C9orf72 or SOD1). Although the present study showed no association between premorbid dietary habits and survival, this lack of association does not exclude a positive effect of a hypercaloric diet on survival after symptom onset as compensation for an increased resting energy expenditure. Our observations emphasize the importance of a comparison in a future phase 3 trial: to establish whether a high-carbohydrate, high-caloric diet is to be preferred to a high-fat, high-caloric diet in ALS. Nevertheless, the present study lends support to the hypothesis that altered energy metabolism may already be present in patients with presymptomatic ALS.

There are several possible explanations for the observed decreased risk of ALS with a higher intake of alcohol, which was not identified by a previous relatively small population-based study including 161 patients. A previous study has shown that a lyophilized extract of red wine, which contains several antioxidant compounds, was able to block glutamate-induced apoptosis in cerebellar granule neurones. Furthermore, an in vivo experiment carried out on mutant SOD1 mice showed that survival in mice fed with lyophilized red wine was significantly increased compared with the control untreated animals. In our study, however, the association between intake of alcohol and the risk of ALS was independent of the intake of red wine (eFigure 2 in the Supplement). Thus, the association cannot be attributed only to the possible protective effect of antioxidants in red wine.

Two previous case-control studies have shown that a high intake of PUFAs is associated with a decreased risk of ALS. The PUFAs have neuroprotective properties because they exert beneficial effects on excitotoxicity, inflammation, oxidative stress. In our present study, we neither observed a significant association between intake of PUFAs and risk of ALS, nor found an association between the risk of ALS and the intake of ω-3 fatty acids, which are a subtype of PUFAs. In a recent cohort study, only a higher intake of this subtype was associated with a decreased risk of ALS. However, a trend toward a decreased risk of ALS with a higher intake of PUFAs (P = .10 for trend) was also noted in our study. Despite the relatively large study sample, the power may have been too small to identify a significant association. In addition, the FFQs differ between the studies, which may have contributed to inconsistent results. The FFQ used in the present study covered all relevant sources of ω-3 fatty acids and other PUFAs, including several types of fish, oils, and supplements.

The present study does not lend support to the hypothesis that dietary antioxidants have a protective effect on development of ALS, which has been suggested, since prior research showed a role for oxidative stress in the pathogenesis of ALS. In the single-nutrient analysis adjusted for confounders, a higher intake of flavonoids was associated with a decreased risk of ALS (P = .002); however, in the multivariable analysis including other nutrients, this association was not significant (P = .29). The present study, therefore, does not confirm previous findings that intake of vitamin E and the antioxidant carotenoids are inversely and independently related to a risk of ALS. Discrepancies with previous findings may be caused by including intake of supplements and analyzing multiple nutrients in one model.

Another strength of our study, which include a relatively large sample size, use of a validated questionnaire, a population-based setting, a control population representative of the general population, correction for multiple comparisons, and a correction for many possible confounders (eg, physical activity), we acknowledge its limitations. Case-control studies using questionnaires are inevitably prone to recall bias. Blinding participants to the study hypotheses with an elaborate FFQ and the short time between the date of ALS diagnosis and the date on which the questionnaire was completed (median, 2.3 months) may have reduced this source of bias in our study. Another limitation is that patients completed the questionnaires after symptom onset and diagnosis. Bulbar symptoms and dietary interventions after diagnosis may affect usual dietary habits and, subsequently, how patients filled in the questionnaire despite the fact that patients were asked to recall their dietary habits during the period before the onset of bulbar signs. In the Netherlands, no dietary interventions are given to patients with ALS who do not have bulbar symptoms; therefore, the chance of a change in dietary habits in patients with spinal-onset ALS is smaller than in those with the bulbar-onset disease. Sensitivity analysis excluding patients with bulbar onset did not essentially change the results, suggesting that the identified associated dietary pattern was not the result of disease-related dietary changes. Only a prospective cohort study would be able to eliminate this source of bias.

Conclusions

The combination of a positive association of a low premorbid BMI and a high fat intake together with prior evidence from ALS SOD1 mouse models and earlier reports on premorbid BMI support a role for increased resting energy expenditure occurring before clinical onset of ALS.
Effect of Presymptomatic Dietary Intake on Amyotrophic Lateral Sclerosis

Original Investigation Research

Conflict of Interest Disclosures: Dr van den Berg received travel grants and consultancy fees from Baxter and serves on the advisory board forrogen and Cytokinetics. Dr Veldink received travel grants from Baxter. No other disclosures were reported.

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