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Consumption of animal products, olive oil and dietary fat and results from the Belgian case–control study on bladder cancer risk

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ABSTRACT

Aim: The Western diet typically consists of high levels of saturated fat from animal products and has been associated with an increased risk of bladder cancer. Whilst olive oil, the predominant fat in the Mediterranean diet, has been associated with many health benefits its role in bladder cancer aetiology is still unknown. Therefore, we investigated the effect of intake of animal products, olive oil and other major dietary fats on bladder cancer risk.

Methods: Dietary data were collected from 200 cases and 386 controls participating in a Belgian case–control study on bladder cancer. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) by comparing the highest with the lowest tertiles of intake between cases and controls using unconditional logistic regression. Adjustment was made for age, sex, smoking characteristics, occupational exposures and calorie intake.

Results: There was a statistically significant inverse association between olive oil intake and bladder cancer consistent with a linear dose–response relationship: middle versus the lowest tertile (OR: 0.62; 95% CI: 0.39–0.99) and the highest versus the lowest tertile (OR: 0.47; 95% CI: 0.28–0.78; p-trend = 0.002). We also observed borderline statistically significant increased odds of bladder cancer for the highest versus the lowest intake of cheese (OR: 1.53; 95% CI: 0.95–2.46; p-trend = 0.08). No potential associations were detected for any other source or type of dietary fat.

Conclusion: We observed evidence for a protective effect by olive oil and a possible increased risk of bladder cancer associated with a high intake of cheese. Our results require further investigation and confirmation by other studies.

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1. Introduction

According to a 2008 global study,[1] Western populations have the highest age-standardised incidence rate of bladder cancer. As exposure to established risk factors such as smoking and occupational carcinogens does not fully explain the observed differences amongst countries other potential risk factors need to be identified.[2]

Although the influence of dietary factors on the risk of bladder cancer has yet to be clearly established,[3] differences in dietary intake may explain part of the variation in incidence observed amongst populations.[1]

A recent Uruguayan case–control study[4] identified a high intake of red meat as one of the major components of a Western dietary pattern which was associated with an increased risk of bladder cancer. Whilst the role of red meat and other foods from animal sources such as fish, chicken, eggs, cheese and milk in bladder cancer aetiology is unclear,[3] it may be that these foods act as surrogate markers for high total and saturated fat intake.[5,6]

Fat, particularly saturated fat from animal products is a major component of the Western diet.[5] However, the current evidence for an association between dietary fat and bladder cancer has been limited and inconclusive.[3] A possible explanation for the lack of evidence is that to date few epidemiological studies,[7–11] have examined the effects of different types of dietary fat on bladder cancer risk. Although, a Serbian case–control study[8] from this group of studies, has reported a protective effect associated with intake of sunflower oil and an increased risk of bladder cancer associated with high consumption of animal fat.

One of the major differences between the Western and Mediterranean diets lies in the sources of dietary fat.[12] Although the Mediterranean diet is characterised by a moderately high intake of dietary fat, this fat is derived predominantly from plant sources such as olive oil with only a small contribution made by animal sources.[12] Contrary to reports that high consumption of dietary fat may induce bladder carcinogenesis through lipid peroxidation and oxidative DNA damage, olive oil has been associated with numerous health benefits including protection against cancer at several body sites.[13] Apart from containing the peroxidation-resistant lipid and monounsaturated fat, oleic acid, olive oil also contains several micronutrients with antioxidant properties including polyphenols, carotenoids and tocopherols (vitamin E).[13] A Greek cross-sectional study of approximately 3000 healthy men and women highlighted its antioxidant potential when it reported a positive correlation between intake of olive oil and total antioxidant capacity ($r = 0.54$; $p = 0.002$).[14] Despite these possible chemopreventive properties we are unaware of any other epidemiological study that has investigated the association between olive oil intake and bladder cancer risk.

In 2003, a possible cluster of increased bladder cancer incidence was identified amongst males in the Belgian province of Limburg.[15] A case–control study was designed to study possible risk factors for increased bladder cancer risk.[16] Previous investigations within this study population have identified inverse associations between bladder cancer and fruit consumption[17] and serum selenium levels.[18] The aim of our present study was to extend investigations into this Belgian study population and examine the association between the consumption of major dietary fats and bladder cancer risk.

2. Subjects and methods

2.1. Study design and subjects

This study design has been described previously in detail.[16] Briefly, a population based case–control study was conducted in the Belgian province of Limburg consisting of 200 bladder cancer cases and 386 healthy controls. All cases included in the study were incident cases histologically confirmed with transitional cell carcinoma (TCC) of the bladder between 1999 and 2004. Cases were derived from the Limburg Cancer Registry (LIKAR) and invited to participate in the study by urologists and general practitioners. Strict Belgian privacy laws prevented direct access to population registers and so controls were selected through a Belgian authority, Kruispuntbank van de Sociale Zekerheid (Crossroads Bank of Social Security). This was done by stratified random sampling of individuals 50 years of age and older from the province of Limburg according to municipality and social economic status. Individuals were eligible for inclusion as controls in the study if they belonged to the Caucasian race (to minimise differences due to genetic polymorphisms), were fluent in the Dutch language (spoken and written) and had no previous diagnosis of bladder cancer or any mental impairment which prevented their participation.[16] Of the selected individuals invited by mail to participate in the study, 26% agreed to take part by returning their signed consent form to the Study Centre.[19] The study was approved by the Ethical Review Board of the Medical School of the Katholieke Universiteit of Leuven and all participants provided written informed consent.

2.2. Data collection

A standardised food frequency questionnaire (FFQ) was sent by mail to all participants in the study. This validated FFQ[20,21] had been developed previously on behalf of the IMMIDIET study[22] and focuses specifically on the Belgian population. The semi-quantitative FFQ contains 322 food items and is linked to the combined contents of three existing food tables (the NEVO table of The Netherlands,[23] the Belgian Nubel table[24] and the IPL table of Francophone Belgians[25]) supplemented with information on the composition of common recipes from the region. Three trained interviewers visited cases and controls at home, checked answers to the FFQ and where necessary clarified and helped participants to answer questions. Usual dietary intake was estimated from food frequencies and quantities reported by participants for the 12 months prior to the interview. Interviewers also obtained information via a structured interview on participants' medical history, lifetime smoking history (never, ex-smoker and current smoker), family history of bladder cancer, lifetime occupational history and residential history for the previous 20 years.

2.3. Exposure assessment

Dietary information obtained from the FFQ was based on a fixed response format consisting of nine categories for frequency of food consumption (never/seldom; 1–3 d/month, 1–7 d/week). For some food items, alternative response options of five frequency categories were provided ((almost) never, sometimes, 50% of the time, most of the time, (almost) always). Intake quantity was determined based on standard household measures (five categories: 1–4, ≫5 table spoons/glasses/slices/portions, etc.; or, alternatively 1–2, 3–4, 5–6, 7–8, ≫9 table spoons, etc.) and an open-ended response option (number of grams/pieces/plates, etc.). Each FFQ was electronically scanned and linked by an adapted version of the Nutritional Analysis of FFQ (NAF) computer software programme developed by Instituto Nazionale Tumori (Epidemiology Unit) in Milan to the combined food composition table. This allowed for conversion of household units into g/mg etc. and the calculation of average daily intakes of food groups, energy and 29 nutrients.

2.4. Statistical analysis

Total intakes of meat, chicken, fish, eggs, milk, cheese and other dietary sources of fat, in particular olive oil, margarine and deep frying fat, were assessed in g/d. Based on these food product intake assessments intake of total fat and subgroups of fat including saturated fat, monounsaturated fat, polyunsaturated fat and linoleic acid were calculated, also in g/d. For the purpose of data analysis intake distributions for all food products were categorised into tertiles, except for some foods with a very low intake level, e.g. butter, crustaceans, duck, horse, game, lamb, offal and vegetable oil. Data from these foods were included in the total meat, fish and dietary fat categories listed above. Cut-off points for tertiles were determined according to distributions amongst controls. Odds ratios (ORs) and 95% confidence intervals (CIs) for the highest versus the lowest tertile of intake between cases and controls were calculated using unconditional logistic regression. Adjustments were made for the following potential confounders: age, sex, smoking (status, history and number of cigarettes smoked), occupational exposure (never versus ever: polycyclic aromatic hydrocarbons (PAHs), aromatic amines) and calorie intake. Adjustment for another potential confounder, dietary cholesterol intake did not influence the results. Similarly, neither did controlling for fruit and selenium (dietary sources only e.g. nuts, grains and cereals) that has been previously reported[17,18] as having inverse associations with bladder cancer in the same study population have any effect on the results.

Likelihood ratio tests were conducted to test for trend by assigning an integer to each tertile of nutrient intake, e.g. 1–3, followed by entering the term as a continuous variable in the model. A test for evidence of departure from a linear trend (non-linearity) was also undertaken using a likelihood ratio test comparing the deviance of a model with a linear term to the deviance of a model with a categorical exposure variable allowing the exposure effect to be non-linear.[26] Given the novel finding relating to olive oil intake, additional statistical analyses were conducted such as the Bonferroni correction and the false positive report probability (FPRP) test to assess the probability that a statistically significant result may actually be a false positive finding.[27] The Stata 10 statistical software programme[28] was used for all analyses and two-sided p-values <0.05 were considered to reflect statistical significance.

3. Results

Data from participants reporting extreme energy intake, that is <1st or >99th percentiles, were excluded from analyses. Consequently, dietary data from a total of 198 cases and 377 controls were included in the analyses of this study.

The study characteristics of the participants are presented in Table 1. There were more men than women in both the case and the control groups: 86% and 60%, respectively. On average cases were older than controls (67.6 ± 9.9 and 64.2 ± 9.6; $p < 0.001$; respectively). A greater percentage of cases were current smokers, had smoked for a longer period and smoked more cigarettes per day than controls. There were no other significant differences between cases and controls.

The fully adjusted ORs and 95% CIs for the highest versus the lowest tertile of total meat, chicken, fish, eggs, milk, cheese, olive oil, margarine and deep frying fat intakes are presented in Table 2. No statistically significant associations, either positive or negative, were observed between total meat consumption or any of the specific meat categories and bladder cancer (data for subtypes not shown). Similarly, we did not detect an association either for consumption of chicken, fish or eggs. Although the point estimate was elevated for both the second and third tertiles of milk consumption, this too was not statistically significant. Borderline statistically significant increased odds of bladder cancer, however, were observed for the highest versus the lowest tertile of cheese intake (OR: 1.53; 95% CI: 0.95–2.46; p-trend = 0.08).

There was a statistically significant inverse association between olive oil intake and bladder cancer risk, with OR = 0.62 (95% CI: 0.39–0.99) for middle versus lowest tertile and OR = 0.47 (95% CI: 0.28–0.78) for the highest versus the lowest tertile of intake (p-trend = 0.002). Additional testing for departure from linearity indicated that this association was consistent with a linear dose–response relationship (data not shown). Moreover, this inverse association between olive oil intake and bladder cancer risk remained statistically significant following the FPRP test with a 26% probability of being false positive for a prior probability of 10% and α of 0.05 and after taking into account the Bonferroni correction ($\alpha = 0.002$) (data not shown).

We did not detect any statistically significant associations between bladder cancer and the other dietary sources of fat: margarine and deep frying oil. Consumption of butter and vegetable oil also contributed to total dietary fat intake but were consumed in quantities too small to be included into the logistic regression model. A comparison of the average intake for both these foods indicated that there were no statistically significant differences between the two groups of participants (data not shown).

Table 1 – Frequency distributions of sex, age, smoking characteristics and occupational exposures of participants in the Belgian case–control study on bladder cancer.

	Cases N (%)	Controls N (%)
Sex		
Women	27 (14)	151 (40)
Men	171 (86)	226 (60)
Age		
<50 years	11 (6)	0
50–60	32 (16)	139 (37)
60–70	70 (35)	136 (36)
70–80	68 (34)	67 (18)
>80 years	17 (9)	34 (9)
Smoking status		
Non-current	32 (16)	156 (41)
Current	166 (84)	221 (59)
Smoking duration		
<10 years	33 (17)	182 (48)
>10 years	165 (83)	194 (52)
Cigarettes per day		
<15/d	86 (43)	259 (69)
>15/d	112 (57)	117 (31)
Occupational exposure[a]		
No	169 (85)	338 (90)
Yes	29 (15)	38 (10)

[a] Based on exposure to polycyclic aromatic hydrocarbons or aromatic amines during the longest performed occupation.[17]

Table 3 shows the fully adjusted ORs and 95% CIs for consumption of major dietary fats: total fat and its subtypes, saturated fat, monounsaturated fat, polyunsaturated fat and linoleic acid and cholesterol. No association was observed between bladder cancer risk and any of these categories of dietary fats.

4. Discussion

4.1. *Main findings*

We investigated the effects of consumption of animal products, olive oil and major dietary fats and observed a statistically significant inverse association between olive oil intake and the risk of bladder cancer consistent with a linear dose–response relationship. We did not detect any associations between bladder cancer risk and any level of meat, chicken, fish, eggs, milk, margarine, deep frying fat, total fat, saturated fat, monounsaturated fat, polyunsaturated fat or linoleic acid intake. A borderline statistically significant positive association, however, was observed between consumption of cheese and bladder cancer.

4.2. *Comparing our results with the existing literature*

To our knowledge this is the first epidemiological study on bladder cancer that has specifically investigated the effect of olive oil consumption. Although, an earlier Japanese case–control study[10] investigated monounsaturated fat intake

Table 2 – Effects of daily intake level of food sources of dietary fat on bladder cancer risk: adjusted odds ratios (ORs) and 95% confidence intervals (CIs).

Tertile of daily intake	Q1	Q2	Q3	*p*-Trend
Total meat[a]	<114.8	114.8–182.5	⩾182.6	
Case/control	65/126	52/125	81/126	
OR (95% CI)	1.0 (Reference)	0.80 (0.48–1.33)	1.11 (0.67–1.83)	0.67
Total fish (g/d)	<24.5	24.5–54.5	⩾54.6	
Case/control	67/118	76/128	50/123	
OR (95% CI)	1.0 (Reference)	1.04 (0.65–1.65)	0.77 (0.47–1.27)	0.33
Eggs (g/d)	<9.8	9.8–20.9	⩾21	
Case/control	59/126	63/124	75/127	
OR (95% CI)	1.0 (Reference)	1.04 (0.63–1.71)	1.02 (0.62–1.67)	0.94
Milk (g/d)	<10.2	10.2–70.0	⩾70.1	
Case/control	53/126	77/125	67/126	
OR (95% CI)	1.0 (Reference)	1.50 (0.93–2.44)	1.45 (0.89–2.37)	0.15
Cheese (g/d)	<24.3	24.3–53.3	⩾53.4	
Case/control	62/124	58/128	77/125	
OR (95% CI)	1.0 (Reference)	1.05 (0.64–1.72)	1.53 (0.95–2.46)	0.08
Olive oil (g/d)	<1.6	1.6–3.8	⩾3.9	
Case/control	106/126	52/125	40/126	
OR (95% CI)	1.0 (Reference)	0.62 (0.39–0.99)	0.47 (0.28–0.78)	0.002
Margarine (g/d)	<8.9	8.9–30.5	⩾30.6	
Case/control	48/125	78/127	72/125	
OR (95% CI)	1.0 (Reference)	1.70 (1.04–2.80)	1.16 (0.70–1.92)	0.64
Deep fry fat (g/d)	<0.7	0.7–1.6	⩾1.7	
Case/control	73/137	54/117	71/123	
OR (95% CI)	1.0 (Reference)	0.77 (0.47–1.26)	1.08 (0.66–1.77)	0.77

ORs: adjusted for sex, age, smoking status (current/non-current), number of cigarettes smoked per day, number of years smoking, occupational exposure to PAHs or aromatic amines and energy intake (kcal).
[a] Total meat = beef, veal, pork, mutton, horse, chicken, duck, unclassified poultry, lamb, game, processed meat and offal.

Table 3 – Effects of intake level of major dietary fats on bladder cancer risk: adjusted odds ratios (ORs) and 95% confidence intervals (CIs).

Tertile of daily intake	Q1	Q2	Q3	*p*-Trend
Total fat (g/d) [a]	22.6–80.8	80.9–114.6	⩾114.7	
Case/control	68/125	62/127	68/125	
OR (95% CI)	1.0 (Reference)	0.91 (0.55–1.50)	1.03 (0.55–1.92)	0.97
Saturated fat (g/d)	6.5–31.3	31.4–45.0	⩾45.1	
Case/control	69/126	55/126	74/125	
OR (95% CI)	1.0 (Reference)	0.86 (0.52–1.43)	1.17 (0.65–2.11)	0.63
Monounsaturated fat (g/d)	7.0–26.6	26.7–37.9	⩾38.0	
Case/control	70/124	60/127	68/125	
OR (95% CI)	1.0 (Reference)	0.86 (0.52–1.43)	1.02 (0.55–1.89)	0.99
Polyunsaturated fat (g/d)	4.5–15.4	15.5–22.7	⩾22.8	
Case/control	71/125	59/127	67/125	
OR (95% CI)	1.0 (Reference)	0.78 (0.48–1.29)	0.80 (0.45–1.42)	0.43
Linoleic acid (g/d)	2.2–10.7	10.8–16.5	⩾16.6	
Case/control	64/125	63/125	71/126	
OR (95% CI)	1.0 (Reference)	1.09 (0.66–1.78)	1.08 (0.62–1.88)	0.78
Cholesterol (mg/d)	46.2–251.9	252.0–387.1	⩾387.2	
Case/control	69/125	75/127	54/125	
OR (95% CI)	1.0 (Reference)	1.11 (0.69–1.80)	0.84 (0.47–1.50)	0.60
Energy (kcal/d)	1108.1–2192.5	2192.6–2904.7	⩾2904.8	
Case/control	65/124	62/126	67/126	
OR (95% CI)	1.0 (Reference)	0.92 (0.56–1.51)	0.99 (0.60–1.65)	0.99

ORs: adjusted for sex, age, smoking status (current/non-current), number of cigarettes smoked per day, number of years smoking, occupational exposure to PAHs or aromatic amines and energy intake (kcal).

[a] Total fat = saturated fat, monounsaturated fat, polyunsaturated fat and linoleic acid.

and found an inverse association with bladder cancer risk. Oleic acid is a monounsaturated fat that forms 70–80% of olive oil's fatty acid content.[29] As a major component of the Mediterranean diet, olive oil has previously been associated with a reduced risk of cardiovascular disease and several cancers.[30] Apart from oleic acid, olive oil is also rich in antioxidants and has been shown to increase the antioxidant capacity in healthy individuals.[14] Furthermore, inverse associations have been reported between the intake of olive oil and biomarkers of oxidative stress and genotoxic exposures[30] (F2-isoprostanes and DNA adducts, respectively), thus demonstrating its ability to reduce lipid peroxidation and subsequent oxidative DNA damage.[13]

Although animal products such as meat, chicken, fish, eggs, milk and cheese are generally considered to be rich sources of dietary fat, particularly saturated fat, we saw no statistically significant associations between intake of any of these foods and bladder cancer risk.[5,6] This disagreed with an early American prospective study[31] that reported increased risk of bladder cancer associated with high intakes of meat, poultry and fish. In contrast to previous studies we did not observe an association for any specific type of meat, either.[5,6] Differences in cooking methods, particularly the type of fat used in cooking, may explain these and other conflicting reports on the effect of consumption of other animal products such as eggs on bladder cancer risk.[4,10] Many fats used in cooking such as polyunsaturated fats are more susceptible to oxidation than olive oil.[13]

Whereas some previous studies have reported inverse associations between bladder cancer and intake of milk[3] and dairy products,[10] other studies, like ours have failed to detect a statistically significant association between bladder cancer risk and milk[31] or cheese[3,31] intakes. It should be noted, however, that the increased odds of bladder cancer we observed for the highest intake of cheese were borderline statistically significant. Regional differences in consumption levels and the fat content of these dairy products may account for the discrepancies observed between studies.[10,12,13]

Unlike previous reports of increased risk of bladder cancer associated with the intake of total fat[6] or saturated fat,[7] we failed to detect any associations. Interestingly, a Spanish study[7] found that the initial positive association observed between monounsaturated fat intake and bladder cancer disappeared following adjustment for saturated fat. Whilst the main source of monounsaturated fat is typically obtained from olive oil in Mediterranean diets,[13] in other populations it may be derived from the same dietary sources as saturated fat, e.g. beef.[12]

In accordance with previous studies[7,10,11] we found no association between intake of polyunsaturated fat and bladder cancer risk. We are unaware of the existence of any other epidemiological studies reporting on the association between dietary intake of the omega-6 polyunsaturated fat, linoleic acid (no effect in our study) and bladder cancer risk. Polyunsaturated fat, similar to other categories of dietary fat comprises of a wide range of different fatty acids derived from both animal and plant sources.[13] Future studies may benefit from differentiating between these dietary sources and from further refining the level of fat intake assessment to include individual fatty acids within these categories of fat.

4.3. *Strengths and weaknesses of our study*

A major strength of our study was that dietary data were collected by means of a detailed, validated[20,21] and population specific FFQ.[22] Although, dietary data were collected only once for the preceding 12-month period, this FFQ has been reported to be an adequate instrument for measuring 'usual dietary intake' for this study population and purpose.[20] Furthermore, it is unlikely that preclinical disease would have changed the dietary behaviour of the subjects.[32]

Whilst recall bias is a major point of concern of the case–control study design in general, we would not expect differential reporting between the cases and control subjects in our study, as diet is generally not considered a putative risk factor for bladder cancer.[3] Non-differential misclassification, if present, would tend to give results biased towards the null hypothesis of no effect, so at the highest an underestimation and never an overestimation of the true association strength.[31] Additionally, there were no statistically significant differences between cases and controls for major dietary factors, calorie intake and total fat, suggesting that any potential recall error did not differ between the two groups.

Apart from the relatively small number of subjects, another problem facing this type of study design is its potential for selection bias due to the low response rate. Invitation by mail is considered a difficult method to achieve high participation rates, however, ours was higher than other epidemiological studies using the same means of recruitment.[33] Furthermore, as reported earlier for the same study population,[17] the control subjects were a fair representation of the general population above 50 years from the province of Limburg in terms of established risk factors for bladder cancer: age, sex and smoking characteristics.

Such a comprehensive investigation into a wide range of foods and nutrients also raises the major concern of multiple testing. We attempted to address this issue by performing additional statistical analyses to check for false-positive results and looking for cross-validation in the existing literature. The inverse association between olive oil and bladder cancer remained statistically significant following additional analyses and therefore appeared to be quite robust. Reports of the many health benefits and components of olive oil suggest that the inverse association we observed is biologically plausible.[13,30] Further investigation however is warranted to determine if and to what extent any beneficial effects can be attributed to olive oil's fatty acid or micronutrient components.

La Vecchia and colleagues,[2] previously reported on the difficulty of disentangling the effects of different dietary components. We attempted to distinguish between the effect of olive oil intake and other potentially 'favourable' dietary factors by controlling for fruit, nuts, grains and cereals and found that these had no influence on our results. When we take into account all the dietary factors examined in the Belgian case–control study population for which there has been some potential association with bladder cancer risk they appear to be some of the key features of a Mediterranean style diet.[13,17,18] These include high consumption of plant foods, using olive oil as the main dietary fat and a low consumption of animal products e.g. cheese. Whilst our results and other studies[12] suggest that the Mediterranean dietary pattern may be anti-carcinogenic, the incidence of bladder cancer is not particularly lower for men from southern Europe.[1] Although, there is a high prevalence of smoking, particularly of black tobacco (air-cured) in countries such as Italy and Spain which has been strongly associated with increased bladder cancer risk and may modify any protective dietary effects.[34]

5. Conclusion

We observed a potentially protective effect from a high intake of olive oil and a suggestive increased risk associated with high cheese consumption. These associations are consistent with two key elements of the Mediterranean diet; olive oil as the main dietary fat and low consumption of animal products such as cheese. Our results need to be confirmed by other studies and further investigation is required to elucidate the biological mechanisms and optimal levels of olive oil and cheese intake.

Conflict of interest statement

None declared.

Role of the funding source

This funding source had no involvement in the study design, collection, analysis and interpretation of data or writing and submission of the manuscript for publication. These activities were the sole responsibility of the authors.

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