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Associations of lifestyle factors and Neuroendocrine Tumor Development: Results from the EPIC cohort

MASTER RESEARCH PROJECT (OP3)
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0. Abstract

The rarity of neuroendocrine tumors (NETs) and their heterogeneous presentation, complicate the identification of risk factors for their development. Results from previously published small observational studies indicate that smoking, alcohol and diabetes may contribute to NET development, but the reported data is conflicting. Additionally, little data is available on diet and physical activity. The aim of the current study is to investigate the association between lifestyle factors and NET development in a large prospective cohort.

Methods: A cohort of in total 450,111 participants from 9 participating countries was established from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Information on lifestyle and diet was obtained at baseline through questionnaires. For this study, lifestyle factors including smoking, alcohol consumption, Mediterranean diet score, body mass index and Cambridge Physical Activity Index were assessed.

Results: 193 carcinoid cases were diagnosed. Smoking was significantly associated with NET development in multivariable analysis in all NETs (HR 1.46, 95% CI 1.02 – 2.11) and gastroenteropancreatic (GEP) NETs (HR 1.58, 95% CI 1.04 – 2.41). Alcohol consumption was not associated with NET development. Hazard ratios for medium (7-10 points) and high adherence (11-18 points) to the Mediterranean diet were 0.71 (95% CI 0.51 – 0.98) and 0.39 (95% CI 0.25 – 0.62) for all NETs, 0.47 (95% CI 0.25 – 0.90) and 0.36 (95% CI 0.15 – 0.86) in lung NETs, and 0.80 (95% CI 0.55 – 1.16) and 0.40 (95% CI 0.23 – 0.69) in GEP NETs. Obesity was statistically not-significantly associated with NET development (HR 1.54, 95% CI 0.99 – 2.41). Physical activity was not found as an associated factor with NET development.

Conclusion: Smoking is strongly associated with NET development in both the entire NET population and GEP NETs in the EPIC cohort. Body mass index increases the risk of NET development. Increased adherence to the Mediterranean diet has a protective association with NETs.

1. Introduction

Neuroendocrine tumors (NETs), a rare tumor entity, develop from diffuse neuroendocrine cells dispersed through the body. These tumors preferably reside in the respiratory or gastrointestinal (GI) tract.(1, 2) The neuroendocrine nature of the cells may cause symptoms due to hormone production.(3, 4) The majority of the NETs, however, are clinically silent and will only be discovered by incidence or when symptoms occur due to mass effects of the tumor.(5)

The etiology of NETs remains unknown. Several genetic syndromes and genetic alterations are associated with the development of NETs, such as Multiple Endocrine Neoplasia (MEN) syndromes or Von Hippel Lindau (VHL) syndrome.(6) Familial history of cancer has also been identified as a risk factor for NET development.(7) Interestingly, research aimed to identify risk factors has been ambiguous towards lifestyle. In a systematic review and meta-analysis, body mass index and diabetes

were named as potential risk factors, while smoking and alcohol consumption may have a tissue-specific effect on NET development.(8, 9) A major limitation of studies into risk factors for NETs, however, are the case-control designs of the studies. Few cohort studies are available, due to the rare nature of the disease. To this date, one cohort study has been performed investigating the role of lifestyle and diet in the development of NET.(10, 11)

The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, is one of the largest ongoing cohort studies designed to detect associations between lifestyle and diseases as cancer and cardiovascular disease.(12) Almost 520,000 participants from 10 countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom) were included. From this highly heterogeneous population, diet and lifestyle characteristics were collected as well as the incidence of cancer during follow up.(13) Due to the nature of the study and the extensive data collection, the EPIC cohort studies have contributed significantly to the knowledge on lifestyle factors and cancer diagnoses.

The current study sought to investigate the association between lifestyle factors as smoking, alcohol consumption, Mediterranean Diet score, body mass index and physical activity and NETs in the EPIC cohort, to contribute to knowledge on NET etiology and potential preventive measures. To our knowledge, this is the largest cohort study on lifestyle and NET performed and the first performed in the EPIC cohort.

2. Methods

2.1 Recruitment of participants

The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort is a multicenter, prospective cohort including participants from 10 countries and 23 centers in Europe (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden and the United Kingdom). Participants were recruited from 1992 till 1999. At recruitment, information on diet, lifestyle characteristics, anthropometric measurements and medical history was collected. Participants are followed over time for the occurrence of cancer, cardiovascular disease and other diseases, as well as overall mortality. The majority of the population was extracted from the general population, with exceptions on all-female cohorts (Utrecht, Florence) or all-vegetarian/vegan cohorts (Oxford).
Dietary intake was assessed by extensive self-administered quantitative questionnaires containing 260 food items, semi-quantitative food-frequency questionnaires or a combination. To overcome differences in data collection, 24 hour dietary recall assessment was collected in representable samples of the population.
Non-dietary information (education, smoking, alcohol consumption, physical activity) was collected from questionnaires. Anthropometric measurements as height, weight, waist and hip circumference were measured according to similar protocols. In France, the United Kingdom (Oxford) and Norway, self-reported height and weight were obtained from all participants.
In total, 521,000 participants were included in the entire EPIC cohort. In 2021, over 109,000 participants were diagnosed with a form of cancer as identified through population registries, health

insurance records, pathology registries or active follow up. A detailed description of the population and data recollection has been published before.(13)

2.2 Exposure definition

For this study, the following factors were assessed: smoking, alcohol consumption, Mediterranean diet score, body mass index and physical activity. Smoking status was defined as never, current or former smoker. Alcohol consumption was categorized as non-drinkers, former drinkers, low consumers (0-6 drinks per month), medium consumers (6-12 drinks per month) and high consumers (12+ drinks per month).

Modified relative Mediterranean diet score was calculated on a scale of 0-18, with 0 as least adherence and 18 as highest adherence to the Mediterranean diet. Scoring was based on intake of 9 key nutritional compounds as registered in the food intake questionnaires. Nutritional compounds scored in tertiles were fruit, vegetable, legume, fish and cereal consumption. The lowest tertile was scored 0, while the highest tertile received a score of 2. Meat and dairy product consumption was ranked based on inverted tertiles: the lowest tertile was scored 2, while the highest tertile was scored 0. Olive oil consumption was scored on a scale of 0-2 based on the median consumption: non-consumers received 0 points, consumers below the median 1 point and consumers above the median 2 points. Alcohol consumption was scored dichotomously: 2 points were given if participants were non-users or low consumers, while 0 points were received if patients were moderate or high consumers (>5 grams/day for women, >10 grams/day for men). Overall Mediterranean diet scores were categorized as low (0-6), medium (7-10) and high (11-18).(14-16)

Participants were categorized according to their calculated body mass indexes for a low-normal (<25), overweight (25-30) and obese (>30) BMI. The Cambridge Physical Activity Index was calculated from the reported work activities (ranging from sedentary to heavy manual) and reported leisure time physical activity.

2.3 Definition of the endpoint

Diagnoses were made based on the International Classification for Diseases in Oncology (ICD-O), designed by the World Health Organization (WHO).(17) For this study, the morphology code “8240/3” (Carcinoid tumor) was used to define neuroendocrine tumor diagnoses. Other morphologic codes, such as “8246” (Neuroendocrine Carcinoma), were excluded as these belong to a different disease entity. Diagnoses were retrieved from national cancer registries, pathology registries and active follow up. Participants were included in the analysis from date of recruitment until diagnosis, end of follow up or death.

2.4 Statistical analysis

Baseline characteristics included age, sex, level of education, body mass index, smoking status, pack years, alcohol consumption, Cambridge physical activity index and Mediterranean diet score. Non-normally distributed variables were assessed as median and inter-quartile range.

Cox proportional hazards regression was used to examine the association between above mentioned lifestyle factors and the development of neuroendocrine tumors, using age as the underlying time scale. All analyses were adjusted for age, sex, country, level of education and mutually adjusted for all lifestyle factors (smoking, alcohol use, body mass index, Mediterranean diet score, physical activity). Models were not stratified for country of origin as too few cases per country were available, and the model could not converge as a result of that. We also present models for women only. Models for men are not presented as the number of cases in men was too little.

All statistical analysis was conducted using R Studio 4.0.3.

3. Results

3.1 Study population

Of over 521,000 participants, a total of 71,213 were excluded because they reported a prevalent cancer (other than non-melanoma skin cancer) at recruitment (n=25,184), had incomplete follow-up (n=4,148), had no lifestyle or dietary information at recruitment (n=6,259), or had an extreme ratio of energy intake to energy requirement (EI/ER), as defined by the top or bottom 1%, (9,573). For this study, Greece was excluded as this center is currently not actively participating in the follow up (n=26,048). A flow chart of the exclusion process is depicted in figure 1. In total, 450,111 participants were included in the current analyses. Baseline characteristics are shown in Table 1. The mean age of the participants was 51 years (SD 9.75), and 29.1% were men. Mean body mass index was 25.6. Of the participants, 22.2% was a current smoker and 27.3% previously smoked with a median of 13.3 pack years (IQR 5.9 – 23.4). Lifetime drinkers, defined as past and current drinkers, made up 57.9% of the population. Most participants drank either over 0-6 beverages per month/0-3 beverages per week (25.4%) or over 6-12 beverages per month/3-12 per week (31.3%). 33.3% of participants was classified as moderately inactive, while 26.7% of patients was classified as moderately active. The mean Mediterranean diet score was 8.6 (SD 2.99), with 47.1% of participants classifying at a medium score and 27.5% classifying at a high score.

Large countries of inclusion were France, the United Kingdom and Denmark. An overview of inclusions per country and center can be found in table 2.

A total of 193 patients were diagnosed with neuroendocrine tumors during a median follow-up time of 15 years (IQR 13 – 16.7 years). Patients were mainly diagnosed in Sweden, Denmark and the United Kingdom. An overview of patients per country can be appreciated in table 3. The majority of patients was diagnosed with NET of the lung or bronchus (n = 50). Secondly, patients were diagnosed with small intestinal (n=48), followed by rectal (n = 23) or cecal/appendiceal (n = 16) NET. 10 patients were diagnosed with pancreatic NET. All anatomical sites are presented in Table 4.

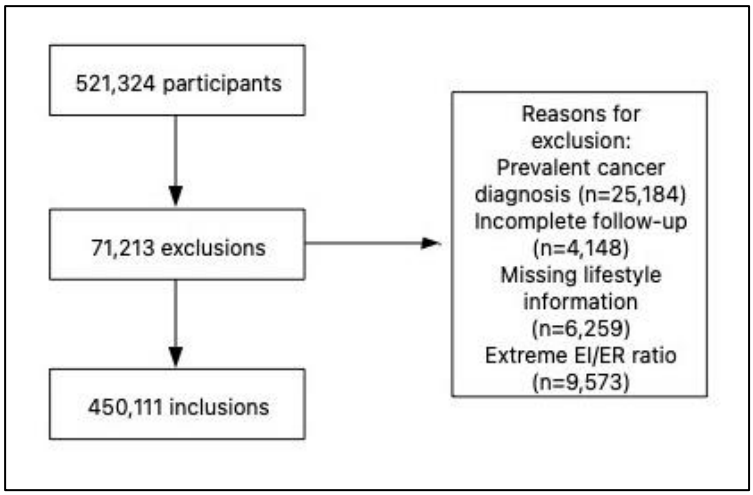

Figure 1 – Flow chart of inclusions. EI/ER: energy intake / energy requirement ratio.

Table 1 – Baseline table including patient characteristics and lifestyle features.

n	450111
Age (mean (SD))	51.13 (9.75)
Sex (n (%))	
Female	318686 (70.8)
Male	131425 (29.2)
Level of education (n (%))	
None	15551 (3.5)
Primary school completed	111064 (25.0)
Technical/professional School	103782 (23.4)
Secondary school	93910 (21.2)
Longer education (incl University)	108931 (24.5)
Not specified	10706 (2.4)
Smoking status (n (%))	
Never	219294 (48.7)
Former	122680 (27.3)
Smoker	99714 (22.2)
Unknown	8423 (1.9)
Pack years (median [IQR])	13.32 [5.93, 23.36]
Alcohol status (n (%))	
Never drinkers	23305 (5.2)
Former drinkers	15443 (3.4)
Drinkers at recruitment	37980 (8.4)
Lifetime drinkers	260534 (57.9)
Unknown	112849 (25.1)
Alcohol consumption (n (%))	
Non drinker	23305 (6.9)
Former	15443 (4.6)
>0-6/month or >0-3/week	85795 (25.4)
>6-12/month or >3-12/week	105650 (31.3)
>12-24	60644 (18.0)
>24-60	38885 (11.5)
>60-96/month or >60/week	5603 (1.7)
>96/month	1937 (0.6)
Mediterranean diet score (mean (SD))	8.60 (2.99)
Mediterranean diet score (categorial) (n (%))	
Low	114223 (25.4)
Medium	211939 (47.1)
High	123949 (27.5)
Body mass index (mean (SD))	25.64 (4.22)
Cambridge physical activity index (n (%))	
Inactive	88032 (19.6)
Moderately inactive	149941 (33.3)
Moderately active	120199 (26.7)
Active	83115 (18.5)
Missing	8824 (2.0)

SD: standard deviation. IQR: Interquartile range.

Table 2 – Distribution of inclusions among countries and centers.

Country (n (%))	
France	67403 (15.0)
Italy	44545 (9.9)
Spain	39989 (8.9)
United Kingdom	75416 (16.8)
The Netherlands	36538 (8.1)
Germany	48557 (10.8)
Sweden	48674 (10.8)
Denmark	55014 (12.2)
Norway	33975 (7.5)
Center of inclusion (n (%))	
France	67403 (15.0)
Florence	12796 (2.8)
Varese	11223 (2.5)
Ragusa	5928 (1.3)
Turin	9645 (2.1)
Naples	4953 (1.1)
Asturias	8279 (1.8)
Granada	7562 (1.7)
Murcia	8214 (1.8)
Navarra	7777 (1.7)
San Sebastian	8157 (1.8)
Cambridge	22798 (5.1)
Oxford	52618 (11.7)
Bilthoven	20992 (4.7)
Utrecht	15546 (3.5)
Heidelberg	23187 (5.2)
Potsdam	25370 (5.6)
Malmo	24373 (5.4)
Umea	24301 (5.4)
Aarhus	16614 (3.7)
Copenhagen	38400 (8.5)
Norway	33975 (7.5)

Table 3 – Diagnoses of carcinoid tumors per country

	Inclusions	**Events**
France	67,392	11
Italy	44,532	13
Spain	39,981	8
United Kingdom	75,388	28
The Netherlands	36,517	21
Germany	48,545	12
Sweden	48,633	41
Denmark	54,973	41
Norway	33,957	18

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Table 4 – Diagnoses of carcinoid tumors per anatomical site

Location	n
Stomach	6
Duodenum	1
Jejunum	3
Ileum	22
Small Intestine (Total)*	48
Cecum and Appendix	16
Colon	5
Rectum	23
Liver and bile ducts	3
Pancreas	10
GI tract	3
Bronchus and Lungs	50
Ovaries	3
Prostate	1
Testis	1
Abdomen and Peritoneum	4
Unknown Primary Site	20

* Small intestine (total) includes duodenum, jejunum, ileum and small intestine (NOS).

3.2 Lifestyle factors and the development of neuroendocrine tumors

All cox regression analyses can be found in table 5 and 6.

3.2.1 Smoking status

Compared to non-smokers, being a smoker was significantly associated with the development of neuroendocrine tumors in multivariable analysis (HR 1.46, 95% CI 1.02 – 2.11). Additional adjustment for country of origin attenuated the associations and these were no longer statistically significant (HR 1.35, 95% CI 0.93 – 1.95). Former smoking was not associated with NETs (adjusted HR 1.14, 95% CI 0.80 – 1.64) (Table 5).

Within the subgroup of gastroenteropancreatic (GEP) NETs, smoking was found as a risk factor (adjusted HR 1.58, 95% CI 1.04 – 2.41 and HR 1.48, 95% CI 0.97 – 2.27 after additional adjustment for country). In lung NETs we found a positive non-significant association between smoking and NET development (adjusted HR 1.29, 95% CI 0.62 – 2.65 and 1.06, 95% CI 0.51 - 2.20) (Table 6)**.** Stratification for sex showed that smoking was not associated with NET development in women in multivariate analysis (HR 1.00, 95% CI 0.47 – 1.16) as compared to non-smokers (Table 7).

3.2.2 Alcohol consumption

Alcohol consumption, as a continuous variable, was not associated with an increased risk for NET development in multivariable analysis (HR 1.00, 95% CI 0.99 – 1.01). Similarly, when analyzed in categories, neither low or high alcohol consumption was significantly associated with the risk of NET development (high vs non/former drinkers: adjusted HR 1.40, 95% CI 0.80 – 2.43 and HR 1.40, 95% CI 0.80 – 2.43 after additional adjustment for country) (Table 5). When stratified by location of the tumor, high alcohol consumption was not associated with lung NET (lung NETs: adjusted HR 0.82, 95% CI 0.33 – 2.06 and HR 0.69, 95% CI 0.33 – 2.06 after adjustment for country). With regard to

GEP NETs, we found a non-significant increased risk of tumor development, (adjusted HR 1.83, 95% CI 0.96 – 3.50 and HR 1.1.91, 95% CI 0.97 – 3.74 after additional adjustment for country) (Table 6). No association between alcohol consumption and NET development was found within the female population (Table 7).

3.2.3 Mediterranean diet

As continuous variable, the Mediterranean diet score was associated with a decreased risk of NET development (HR 0.92, 95% CI 0.87-0.97 and HR 0.95, 95% CI 0.89 – 1.02 after adjustment for country) (Table 5). In subgroup analysis for both lung and GEP NETs, similar results were found (Table 6).

When categorized in low, medium and high adherence to the MED, the association with a decreased risk of neuroendocrine tumors was strongest with high adherence to the Mediterranean diet. Medium adherence to the Mediterranean diet score was significantly associated with a decreased risk of NET development in multivariable analysis (HR 0.71, 95% CI 0.51 – 0.98 and HR 0.84, 95% CI 0.60 – 1.18 after adjustment for country). High adherence to the Mediterranean diet score decreased the risk of NET development in multivariable analysis (HR 0.39 (95% CI 0.25 – 0.62). Additional adjustment for country of origin attenuated the association (HR 0.60, 95% CI 0.35 – 1.04) (Table 5).

Medium and high adherence to the Mediterranean diet was associated separately with lung NETs with adjusted HRs of respectively 0.47 (95% CI 0.25 – 0.90) and 0.36 (95% CI 0.15 – 0.86) in multivariable analysis. GEP NETs were significantly associated with high adherence to the Mediterranean diet only (adjusted HR 0.40, 95% CI 0.23 – 0.69). After adjustment for country, all associations attenuated and were non-significant (Table 6).

In the analysis within the female population, medium adherence to the Mediterranean diet (HR 1.12, 95% CI 0.58 – 2.18) was not associated with NET development. High Mediterranean diet score (HR 0.63, 95% CI 0.25 – 1.69) was associated with a decreased risk of NET development, though not statistically significant (Table 7).

3.2.4 Body mass index

Body mass index was not associated with NET development as a continuous variable (HR 1.02, 95% CI 0.99 – 1.06) with all NETs as well as with lung and GEP NETs separately. When categorized, being overweight (BMI 25-30 kg/m^2) was not significantly associated with an increased risk for NETs as compared to a BMI <25 kg/m^2 (adjusted HR 1.18, 95% CI 0.82 – 1.68). Having a BMI above 30 kg/m^2 (obese) was statistically non-significantly associated with the development of NET (adjusted HR 1.54, 95% CI 0.99 – 2.41), which was significant after additional adjustment for country (HR 1.68, 95% CI 1.07 – 2.64) (Table 5). Similar associations were observed with lung and GEP NETs (Table 6).

Obesity was not associated with NET development in the female population (Table 7).

3.2.5 Physical activity

Being moderately inactive, moderately active, or active, based on the Cambridge Physical Activity Index, was not associated with NET development risk compared to inactivity in all NETs, as well as in lung and GEP NETs separately. An overview of the (un)adjusted HRs can be found in table 5, 6 and 7.

Table 5 – Unstratified hazard ratios for smoking status, alcohol consumption, Mediterranean diet, body mass index and Cambridge physical activity index in relation to the development of NETs. Univariable hazard ratios as well as multivariable hazard ratios are provided.

Variable	Levels	N	Cases (n)	Univariable HR	Corrected for Patient Characteristics *	Multivariable HR **	Multivariable HR + Country ***
Smoking status	*Never*	219294	79	REF	REF	REF	REF
	Former	122680	54	1.18 (0.83 - 1.67)	1.21 (0.84 - 1.73)	1.16 (0.81 - 1.67)	1.06 (0.73 - 1.52)
	Smoker	99714	55	**1.67 (1.18 - 2.36)**	**1.61 (1.13 - 2.31)**	**1.49 (1.03 - 2.14)**	1.35 (0.93 - 1.95)
Alcohol consumption (continuous)	g/day	433238	187	1.00 (0.99 - 1.01)	1.00 (0.99 - 1.01)	1.00 (0.99 - 1.01)	1.00 (0.99 - 1.01)
Alcohol consumption (categorical)	*Non/Former drinker*	38748	20	REF	REF	REF	REF
	Low consumer (0-6 per month)	85795	66	1.55 (0.94 - 2.56)	1.54 (0.93 - 2.56)	1.47 (0.88 - 2.46)	1.34 (0.80 - 2.25)
	Medium consumer (6-12 per month)	105650	42	1.11 (0.65 - 1.89)	1.10 (0.64 - 1.89)	1.20 (0.69 - 2.08)	1.09 (0.62 - 1.91)
	High consumer (12+ per month)	107069	65	1.39 (0.84 - 2.29)	1.29 (0.77 - 2.19)	1.40 (0.82 - 2.83)	1.40 (0.80 - 2.43)
Mediterranean diet (continuous)		450111	193	**0.89 (0.85 - 0.94)**	**0.90 (0.85 - 0.94)**	**0.90 (0.86 - 0.95)**	0.95 (0.89 - 1.02)
Mediterranean diet (categorical)	*Low*	114223	76	REF	REF	REF	REF
	Medium	211939	89	**0.68 (0.48 - 0.89)**	**0.69 (0.50 - 0.94)**	**0.71 (0.51 - 0.98)**	0.84 (0.60 - 1.18)
	High	123949	28	**0.37 (0.24 - 0.57)**	**0.37 (0.24 - 0.58)**	**0.39 (0.25 - 0.62)**	0.60 (0.35 - 1.04)
Body mass index (continuous)		350955	161	1.03 (1.00 - 1.06)	1.02 (0.98 - 1.06)	1.02 (0.99 - 1.06)	1.02 (0.98 - 1.06)
Body mass index (categorical)	*<25*	178958	63	REF	REF	REF	REF
	25-30	135840	70	1.24 (0.88 - 1.75)	1.14 (0.81 - 1.63)	1.18 (0.83 - 1.68)	1.23 (0.86 - 1.75)
	>30	50751	33	**1.56 (1.02 - 2.38)**	1.47 (0.95 - 2.29)	1.54 (0.99 - 2.41)	**1.68 (1.07 - 2.64)**
Cambridge physical activity index	*Inactive*	88032	45	REF	REF	REF	REF
	Moderately inactive	149941	55	0.83 (0,54 - 1.19)	0.73 (0.48 - 1.09)	0.80 (0.53 - 1.20)	0.75 (0.50 - 1.13)
	Moderately active	120199	56	1.14 (0.76 - 1.69)	0.90 (0.58 - 1.38)	1.09 (0.72 - 1.64)	0.96 (0.62 - 1.47)
	Active	83115	32	0.90 (0,57 - 1,42)	0.64 (0.39 - 1.04)	0.80 (0.49 - 1.28)	0.68 (0.42 - 1.12

HR: hazard ratio. REF: Reference.

*** Patient characteristics: sex, age and level of education.**

**** Multivariable: adjusted for sex, age, level of education, smoking status, alcohol consumption, Mediterranean diet score, BMI and/or Cambridge physical activity index.**

***** Additionally adjusted for country of inclusion.**

Table 6 – Unstratified hazard ratios for smoking status, alcohol consumption, Mediterranean diet, body mass index and Cambridge physical activity index in relation to the development of lung and GEP NETs separately.

Variable	Levels	Cases (Lung)	Multivariable HR **	Multivariable HR + Country ***		Cases (GEP)	Multivariable HR **	Multivariable HR + Country ***
Smoking status	*Never*	23	REF	REF		56	REF	REF
	Former	13	1.09 (0.53 - 2.21)	0.98 (0.48 - 2.02)		41	1.21 (0.80 - 1.83)	1.10 (0.72 - 1.66)
	Smoker	13	1.29 (0.62 - 2.65)	1.06 (0.51 - 2.20)		42	**1.58 (1.04 - 2.41)**	1.48 (0.97 - 2.27)
Alcohol consumption (continuous)	g/day	50	0.99 (0.96 - 1.01)	0.98 (0.96 - 1.01)		143	1.00 (0.99 - 1.01)	1.01 (1.00 - 1.02)
Alcohol consumption (categorical)	*Non/Former drinker*	8	REF	REF		12	REF	REF
	Low consumer (0-6 per month)	20	1.09 (0.47 - 2.56)	1.04 (0.43 - 2.47)		46	1.75 (0.92 - 3.33)	1.56 (0.82 - 2.98)
	Medium consumer (6-12 per month)	9	0.69 (0.25 - 1.87)	0.61 (0.21 - 1.73)		33	1.54 (0.78 - 3.03)	1.39 (0.70 - 2.77)
	High consumer (12+ per month)	13	0.82 (0.33 - 2.06)	0.69 (0.26 - 1.86)		52	1.83 (0.96 - 3.50)	1.91 (0.97 - 3.74)
Mediterranean diet (continuous)		50	**0.87 (0.79 - 0.97)**	0.92 (0.81 - 1.04)		143	**0.91 (0.86 - 0.97)**	0.96 (0.90 - 1.04)
Mediterranean diet (categorical)	*Low*	23	REF	REF		53	REF	REF
	Medium	20	**0.47 (0.25 - 0.90)**	0.58 (0.29 - 1.15)		69	0.80 (0.55 - 1.16)	0.93 (0.63 - 1.38)
	High	7	**0.36 (0.15 - 0.86)**	0.53 (0.18 - 1.54)		21	**0.40 (0.23 - 0.69)**	0.63 (0.34 - 1.17)
Body mass index (continuous)		50	1.03 (0.96 - 1.10)	1.02 (0.95 - 1.10)		143	1.02 (0.98 - 1.07)	1.03 (0.99 - 1.08)
Body mass index (categorical)	*<25*	15	REF	REF		48	REF	REF
	25-30	21	1.37 (0.69 - 1.96)	1.34 (0.67 - 2.68)		49	1.15 (0.77 - 1.72)	1.21 (0.81 - 1.83)
	>30	10	1.69 (0.72 - 3.96)	1.65 (0.70 - 3.91)		23	1.52 (0.91 - 2.55)	**1.71 (1.01 - 2.89)**
Cambridge physical activity index	*Inactive*	12	REF	REF		33	REF	REF
	Moderately inactive	12	0.84 (0.36 - 1.96)	0.78 (0.33 - 1.85)		43	0.78 (0.49 - 1.25)	0.75 (0.47 - 1.20)
	Moderately active	14	1.21 (0.51 - 2.83)	1.08 (0.45 - 2.60)		42	1.05 (0.66 - 1.69)	0.93 (0.57 - 1.52)
	Active	10	1.21 (0.49 - 3.00)	0.90 (0.35 - 2.29)		22	0.69 (0.39 - 1.21)	0.63 (0.35 - 1.12)

HR: hazard ratio. REF: Reference. GEP: Gastroenteropancreatic.

*** Patient characteristics: sex, age and level of education.**

**** Multivariable: adjusted for sex, age, level of education, smoking status, alcohol consumption, Mediterranean diet score, BMI and/or Cambridge physical activity index.**

***** Additionally adjusted for country of inclusion.**

Table 7 – Unstratified hazard ratios for smoking status, alcohol consumption, Mediterranean diet, body mass index and Cambridge physical activity index in relation to the development of NETs within the female population. Univariable hazard ratios as well as multivariable hazard ratios are provided.

Variable	Levels	N	Cases (n)	Univariable HR	Corrected for Patient Characteristics *	Multivariable HR **
Smoking status	*Never*	175024	60	REF	REF	REF
	Former	74372	28	1.05 (0.50 - 2.20)	1.17 (0.55 - 2.48)	1.20 (0.57 - 2.57)
	Smoker	62133	29	0.91 (0.43 - 1.91)	0.99 (0.47 - 2.11)	1.00 (0.47 - 2.16)
Alcohol consumption (continuous)	g/day	433238	122	0.99 (0.97 - 1.01)	0.99 (0.97 - 1.01)	0.99 (0.97 - 1.01)
Alcohol consumption (categorical)	*Non/Former drinker*	38748	18	REF	REF	REF
	Low consumer (0-6 per month)	85795	51	0.77 (0.16 - 3.71)	0.78 (0.16 - 3.75)	0.72 (0.15 - 3.51)
	Medium consumer (6-12 per month)	105650	32	0.51 (0.10 - 2.60)	0.53 (0.10 - 2.70)	0.56 (0.11 - 2.90)
	High consumer (12+ per month)	107069	21	0.30 (0.06 - 1.40)	0.34 (0.07 - 1.62)	0.33 (0.07 - 1.61)
Mediterranean diet (continuous)		450111	122	**0.90 (0.81 - 0.99)**	**0.90 (0.82 - 1.00)**	0.93 (0.84 - 1.03)
Mediterranean diet (categorical)	*Low*	114223	43	REF	REF	REF
	Medium	211939	63	1.00 (0.52 - 1.91)	0.99 (0.51 - 1.90)	1.12 (0.58 - 2.18)
	High	123949	16	0.46 (0.19 - 1.11)	0.48 (0.19 - 1.19)	0.63 (0.25 - 1.59)
Body mass index (continuous)		350955	161	0.98 (0.91 - 1.06)	0.97 (0.92 - 1.05)	0.97 (0.89 - 1.05)
Body mass index	*<25*	178958	43	REF	REF	REF
	25-30	135840	32	0.83 (0.41 - 1.69)	0.80 (0.39 - 1.66)	0.79 (0.38 - 1.64)
	>30	50751	20	0.99 (0.41 - 2.39)	0.88 (0.36 - 2.20)	0.86 (0.34 - 2.14)
Cambridge physical activity index	*Inactive*	88032	29	REF	REF	REF
	Moderately inactive	149941	36	1.06 (0.46 - 2.43)	1.20 (0.52 - 2.80)	1.14 (0.49 - 2.67)
	Moderately active	120199	34	0.85 (0.37 - 1.94)	0.95 (0.41 - 2.20)	0.85 (0.36 - 1.98)
	Active	83115	19	1.33 (0.52 - 3.41)	1.64 (0.62 - 4.33)	1.50 (0.56 - 4.00)

HR: hazard ratio. REF: Reference.

*** Patient characteristics: sex, age and level of education.**

**** Multivariable: adjusted for sex, age, level of education, smoking status, alcohol consumption, Mediterranean diet score, BMI and/or Cambridge physical activity index.**

***** Additionally adjusted for country of inclusion.**

4. Discussion

The EPIC study is one of the largest cohort studies investigating the role of lifestyle and nutrition in the development of cancer. As the implication of lifestyle in cancers has gained more scientific appreciation, knowledge from this study has become increasingly important. However, information on NETs and lifestyle is sparse. Therefore, the aim of this study was to investigate the association between lifestyle factors and NETs within the EPIC cohort. The present study is the largest cohort study investigating the interaction between lifestyle factors and NET development. We have assessed the association between smoking, alcohol consumption, Mediterranean diet score, body mass index, physical activity and NETs in general, lung NETs and GEP NETs specifically.

Smoking was identified as a risk factor for NET development in both univariable and multivariable analyses, increasing the odds of NET development within the entire group of NETs. Previous research has associated smoking with small intestinal (SI) NET in particular, while the relationship with lung NET (often referred to as typical or atypical carcinoids) is not clear.(18, 19) In our study, we were not able to show an association between lung NETs and smoking (HR 1.29, 95% CI 0.62 – 2.65), most likely due to a low statistical power. This finding, however, is in line with present literature. Similar to current literature, smoking seemed to be associated with GEP NET development in our study.

Alcohol was not identified as a risk factor within the EPIC cohort as continuous variable or categorized according to consumption per month in our study. We did not find an association with NETs in general, nor with lung or GEP NETs. Contrasting with our findings, previous literature has reported on alcohol use and abuse as a risk factor for pancreatic or rectal NETs. (20, 21)

Most interesting, Mediterranean diet was associated with a decreased risk of NET development in our study. Diet has been a relatively unexplored variable within NET research. Our study points towards high adherence to the Mediterranean diet as an important protective factor, indicating diet can be an important strategy in cancer prevention. The Mediterranean diet is characterized by high consumption of fruits, vegetables, legumes, fish, non-refined cereals and olive oil, as well as low consumption of meat, dairy products and alcohol, and has been studied extensively in the PREDIMED studies (22). From the PREDIMED studies, it was concluded that the Mediterranean diet was associated with a decreased risk of development of all sorts of metabolic diseases, including diabetes and the metabolic syndrome.(23)

The contents of the Mediterranean diet are high in fiber, polyunsaturated fats and phytosterols.(24) Additionally, the contents of the Mediterranean diet are believed to have high anti-oxidant effects. Hence, the overall profile of the Mediterranean diet has been described as anti-inflammatory, anti-oxidative and lipid-modulating.(24) In regards to cancer biology, the Mediterranean diet has been stated to alter hormonal and growth factor pathways, contributing to a protective effect.(24) The present study has shown that the Mediterranean diet might have similar protective effects in neuroendocrine tumors.

Our results within the female population suggest that Mediterranean diet may be associated with a decreased risk of NET development, especially in the population with high adherence to this diet. However, due to the small statistical power, we report wide confidence intervals and no strong conclusions can be drawn from these results.

The role of body mass index in NET development has been a topic of discussion, with a previously published cohort study suggesting a NET-promoting role for obesity. In our study, we found that being overweight (a BMI of 25-30) was not associated with NET development, while obesity was identified as a risk factor. Our results suggest that obesity may indeed play a promoting role in NETs as well, with a seeming preference for GEP NET development. For several types of cancers, both overweight and obesity have been identified as risk factors, even showing a trend with increasing BMI.(25, 26) Potential mechanisms of disease linking obesity and cancer in general that have been suggested here are low grade chronic inflammation, altered hormonal status and perturbations of the microenvironment, all leading to a tumor promoting environment.(27) It can be assumed that these factors may play a role in (GEP) NETs as well.

We were unable to show an association between physical activity and NET development. We did observe that in the moderately active population the hazard for developing NET was seemingly increased. This can be due to the small sample size, but another possibility could be that participants tend to over-report their activity. This could bias our results.

Despite being the largest prospective cohort studied assessing the association between lifestyle and NET development so far, it is still a small case group. This contributes to a statistical power too small for our stratified analyses. Hence, we were unable to stratify for sex or country. Instead we performed an additional adjustment for country. From this analysis, we observed an attenuation of our results, as well as a shift in confidence intervals. A potential explanation could be that this adjustment corrects for residual confounding, altering the effect of lifestyle. As different EPIC centers have included different populations (e.g. Utrecht and Florence included only women, while Oxford included a fully vegetarian cohort), this may very well be the case.(13) However, due to differences observed in frequencies of NET diagnoses in the included countries, we hypothesize that the problem lies within the diagnostics of NETs. Potentially, NET diagnoses are more commonly made in countries with expertise centers or countries with a population screening program, including colonoscopies. The latter thought is supported by the high number of rectal NET, which are most commonly diagnosed with colonoscopies. A majority of rectal NET was diagnosed in Germany (n=10), a country that provides routine colonoscopy as part of the colorectal cancer screening program, further supporting this hypothesis.(28, 29)

It would be of great interest to investigate to what extend lifestyle factors are related to tumor grade and disease stage. Similarly, knowledge on the effect of lifestyle on treatment effect and natural course could contribute considerably to the efficacy of treatment modules. With regards to dietary

exposure, the information within the EPIC cohort could provide knowledge on the association with certain nutritional compounds through in depth analysis.

The present study shows that smoking is strongly associated with NET development within the entire NET population and GEP NETs in the EPIC cohort. Similarly, BMI increases the risk of NET development independent of country. Lastly, we observed that increased adherence to the Mediterranean diet has a protective association with NETs. So far, this has been the largest prospective cohort study looking at lifestyle and NET development. The observations from our study provide support for further research into lifestyle and NETs with regard to natural course, disease stage and treatment response, eventually contributing to preventive measures.

5. List of abbreviations

Below, a list of abbreviations used in the article can be found.

BMI	Body mass index
CI	Confidence interval
EPIC	European Prospective Investigation into Cancer and Nutrition
e.g.	Exempli gratia
EI/ER	Energy intake/Energy requirement
GEP-NET	Gastroenteropancreatic NET
GI	Gastrointestinal
HR	Hazard ratio
ICD-O	International Classification for Diseases in Oncology
IQR	Interquartile range
MEN	Multiple endocrine neoplasia
NET	Neuroendocrine tumor
PREDIMED	Prevention with Mediterranean Diet
REF	Reference
SD	Standard deviation
SI-NET	Small intestinal NET
VHL	Von Hippel Lindau
WHO	World Health Organization

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