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Changes in lifestyle and risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition

--Manuscript Draft--

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Full Title:	Changes in lifestyle and risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition
Article Type:	Article
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Abstract:	Introductio n: We investigated the impact of changes in lifestyle habits on colorectal cancer (CRC) risk in a multi-country European cohort. Methods: We used baseline and follow-up questionnaire data from the EPIC cohort to assess changes in lifestyle habits and their associations with CRC development. We calculated a healthy lifestyle index (HLI) score based on smoking status, alcohol consumption, body mass index and physical activity collected at the two timepoints. HLI ranged from 0 (most unfavourable) to 16 (most favourable). We estimated the association between HLI changes and CRC risk using Cox regression models and reported hazard ratios (HR) with 95% confidence intervals (CI). Results : Among 295,865 participants, 2,799 CRC cases were observed over a median of 7.8 years. Median time between questionnaires was 5.7 years. Each unit increase in HLI from the baseline to the follow-up assessment was associated with a statistically significant 3% lower CRC risk. Among participants in the top tertile at baseline (HLI>11), those in the bottom tertile at follow-up (HLI≤9) had a higher CRC risk (HR 1.34; 95%CI 1.02-1.75) than those remaining in the top tertile. Among individuals in the bottom tertile at baseline, those in the top tertile at follow-up had a lower risk (HR 0.77; 95%CI 0.59-1.00) than those remaining in the bottom tertile. Discussion: Improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. These results justify and support recommendations for healthy lifestyle changes and healthy lifestyle maintenance for CRC prevention.
Response to Reviewers:	Reviewer #1: 1.This paper presents a valuable analysis of the association between key lifestyle factors and colorectal cancer(CRC) using data from the EPIC cohort. The paper is generally well written with nice figures. We thank the Reviewer for these positive remarks 2.It would be useful to highlight more that the work is on change…. and more might be made of risk in people who maintain healthy habits (if numbers allowed analysis) who would have scored 0 in terms of change. Reply: In line with the Reviewer's view, the title, the abstract, the introduction and the discussion emphasize that the focus of this study is on lifestyle change. Also, to avoid confusion on the exposure of interest, we did not present any result on the association between lifestyle per se, collected at one point in time, and the risk of colorectal cancer (CRC). As for the second part of the comment, according to our analysis based on tertiles of HLI, people who maintained healthy habits were those with an HLI score of 12 or more at baseline and an HLI score of 12 or more at follow-up (figure 3c, dotted line). We already reported that individuals who reduced their HLI score from 12 or more at baseline to 9 or less at follow-up had a significantly higher risk of CRC compared to those who maintained healthy habits (HR 1.34 (1.02−1.75); figure 3c). Following the reviewer's suggestion, we can now estimate that those who maintained healthy habits had a lower risk of CRC compared to all the other individuals grouped together (HR 0.82 (0.73-0.92)), further supporting our conclusions that having a healthy lifestyle, and maintaining it, is important for CRC prevention. However, all the other individuals grouped together represent a very heterogeneous group, with different baseline HLI scores and different change patterns. So, we would like not to report this estimate in the manuscript, as it does not really add evidence on the impact of lifestyle changes on CRC risk, which is the main interest of the paper.

Changes to the manuscript: none

3.Excellent international cohort with a wide variety of lifestyle patterns. Good follow up data for the lifestyles presented Excellent life stage to study to support evidence for lifestyle change as they move into retirement.
We thank again the Reviewer for these positive remarks

4.Similar work has been reported from the Nurses study the novelty of the current work needs to be stressed
Reply: While previous studies reported on the association between changes in one lifestyle behaviour (e.g. smoking or BMI) and CRC risk, our study examined for the first time the association between multifactorial lifestyle changes and the risk of CRC. As emphasized in the discussion, this represents the main novelty of the study. Following the Reviewer's suggestion, we added an important novelty, which is that we showed that improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. Other studies reported significant associations in one direction only, e.g. that smoking cessation or increasing physical activity were inversely related to CRC risk.
In looking for additional published evidence, a study that we had previously missed is now briefly described in the discussion in the revised version of our manuscript.
Changes to the manuscript: the text in the discussion was amended and now reads:
"An important novel result of our study is that lifestyle changes can affect CRC risk in both directions: improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. This is a clear message that practicing clinicians and gastroenterologists could give to their patients and to CRC screening participants to improve CRC prevention."

We also added a reference and a comment:
"Similar results indicating that body weight gains in early adulthood, but not late adulthood, was positively associated with CRC risk were found in the Nurses' Health Study and Health Professionals Follow-up (19)"
New ref #19 Song M et al. Adulthood Weight Change and Risk of Colorectal Cancer in the Nurses' Health Study and Health Professionals Follow-up Study. Cancer Prev Res (Phila). 2015 Jul;8(7):620-7.

5.The lack of data on dietary change is a weakness given the evidence relating this to CRC and some comments should be provided on the potential of dietary changes to influence CRC risk Possible amendments and discussion points
Reply: We agree with the Reviewer and added a comment in the discussion.
Changes to the manuscript (in Italic): "Our study has some limitations. We acknowledge that the lack of data on diet collected during follow-up may have led to inadequately adjusted risk estimates and residual confounding. For example, if improvements in diet were associated with both improvements in the HLI score and a decreased CRC risk, then we might have overestimated the association between HLI score and CRC risk. The collection and harmonization of dietary data at follow-up is currently ongoing in EPIC".

6.All lifestyle variables are weighted equally - it is possible that smoking might be weighted higher- some discussion of the decision for equal weightings should be provided
Reply: We agree with the Reviewer, and we added a comment in the discussion to clarify our choice.
Changes to the manuscript: "For sake of consistency, a scoring system that was used previously in EPIC publications was used in this study. While specific components, for example smoking or obesity, might weight more in the computation of the HLI, this approach has the advantage of ensuring comparability across studies and according to different cancer and other disease outcomes".

7.Some comments are made re importance of alcohol in younger adults but the youngest here is 55.9 years
Reply: As stated in the materials "[…] 521,323 participants mostly aged from 35 to 70 years were recruited". In order to clarify this point, we added the mean age of the young group in the results section.

Changes to the manuscript (changes in Italic): “Increases in the alcohol score (i.e., decreases in alcohol consumption) were significantly associated with a lower risk of CRC in participants aged 55 or younger (mean age 46 years) at baseline”.

8.The mean BMI is lower that that reported by many national studies which reminds us that some indication of representation should be reported.
Reply: The Reviewer is correct that study participants recruited in some EPIC centres were not representatitive of the general population due to healthy cohort effects. This calls for cautious interpretation of our findings. Nonetheless, while the distribution of healthy behaviors might lack validity on an absolute scale, with an over representation of non-smokers and/or an under-representation of overweight/obese participants, in this study we examined the relationship between adherence to a healthy lifestyle based on a relative scale, i.e. by focusing on lifestyle changes. As a result, our findings on a benefit of adopting healthy choices during adulthood with respect to CRC risk might have a larger impact in the general population, characterized by less healthy profiles.
Changes to the manuscript: we added in the limitations paragraph that: “EPIC participants might not be representative of the general population due to healthy cohort effects, and this warrants cautious interpretation of our findings. However, we can speculate that our findings on the benefit of adopting healthy choices during adulthood might have a larger impact on CRC risk in the general population, characterized by less healthy profiles”.

9.It would be useful to have an analysis by socio-economic position - is it always the wealthiest in society who can make lifestyle changes?
Reply: In line with the Reviewer’s suggestion, participants’ education, as a proxy for socio-economic position, was categorized in three levels: 1) none or primary school; 2) technical, professional or secondary; and 3) university or higher. In men, we found that the HLI score change was increasingly larger among participants with higher education, with mean HLI change equal to 0.11 for the lowest education group to 0.20 for the highest. In women, negative HLI changes were observed in all education groups, with smaller HLI score decrease for women with higher education: mean HLI change ranged from -0.29 for the lowest education to -0.18 for the highest, with a significant trend. We added these results in the results section.

Changes to the manuscript: “We observed larger mean HLI score changes in men with higher education (HLI change=0.20) compared to men with lower education (0.11); we observed smaller HLI decreases in women with higher education (-0.18), compared to women with lower education (-0.29)”.

10.More should be made of the age at which these changes are happening (i.e., people in their mid 50's to mid 60's) and how this might be used in health promotion/worksite programmes/pre-retirement programmes etc
Reply: According to the Reviewer’s suggestion to examine whether associations were dependent on the age of change, we carried out extra statistical analyses to evaluate HLI change and CRC risk associations by groups defined by the age at follow-up. One one-unit increase in HLI was associated with a 3% lower risk of CRC among participants with age at follow-up below or above 65 years, i.e. approximately the retirement age, with HR equal to 0.97; 95% CI 0.94-1.00 and equal to 0.97; 95% CI 0.94,1.01, p for heterogeneity 0.884, respectively. These results do not seem to add evidence to the implementation of health promotion programmes at specific ages.

Changes to the manuscript: None

Reviewer #2:
This paper is a well-written original article assessing the prognostic impact of lifestyle-change in patients with colorectal cancer. Preference history, body composition, and physical activity have been reported to be associated with carcinogenesis and cancer progression in various types of cancer. This study is clinically very meaningful in that it is an international, multi-center, large prospective study. In addition, the selection of analysis targets and analysis methods are appropriate.

However, there are some points to be additionally commented or revised.

We thank the Reviewer for these positive remarks and feedback.

Comments;
1.This is a study where when to follow up can have a significant impact on the outcome. A detailed explanation of the criteria for when to follow up is needed.
Reply: The Reviewer raises an important point. There is growing interest in cancer epidemiology towards assessing lifestyle behaviour of study participants repeatedly over time. In this study, however, associations between lifestyle changes and colorectal risk were similar with respect to the age at diagnosis and the age of change, based on the available information at baseline and at follow-up. Therefore, there was no clear evidence on the opportunity of re-assessing exposure at specific ages, before, during or after retirement age, for example, at least in relation to CRC. Also, the collection (and centralization) of lifestyle measurements in EPIC was mainly finalised based on local resources available in each recruitment centre, rather than targeting specific age groups.
Changes to the manuscript: none

2.According to the multi-step carcinogenesis theory of colorectal cancer carcinogenesis, endoscopic resection at the time of benign polyps can reduce carcinogenesis. In this regard, the habit of visiting a medical institution on a daily basis may contribute to the early detection of benign polyps. For example, patients who are being followed up for other comorbidities may have a better chance of benign polyp detection during routine examinations. If data on the presence of comorbidities are available, this should also be analysed.
Reply:, We agree with the Reviewer's remark that individuals who go more often to the doctor for comorbidities might also be more health conscious and attend CRC screening more often than people without comorbidities. On the other hand, certain comorbidities are associated with lower participation in CRC screening. It is hard to anticipate whether the presence of certain comorbidities, whose information is challenging to collect in observational epidemiological studies, might affect CRC prevention.
Changes to the manuscript: none

3.Author mentioned that reducing alcohol from early adulthood to middle age was associated with a reduced risk of CRC, but not in older adulthood. However, significant bias can exist in these data. Firstly, older people who often developed CRC might refrain from alcohol. Secondly, people who were 55 years old or younger at base line might develop CRC without refraining from alcohol after completing the follow-up questionnaire. In order to reveal the difference between younger people and older, author should show the table of CRC patients compered by generations for alcohol score and follow up times.
Reply: The Reviewer refers to the possibility that our analysis (Table 2, model 3) might be affected by reverse causation, which might have biased association estimates differently in younger and older participants. To test this, we re-ran model 3 after excluding the first two years of follow-up. We obtained results that were similar to the main analysis, with HR=0.92 (0.85,1.00) and 0.98 (0.91,1.05) among younger (≤55 years) and older (>55 years) participants, respectively. These results seem to suggest that preventive measures for reducing alcohol consumption should target younger people more forcefully is still valid.
Changes to the manuscript: none

4.Figure 2 shows that HLI values vary widely from country to country. What factors are associated with these differences?
Reply: As we discussed in the manuscript, countries with the highest HLI score at baseline (e.g., Norway and United Kingdom) showed a decrease in HLI score at follow-up, while countries with the lowest HLI score at baseline (e.g., Denmark and Sweden) displayed an increase in HLI score at follow-up. Also, countries with the highest mean age at baseline (e.g., Denmark, Sweden and France) had the most favourable HLI score changes. We tested if country-specific levels of education (the only proxy available for socioeconomic status) could explain the differences in the country-specific HLI changes observed, but it was not the case. We edited the discussion as follows.

	Changes to the manuscript: “Unlike baseline age and HLI score, educational levels did not explain the differences in the country-specific HLI changes” Reviewer #3: Botteri et al present a multinational study of the EPIC cohort looking at lifestyle change and CRC risk. I think the study is rigorously done, and the findings and message is pretty simple- worsening of healthy lifestyle factors (tobacco, alcohol, BMI, physical activity) is associated with increased risk while improvement is associated with decreased risk. While there are certainly other papers on this and related topics (including from US data from Nurse's health study) and some limitations of observational design, I think this would be of interest to practicing clinicians, and useful for giving advice to patients after colonoscopy. We thank the Reviewer for these positive remarks and the following comments Specific comments: 1) I would like to see the authors include absolute risk differences and incidences--results and tables are mainly hazard ratios. Otherwise I agree that although it's not the most novel, it is rigorously done and clinically relevant. Reply: We thank the reviewer for his/her suggestion. CRC incidences were estimated and reported in the results section for 4 indicative groups of study participants: those who maintained a low HLI score (baseline ≤ 9, follow-up ≤ 9) those who increased it (baseline ≤ 9, follow-up ≥ 12), those who maintained a good HLI score (baseline ≥ 12, follow-up ≥ 12) and those who reduced it (baseline ≥ 12, follow-up ≤ 9). Changes to the manuscript: The method section was updated with the following description: “Crude CRC incidence rates were calculated as the number of CRCs divided by the sum of person-years”. The result section now reads (changes in italic): “Among participants with a baseline score of HLI≤9 (bottom tertile), those with follow-up score of HLI≥12 (top tertile) had a lower risk of CRC (Figure 3a; HR 0.77; 95% CI 0.59-1.00) than those with a follow-up score of HLI≤9. The crude CRC incidence rates in the two groups were 134 and 162 per 100,000 person-years, respectively. Among participants with a baseline score of HLI≥12, those with a follow-up score of HLI≤9 had a higher risk of CRC (Figure 3c; HR 1.34; 95% CI 1.02-1.75) compared to those with follow-up score of HLI≥12. The crude CRC incidence rates in the two groups were 119 and 86 per 100,000 person-years, respectively”. 2) There are numerous authors listed; While this is not necessarily unreasonable for a large multicenter study such as EPIC, the corresponding author should confirm that all met this journal's criteria for authorship. We confirm that all Authors contributed to the authorship criteria of the American Journal of Gastroenterology. 3) Perhaps the authors could enhance the clinical implications and next steps in the discussion. Reply We added the following sentence in the discussion section. Changes to the manuscript: “An important novel result of our study is that lifestyle changes can affect CRC risk in both directions: improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. We believe this represents a clear and simple message that practicing clinicians and gastroenterologists could give to their patients or to CRC screening participants to improve CRC prevention.”
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Opposed Reviewers:	

Dear Editor,

Please find enclosed an original research manuscript entitled “Changes in lifestyle and risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition”.

In planning cancer preventive strategies, researchers and policy makers should be aware not only of the impact of lifestyle behaviours on cancer risk, but also how changing lifestyle in the recommended direction will affect cancer risk. Nonetheless, how lifestyle changes affect the risk of cancer, and specifically colorectal cancer, remains largely unexplored.

We conducted the first study, to our knowledge, to investigate the association between multifactorial lifestyle changes and the risk of colorectal cancer. We measured changes in smoking habits, alcohol consumption, body mass index and physical activity in approximately 300,000 participants from the large-scale EPIC cohort, using a baseline and follow-up questionnaire data. We found that improving adherence to a healthy lifestyle was inversely associated with colorectal cancer risk, while worsening adherence raised cancer risk.

These results support recommendations for healthy lifestyle changes and healthy lifestyle maintenance for colorectal cancer prevention. An important message is that, even in people with poor lifestyle habits in middle age, it is not too late to adopt a healthier lifestyle, as this could bring substantial health benefits for colorectal cancer prevention.

We believe that the manuscript will be of broad interest to your readership and will have a significant impact on the scientific community and the public.

This study is not under consideration elsewhere and all authors have appropriately contributed to the work. The authors declare that they have no competing interests. The highest standards of ethical guidelines were followed in conducting this study. We hope that you will find the manuscript suitable for publication in American Journal of Gastroenterology.

Yours sincerely,

Edoardo Botteri and Pietro Ferrari

Changes in lifestyle and risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition

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Abstract

Introduction: We investigated the impact of changes in lifestyle habits on colorectal cancer (CRC) risk in a multi-country European cohort.

Methods: We used baseline and follow-up questionnaire data from the EPIC cohort to assess changes in lifestyle habits and their associations with CRC development. We calculated a healthy lifestyle index (HLI) score based on smoking status, alcohol consumption, body mass index and physical activity collected at the two timepoints. HLI ranged from 0 (most unfavourable) to 16 (most favourable). We estimated the association between HLI changes and CRC risk using Cox regression models and reported hazard ratios (HR) with 95% confidence intervals (CI).

Results: Among 295,865 participants, 2,799 CRC cases were observed over a median of 7.8 years. Median time between questionnaires was 5.7 years. Each unit increase in HLI from the baseline to the follow-up assessment was associated with a statistically significant 3% lower CRC risk. Among participants in the top tertile at baseline (HLI>11), those in the bottom tertile at follow-up (HLI≤9) had a higher CRC risk (HR 1.34; 95%CI 1.02-1.75) than those remaining in the top tertile. Among individuals in the bottom tertile at baseline, those in the top tertile at follow-up had a lower risk (HR 0.77; 95%CI 0.59-1.00) than those remaining in the bottom tertile.

Discussion: Improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. These results justify and support recommendations for healthy lifestyle changes and healthy lifestyle maintenance for CRC prevention.

Keywords: Colorectal cancer; Lifestyle; Prevention; Cohort study

Study highlights

What is known

- Leading a healthy lifestyle reduces the risk of colorectal cancer (CRC)
- There is limited knowledge on the impact of lifestyle changes on the risk of CRC

What is new here

- We measured changes in lifestyle among 300.000 participants in the EPIC cohort, using a baseline and a follow-up questionnaire
- Improving adherence to a healthy lifestyle was inversely associated with CRC risk
- Worsening adherence was positively associated with CRC risk
- These results justify recommendations for healthy lifestyle changes and healthy lifestyle maintenance for CRC prevention

Introduction

Worldwide, colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer death, with an estimated 1.9 million new cases and 0.9 million deaths in 2020 (1). There is clear evidence that five healthy lifestyle behaviours, namely non-smoking, avoiding being overweight, being physically active, consuming no or low amounts of alcohol and having a healthy diet, reduce the risk of CRC (2, 3). The number of healthy behaviours is inversely associated with CRC risk (4-6). In the European Prospective Investigation into Cancer (EPIC), when combining these five behaviours in a lifestyle index, the risk of CRC was 12% lower for each additional healthy behaviour (4). In the Nurses' Health Study and Health Professionals Follow-up Study, it was also shown that an increasing healthy lifestyle index score, based on the same five behaviours, was associated with a reduced CRC risk independently from participation in endoscopic screening (6).

In planning cancer preventive strategies, researchers and policy makers should be aware not only of the impact of lifestyle behaviours on cancer risk, but also how changing lifestyle in the recommended direction will affect cancer risk. Nonetheless, how lifestyle changes affect the risk of cancer, and specifically CRC, remains largely unexplored. A randomized trial showed that a successful intervention on smoking cessation and diet improvement can reduce the risk of lifestyle-related cancers in men with high risk for cancer (7). Observational studies have shown that increasing physical activity levels during adult life are associated with lower cancer mortality (8, 9), and that improving the cardiorespiratory physical fitness is associated with reduced cancer risk and mortality (10). A recent observational study in Swedish women suggested that lifestyle improvements are associated with a lower risk of lifestyle-related cancers (11). With regard to the specific risk of CRC, a 2020 meta-analysis indicated that smoking cessation significantly reduces the risk of CRC after 25 years since quitting (2), and a 2015 meta-analysis showed that large weight gains from early adulthood to midlife are

associated with increased risk of CRC (12, 13). Changes in other lifestyle factors related to CRC risk have not been examined.

With the aim of contributing to the limited knowledge base on lifestyle changes and risk of CRC, in this study we investigated the effect of changes in smoking habits, body mass index (BMI), physical activity level, alcohol consumption, and a lifestyle index which combined those four factors, on the subsequent incidence of CRC in the EPIC cohort.

Methods

From 1992 to 2000, 521,323 participants mostly aged from 35 to 70 years were recruited, mostly from the general population, across 23 centres in 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. The rationale, study design, and methods for EPIC have been described in detail elsewhere (14). All participants completed a lifestyle questionnaire at baseline and provided informed consent to participate in the study. Ethical approval was obtained from participating centres and the IARC ethics committee (reference number 20-02).

We initially excluded 28,561 participants from Greece due to administrative and data-use restrictions, 24,550 participants with prevalent cancer at baseline, 9,064 with extreme energy intakes (i.e., below the 1st and above the 99th percentiles of the energy intake over energy requirement ratio distribution) and 3,137 without follow-up after the baseline questionnaire (Figure 1). After an average of 7 years (range 2-17) from recruitment, a second lifestyle questionnaire was administered during follow-up. Since the main exposure of interest of the current analysis was lifestyle changes, we further excluded 16,816 participants with cancer before the follow-up questionnaire and 100,828 participants for whom the follow-up lifestyle assessment questionnaire data was not available in the centralized EPIC dataset in October 2020. We then excluded 3,426 participants for whom no follow-up time was available after the follow-up questionnaire date. We additionally excluded 5,900 and 11,419 participants for whom information about the four lifestyle factors of interest - smoking status, alcohol consumption, BMI and physical activity - was missing at the baseline questionnaire and at the follow-up questionnaire, respectively. We finally excluded 21,757 participants for whom information of at least one of the four factors of interest was missing both at baseline and follow-up. Hence, the final analytic dataset included 295,865 participants. Participants were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Exposure assessment

Four lifestyle factors were investigated: smoking status, alcohol consumption, BMI and physical activity. For each factor, scores ranging from 0 to 4 were assigned to increasingly healthy categories of behaviour (Supplementary Figure 1). The "healthiest" behaviours were never smoking (never smoked=4 points, smoke cessation>10 years=3, smoke cessation≤10 years=2, current smoking≤15 cigarettes/day=1, current smoking>15 cigarettes/day=0), low consumption of alcohol (<6.0 g/day=4 points, 6.0–11.9=3, 12.0–23.9=2, 24.0–59.9=1, ≥60=0), top quintile of physical activity based on recreational and household metabolic equivalent of task units (METs) (5th quintile=4 points, 4th quintile=3, 3rd quintile=2, 2nd quintile=1, 1st quintile=0) and low BMI (<22=4 points, 22-23.9=3, 24–25.9=2, 26–29.9=1, ≥30=0). A healthy lifestyle index (HLI) was obtained by summing the scores of each lifestyle factor, thus ranging from 0 to 16. Changes in the HLI from the baseline questionnaire to the follow-up questionnaire was our main exposure of interest.

Information on diet was available only at baseline and was therefore not included in the HLI for the current analysis. Intakes of 6 dietary factors – namely cereal fiber, red and processed meat, the ratio of polyunsaturated to saturated fat, margarine, glycemic load, and fruits and vegetables - were combined only at baseline in a diet score (15), which was used as an adjustment variable in all analyses.

Outcome assessment

Cases of CRC were identified through population cancer registries in Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. A combination of methods

were used, including health insurance records, contacts with cancer and pathology registries, and active follow-up of EPIC participants and their next of kin in France and Germany.

CRC cases were defined as carcinomas with topography codes C18, C19 and C20 according to the 10th Revision of the International Statistical Classification of Diseases, Injuries and Causes of Death. In addition to CRC, we also examined associations for the following subsites: proximal colon (C18.0-C18.5), distal colon (C18.6-C18.7) and rectum (C19-C20). When analysing colorectum subsites, CRC coded as C18.8 (overlapping more than one subsite) and C18.9 (unspecified subsite) were censored.

Statistical methods

Categorical variables were summarized as frequencies and percentages, and continuous variables as means, medians, standard deviations (SD) and interquartile ranges. In forest plots, HLI and HLI changes were summarized as mean values. In survival analyses, participants were followed from return of follow-up questionnaire until any first cancer, excluding non-melanoma skin cancer, death, emigration, or end of follow-up, whichever came first. Kaplan-Meier survival curves were constructed, separately by tertiles of HLI at baseline and stratified by HLI at follow-up. Multivariable Cox proportional hazards regression models, using participants' age as the underlying time scale, were used to estimate hazard ratios (HR) and the corresponding 95% confidence intervals (CI). We used two decimals for the CIs, but we reported three decimals in some cases to show full statistical significance. The models were stratified by study centre, age at recruitment rounded to one year and sex, and adjusted for the highest education level achieved (none or primary; technical, professional or secondary; university or higher; missing), diet score at baseline, and the calendar date of follow-up

questionnaire. The models with lifestyle changes as the main exposure were additionally adjusted for the continuous HLI score at baseline.

To estimate the association between lifestyle changes and risk of CRC, we used the difference between the HLI score at follow-up and the HLI score at baseline both as a continuous variable (Model 1) and a categorical variable (according to seven groups: ≤-3, -2, -1, 0, 1, 2, and ≥3; Model 2). In an additional model, we estimated the associations between the changes in the HLI's individual four components (mutually adjusted; Model 3) and CRC risk. Similar models were conducted stratified by sex, by age (≤55 or >55 years at baseline) and by time between questionnaires (≤median or >median value). We investigated heterogeneity of the estimates between the strata using the Cochran's Q test. HRs were presented by colorectal subsite. For example, when proximal colon cancer was the outcome of interest, the observations of the participants who had a diagnosis of distal colon cancer and rectum cancer were censored at the date of diagnosis.

Due to the small proportion of participants with complete information on all the four components of the HLI at both questionnaires, for the main analysis we used a multivariate normal missing imputation (MI) model, which included baseline and follow-up smoking status, alcohol consumption, BMI, and physical activity, and relevant covariates: study centre, sex, educational level, age at follow-up questionnaire, time between questionnaires (log-transformed), diet score at baseline, CRC status, and the time to event or censorship (log-transformed). For the ordinal variables, we followed the projected distance rounding method, based on indicators. We generated 15 imputed datasets, analysed each dataset individually, and then combined the estimates using the Rubin's rules (16, 17). As a sensitivity analysis, we conducted a complete-case analysis limited to individuals with non-missing data for the four components in both questionnaires (Figure 1). Further sensitivity analyses were performed starting the observation time one year and two years after the follow-up questionnaire, in order

to reduce the risk of potential reverse causation caused by changes in lifestyle due to early symptoms of undiagnosed CRC.

Results with p-value <0.05 were considered statistically significant. Analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) and R software, version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Among 295,865 participants, 2,799 CRC cases were observed over a median of 7.8 years. Median time between the baseline and the follow-up questionnaire was 5.7 years (mean 7.0 years, interquartile range 5.0-9.9). Follow-up characteristics of 212,719 women and 83,146 men included in the study are reported in Table 1, overall and according to HLI score changes. The median age, BMI, physical activity METs and alcohol consumption among women were 58.6 years, 24.5, 76.0, 4.1 grams/day (g/d), respectively, and 59.2 years, 26.4, 60.0, and 13.5 g/d, respectively among men. There were 15.1% current smokers among women and 21.0% among men.

Mean HLI score at baseline and at follow-up were 10.04 (SD 2.8) and 9.95 (SD 2.7) units, respectively (Figure 2). Mean HLI score change was -0.09 units (SD 2.1). The largest positive HLI change was observed in participants from Denmark, while the largest negative HLI change was observed in participants from Norway. Men had more favourable changes compared to women, overall and in each country. We observed larger mean HLI score changes in men with higher education (HLI change=0.20) compared to men with lower education (0.11); we observed smaller HLI decreases in women with higher education (-0.18), compared to women with lower education (-0.29).

The association between lifestyle changes and the risk of CRC is shown in Table 2. A one-unit increase in HLI from baseline to follow-up was associated with a 3% lower risk of CRC (Model 1; HR=0.97; 95% CI 0.95-0.997). Similar inverse associations, though not statistically significant, were observed for the risk of proximal colon cancer (HR 0.96; 95% CI 0.92-1.00), distal colon cancer (HR 0.98; 95% CI 0.94-1.03) and rectal cancer (HR 0.98; 95% CI 0.94-1.02). Compared to no change in HLI, decrements of ≥3 units were associated with higher CRC risk (HR 1.21; 95% CI 1.02-1.43), while increments of ≥3 units were associated

with a lower risk of CRC (HR 0.88; 95% CI 0.74-1.05; Model 2). Increases in the alcohol, BMI and physical activity scores, but not in the smoking score, showed a trend towards an inverse association with CRC risk (Model 3). Increases in the alcohol score (i.e., decreases in alcohol consumption) were significantly associated with a lower risk of CRC in participants aged 55 or younger (mean age 46 years) at baseline. Increases in the physical activity score were significantly associated with a lower risk of proximal colon cancer. Increases in the smoking score were significantly associated with a higher risk of CRC in participants who were younger at baseline and with a higher risk of distal colon cancer. We found that a one-unit increase in HLI from baseline to follow-up was associated with a 3% lower risk of CRC in individuals with time between questionnaires ≤ 5.7 years (HR 0.97; 95% CI 0.95-1.00) and with time between questionnaires > 5.7 years (HR 0.97; 95% CI 0.93-1.01; p for heterogeneity 0.971).

Among participants with a baseline score of HLI≤9 (bottom tertile), those with follow-up score of HLI≥12 (top tertile) had a lower risk of CRC (Figure 3a; HR 0.77; 95% CI 0.59-1.00) than those with a follow-up score of HLI≤9. The crude CRC incidence rates in the two groups were 134 and 162 per 100,000 person-years, respectively. Among participants with a baseline score of HLI≥12, those with a follow-up score of HLI≤9 had a higher risk of CRC (Figure 3c; HR 1.34; 95% CI 1.02-1.75) compared to those with follow-up score of HLI≥12. The crude CRC incidence rates in the two groups were 119 and 86 per 100,000 person-years, respectively.

Compared to participants in the MI analysis, participants in the complete-case analysis were younger (mean age 55.3 vs. 58.3 years) and included a larger proportion of men (34.7% vs 28.1%) at the follow-up questionnaire. In the complete-case analysis the associations were generally stronger than in the MI analysis (Supplementary Table 1). For example, the HR for CRC for one unit increase in the HLI change was 0.95 (95% CI 0.92-0.99). Significant associations were observed also in women, in younger individuals and for the proximal subsite.

The complete-case analysis for the mean HLI changes stratified by sex and country is reported in the Supplementary Figure 2. The results did not change substantially when the first year and the first two years of follow-up were excluded from the analysis (data not shown).

Discussion

In this analysis conducted in the EPIC cohort, lifestyle behaviours were assessed twice, at baseline and once during follow-up. We showed that lifestyle changes across several years between these two timepoints were associated with the subsequent risk of CRC. Specifically, each unit increment in the HLI score (i.e., towards a healthier lifestyle) was associated with a significant 3% lower risk of CRC, after adjustment for baseline HLI. When HLI score was analysed in tertiles, improvement from an unfavourable lifestyle (score 0-9) to a favourable one (score 12-16) was associated with a 23% lower risk of CRC, compared to no change. On the other hand, a decline from a favourable lifestyle (score 12-16) to an unfavourable one (score 0-9) was associated with a 34% higher risk of CRC, compared to no change.

Similar associations between HLI changes and CRC risk were observed in men and women, in different age groups, and for specific cancer sites, although the associations were only statistically significant in men. The complete-case analysis showed generally stronger results than the MI analysis, with a statistically significant association between an increase in HLI score and a lower risk of CRC in the whole population, in women, in individuals of 55 years of age or younger at baseline and for the proximal subsite. To further evaluate the beneficial effect of an increase in HLI score and the detrimental effect of a decrease in HLI score, we divided the population according to tertiles of baseline HLI score. Notably, changing from an unfavourable to a favourable lifestyle was inversely associated with the risk of CRC, while changing from a favourable to an unfavourable lifestyle was positively associated with the risk of CRC.

Changes in BMI score from baseline to follow-up showed a trend towards an association with CRC risk. In two previous EPIC studies, bodyweight gain from age 20 to 50 years was associated with an increased risk of CRC (14), while weight changes after age 50

were not (18). In a 2015 meta-analysis, which included those two EPIC studies, the authors found that large bodyweight gains from early adulthood to midlife were associated with increased risk of CRC, whereas no association was found for large bodyweight gains from midlife to late in life, or for moderate bodyweight gains or weight loss at any age (12). Similar results indicating that body weight gains in early adulthood, but not late adulthood, was positively associated with CRC risk were found in the Nurses' Health Study and Health Professionals Follow-up (19). A 2019 study among 81,388 individuals, aged 55 to 74 years, did not show a clear association between bodyweight change and the risk of CRC (20). This evidence suggests that there might be an effect of bodyweight gain in early adulthood, but not in older adulthood, on the risk ok CRC. In this study, we did not find an interaction between age at baseline and bodyweight gain, possibly due to the relatively high mean age in our population at follow-up.

We found that decreasing alcohol consumption was associated with a lower risk of CRC, especially in younger individuals, when adjusted for the other components in the HLI as well as possible confounders. To the best of our knowledge, no previous studies reported evidence on this association. Our findings suggest that preventive measures for reducing alcohol consumption should target people at a young age more forcefully.

We found that increasing levels of physical activity were associated with a lower risk of CRC, specifically proximal cancer. Moreover, in the complete-case analysis, we found statistically significant associations between changes in physical activity level and risk of CRC in the overall population as well as in younger individuals. Like for alcohol reduction, our results suggest that it might be important to promote physical activity early in life.

We observed that increases in the smoking score, equivalent to reducing smoking levels, was associated with an increased risk of CRC, particularly in the younger individuals

and for distal cancer. This may be a result of reverse causation, whereby participants who quit smoking or reduced the number of cigarettes may have experienced early symptoms of CRC. Changes in tobacco exposure were associated with risk of CRC even after exclusion of the first two years of follow-up. Notably, changes in smoking habits performed a marginal influence on the association between HLI and CRC in our study, as only a small proportion of the population changed their smoking habits in this study.

Our study shows country- and sex- specific differences in HLI score changes. In general, countries with the highest HLI score at baseline (e.g., Norway and United Kingdom) showed a decrease in HLI score at follow-up, while countries with the lowest HLI score at baseline (e.g., Denmark and Sweden) had a higher HLI score at follow-up. Also, countries with the highest mean age at baseline (e.g., Denmark, Sweden and France) had the most favourable HLI score changes. Unlike baseline age and HLI score, educational levels did not explain the differences in the country-specific HLI changes. Within each country that recruited both men and women, women had a higher HLI score at baseline than men, and women showed lower increases or higher decreases in HLI score at follow-up compared to men. This may have occurred since men, starting with a lower HLI score than women, have more room to improve their lifestyle. Country-specific differences in the questionnaires and their updates at follow-up may also explain the difference between countries. An increase in smoking among Norwegian women has been of concern in recent decades (21) and may explain the worsening in the HLI score in the Norwegian population. However, studies on time trends have shown that the lifestyle of European populations, both in men and women, has generally improved during the last decades (22, 23). Altogether, it is difficult to disentangle the contributions of these different changes in score components to our results.

To our knowledge, this is the first study to report an association between multifactorial lifestyle changes and the risk of CRC. According to our results, changing lifestyle habits in

adult life is significantly associated with risk of CRC. If confirmed by other studies, this observation may provide strong evidence to design intervention studies for CRC prevention targeting middle-aged adults, and other research on preventive strategies, which is urgently needed given the scale of the CRC burden (24). An important novel result of our study is that lifestyle changes can affect CRC risk in both directions: improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. This is a clear message that practicing clinicians and gastroenterologists could give to their patients and to CRC screening participants to improve CRC prevention. The large sample size and the prospective multi-country and multi-centre design of the EPIC cohort are major strengths of the study, including that the results were consistent across different analytical strategies. For sake of consistency, a scoring system that was used previously in EPIC publications was used in this study. While specific components, for example smoking or obesity, might weight more in the computation of the HLI, this approach has the advantage of ensuring comparability across studies and according to different cancer and other disease outcomes.

Our study has some limitations. We acknowledge that the lack of data on diet collected during follow-up may have led to inadequately adjusted risk estimates and residual confounding. For example, if improvements in diet were associated with both improvements in the HLI score and a decreased CRC risk, then we might have overestimated the association between HLI score and CRC risk. The collection and harmonization of dietary data at follow-up is currently ongoing in EPIC. Furthermore, socioeconomic status impacts both lifestyle and CRC risk, and the use of only educational level as a proxy for socioeconomic status may also have led to residual confounding. EPIC participants might not be representative of the general population due to healthy cohort effects, and this warrants cautious interpretation of our findings. However, we can speculate that our findings on the benefit of adopting healthy

choices during adulthood might have a larger impact on CRC risk in the general population, characterized by less healthy profiles. Moreover, the HLI score may be too simplistic, assuming equal associations between each lifestyle factor and CRC risk. The HLI score may therefore not accurately capture the complex relationship between lifestyle habits and risk of CRC. However, the main aim of this study was to investigate the association between changes in a multifactorial index summarizing information on major lifestyle factors and the risk of CRC.

Conclusion

This large European cohort study used longitudinal data to show for the first time that changes in lifestyle habits in adult life are associated with the risk of CRC. Favourable changes were associated with a reduced risk of CRC, while unfavourable changes were associated with an increased risk of CRC.

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Table 1. Characteristics of the cohort at follow-up questionnaire according to the healthy lifestyle index difference, in males (n=83146) and females (n=212719)

		Overall	HLI difference							
			≤-3	-2	-1	0	1	2	≥3	Missing
Males	**n**	83146	5096	5582	8645	10301	8128	5116	4801	35477
	BMI (kg/m^2)	26.4 (24.4-28.7)	26.7 (24.7-29.1)	26.5 (24.5-28.9)	26.5 (24.4-28.8)	26.5 (24.5-28.8)	26.5 (24.5-29.0)	26.5 (24.6-28.9)	26.7 (24.5-29.1)	26.2 (24.2-28.4)
	METS recreational and household activity	60.0 (35.5-92.0)	34.5 (21.0-50.4)	44.0 (27.0-64.0)	51.5 (30.0-78.6)	58.1 (33.0-90.5)	65.0 (38.5-100.1)	73.5 (50.0-105.9)	84.0 (60.0-111.6)	65.5 (40.5-98.0)
	Alcohol (g/d)	13.5 (4.3-28.7)	19.6 (8.2-35.4)	16.1 (6.7-31.3)	15.1 (5.3-31.2)	13.5 (3.5-30.0)	13.0 (3.7-27.6)	11.3 (3.5-25.0)	8.2 (1.4-22.1)	13.2 (4.8-27.1)
	Smoker									
	Never	28005 (34.7)	1847 (36.2)	2225 (39.9)	3490 (40.4)	4015 (39.0)	2965 (36.5)	1754 (34.3)	1432 (29.8)	10277 (31.1)
	Former	35716 (44.3)	1976 (38.8)	2257 (40.4)	3517 (40.7)	4422 (42.9)	3705 (45.6)	2458 (48.0)	2464 (51.3)	14917 (45.2)
	Current	16985 (21.0)	1273 (25.0)	1100 (19.7)	1638 (18.9)	1864 (18.1)	1458 (17.9)	904 (17.7)	905 (18.9)	7843 (23.7)
	Age (years)	59.2 (53.3-65.1)	55.8 (48.7-64.5)	55.9 (49.0-63.6)	56.6 (49.4-63.7)	57.2 (50.0-64.1)	57.5 (50.4-64.3)	57.8 (50.8-64.1)	58.2 (50.8-63.8)	61.0 (57.0-66.2)
	Highest school level									
	None or primary	27053 (33.4)	1430 (29.4)	1543 (28.8)	2315 (27.9)	3082 (30.9)	2648 (33.5)	1771 (35.5)	1959 (41.5)	12305 (35.2)
	Technical, professional or secondary	29521 (36.4)	1796 (36.9)	1867 (34.8)	3042 (36.6)	3475 (34.9)	2747 (34.8)	1752 (35.1)	1613 (34.2)	13229 (37.9)
	University or higher	24485 (30.2)	1635 (33.6)	1956 (36.5)	2953 (35.5)	3414 (34.2)	2505 (31.7)	1467 (29.4)	1147 (24.3)	9408 (26.9)
	Diet score at baseline	27 (23-32)	27 (23-32)	28 (23-32)	28 (23-33)	27 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)	27 (22-32)
	HLI score at baseline	9 (7-11)	11 (9-12)	10 (8-12)	10 (8-11)	9 (7-11)	8 (6-10)	8 (6-9)	6 (5-8)	8 (6-10)
	HLI score at follow-up	9 (7-11)	7 (5-9)	8 (6-10)	9 (7-10)	9 (7-11)	9 (7-11)	10 (8-11)	10 (8-12)	9 (7-11)
Females	**n**	212719	12091	12747	18724	20836	13882	6887	4466	123086
	BMI (kg/m^2)	24.5 (22.2-27.6)	25.4 (23.0-28.3)	25.2 (22.7-28.4)	25.1 (22.6-28.4)	25.4 (22.6-28.9)	25.0 (22.5-28.4)	24.6 (22.3-27.8)	24.2 (21.9-27.3)	24.1 (21.9-27.0)
	METS recreational and household activity	76.0 (46.4-115.3)	48.6 (33.0-69.0)	66.0 (42.0-91.5)	84.0 (51.3-116.5)	105.0 (64.5-144.0)	111.0 (75.3-149.2)	115.5 (83.5-151.5)	130.0 (99.0-157.5)	62.6 (39.4-93.0)
	Alcohol (g/d)	4.1 (0.6-11.8)	6.8 (0.8-15.8)	4.0 (0.1-12.4)	2.4 (0.0-9.3)	1.6 (0.0-7.7)	2.1 (0.0-8.1)	2.4 (0.0-8.2)	2.5 (0.0-7.7)	5.7 (1.5-13.7)
	Smoker									
	Never	110648 (56.1)	7033 (58.2)	7954 (62.4)	12064 (64.4)	13958 (67.0)	8851 (63.8)	4090 (59.4)	2386 (53.4)	54312 (50.5)
	Former	56793 (28.8)	2784 (23.0)	2856 (22.4)	3997 (21.3)	4155 (19.9)	3133 (22.6)	1801 (26.2)	1323 (29.6)	36744 (34.2)
	Current	29765 (15.1)	2274 (18.8)	1937 (15.2)	2663 (14.2)	2723 (13.1)	1898 (13.7)	996 (14.5)	757 (17.0)	16517 (15.4)

Age (years)	58.6 (52.1-64.7)	53.9 (45.4-63.3)	54.3 (46.3-62.9)	54.4 (46.3-62.4)	54.3 (46.5-62.2)	54.6 (46.6-62.3)	54.8 (46.4-62.5)	54.4 (45.4-62.3)	60.1 (56.3-66.0)
Highest school level									
None or primary	58515 (28.6)	3120 (27.8)	3718 (31.2)	6365 (36.3)	8630 (43.7)	5780 (43.8)	2680 (40.7)	1743 (40.9)	26479 (22.1)
Technical, professional or secondary	95922 (46.9)	5065 (45.2)	4927 (41.4)	6686 (38.1)	6689 (33.9)	4449 (33.7)	2345 (35.6)	1543 (36.2)	64218 (53.6)
University or higher	49934 (24.4)	3020 (27.0)	3268 (27.4)	4499 (25.6)	4441 (22.5)	2960 (22.4)	1563 (23.7)	978 (22.9)	29205 (24.4)
Diet score at baseline	28 (23-32)	26 (22-31)	27 (23-31)	27 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)
HLI score at baseline	11 (9-12)	12 (11-14)	12 (10-13)	12 (10-13)	12 (10-13)	11 (9-12)	10 (8-11)	9 (7-10)	10 (8-12)
HLI score at follow-up	11 (9-12)	9 (8-10)	10 (8-11)	11 (9-12)	12 (10-13)	12 (10-13)	12 (10-13)	12 (11-14)	10 (9-12)

HLI: healthy lifestyle index. BMI: body mass index. METs: metabolic equivalent of task. Continuous variables are summarized as median (interquartile range), categorical variables as n (%).

Table 2. Association between lifestyle changes from baseline to follow-up and risk of colorectal cancer

				By sex*		By age at baseline°		By site		
			Overall	Males	Females	Age ≤55	Age >55	Proximal	Distal	Rectal
		n (CRC events)	295865 (2799)	83146 (1299)	212719 (1500)	194317 (1147)	101548 (1652)	295865 (907)	295865 (769)	295865 (985)
Model 1	Difference in continuous HLI score	1unit increase	**0.97 (0.95-0.997)**	**0.97 (0.93-0.998)**	0.98 (0.95-1.01)	0.97 (0.93-1.01)	0.97 (0.95-1.00)	0.96 (0.92-1.00)	0.98 (0.94-1.03)	0.98 (0.94-1.02)
Model 2	Difference in categorical HLI score	≤ -3 vs 0	**1.21 (1.02-1.43)**	**1.31 (1.01-1.69)**	1.15 (0.92-1.42)	**1.32 (1.02-1.70)**	1.14 (0.92-1.42)	1.21 (0.90-1.64)	1.33 (0.99-1.79)	1.11 (0.83-1.47)
		-2 vs 0	0.95 (0.77-1.16)	0.95 (0.72-1.24)	0.94 (0.72-1.24)	1.07 (0.79-1.46)	0.86 (0.68-1.10)	1.09 (0.78-1.52)	0.93 (0.64-1.34)	0.82 (0.62-1.09)
		-1 vs 0	0.99 (0.83-1.17)	0.98 (0.78-1.25)	0.98 (0.80-1.21)	0.97 (0.77-1.21)	1.00 (0.81-1.24)	1.09 (0.82-1.45)	0.96 (0.71-1.29)	0.92 (0.72-1.17)
		1 vs 0	1.00 (0.85-1.17)	1.00 (0.80-1.25)	1.01 (0.82-1.23)	1.02 (0.80-1.29)	0.99 (0.82-1.2)	1.07 (0.81-1.42)	1.03 (0.77-1.38)	0.92 (0.73-1.16)
		2 vs 0	0.95 (0.81-1.12)	0.96 (0.77-1.20)	0.94 (0.75-1.19)	0.99 (0.77-1.28)	0.93 (0.75-1.14)	1.08 (0.79-1.47)	1.08 (0.76-1.54)	0.78 (0.59-1.03)
		≥ 3 vs 0	0.88 (0.74-1.05)	0.86 (0.69-1.07)	0.91 (0.70-1.18)	0.96 (0.73-1.26)	0.84 (0.68-1.04)	0.83 (0.61-1.14)	0.99 (0.71-1.37)	0.86 (0.66-1.12)
Model 3	Continuous difference in Smoking score	1unit increase	1.04 (0.94-1.14)	1.05 (0.92-1.19)	1.03 (0.89-1.19)	**1.17 (1.02-1.34)**	0.93 (0.81-1.06)	0.99 (0.83-1.18)	**1.26 (1.06-1.51)**	0.93 (0.79-1.09)
	Continuous difference in Alcohol score	1unit increase	0.97 (0.92-1.02)	0.97 (0.90-1.04)	0.97 (0.90-1.04)	**0.92 (0.86-0.999)**	1.00 (0.93-1.07)	0.96 (0.88-1.06)	0.95 (0.86-1.05)	0.98 (0.91-1.07)
	Continuous difference in BMI score	1unit increase	0.97 (0.91-1.03)	0.93 (0.84-1.02)	0.99 (0.92-1.07)	0.98 (0.90-1.07)	0.96 (0.88-1.04)	1.00 (0.90-1.10)	0.91 (0.81-1.02)	0.99 (0.89-1.09)
	Continuous difference in Physical Activity score	1unit increase	0.97 (0.93-1.01)	0.96 (0.91-1.01)	0.98 (0.93-1.03)	0.97 (0.91-1.03)	0.97 (0.93-1.01)	**0.94 (0.88-0.998)**	0.99 (0.93-1.05)	1.00 (0.95-1.07)

CRC: colorectal cancer. HLI: healthy lifestyle index. BMI: body mass index. Bold font indicates statistical significance (p<0.05). Models 1 and 2 are stratified by study centre, age and sex, and adjusted for education, diet score at baseline, continuous healthy lifestyle index (HLI) score at baseline, and calendar year of follow-up questionnaire. Model 3 is stratified by study centre, age and sex, and adjusted for education, diet score at baseline, continuous index components scores at baseline, and date of follow-up questionnaire; differences for the single index components are mutually adjusted. * p-values for heterogeneity between males and females: difference in continuous HLI score, p=0.565; difference in categorical HLI score, p=0.495; smoking, p=0.846; alcohol, p=0.980; BMI, p=0.289; physical activity, p=0.664. ° p-values for heterogeneity between age groups: continuous HLI score, p=0.859; categorical HLI score, p=0.757; smoking, p=0.018; alcohol p=0.148; BMI, p=0.681; physical activity, p=0.958.

Figure 1. Selection of the population

Figure 2. Changes in the healthy lifestyle index score by country and sex

Figure 3. Survival analysis by tertiles of the healthy lifestyle index score at follow up in: A) participants in the bottom tertile at baseline (healthy lifestyle index <10); B) participants in the middle tertile at baseline (healthy lifestyle index of 10 and 11); C) participants in the top tertile at baseline (healthy lifestyle index >11).

Supplementary Figure 1. Scoring system implemented to combine the four lifestyle factors into the heathy lifestyle index

Supplementary Figure 2. Changes in the healthy lifestyle index score by country and sex in the complete-case analysis

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Changes in lifestyle and risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition

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Abstract

Introduction: We investigated the impact of changes in lifestyle habits on colorectal cancer (CRC) risk in a multi-country European cohort.

Methods: We used baseline and follow-up questionnaire data from the EPIC cohort to assess changes in lifestyle habits and their associations with CRC development. We calculated a healthy lifestyle index (HLI) score based on smoking status, alcohol consumption, body mass index and physical activity collected at the two timepoints. HLI ranged from 0 (most unfavourable) to 16 (most favourable). We estimated the association between HLI changes and CRC risk using Cox regression models and reported hazard ratios (HR) with 95% confidence intervals (CI).

Results: Among 295,865 participants, 2,799 CRC cases were observed over a median of 7.8 years. Median time between questionnaires was 5.7 years. Each unit increase in HLI from the baseline to the follow-up assessment was associated with a statistically significant 3% lower CRC risk. Among participants in the top tertile at baseline (HLI>11), those in the bottom tertile at follow-up (HLI≤9) had a higher CRC risk (HR 1.34; 95%CI 1.02-1.75) than those remaining in the top tertile. Among individuals in the bottom tertile at baseline, those in the top tertile at follow-up had a lower risk (HR 0.77; 95%CI 0.59-1.00) than those remaining in the bottom tertile.

Discussion: Improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. These results justify and support recommendations for healthy lifestyle changes and healthy lifestyle maintenance for CRC prevention.

Keywords: Colorectal cancer; Lifestyle; Prevention; Cohort study

Study highlights

What is known

- Leading a healthy lifestyle reduces the risk of colorectal cancer (CRC)
- There is limited knowledge on the impact of lifestyle changes on the risk of CRC

What is new here

- We measured changes in lifestyle among 300.000 participants in the EPIC cohort, using a baseline and a follow-up questionnaire
- Improving adherence to a healthy lifestyle was inversely associated with CRC risk
- Worsening adherence was positively associated with CRC risk
- These results justify recommendations for healthy lifestyle changes and healthy lifestyle maintenance for CRC prevention

Introduction

Worldwide, colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer death, with an estimated 1.9 million new cases and 0.9 million deaths in 2020 (1). There is clear evidence that five healthy lifestyle behaviours, namely non-smoking, avoiding being overweight, being physically active, consuming no or low amounts of alcohol and having a healthy diet, reduce the risk of CRC (2, 3). The number of healthy behaviours is inversely associated with CRC risk (4-6). In the European Prospective Investigation into Cancer (EPIC), when combining these five behaviours in a lifestyle index, the risk of CRC was 12% lower for each additional healthy behaviour (4). In the Nurses' Health Study and Health Professionals Follow-up Study, it was also shown that an increasing healthy lifestyle index score, based on the same five behaviours, was associated with a reduced CRC risk independently from participation in endoscopic screening (6).

In planning cancer preventive strategies, researchers and policy makers should be aware not only of the impact of lifestyle behaviours on cancer risk, but also how changing lifestyle in the recommended direction will affect cancer risk. Nonetheless, how lifestyle changes affect the risk of cancer, and specifically CRC, remains largely unexplored. A randomized trial showed that a successful intervention on smoking cessation and diet improvement can reduce the risk of lifestyle-related cancers in men with high risk for cancer (7). Observational studies have shown that increasing physical activity levels during adult life are associated with lower cancer mortality (8, 9), and that improving the cardiorespiratory physical fitness is associated with reduced cancer risk and mortality (10). A recent observational study in Swedish women suggested that lifestyle improvements are associated with a lower risk of lifestyle-related cancers (11). With regard to the specific risk of CRC, a 2020 meta-analysis indicated that smoking cessation significantly reduces the risk of CRC after 25 years since quitting (2), and a 2015 meta-analysis showed that large weight gains from early adulthood to midlife are

associated with increased risk of CRC (12, 13). Changes in other lifestyle factors related to CRC risk have not been examined.

With the aim of contributing to the limited knowledge base on lifestyle changes and risk of CRC, in this study we investigated the effect of changes in smoking habits, body mass index (BMI), physical activity level, alcohol consumption, and a lifestyle index which combined those four factors, on the subsequent incidence of CRC in the EPIC cohort.

Methods

From 1992 to 2000, 521,323 participants mostly aged from 35 to 70 years were recruited, mostly from the general population, across 23 centres in 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. The rationale, study design, and methods for EPIC have been described in detail elsewhere (14). All participants completed a lifestyle questionnaire at baseline and provided informed consent to participate in the study. Ethical approval was obtained from participating centres and the IARC ethics committee (reference number 20-02).

We initially excluded 28,561 participants from Greece due to administrative and data-use restrictions, 24,550 participants with prevalent cancer at baseline, 9,064 with extreme energy intakes (i.e., below the 1st and above the 99th percentiles of the energy intake over energy requirement ratio distribution) and 3,137 without follow-up after the baseline questionnaire (Figure 1). After an average of 7 years (range 2-17) from recruitment, a second lifestyle questionnaire was administered during follow-up. Since the main exposure of interest of the current analysis was lifestyle changes, we further excluded 16,816 participants with cancer before the follow-up questionnaire and 100,828 participants for whom the follow-up lifestyle assessment questionnaire data was not available in the centralized EPIC dataset in October 2020. We then excluded 3,426 participants for whom no follow-up time was available after the follow-up questionnaire date. We additionally excluded 5,900 and 11,419 participants for whom information about the four lifestyle factors of interest - smoking status, alcohol consumption, BMI and physical activity - was missing at the baseline questionnaire and at the follow-up questionnaire, respectively. We finally excluded 21,757 participants for whom information of at least one of the four factors of interest was missing both at baseline and follow-up. Hence, the final analytic dataset included 295,865 participants. Participants were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Exposure assessment

Four lifestyle factors were investigated: smoking status, alcohol consumption, BMI and physical activity. For each factor, scores ranging from 0 to 4 were assigned to increasingly healthy categories of behaviour (Supplementary Figure 1). The "healthiest" behaviours were never smoking (never smoked=4 points, smoke cessation>10 years=3, smoke cessation≤10 years=2, current smoking≤15 cigarettes/day=1, current smoking>15 cigarettes/day=0), low consumption of alcohol (<6.0 g/day=4 points, 6.0–11.9=3, 12.0–23.9=2, 24.0–59.9=1, ≥60=0), top quintile of physical activity based on recreational and household metabolic equivalent of task units (METs) (5th quintile=4 points, 4th quintile=3, 3rd quintile=2, 2nd quintile=1, 1st quintile=0) and low BMI (<22=4 points, 22-23.9=3, 24–25.9=2, 26–29.9=1, ≥30=0). A healthy lifestyle index (HLI) was obtained by summing the scores of each lifestyle factor, thus ranging from 0 to 16. Changes in the HLI from the baseline questionnaire to the follow-up questionnaire was our main exposure of interest.

Information on diet was available only at baseline and was therefore not included in the HLI for the current analysis. Intakes of 6 dietary factors – namely cereal fiber, red and processed meat, the ratio of polyunsaturated to saturated fat, margarine, glycemic load, and fruits and vegetables - were combined only at baseline in a diet score (15), which was used as an adjustment variable in all analyses.

Outcome assessment

Cases of CRC were identified through population cancer registries in Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. A combination of methods

were used, including health insurance records, contacts with cancer and pathology registries, and active follow-up of EPIC participants and their next of kin in France and Germany.

CRC cases were defined as carcinomas with topography codes C18, C19 and C20 according to the 10th Revision of the International Statistical Classification of Diseases, Injuries and Causes of Death. In addition to CRC, we also examined associations for the following subsites: proximal colon (C18.0-C18.5), distal colon (C18.6-C18.7) and rectum (C19-C20). When analysing colorectum subsites, CRC coded as C18.8 (overlapping more than one subsite) and C18.9 (unspecified subsite) were censored.

Statistical methods

Categorical variables were summarized as frequencies and percentages, and continuous variables as means, medians, standard deviations (SD) and interquartile ranges. In forest plots, HLI and HLI changes were summarized as mean values. In survival analyses, participants were followed from return of follow-up questionnaire until any first cancer, excluding non-melanoma skin cancer, death, emigration, or end of follow-up, whichever came first. Kaplan-Meier survival curves were constructed, separately by tertiles of HLI at baseline and stratified by HLI at follow-up. Multivariable Cox proportional hazards regression models, using participants' age as the underlying time scale, were used to estimate hazard ratios (HR) and the corresponding 95% confidence intervals (CI). We used two decimals for the CIs, but we reported three decimals in some cases to show full statistical significance. The models were stratified by study centre, age at recruitment rounded to one year and sex, and adjusted for the highest education level achieved (none or primary; technical, professional or secondary; university or higher; missing), diet score at baseline, and the calendar date of follow-up

questionnaire. The models with lifestyle changes as the main exposure were additionally adjusted for the continuous HLI score at baseline.

To estimate the association between lifestyle changes and risk of CRC, we used the difference between the HLI score at follow-up and the HLI score at baseline both as a continuous variable (Model 1) and a categorical variable (according to seven groups: ≤-3, -2, -1, 0, 1, 2, and ≥3; Model 2). In an additional model, we estimated the associations between the changes in the HLI's individual four components (mutually adjusted; Model 3) and CRC risk. Similar models were conducted stratified by sex, by age (≤55 or >55 years at baseline) and by time between questionnaires (≤median or >median value). We investigated heterogeneity of the estimates between the strata using the Cochran's Q test. HRs were presented by colorectal subsite. For example, when proximal colon cancer was the outcome of interest, the observations of the participants who had a diagnosis of distal colon cancer and rectum cancer were censored at the date of diagnosis.

Due to the small proportion of participants with complete information on all the four components of the HLI at both questionnaires, for the main analysis we used a multivariate normal missing imputation (MI) model, which included baseline and follow-up smoking status, alcohol consumption, BMI, and physical activity, and relevant covariates: study centre, sex, educational level, age at follow-up questionnaire, time between questionnaires (log-transformed), diet score at baseline, CRC status, and the time to event or censorship (log-transformed). For the ordinal variables, we followed the projected distance rounding method, based on indicators. We generated 15 imputed datasets, analysed each dataset individually, and then combined the estimates using the Rubin's rules (16, 17). As a sensitivity analysis, we conducted a complete-case analysis limited to individuals with non-missing data for the four components in both questionnaires (Figure 1). Further sensitivity analyses were performed starting the observation time one year and two years after the follow-up questionnaire, in order

to reduce the risk of potential reverse causation caused by changes in lifestyle due to early symptoms of undiagnosed CRC.

Results with p-value <0.05 were considered statistically significant. Analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) and R software, version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Among 295,865 participants, 2,799 CRC cases were observed over a median of 7.8 years. Median time between the baseline and the follow-up questionnaire was 5.7 years (mean 7.0 years, interquartile range 5.0-9.9). Follow-up characteristics of 212,719 women and 83,146 men included in the study are reported in Table 1, overall and according to HLI score changes. The median age, BMI, physical activity METs and alcohol consumption among women were 58.6 years, 24.5, 76.0, 4.1 grams/day (g/d), respectively, and 59.2 years, 26.4, 60.0, and 13.5 g/d, respectively among men. There were 15.1% current smokers among women and 21.0% among men.

Mean HLI score at baseline and at follow-up were 10.04 (SD 2.8) and 9.95 (SD 2.7) units, respectively (Figure 2). Mean HLI score change was -0.09 units (SD 2.1). The largest positive HLI change was observed in participants from Denmark, while the largest negative HLI change was observed in participants from Norway. Men had more favourable changes compared to women, overall and in each country. We observed larger mean HLI score changes in men with higher education (HLI change=0.20) compared to men with lower education (0.11); we observed smaller HLI decreases in women with higher education (-0.18), compared to women with lower education (-0.29).

The association between lifestyle changes and the risk of CRC is shown in Table 2. A one-unit increase in HLI from baseline to follow-up was associated with a 3% lower risk of CRC (Model 1; HR=0.97; 95% CI 0.95-0.997). Similar inverse associations, though not statistically significant, were observed for the risk of proximal colon cancer (HR 0.96; 95% CI 0.92-1.00), distal colon cancer (HR 0.98; 95% CI 0.94-1.03) and rectal cancer (HR 0.98; 95% CI 0.94-1.02). Compared to no change in HLI, decrements of ≥3 units were associated with higher CRC risk (HR 1.21; 95% CI 1.02-1.43), while increments of ≥3 units were associated

with a lower risk of CRC (HR 0.88; 95% CI 0.74-1.05; Model 2). Increases in the alcohol, BMI and physical activity scores, but not in the smoking score, showed a trend towards an inverse association with CRC risk (Model 3). Increases in the alcohol score (i.e., decreases in alcohol consumption) were significantly associated with a lower risk of CRC in participants aged 55 or younger (mean age 46 years) at baseline. Increases in the physical activity score were significantly associated with a lower risk of proximal colon cancer. Increases in the smoking score were significantly associated with a higher risk of CRC in participants who were younger at baseline and with a higher risk of distal colon cancer. We found that a one-unit increase in HLI from baseline to follow-up was associated with a 3% lower risk of CRC in individuals with time between questionnaires ≤ 5.7 years (HR 0.97; 95% CI 0.95-1.00) and with time between questionnaires > 5.7 years (HR 0.97; 95% CI 0.93-1.01; p for heterogeneity 0.971).

Among participants with a baseline score of HLI≤9 (bottom tertile), those with follow-up score of HLI≥12 (top tertile) had a lower risk of CRC (Figure 3a; HR 0.77; 95% CI 0.59-1.00) than those with a follow-up score of HLI≤9. The crude CRC incidence rates in the two groups were 134 and 162 per 100,000 person-years, respectively. Among participants with a baseline score of HLI≥12, those with a follow-up score of HLI≤9 had a higher risk of CRC (Figure 3c; HR 1.34; 95% CI 1.02-1.75) compared to those with follow-up score of HLI≥12. The crude CRC incidence rates in the two groups were 119 and 86 per 100,000 person-years, respectively.

Compared to participants in the MI analysis, participants in the complete-case analysis were younger (mean age 55.3 vs. 58.3 years) and included a larger proportion of men (34.7% vs 28.1%) at the follow-up questionnaire. In the complete-case analysis the associations were generally stronger than in the MI analysis (Supplementary Table 1). For example, the HR for CRC for one unit increase in the HLI change was 0.95 (95% CI 0.92-0.99). Significant associations were observed also in women, in younger individuals and for the proximal subsite.

The complete-case analysis for the mean HLI changes stratified by sex and country is reported in the Supplementary Figure 2. The results did not change substantially when the first year and the first two years of follow-up were excluded from the analysis (data not shown).

Discussion

In this analysis conducted in the EPIC cohort, lifestyle behaviours were assessed twice, at baseline and once during follow-up. We showed that lifestyle changes across several years between these two timepoints were associated with the subsequent risk of CRC. Specifically, each unit increment in the HLI score (i.e., towards a healthier lifestyle) was associated with a significant 3% lower risk of CRC, after adjustment for baseline HLI. When HLI score was analysed in tertiles, improvement from an unfavourable lifestyle (score 0-9) to a favourable one (score 12-16) was associated with a 23% lower risk of CRC, compared to no change. On the other hand, a decline from a favourable lifestyle (score 12-16) to an unfavourable one (score 0-9) was associated with a 34% higher risk of CRC, compared to no change.

Similar associations between HLI changes and CRC risk were observed in men and women, in different age groups, and for specific cancer sites, although the associations were only statistically significant in men. The complete-case analysis showed generally stronger results than the MI analysis, with a statistically significant association between an increase in HLI score and a lower risk of CRC in the whole population, in women, in individuals of 55 years of age or younger at baseline and for the proximal subsite. To further evaluate the beneficial effect of an increase in HLI score and the detrimental effect of a decrease in HLI score, we divided the population according to tertiles of baseline HLI score. Notably, changing from an unfavourable to a favourable lifestyle was inversely associated with the risk of CRC, while changing from a favourable to an unfavourable lifestyle was positively associated with the risk of CRC.

Changes in BMI score from baseline to follow-up showed a trend towards an association with CRC risk. In two previous EPIC studies, bodyweight gain from age 20 to 50 years was associated with an increased risk of CRC (14), while weight changes after age 50

were not (18). In a 2015 meta-analysis, which included those two EPIC studies, the authors found that large bodyweight gains from early adulthood to midlife were associated with increased risk of CRC, whereas no association was found for large bodyweight gains from midlife to late in life, or for moderate bodyweight gains or weight loss at any age (12). Similar results indicating that body weight gains in early adulthood, but not late adulthood, was positively associated with CRC risk were found in the Nurses' Health Study and Health Professionals Follow-up (19). A 2019 study among 81,388 individuals, aged 55 to 74 years, did not show a clear association between bodyweight change and the risk of CRC (20). This evidence suggests that there might be an effect of bodyweight gain in early adulthood, but not in older adulthood, on the risk ok CRC. In this study, we did not find an interaction between age at baseline and bodyweight gain, possibly due to the relatively high mean age in our population at follow-up.

We found that decreasing alcohol consumption was associated with a lower risk of CRC, especially in younger individuals, when adjusted for the other components in the HLI as well as possible confounders. To the best of our knowledge, no previous studies reported evidence on this association. Our findings suggest that preventive measures for reducing alcohol consumption should target people at a young age more forcefully.

We found that increasing levels of physical activity were associated with a lower risk of CRC, specifically proximal cancer. Moreover, in the complete-case analysis, we found statistically significant associations between changes in physical activity level and risk of CRC in the overall population as well as in younger individuals. Like for alcohol reduction, our results suggest that it might be important to promote physical activity early in life.

We observed that increases in the smoking score, equivalent to reducing smoking levels, was associated with an increased risk of CRC, particularly in the younger individuals

and for distal cancer. This may be a result of reverse causation, whereby participants who quit smoking or reduced the number of cigarettes may have experienced early symptoms of CRC. Changes in tobacco exposure were associated with risk of CRC even after exclusion of the first two years of follow-up. Notably, changes in smoking habits performed a marginal influence on the association between HLI and CRC in our study, as only a small proportion of the population changed their smoking habits in this study.

Our study shows country- and sex- specific differences in HLI score changes. In general, countries with the highest HLI score at baseline (e.g., Norway and United Kingdom) showed a decrease in HLI score at follow-up, while countries with the lowest HLI score at baseline (e.g., Denmark and Sweden) had a higher HLI score at follow-up. Also, countries with the highest mean age at baseline (e.g., Denmark, Sweden and France) had the most favourable HLI score changes. Unlike baseline age and HLI score, educational levels did not explain the differences in the country-specific HLI changes. Within each country that recruited both men and women, women had a higher HLI score at baseline than men, and women showed lower increases or higher decreases in HLI score at follow-up compared to men. This may have occurred since men, starting with a lower HLI score than women, have more room to improve their lifestyle. Country-specific differences in the questionnaires and their updates at follow-up may also explain the difference between countries. An increase in smoking among Norwegian women has been of concern in recent decades (21) and may explain the worsening in the HLI score in the Norwegian population. However, studies on time trends have shown that the lifestyle of European populations, both in men and women, has generally improved during the last decades (22, 23). Altogether, it is difficult to disentangle the contributions of these different changes in score components to our results.

To our knowledge, this is the first study to report an association between multifactorial lifestyle changes and the risk of CRC. According to our results, changing lifestyle habits in

adult life is significantly associated with risk of CRC. If confirmed by other studies, this observation may provide strong evidence to design intervention studies for CRC prevention targeting middle-aged adults, and other research on preventive strategies, which is urgently needed given the scale of the CRC burden (24). An important novel result of our study is that lifestyle changes can affect CRC risk in both directions: improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. This is a clear message that practicing clinicians and gastroenterologists could give to their patients and to CRC screening participants to improve CRC prevention. The large sample size and the prospective multi-country and multi-centre design of the EPIC cohort are major strengths of the study, including that the results were consistent across different analytical strategies. For sake of consistency, a scoring system that was used previously in EPIC publications was used in this study. While specific components, for example smoking or obesity, might weight more in the computation of the HLI, this approach has the advantage of ensuring comparability across studies and according to different cancer and other disease outcomes.

Our study has some limitations. We acknowledge that the lack of data on diet collected during follow-up may have led to inadequately adjusted risk estimates and residual confounding. For example, if improvements in diet were associated with both improvements in the HLI score and a decreased CRC risk, then we might have overestimated the association between HLI score and CRC risk. The collection and harmonization of dietary data at follow-up is currently ongoing in EPIC. Furthermore, socioeconomic status impacts both lifestyle and CRC risk, and the use of only educational level as a proxy for socioeconomic status may also have led to residual confounding. EPIC participants might not be representative of the general population due to healthy cohort effects, and this warrants cautious interpretation of our findings. However, we can speculate that our findings on the benefit of adopting healthy

choices during adulthood might have a larger impact on CRC risk in the general population, characterized by less healthy profiles. Moreover, the HLI score may be too simplistic, assuming equal associations between each lifestyle factor and CRC risk. The HLI score may therefore not accurately capture the complex relationship between lifestyle habits and risk of CRC. However, the main aim of this study was to investigate the association between changes in a multifactorial index summarizing information on major lifestyle factors and the risk of CRC.

Conclusion

This large European cohort study used longitudinal data to show for the first time that changes in lifestyle habits in adult life are associated with the risk of CRC. Favourable changes were associated with a reduced risk of CRC, while unfavourable changes were associated with an increased risk of CRC.

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Table 1. Characteristics of the cohort at follow-up questionnaire according to the healthy lifestyle index difference, in males (n=83146) and females (n=212719)

		Overall	HLI difference							
			≤-3	**-2**	**-1**	**0**	**1**	**2**	**≥3**	**Missing**
Males	**n**	83146	5096	5582	8645	10301	8128	5116	4801	35477
	BMI (kg/m^2)	26.4 (24.4-28.7)	26.7 (24.7-29.1)	26.5 (24.5-28.9)	26.5 (24.4-28.8)	26.5 (24.5-28.8)	26.5 (24.5-29.0)	26.5 (24.6-28.9)	26.7 (24.5-29.1)	26.2 (24.2-28.4)
	METS recreational and household activity	60.0 (35.5-92.0)	34.5 (21.0-50.4)	44.0 (27.0-64.0)	51.5 (30.0-78.6)	58.1 (33.0-90.5)	65.0 (38.5-100.1)	73.5 (50.0-105.9)	84.0 (60.0-111.6)	65.5 (40.5-98.0)
	Alcohol (g/d)	13.5 (4.3-28.7)	19.6 (8.2-35.4)	16.1 (6.7-31.3)	15.1 (5.3-31.2)	13.5 (3.5-30.0)	13.0 (3.7-27.6)	11.3 (3.5-25.0)	8.2 (1.4-22.1)	13.2 (4.8-27.1)
	Smoker									
	Never	28005 (34.7)	1847 (36.2)	2225 (39.9)	3490 (40.4)	4015 (39.0)	2965 (36.5)	1754 (34.3)	1432 (29.8)	10277 (31.1)
	Former	35716 (44.3)	1976 (38.8)	2257 (40.4)	3517 (40.7)	4422 (42.9)	3705 (45.6)	2458 (48.0)	2464 (51.3)	14917 (45.2)
	Current	16985 (21.0)	1273 (25.0)	1100 (19.7)	1638 (18.9)	1864 (18.1)	1458 (17.9)	904 (17.7)	905 (18.9)	7843 (23.7)
	Age (years)	59.2 (53.3-65.1)	55.8 (48.7-64.5)	55.9 (49.0-63.6)	56.6 (49.4-63.7)	57.2 (50.0-64.1)	57.5 (50.4-64.3)	57.8 (50.8-64.1)	58.2 (50.8-63.8)	61.0 (57.0-66.2)
	Highest school level									
	None or primary	27053 (33.4)	1430 (29.4)	1543 (28.8)	2315 (27.9)	3082 (30.9)	2648 (33.5)	1771 (35.5)	1959 (41.5)	12305 (35.2)
	Technical, professional or secondary	29521 (36.4)	1796 (36.9)	1867 (34.8)	3042 (36.6)	3475 (34.9)	2747 (34.8)	1752 (35.1)	1613 (34.2)	13229 (37.9)
	University or higher	24485 (30.2)	1635 (33.6)	1956 (36.5)	2953 (35.5)	3414 (34.2)	2505 (31.7)	1467 (29.4)	1147 (24.3)	9408 (26.9)
	Diet score at baseline	27 (23-32)	27 (23-32)	28 (23-32)	28 (23-33)	27 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)	27 (22-32)
	HLI score at baseline	9 (7-11)	11 (9-12)	10 (8-12)	10 (8-11)	9 (7-11)	8 (6-10)	8 (6-9)	6 (5-8)	8 (6-10)
	HLI score at follow-up	9 (7-11)	7 (5-9)	8 (6-10)	9 (7-10)	9 (7-11)	9 (7-11)	10 (8-11)	10 (8-12)	9 (7-11)
Females	**n**	212719	12091	12747	18724	20836	13882	6887	4466	123086
	BMI (kg/m^2)	24.5 (22.2-27.6)	25.4 (23.0-28.3)	25.2 (22.7-28.4)	25.1 (22.6-28.4)	25.4 (22.6-28.9)	25.0 (22.5-28.4)	24.6 (22.3-27.8)	24.2 (21.9-27.3)	24.1 (21.9-27.0)
	METS recreational and household activity	76.0 (46.4-115.3)	48.6 (33.0-69.0)	66.0 (42.0-91.5)	84.0 (51.3-116.5)	105.0 (64.5-144.0)	111.0 (75.3-149.2)	115.5 (83.5-151.5)	130.0 (99.0-157.5)	62.6 (39.4-93.0)
	Alcohol (g/d)	4.1 (0.6-11.8)	6.8 (0.8-15.8)	4.0 (0.1-12.4)	2.4 (0.0-9.3)	1.6 (0.0-7.7)	2.1 (0.0-8.1)	2.4 (0.0-8.2)	2.5 (0.0-7.7)	5.7 (1.5-13.7)
	Smoker									
	Never	110648 (56.1)	7033 (58.2)	7954 (62.4)	12064 (64.4)	13958 (67.0)	8851 (63.8)	4090 (59.4)	2386 (53.4)	54312 (50.5)
	Former	56793 (28.8)	2784 (23.0)	2856 (22.4)	3997 (21.3)	4155 (19.9)	3133 (22.6)	1801 (26.2)	1323 (29.6)	36744 (34.2)
	Current	29765 (15.1)	2274 (18.8)	1937 (15.2)	2663 (14.2)	2723 (13.1)	1898 (13.7)	996 (14.5)	757 (17.0)	16517 (15.4)

Age (years)	58.6 (52.1-64.7)	53.9 (45.4-63.3)	54.3 (46.3-62.9)	54.4 (46.3-62.4)	54.3 (46.5-62.2)	54.6 (46.6-62.3)	54.8 (46.4-62.5)	54.4 (45.4-62.3)	60.1 (56.3-66.0)
Highest school level									
None or primary	58515 (28.6)	3120 (27.8)	3718 (31.2)	6365 (36.3)	8630 (43.7)	5780 (43.8)	2680 (40.7)	1743 (40.9)	26479 (22.1)
Technical, professional or secondary	95922 (46.9)	5065 (45.2)	4927 (41.4)	6686 (38.1)	6689 (33.9)	4449 (33.7)	2345 (35.6)	1543 (36.2)	64218 (53.6)
University or higher	49934 (24.4)	3020 (27.0)	3268 (27.4)	4499 (25.6)	4441 (22.5)	2960 (22.4)	1563 (23.7)	978 (22.9)	29205 (24.4)
Diet score at baseline	28 (23-32)	26 (22-31)	27 (23-31)	27 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)	28 (23-32)
HLI score at baseline	11 (9-12)	12 (11-14)	12 (10-13)	12 (10-13)	12 (10-13)	11 (9-12)	10 (8-11)	9 (7-10)	10 (8-12)
HLI score at follow-up	11 (9-12)	9 (8-10)	10 (8-11)	11 (9-12)	12 (10-13)	12 (10-13)	12 (10-13)	12 (11-14)	10 (9-12)

HLI: healthy lifestyle index. BMI: body mass index. METs: metabolic equivalent of task. Continuous variables are summarized as median (interquartile range), categorical variables as n (%).

Table 2. Association between lifestyle changes from baseline to follow-up and risk of colorectal cancer

				By sex*		By age at baseline°		By site		
			Overall	Males	Females	Age ≤55	Age >55	Proximal	Distal	Rectal
		n (CRC events)	295865 (2799)	83146 (1299)	212719 (1500)	194317 (1147)	101548 (1652)	295865 (907)	295865 (769)	295865 (985)
Model 1	Difference in continuous HLI score	1unit increase	**0.97 (0.95-0.997)**	**0.97 (0.93-0.998)**	0.98 (0.95-1.01)	0.97 (0.93-1.01)	0.97 (0.95-1.00)	0.96 (0.92-1.00)	0.98 (0.94-1.03)	0.98 (0.94-1.02)
Model 2	Difference in categorical HLI score	≤ -3 vs 0	**1.21 (1.02-1.43)**	**1.31 (1.01-1.69)**	1.15 (0.92-1.42)	**1.32 (1.02-1.70)**	1.14 (0.92-1.42)	1.21 (0.90-1.64)	1.33 (0.99-1.79)	1.11 (0.83-1.47)
		-2 vs 0	0.95 (0.77-1.16)	0.95 (0.72-1.24)	0.94 (0.72-1.24)	1.07 (0.79-1.46)	0.86 (0.68-1.10)	1.09 (0.78-1.52)	0.93 (0.64-1.34)	0.82 (0.62-1.09)
		-1 vs 0	0.99 (0.83-1.17)	0.98 (0.78-1.25)	0.98 (0.80-1.21)	0.97 (0.77-1.21)	1.00 (0.81-1.24)	1.09 (0.82-1.45)	0.96 (0.71-1.29)	0.92 (0.72-1.17)
		1 vs 0	1.00 (0.85-1.17)	1.00 (0.80-1.25)	1.01 (0.82-1.23)	1.02 (0.80-1.29)	0.99 (0.82-1.2)	1.07 (0.81-1.42)	1.03 (0.77-1.38)	0.92 (0.73-1.16)
		2 vs 0	0.95 (0.81-1.12)	0.96 (0.77-1.20)	0.94 (0.75-1.19)	0.99 (0.77-1.28)	0.93 (0.75-1.14)	1.08 (0.79-1.47)	1.08 (0.76-1.54)	0.78 (0.59-1.03)
		≥ 3 vs 0	0.88 (0.74-1.05)	0.86 (0.69-1.07)	0.91 (0.70-1.18)	0.96 (0.73-1.26)	0.84 (0.68-1.04)	0.83 (0.61-1.14)	0.99 (0.71-1.37)	0.86 (0.66-1.12)
Model 3	Continuous difference in Smoking score	1unit increase	1.04 (0.94-1.14)	1.05 (0.92-1.19)	1.03 (0.89-1.19)	**1.17 (1.02-1.34)**	0.93 (0.81-1.06)	0.99 (0.83-1.18)	**1.26 (1.06-1.51)**	0.93 (0.79-1.09)
	Continuous difference in Alcohol score	1unit increase	0.97 (0.92-1.02)	0.97 (0.90-1.04)	0.97 (0.90-1.04)	**0.92 (0.86-0.999)**	1.00 (0.93-1.07)	0.96 (0.88-1.06)	0.95 (0.86-1.05)	0.98 (0.91-1.07)
	Continuous difference in BMI score	1unit increase	0.97 (0.91-1.03)	0.93 (0.84-1.02)	0.99 (0.92-1.07)	0.98 (0.90-1.07)	0.96 (0.88-1.04)	1.00 (0.90-1.10)	0.91 (0.81-1.02)	0.99 (0.89-1.09)
	Continuous difference in Physical Activity score	1unit increase	0.97 (0.93-1.01)	0.96 (0.91-1.01)	0.98 (0.93-1.03)	0.97 (0.91-1.03)	0.97 (0.93-1.01)	**0.94 (0.88-0.998)**	0.99 (0.93-1.05)	1.00 (0.95-1.07)

CRC: colorectal cancer. HLI: healthy lifestyle index. BMI: body mass index. Bold font indicates statistical significance (p<0.05). Models 1 and 2 are stratified by study centre, age and sex, and adjusted for education, diet score at baseline, continuous healthy lifestyle index (HLI) score at baseline, and calendar year of follow-up questionnaire. Model 3 is stratified by study centre, age and sex, and adjusted for education, diet score at baseline, continuous index components scores at baseline, and date of follow-up questionnaire; differences for the single index components are mutually adjusted. * p-values for heterogeneity between males and females: difference in continuous HLI score, p=0.565; difference in categorical HLI score, p=0.495; smoking, p=0.846; alcohol, p=0.980; BMI, p=0.289; physical activity, p=0.664. ° p-values for heterogeneity between age groups: continuous HLI score, p=0.859; categorical HLI score, p=0.757; smoking, p=0.018; alcohol p=0.148; BMI, p=0.681; physical activity, p=0.958.

Figure 1. Selection of the population

Figure 2. Changes in the healthy lifestyle index score by country and sex

Figure 3. Survival analysis by tertiles of the healthy lifestyle index score at follow up in: A) participants in the bottom tertile at baseline (healthy lifestyle index <10); B) participants in the middle tertile at baseline (healthy lifestyle index of 10 and 11); C) participants in the top tertile at baseline (healthy lifestyle index >11).

Supplementary Figure 1. Scoring system implemented to combine the four lifestyle factors into the heathy lifestyle index

Supplementary Figure 2. Changes in the healthy lifestyle index score by country and sex in the complete-case analysis

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Figure 1

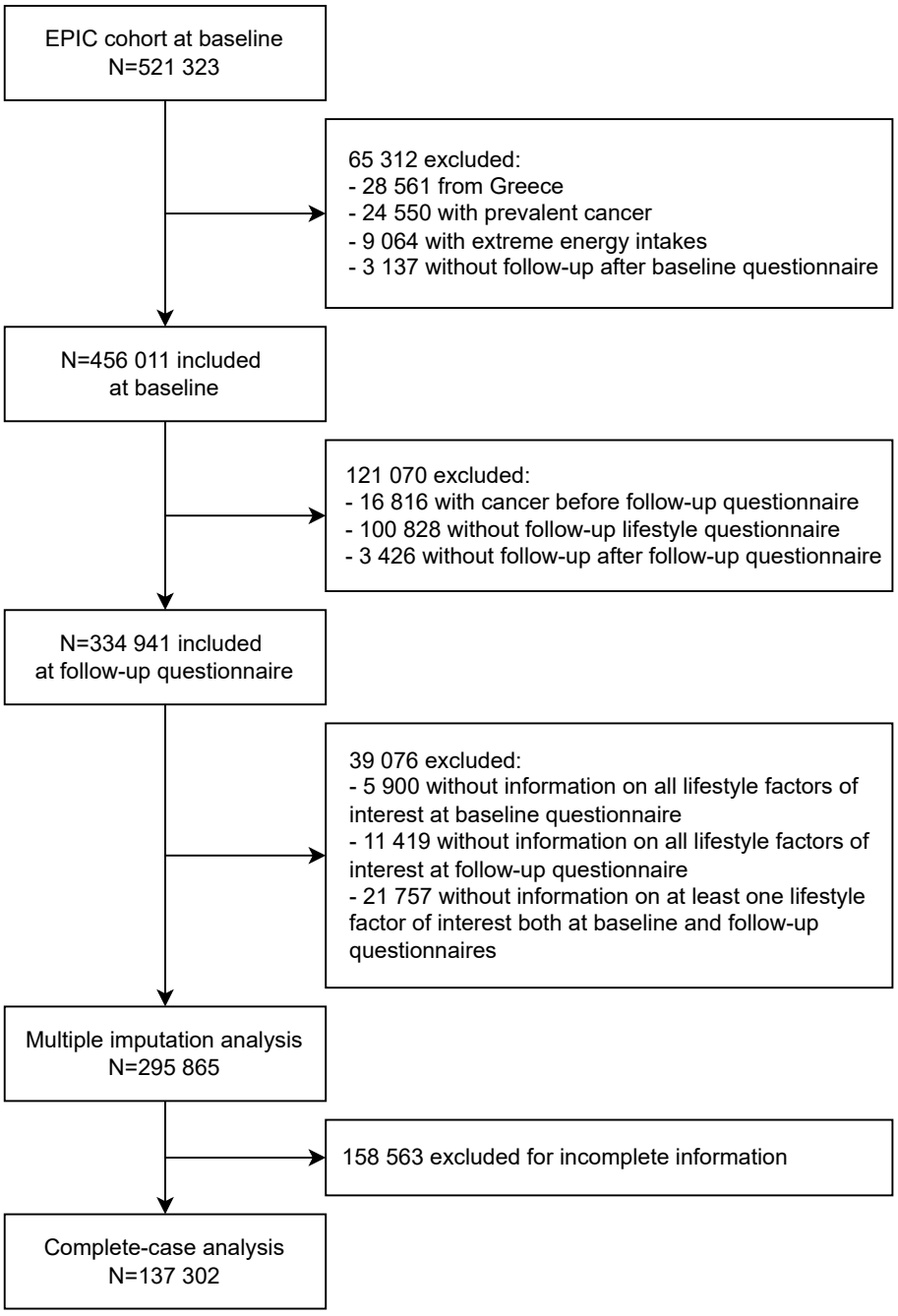

EPIC cohort at baseline
N=521 323
65 312 excluded:
- 28 561 from Greece
- 24 550 with prevalent cancer
- 9 064 with extreme energy intakes
- 3 137 without follow-up after baseline questionnaire
N=456 011 included
at baseline
121 070 excluded:
- 16 816 with cancer before follow-up questionnaire
- 100 828 without follow-up lifestyle questionnaire
- 3 426 without follow-up after follow-up questionnaire
N=334 941 included
at follow-up questionnaire
39 076 excluded:
- 5 900 without information on all lifestyle factors of interest at baseline questionnaire
- 11 419 without information on all lifestyle factors of interest at follow-up questionnaire
- 21 757 without information on at least one lifestyle factor of interest both at baseline and follow-up questionnaires
Multiple imputation analysis
N=295 865
158 563 excluded for incomplete information
Complete-case analysis
N=137 302

Figure 2

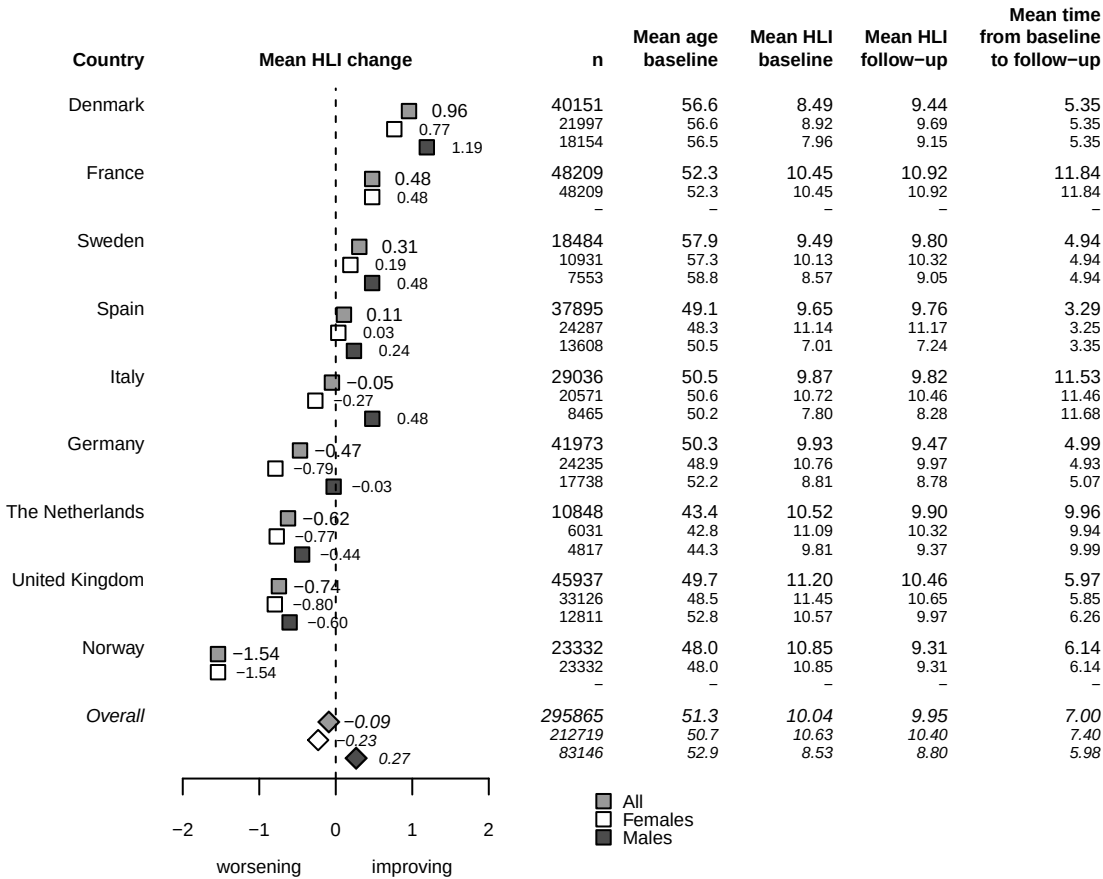

Country
Mean HLI change
n
Mean age baseline
Mean HLI baseline
Mean HLI follow−up
Mean time from baseline to follow−up
Denmark
0.96
0.77
1.19
40151
21997
18154
56.6
56.6
56.5
8.49
8.92
7.96
9.44
9.69
9.15
5.35
5.35
5.35
France
0.48
0.48
48209
48209
–
52.3
52.3
–
10.45
10.45
–
10.92
10.92
–
11.84
11.84
–
Sweden
0.31
0.19
0.48
18484
10931
7553
57.9
57.3
58.8
9.49
10.13
8.57
9.80
10.32
9.05
4.94
4.94
4.94
Spain
0.11
0.03
0.24
37895
24287
13608
49.1
48.3
50.5
9.65
11.14
7.01
9.76
11.17
7.24
3.29
3.25
3.35
Italy
−0.05
−0.27
0.48
29036
20571
8465
50.5
50.6
50.2
9.87
10.72
7.80
9.82
10.46
8.28
11.53
11.46
11.68
Germany
−0.47
−0.79
−0.03
41973
24235
17738
50.3
48.9
52.2
9.93
10.76
8.81
9.47
9.97
8.78
4.99
4.93
5.07
The Netherlands
−0.62
−0.77
−0.44
10848
6031
4817
43.4
42.8
44.3
10.52
11.09
9.81
9.90
10.32
9.37
9.96
9.94
9.99
United Kingdom
−0.74
−0.80
−0.60
45937
33126
12811
49.7
48.5
52.8
11.20
11.45
10.57
10.46
10.65
9.97
5.97
5.85
6.26
Norway
−1.54
−1.54
23332
23332
–
48.0
48.0
–
10.85
10.85
–
9.31
9.31
–
6.14
6.14
–
Overall
−0.09
−0.23
0.27
295865
212719
83146
51.3
50.7
52.9
10.04
10.63
8.53
9.95
10.40
8.80
7.00
7.40
5.98
−2
−1
0
1
2
worsening
improving
All
Females
Males

Figure 3a

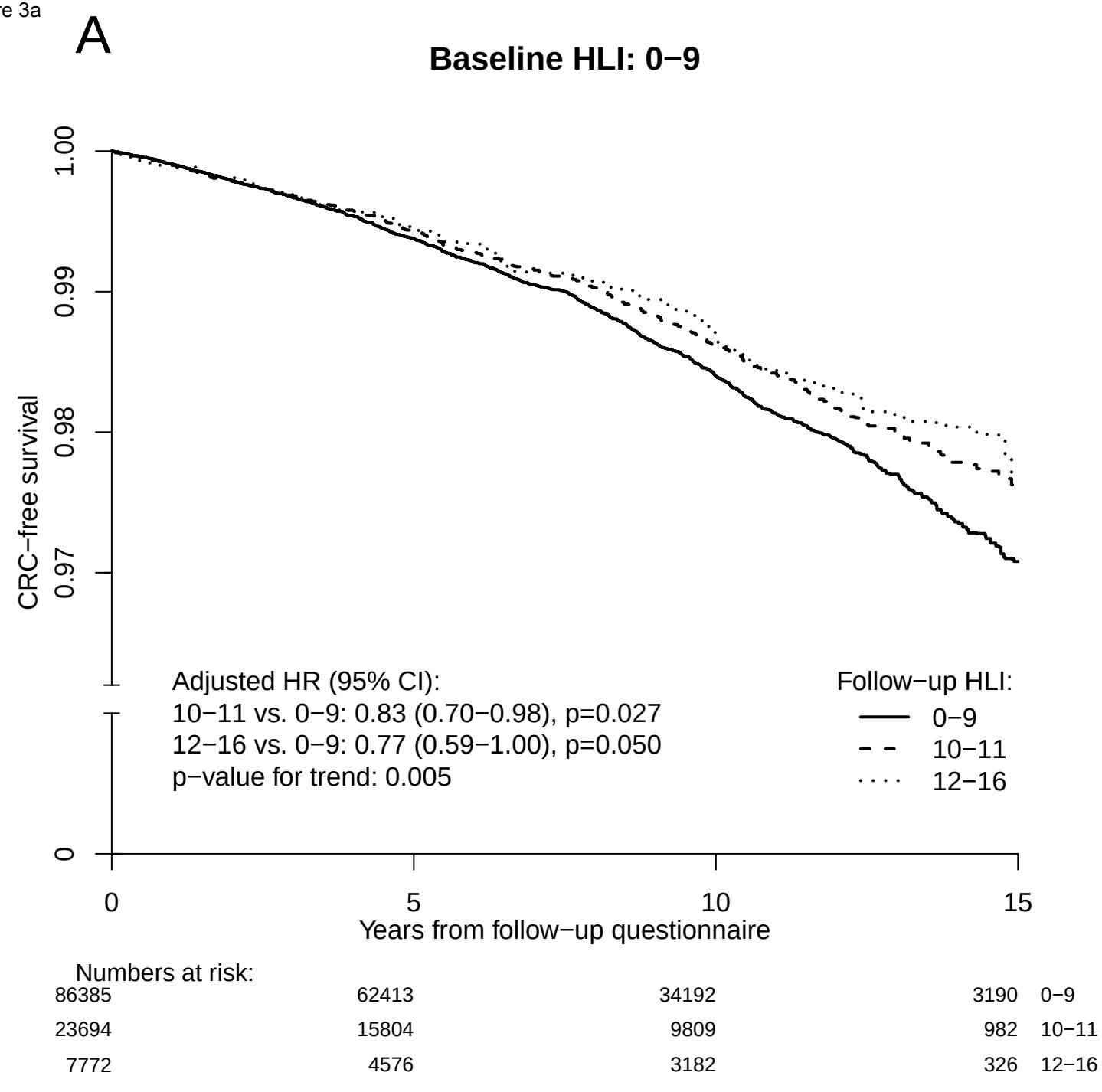

A
Baseline HLI: 0−9
CRC−free survival
1.00
0.99
0.98
0.97
0
Adjusted HR (95% CI):
10−11 vs. 0−9: 0.83 (0.70−0.98), p=0.027
12−16 vs. 0−9: 0.77 (0.59−1.00), p=0.050
p−value for trend: 0.005
Follow−up HLI:
0−9
10−11
12−16
0
5
10
15
Years from follow−up questionnaire
Numbers at risk:
86385 62413 34192 3190 0−9
23694 15804 9809 982 10−11
7772 4576 3182 326 12−16

Figure 3b

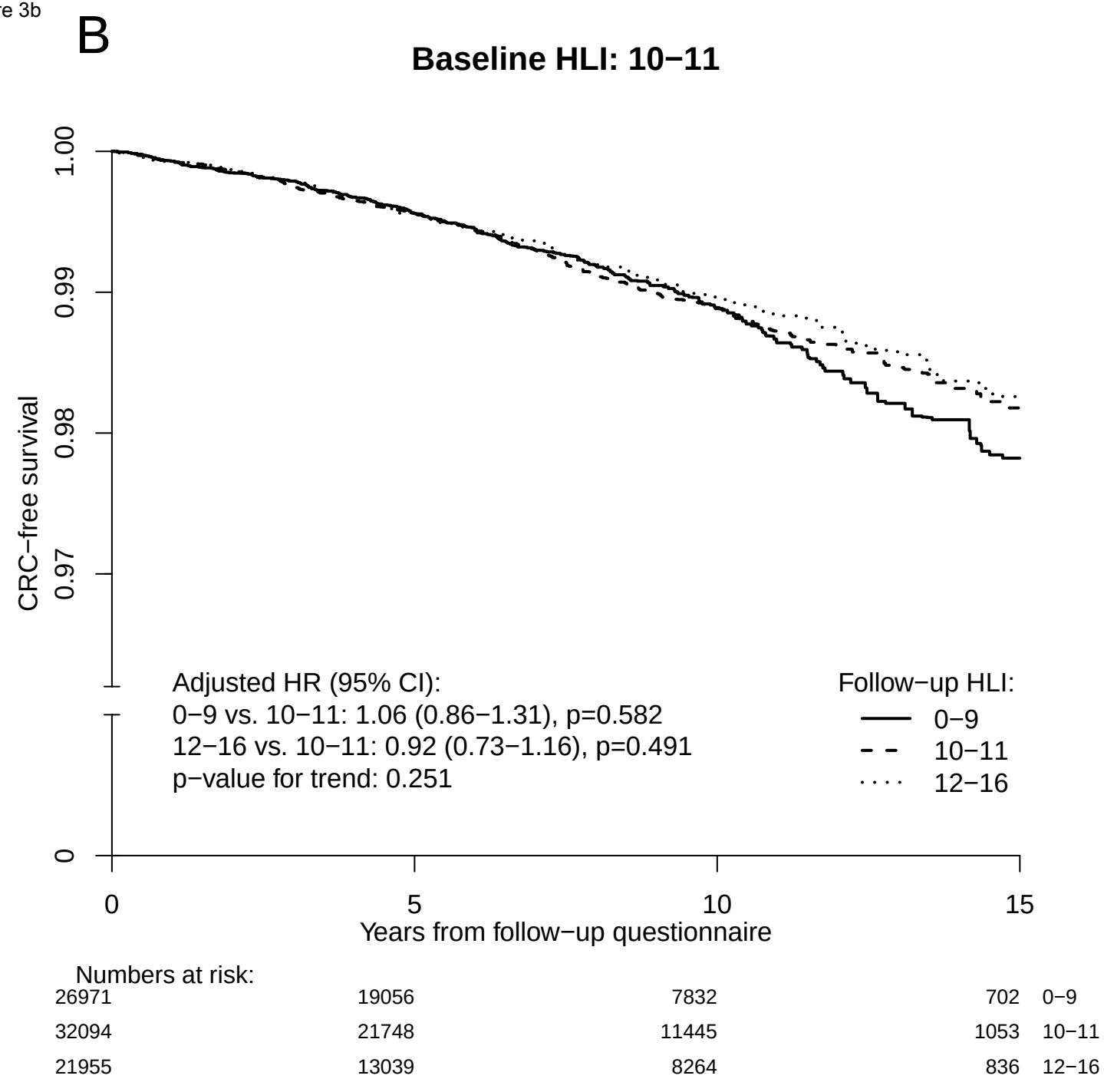
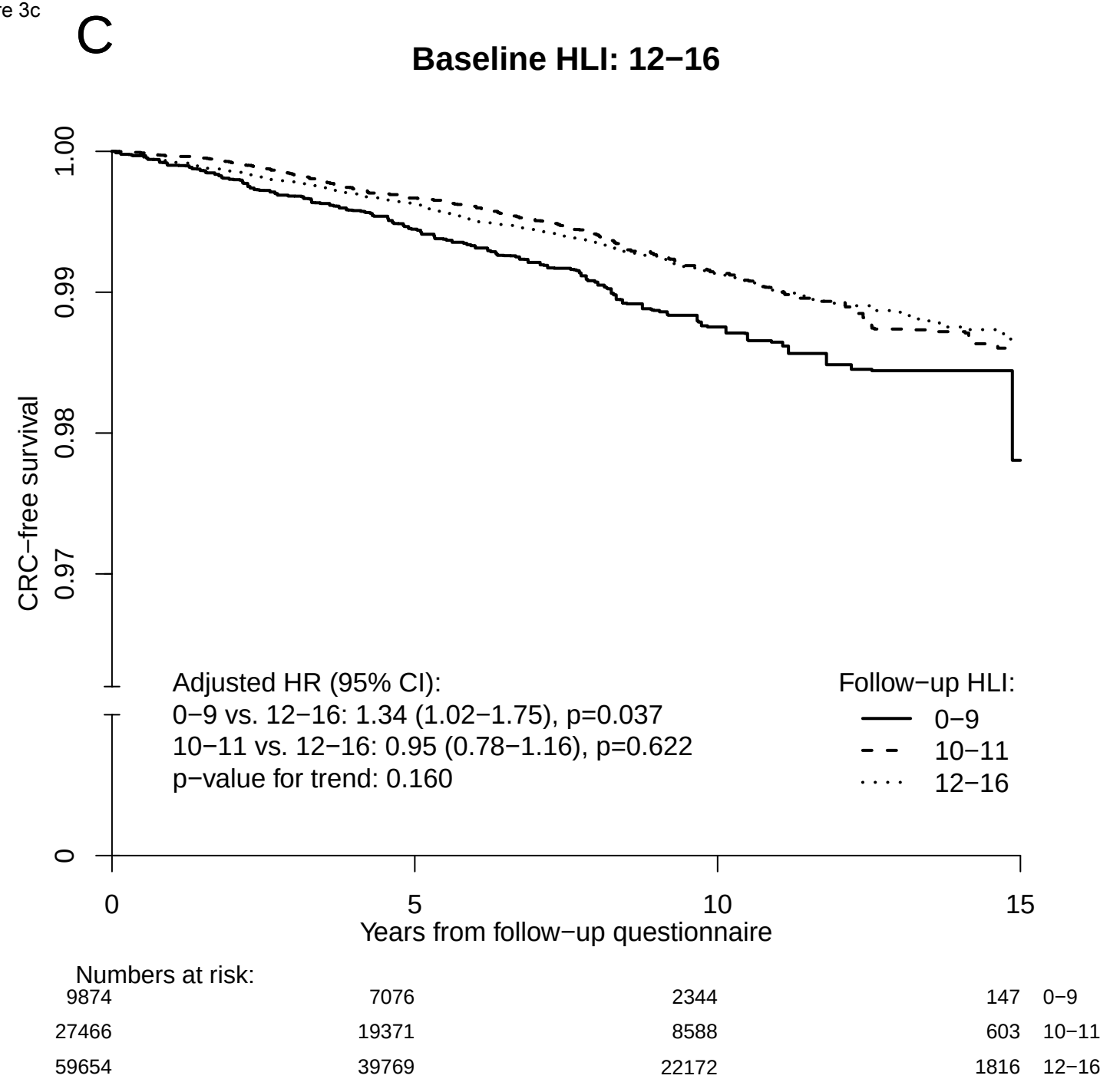

B
Baseline HLI: 10−11
CRC−free survival
1.00
0.99
0.98
0.97
0
Adjusted HR (95% CI):
0−9 vs. 10−11: 1.06 (0.86−1.31), p=0.582
12−16 vs. 10−11: 0.92 (0.73−1.16), p=0.491
p−value for trend: 0.251
Follow−up HLI:
0−9
10−11
12−16
0
5
10
15
Years from follow−up questionnaire
Numbers at risk:
26971 19056 7832 702 0−9
32094 21748 11445 1053 10−11
21955 13039 8264 836 12−16

Figure 3c


C
Baseline HLI: 12−16
1.00
0.99
0.98
0.97
0
CRC−free survival
Adjusted HR (95% CI):
0−9 vs. 12−16: 1.34 (1.02−1.75), p=0.037
10−11 vs. 12−16: 0.95 (0.78−1.16), p=0.622
p−value for trend: 0.160
Follow−up HLI:
0−9
10−11
12−16
0
5
10
15
Years from follow−up questionnaire
Numbers at risk:
9874 7076 2344 147 0−9
27466 19371 8588 603 10−11
59654 39769 22172 1816 12−16

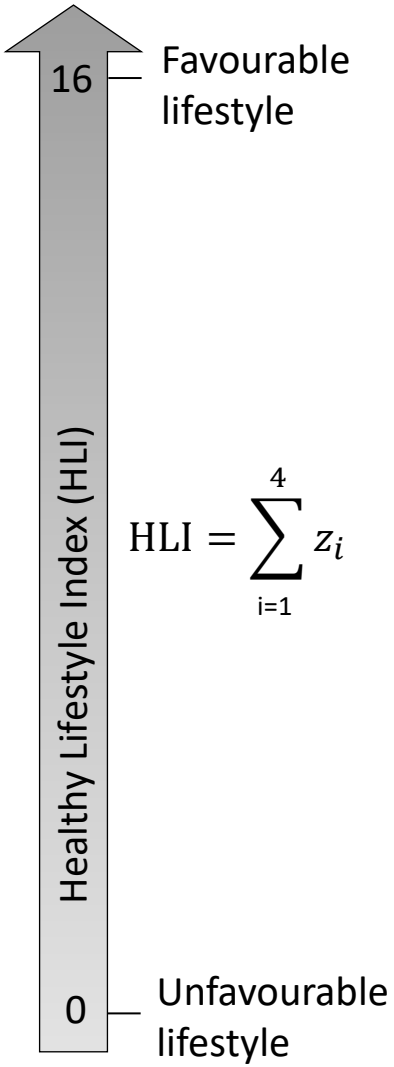
Supplementary Table 1. Complete-case analysis: association between lifestyle changes from baseline to follow-up and risk of colorectal cancer

				By sex		By age at baseline		By site		
			Overall	Males	Females	Age ≤55	Age >55	Proximal	Distal	Rectal
		N (CRC events)	137302 (1219)	47669 (584)	89633 (635)	94563 (533)	42739 (686)	137302 (367)	137302 (361)	137302 (418)
Model 1	Difference in continuous HLI score	1unit increase	**0.95 (0.92-0.99)**	0.96 (0.92-1.00)	**0.95 (0.90-0.99)**	**0.93 (0.89-0.98)**	0.97 (0.93-1.02)	**0.93 (0.87-0.98)**	0.96 (0.90-1.02)	0.98 (0.93-1.04)
Model 2	Difference in categorical HLI score	≤ -3 vs 0	**1.43 (1.17-1.75)**	1.31 (0.97-1.77)	**1.54 (1.17-2.02)**	**1.67 (1.23-2.28)**	1.28 (0.99-1.67)	**1.80 (1.24-2.61)**	**1.70 (1.17-2.48)**	1.06 (0.76-1.49)
		-2 vs 0	0.94 (0.76-1.16)	0.86 (0.62-1.18)	1.01 (0.76-1.35)	1.29 (0.95-1.75)	**0.72 (0.53-0.97)**	1.41 (0.97-2.07)	0.92 (0.61-1.39)	**0.69 (0.48-0.999)**
		-1 vs 0	1.07 (0.89-1.27)	0.94 (0.72-1.23)	1.18 (0.93-1.50)	1.01 (0.77-1.35)	1.09 (0.87-1.38)	**1.47 (1.06-2.06)**	1.04 (0.74-1.47)	0.90 (0.67-1.20)
		1 vs 0	1.05 (0.88-1.26)	0.95 (0.74-1.24)	1.16 (0.90-1.49)	1.17 (0.89-1.54)	0.98 (0.77-1.25)	1.24 (0.87-1.77)	1.15 (0.82-1.60)	0.88 (0.65-1.19)
		2 vs 0	1.01 (0.81-1.26)	1.01 (0.75-1.35)	0.98 (0.70-1.37)	1.00 (0.71-1.40)	1.02 (0.76-1.37)	1.47 (0.99-2.20)	1.20 (0.82-1.77)	0.70 (0.47-1.05)
		≥ 3 vs 0	0.83 (0.65-1.07)	0.74 (0.54-1.03)	0.97 (0.64-1.45)	0.89 (0.61-1.30)	0.80 (0.57-1.12)	0.91 (0.55-1.50)	0.97 (0.63-1.49)	0.74 (0.48-1.13)
Model 3	Difference in continuous smoking score	1unit increase	1.07 (0.93-1.22)	1.08 (0.91-1.28)	1.07 (0.85-1.34)	1.15 (0.96-1.37)	0.99 (0.81-1.22)	1.04 (0.81-1.33)	1.24 (0.98-1.57)	0.95 (0.76-1.20)
	Difference in continuous alcohol score	1unit increase	0.96 (0.89-1.02)	0.96 (0.89-1.05)	0.95 (0.85-1.06)	**0.85 (0.77-0.94)**	1.04 (0.96-1.14)	0.93 (0.82-1.05)	0.95 (0.84-1.07)	0.98 (0.88-1.10)
	Difference in continuous BMI score	1unit increase	0.95 (0.87-1.05)	0.97 (0.83-1.12)	0.94 (0.83-1.06)	1.02 (0.89-1.18)	0.90 (0.79-1.02)	0.98 (0.83-1.16)	0.87 (0.73-1.04)	0.99 (0.84-1.16)
	Difference in continuous physical activity score	1 unit increase	**0.94 (0.89-0.98)**	0.94 (0.88-1.00)	0.94 (0.88-1.00)	**0.92 (0.86-0.99)**	0.95 (0.89-1.01)	**0.88 (0.81-0.96)**	0.96 (0.88-1.05)	0.99 (0.92-1.08)

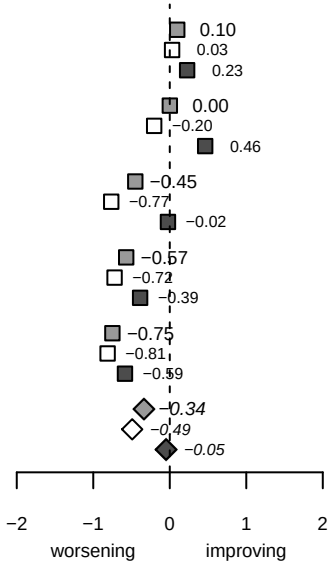
CRC: colorectal cancer. HLI: healthy lifestyle index. BMI: body mass index. Bold font indicates statistical significance (p<0.05). Models 1 and 2 are stratified by study centre, age and sex, and adjusted for education, diet score at baseline, continuous healthy lifestyle index (HLI) score at baseline, and calendar year of follow-up questionnaire. Model 3 is stratified by study centre, age and sex, and adjusted for education, diet score at baseline, continuous index components scores at baseline, and date of follow-up questionnaire; differences for the single index components are mutually adjusted. * p-values for heterogeneity between males and females: difference in continuous HLI score, p=0.698; difference in categorical HLI score, p=0.899; smoking, p=0.952; alcohol, p=0.832; BMI, p=0.739; physical activity, p=0.990. ° p-values for heterogeneity between age groups: continuous HLI score, p=0.230; categorical HLI score, p=0.207; smoking, p=0.298; alcohol p=0.003; BMI, p=0.178; physical activity, p=0.591.

Supplementary Figure 1

Modifiable lifestyle factors (z_i)	Levels of exposure	Level Score
Smoking status	Never	4
	Ex-smokers > 10 years	3
	Ex-smokers ≤ 10 years	2
	Current ≤15 cig/day	1
	Current > 15 cig/day	0
Alcohol intake (in g/day)	< 6	4
	6 - 11.9	3
	12 - 23.9	2
	24 - 59.9	1
	≥ 60	0
BMI (in kg/m^2)	< 22	4
	22 - 23.9	3
	24 - 25.9	2
	26 - 29.9	1
	≥ 30	0
Physical activity (in MET-hours/week)	5th quintile (M: >96 ; W : >134)	4
	4th quintile (M: 67-96 ; W: 96-134)	3
	3rd quintile (M: 47-67 ; W: 69-96)	2
	2nd quintile (M: 30-47 ; W: 45-69)	1
	1st quintile (M: <30 ; W: <45)	0



Supplementary Figure 2



Country	n	Mean age baseline	Mean HLI baseline	Mean HLI follow−up	Mean time from baseline to follow−up
Spain	35890	49.0	9.75	9.85	3.28
	23617	48.3	11.16	11.19	3.25
	12273	50.4	7.04	7.26	3.34
Italy	17840	50.9	10.20	10.21	11.05
	12365	51.1	11.14	10.93	10.91
	5475	50.4	8.10	8.56	11.37
Germany	39262	50.3	9.94	9.49	5.00
	22612	48.9	10.77	10.00	4.95
	16650	52.2	8.82	8.80	5.08
The Netherlands	6139	43.7	10.71	10.14	9.56
	3345	42.9	11.28	10.56	9.51
	2794	44.6	10.03	9.64	9.62
United Kingdom	38171	49.5	11.29	10.54	5.93
	27694	48.3	11.53	10.72	5.82
	10477	52.7	10.65	10.07	6.23
Overall	*137302*	*49.5*	*10.34*	*10.00*	*5.80*
	89633	*48.6*	*11.18*	*10.69*	*5.76*
	47669	*51.2*	*8.75*	*8.70*	*5.87*

Reviewer #1:

1. This paper presents a valuable analysis of the association between key lifestyle factors and colorectal cancer(CRC) using data from the EPIC cohort. The paper is generally well written with nice figures.

   We thank the Reviewer for these positive remarks

2. It would be useful to highlight more that the work is on change…. and more might be made of risk in people who maintain healthy habits (if numbers allowed analysis) who would have scored 0 in terms of change.

   Reply: In line with the Reviewer's view, the title, the abstract, the introduction and the discussion emphasize that the focus of this study is on lifestyle change. Also, to avoid confusion on the exposure of interest, we did not present any result on the association between lifestyle *per se*, collected at one point in time, and the risk of colorectal cancer (CRC).

   As for the second part of the comment, according to our analysis based on tertiles of HLI, people who maintained healthy habits were those with an HLI score of 12 or more at baseline and an HLI score of 12 or more at follow-up (figure 3c, dotted line). We already reported that individuals who reduced their HLI score from 12 or more at baseline to 9 or less at follow-up had a significantly higher risk of CRC compared to those who maintained healthy habits (HR 1.34 (1.02−1.75); figure 3c). Following the reviewer's suggestion, we can now estimate that those who maintained healthy habits had a lower risk of CRC compared to all the other individuals grouped together (HR 0.82 (0.73-0.92)), further supporting our conclusions that having a healthy lifestyle, and maintaining it, is important for CRC prevention. However, all the other individuals grouped together represent a very heterogeneous group, with different baseline HLI scores and different change patterns. So, we would like not to report this estimate in the manuscript, as it does not really add evidence on the impact of lifestyle changes on CRC risk, which is the main interest of the paper.

   Changes to the manuscript: none

3. Excellent international cohort with a wide variety of lifestyle patterns. Good follow up data for the lifestyles presented Excellent life stage to study to support evidence for lifestyle change as they move into retirement.

   We thank again the Reviewer for these positive remarks

4. Similar work has been reported from the Nurses study the novelty of the current work needs to be stressed

   Reply: While previous studies reported on the association between changes in one lifestyle behaviour (e.g. smoking or BMI) and CRC risk, our study examined for the first time the association between multifactorial lifestyle changes and the risk of CRC. As emphasized in the discussion, this represents the main novelty of the study. Following the Reviewer's suggestion, we added an important novelty, which is that we showed that improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. Other studies reported significant associations in one direction only, e.g. that smoking cessation or increasing physical activity were inversely related to CRC risk.
   In looking for additional published evidence, a study that we had previously missed is now briefly described in the discussion in the revised version of our manuscript.

   Changes to the manuscript: the text in the discussion was amended and now reads: "*An important novel result of our study is that lifestyle changes can affect CRC risk in both directions: improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. This is a clear message that practicing clinicians and gastroenterologists could give to their patients and to CRC screening participants to improve CRC prevention.*"

   We also added a reference and a comment:
   "*Similar results indicating that body weight gains in early adulthood, but not late adulthood, was positively associated with CRC risk were found in the Nurses' Health Study and Health Professionals Follow-up (19)*"
   New ref #19 *Song M et al. Adulthood Weight Change and Risk of Colorectal Cancer in the Nurses' Health Study and Health Professionals Follow-up Study. Cancer Prev Res (Phila). 2015 Jul;8(7):620-7.*

5. The lack of data on dietary change is a weakness given the evidence relating this to CRC and some comments should be provided on the potential of dietary changes to influence CRC risk Possible amendments and discussion points

   Reply: We agree with the Reviewer and added a comment in the discussion.

   Changes to the manuscript (in *Italic*): "*Our study has some limitations. We* acknowledge that the lack of data on diet collected during follow-up may have led to inadequately adjusted risk

estimates and residual confounding. *For example, if improvements in diet were associated with both improvements in the HLI score and a decreased CRC risk, then we might have overestimated the association between HLI score and CRC risk*. The collection and harmonization of dietary data at follow-up is currently ongoing in EPIC".

6. All lifestyle variables are weighted equally - it is possible that smoking might be weighted higher- some discussion of the decision for equal weightings should be provided

   Reply: We agree with the Reviewer, and we added a comment in the discussion to clarify our choice.

   Changes to the manuscript: "*For sake of consistency, a scoring system that was used previously in EPIC publications was used in this study. While specific components, for example smoking or obesity, might weight more in the computation of the HLI, this approach has the advantage of ensuring comparability across studies and according to different cancer and other disease outcomes*".

7. Some comments are made re importance of alcohol in younger adults but the youngest here is 55.9 years

   Reply: As stated in the materials "[…] 521,323 participants mostly aged from 35 to 70 years were recruited". In order to clarify this point, we added the mean age of the young group in the results section.

   Changes to the manuscript (changes in *Italic*): "Increases in the alcohol score (i.e., decreases in alcohol consumption) were significantly associated with a lower risk of CRC in participants aged 55 or younger *(mean age 46 years)* at baseline".

8. The mean BMI is lower that that reported by many national studies which reminds us that some indication of representation should be reported.

   Reply: The Reviewer is correct that study participants recruited in some EPIC centres were not representatitive of the general population due to healthy cohort effects. This calls for cautious interpretation of our findings. Nonetheless, while the distribution of healthy behaviors might lack validity on an absolute scale, with an over representation of non-smokers and/or an under-representation of overweight/obese participants, in this study we examined the relationship between adherence to a healthy lifestyle based on a relative scale, i.e. by focusing on lifestyle changes. As a result, our findings on a benefit of adopting healthy choices during adulthood with

respect to CRC risk might have a larger impact in the general population, characterized by less healthy profiles.

Changes to the manuscript: we added in the limitations paragraph that**: "***EPIC participants might not be representative of the general population due to healthy cohort effects, and this warrants cautious interpretation of our findings. However, we can speculate that our findings on the benefit of adopting healthy choices during adulthood might have a larger impact on CRC risk in the general population, characterized by less healthy profiles*".

9. It would be useful to have an analysis by socio-economic position - is it always the wealthiest in society who can make lifestyle changes?

   Reply: In line with the Reviewer's suggestion, participants' education, as a proxy for socio-economic position, was categorized in three levels: 1) none or primary school; 2) technical, professional or secondary; and 3) university or higher. In men, we found that the HLI score change was increasingly larger among participants with higher education, with mean HLI change equal to 0.11 for the lowest education group to 0.20 for the highest. In women, negative HLI changes were observed in all education groups, with smaller HLI score decrease for women with higher education: mean HLI change ranged from -0.29 for the lowest education to -0.18 for the highest, with a significant trend. We added these results in the results section.

   Changes to the manuscript: "*We observed larger mean HLI score changes in men with higher education (HLI change=0.20) compared to men with lower education (0.11); we observed smaller HLI decreases in women with higher education (-0.18), compared to women with lower education (-0.29)*".

10. More should be made of the age at which these changes are happening (i.e., people in their mid 50's to mid 60's) and how this might be used in health promotion/worksite programmes/pre-retirement programmes etc

    Reply: According to the Reviewer's suggestion to examine whether associations were dependent on the age of change, we carried out extra statistical analyses to evaluate HLI change and CRC risk associations by groups defined by the age at follow-up. One one-unit increase in HLI was associated with a 3% lower risk of CRC among participants with age at follow-up below or above 65 years, i.e. approximately the retirement age, with HR equal to 0.97; 95% CI 0.94-1.00 and

equal to 0.97; 95% CI 0.94,1.01, p for heterogeneity 0.884, respectively. These results do not seem to add evidence to the implementation of health promotion programmes at specific ages.

Changes to the manuscript: None

Reviewer #2:

This paper is a well-written original article assessing the prognostic impact of lifestyle-change in patients with colorectal cancer. Preference history, body composition, and physical activity have been reported to be associated with carcinogenesis and cancer progression in various types of cancer. This study is clinically very meaningful in that it is an international, multi-center, large prospective study. In addition, the selection of analysis targets and analysis methods are appropriate.

However, there are some points to be additionally commented or revised.

We thank the Reviewer for these positive remarks and feedback.

Comments;

1. This is a study where when to follow up can have a significant impact on the outcome. A detailed explanation of the criteria for when to follow up is needed.

Reply: The Reviewer raises an important point. There is growing interest in cancer epidemiology towards assessing lifestyle behaviour of study participants repeatedly over time. In this study, however, associations between lifestyle changes and colorectal risk were similar with respect to the age at diagnosis and the age of change, based on the available information at baseline and at follow-up. Therefore, there was no clear evidence on the opportunity of re-assessing exposure at specific ages, before, during or after retirement age, for example, at least in relation to CRC. Also, the collection (and centralization) of lifestyle measurements in EPIC was mainly finalised based on local resources available in each recruitment centre, rather than targeting specific age groups.

Changes to the manuscript: none

2. According to the multi-step carcinogenesis theory of colorectal cancer carcinogenesis, endoscopic resection at the time of benign polyps can reduce carcinogenesis. In this regard, the habit of visiting a medical institution on a daily basis may contribute to the early detection of benign

polyps. For example, patients who are being followed up for other comorbidities may have a better chance of benign polyp detection during routine examinations. If data on the presence of comorbidities are available, this should also be analysed.

Reply:, We agree with the Reviewer's remark that individuals who go more often to the doctor for comorbidities might also be more health conscious and attend CRC screening more often than people without comorbidities. On the other hand, certain comorbidities are associated with lower participation in CRC screening. It is hard to anticipate whether the presence of certain comorbidities, whose information is challenging to collect in observational epidemiological studies, might affect CRC prevention.

Changes to the manuscript: none

3. Author mentioned that reducing alcohol from early adulthood to middle age was associated with a reduced risk of CRC, but not in older adulthood. However, significant bias can exist in these data. Firstly, older people who often developed CRC might refrain from alcohol. Secondly, people who were 55 years old or younger at base line might develop CRC without refraining from alcohol after completing the follow-up questionnaire. In order to reveal the difference between younger people and older, author should show the table of CRC patients compered by generations for alcohol score and follow up times.

Reply: The Reviewer refers to the possibility that our analysis (Table 2, model 3) might be affected by reverse causation, which might have biased association estimates differently in younger and older participants. To test this, we re-ran model 3 after excluding the first two years of follow-up. We obtained results that were similar to the main analysis, with HR=0.92 (0.85,1.00) and 0.98 (0.91,1.05) among younger (≤55 years) and older (>55 years) participants, respectively. These results seem to suggest that preventive measures for reducing alcohol consumption should target younger people more forcefully is still valid.

Changes to the manuscript: none

4. Figure 2 shows that HLI values vary widely from country to country. What factors are associated with these differences?

Reply: As we discussed in the manuscript, countries with the highest HLI score at baseline (e.g., Norway and United Kingdom) showed a decrease in HLI score at follow-up, while countries with the lowest HLI score at baseline (e.g., Denmark and Sweden) displayed an increase in HLI score at follow-up. Also, countries with the highest mean age at baseline (e.g., Denmark, Sweden and France) had the most favourable HLI score changes. We tested if country-specific levels of education (the only

proxy available for socioeconomic status) could explain the differences in the country-specific HLI changes observed, but it was not the case. We edited the discussion as follows.

Changes to the manuscript: "*Unlike baseline age and HLI score, educational levels did not explain the differences in the country-specific HLI changes"*

Reviewer #3:

Botteri et al present a multinational study of the EPIC cohort looking at lifestyle change and CRC risk. I think the study is rigorously done, and the findings and message is pretty simple- worsening of healthy lifestyle factors (tobacco, alcohol, BMI, physical activity) is associated with increased risk while improvement is associated with decreased risk. While there are certainly other papers on this and related topics (including from US data from Nurse's health study) and some limitations of observational design, I think this would be of interest to practicing clinicians, and useful for giving advice to patients after colonoscopy.

We thank the Reviewer for these positive remarks and the following comments

Specific comments:

1) I would like to see the authors include absolute risk differences and incidences-- results and tables are mainly hazard ratios. Otherwise I agree that although it's not the most novel, it is rigorously done and clinically relevant.

Reply: We thank the reviewer for his/her suggestion. CRC incidences were estimated and reported in the results section for 4 indicative groups of study participants: those who maintained a low HLI score (baseline ≤ 9, follow-up ≤ 9) those who increased it (baseline ≤ 9, follow-up ≥ 12), those who maintained a good HLI score (baseline ≥ 12, follow-up ≥ 12) and those who reduced it (baseline ≥ 12, follow-up ≤ 9).

Changes to the manuscript:

The method section was updated with the following description: "*Crude CRC incidence rates were calculated as the number of CRCs divided by the sum of person-years*".

The result section now reads (changes in *italic*): "Among *participants* with a baseline score of HLI≤9 (bottom tertile), those with follow-up score of *HLI≥12* (top tertile) had a lower risk of CRC (Figure 3a; HR 0.77; 95% CI 0.59-1.00) than those with a follow-up score of HLI≤9. *The crude CRC incidence rates in the two groups were 134 and 162 per 100,000 person-years, respectively.* Among *participants* with

a baseline score of *HLI≥12*, those with a follow-up score of HLI≤9 had a higher risk of CRC (Figure 3c; HR 1.34; 95% CI 1.02-1.75) compared to those with follow-up score of *HLI≥12*. *The crude CRC incidence rates in the two groups were 119 and 86 per 100,000 person-years, respectively*".

2) There are numerous authors listed; While this is not necessarily unreasonable for a large multicenter study such as EPIC, the corresponding author should confirm that all met this journal's criteria for authorship.

We confirm that all Authors contributed to the authorship criteria of the American Journal of Gastroenterology.

3) Perhaps the authors could enhance the clinical implications and next steps in the discussion.

Reply We added the following sentence in the discussion section.

Changes to the manuscript: "An important novel result of our study is that lifestyle changes can affect CRC risk in both directions: improving adherence to a healthy lifestyle was inversely associated with CRC risk, while worsening adherence was positively associated with CRC risk. We believe this represents a clear and simple message that practicing clinicians and gastroenterologists could give to their patients or to CRC screening participants to improve CRC prevention."

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9-11
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	9-11
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	11-13
Bias	9	Describe any efforts to address potential sources of bias	11-13
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11-13
		(*b*) Describe any methods used to examine subgroups and interactions	12
		(*c*) Explain how missing data were addressed	12
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	12-13
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14 +Fig1
		(b) Give reasons for non-participation at each stage	Fig1
		(c) Consider use of a flow diagram	Fig1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14 +Tab1
		(b) Indicate number of participants with missing data for each variable of interest	Tab1
		(c) Summarise follow-up time (eg, average and total amount)	14
Outcome data	15*	Report numbers of outcome events or summary measures over time	14-15 +Tab2

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	14-15 +Tab2 Tab2
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14-15 + Tab2 + Suppl Tab1
Discussion			
Key results	18	Summarise key results with reference to study objectives	16-19
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16-19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.