Original Article

The Combined Effects of Healthy Lifestyle Behaviors on All-Cause Mortality: The Golestan Cohort Study

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Abstract

Background: Most studies that have evaluated the association between combined lifestyle factors and mortality outcomes have been conducted in populations of developed countries.

Objectives: The aim of this study was to examine the association between combined lifestyle scores and risk of all-cause and cause-specific mortality for the first time among Iranian adults.

Methods: The study population included 50,045 Iranians, 40 – 75 years of age, who were enrolled in the Golestan Cohort Study, between 2004 and 2008. The lifestyle risk factors used in this study included cigarette smoking, physical inactivity, and Alternative Healthy Eating Index. The lifestyle score ranged from zero (non-healthy) to 3 (most healthy) points. From the study baseline up to analysis, a total of 4691 mortality cases were recorded. Participants with chronic diseases at baseline, outlier reports of calorie intake, missing data, and body mass index of less than 18.5 were excluded from the analyses. Cox regression models were fitted to establish the association between combined lifestyle scores and mortality outcomes.

Results: After implementing the exclusion criteria, data from 40,708 participants were included in analyses. During 8.08 years of follow-up, 3,039 cases of all-cause mortality were recorded. The adjusted hazard ratio of a healthy lifestyle score, compared with non-healthy lifestyle score, was 0.68 (95% CI: 0.54, 0.86) for all-cause mortality, 0.53 (95% CI: 0.37, 0.77) for cardiovascular mortality, and 0.82 (95% CI: 0.53, 1.26) for mortality due to cancer. When we excluded the first two years of follow up from the analysis, the protective association between healthy lifestyle score and cardiovascular death did not change much 0.55 (95% CI: 0.36, 0.84), but the inverse association with all-cause mortality became weaker 0.72 (95% CI: 0.55, 0.94), and the association with cancer mortality was non-significant 0.92 (95% CI: 0.58, 1.48). In the gender-stratified analysis, we found an inverse strong association between adherence to healthy lifestyle and mortality from all causes and cardiovascular disease in either gender, but no significant relationship was seen with mortality from cancer in men or women. Stratified analysis of BMI status revealed an inverse significant association between adherence to healthy lifestyle and mortality from all causes, cardiovascular disease and cancer among non-obese participants.

Conclusion: We found evidence indicating that adherence to a healthy lifestyle, compared to non-healthy lifestyle, was associated with decreased risk of all-cause mortality and mortality from cardiovascular diseases in Iranian adults.

Keyword: Alternative healthy eating index, Golestan cohort study, life style score, mortality

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Introduction

Several studies have shown that unhealthy eating habit, physical inactivity, obesity, alcohol consumption and cigarette smoking are associated with an increased risk of mortality.[1] However, the magnitude of the effects of these behaviors, both individually and in combination, on overall health has yet remained unknown.[2]

Earlier prospective cohort studies have examined the combined effects of lifestyle factors on various outcomes.[2–6] Adherence to

a healthy lifestyle has been linked with a lower risk of mortality. However, there is still no scientific consensus as to how to define a healthy behavior combining all lifestyle risk factors.[1] This is particularly important in terms of incorporating a healthy diet in the score. Most studies that assessed lifestyle score have considered a healthy diet as high intake of fruits and vegetables.[5,7] Some investigations have used healthy eating index to define healthy eating.[8] It must also be kept in mind that most previous studies on lifestyle score and mortality have used data from a food frequency questionnaire to assess long term dietary intakes. We are aware of only one study that has used alternative healthy eating Index (AHEI), as a measure of a healthy diet, to examine the link between a healthy lifestyle and mortality.[2]

Almost all previous studies on the association of healthy lifestyle and mortality came from western nations and there is no report available on this association from developing countries, in particular from Middle Eastern countries, where many components of lifestyle may be different from those in developed countries.[1] In addition, the results of earlier studies have not been controlled for some potential confounding factors, including socioeconomic status.[2,8–12] We therefore constructed a composite score for a healthy lifestyle in a developing country and aimed to examine the association between a healthy lifestyle and mortality from all causes, cardiovascular disease, and cancer in a prospective cohort study in Iranian adults.

Methods

Study population

A total of 50,045 cancer-free subjects, aged 40 – 75 years, residing in Gonbad, Aq-Qala and Kalaleh counties in Golestan Province were enrolled between January 2004 and June 2008 in the Golestan Cohort Study (GCS). The majority of people in the study catchment area were of Turkmen ethnicity. All participants were asked to provide a written informed consent. The ethical review boards of the Digestive Disease Research Institute (DDRI) of Tehran University of Medical Sciences, the US National Cancer Institute (NCI) and the International Agency for Research on Cancer approved the study.[13,14]

In the current analysis, we excluded participants who reported a total daily energy intake of < 800 kcal (n = 271) and > 4200 kcal (n = 219), those with self-reports of cardiovascular disease (n = 3015) or type 2 diabetes (n = 3412) at the study baseline, and those with missing data on required variables (n = 699). We also excluded subjects with a body mass index (BMI) of < 18.5 (n = 2345), because they might have lost weight secondary to preclinical diseases. Finally, 40,708 participants were included in this analysis. Of this population, 3039 cases of death were identified from the study baseline until June 1, 2015. Mean duration of follow up was 8.03 years.

Assessment of exposure

Usual dietary intakes of study participants were assessed with a 116-item validated semi-quantitative food frequency questionnaire (FFQ). Participants were asked to report their usual consumption frequency of a certain serving of foods in the preceding year on a daily, weekly or monthly basis through a face-to-face interview method. Energy and nutrient intakes of each participant were calculated using the United States Department of Agriculture (USDA) food composition database that was modified for Iranian foods[15,16] used in previous studies.[17–19]A previous year-long validation study of this FFQ revealed good correlations between dietary intakes assessed by FFQ and those obtained from 24-days (two recalls in each month of a year) of 24-h dietary recalls in this population. The reliability of the FFQ was assessed by comparing nutrient intakes obtained from the FFQ on two occasions, one year apart. The correlation coefficients for the reliability of the FFQ for dietary vitamin E, β-Carotene and vitamin C were 0.78, 0.84 and 0.83, respectively. The energy-adjusted correlation coefficients between the dietary intakes were obtained from the FFQ and those from the multiple 24-h dietary recalls were 0.65 for vitamin E, 0.68 for β-Carotene and 0.65 for vitamin C. Intra-class correlation coefficients were used to measure reproducibility of FFQ ranged from 0.66 for PUFA to 0.89 for Retinol. Overall, we found that the FFQ is a reasonably valid measure of the average long-term dietary intakes.[17]

Data on smoking, as well as on the starting and stopping ages and amount used in different time periods, which captured changes in use over time. Subjects were considered to be tobacco users if they had ever used cigarettes at least once a week within a 6 month periodor more.[20,21] To assess physical activity, participants were requested to report their physical activity that spent per day in light (e.g. Walking), moderate (e.g. Playing volleyball), and vigorous (e.g. Running) activities.[13,14]

Classification of low risk categories

In the present study the previously designed alternative healthy eating index (AHEI) was applied to examine the adherence of the study population to the healthy diet. The original AHEI was composed of 9 components (fruit, vegetables, nuts and soy, the ratio of white to red meat, cereal fiber, trans fatty acids, the ratio of polyunsaturated fatty acids to saturated fatty acids, long-term multivitamin use, and alcohol consumption).[1,22,23] In the current study, we modified the original AHEI by including only seven of the nine components: multivitamin intake was not included due to the lack of information in the original dataset. We also did not include alcohol intake, because this is not commonly consumed in this population (3.3% of the whole population had reported consuming alcohol). In addition, cereal fiber in the original AHEI was replaced by total dietary fiber in the current study; because data on cereal fiber was not available separately. Furthermore, since consumption of legumes among Iranians is common,[24] legumes were listed in the category of "nuts, soy and legumes". To construct the index, energy-adjusted intakes of the mentioned components was obtained based on residual methods.[25] Then, participants were classified based on the decile categories of energy-adjusted intakes of these components. Individuals in the highest decile of fruits, vegetables, nuts and legumes, the ratio of white to red meat, dietary fiber, as well as the ratio of polyunsaturated fatty acids to saturated fatty acids were given the score of 10 and those in the lowest decile of these items were given the score of 1. Individuals in the second, third and other deciles of these components were given the corresponding score. Regarding trans fatty acids, for which we used hydrogenated fats instead, the lowest decile was given a score of 10 and the highest decile was given the score of 1. Those in other deciles were given reverse score. To compute the AHEI, the scores for individual items were added, resulting in a minimum score of 7 and a maximum score of 68. Participants in the highest 40% of AHEI (upper two fifths) were considered to have a healthy diet.[23]

For physical activity, the low risk group was defined as those who had reported the average of at least 30 minutes a day of physical activity of moderate and vigorous intensity (requiring > 3 metabolic equivalents an hour, including volleyball, horse running). Current cigarette smoking was considered for smoking. The low risk group was defined as those who had never smoked. To calculate the life style score, we added scores of AHEI, physical activity, and smoking for individual items, resulting in a minimum score of 0 and a maximum score of 3.

Assessment of outcome

Mortality ascertainment details have been given previously.[20] Briefly, all study participants were actively followed by annual telephone calls. Detailed questions were asked regarding their health status and hospitalization or outpatient proceedings. Following seven non-successful attempts during a two-week period, researchers contacted friends or local health workers of participants who were inaccessible through the follow up. Our follow up method has been shown to be 99% complete for this cohort. For more acquisition of a reported death, a general practitioner from the follow-up team visited the home of the dead person and completed a validated verbal autopsy questionnaire by interviewing the closest relative of the dead person. Follow-up team gathered all available and relevant medical documents from hospitals or pathology centers, either within the province or neighboring provinces. In addition to the verbal autopsy, extensive medical documents were retrieved. Previous studies demonstrated great accuracy (all measures of accuracy above 81%) and reliability ($\kappa > 0.75$), for this verbal autopsy. Two separate internists independently reviewed all documents, and the cause of death was distinguished with ICD-10 codes (international classification of diseases, 10th revision) due to circulatory, cancer, respiratory, digestive, infectious, external (mainly from motor vehicle crashes or other unintentional injury and suicide), unknown, or other. For concordant diagnosis results, a third, more experienced internist reviewed all documents and the two initial diagnoses and made the final diagnosis.[13,20]

Assessment of covariates

A structured general questionnaire was used by trained interviewers to collect data on age (continuous), gender (male, female), residence area (urban, rural), education (illiterate, literate), house area (continuous), marital status (married, single, other), ethnicity (Turkmen, Non Turkmen), alcohol consumption (yes, no), and opium use (yes, no). Data on anthropometric measurements, including height, weight, waist and hip circumferences were also measured according to standard protocols. BMI was also calculated. To assess the wealth score, information on ownership of car or motorbike, black and white TV, color TV, refrigerator, freezer, vacuum cleaner, and washing machine, as well as having a bath in the home were recorded. All these information has been previously used to estimate socioeconomic status in the Golestan Province.[26]

Statistical analysis

To ensure normal distribution of variables, histogram and Kolmogorov-Smirnov statistical test were applied. The log-transformation was applied for non-normally distributed variables. Comparison of continuous variables across categories of lifestyle scores was performed using one-way ANOVA. Distribution of subjects in terms of categorical variables across categories of lifestyle score was assessed using a Chi-square test. Proportional hazards assumptions were evaluated by testing the significance of time-dependent interaction terms for all variables. Hazard ratios (HRs) and 95% confidence intervals were computed using Cox proportional hazards regression to estimate unadjusted and adjusted hazard ratios and 95% confidence intervals for all-cause and specific-cause deaths in relation to the assumed exposures. Follow-up time was used as the underlying time metric. Different regression models were constructed controlling for age (continuous), sex (categorical), residential area (urban/rural), score of a wealth score (continuous), ethnicity (Turkmen, non-Turkmen), and BMI (>=30, < 30). The linear trend of HRs across increasing lifestyle score was examined by considering the lifestyle score as an ordinal variable in the analysis. In all regression models, the lowest lifestyle score was considered as a reference. In a sensitivity analysis, the first two years of follow-up were excluded from the analysis. In addition, stratified analyses were done by gender and BMI status.

Results

During a median of 8.08 years of follow-up and 326711 person years, 3039 deaths were documented from all causes, including 797 from cancer, 1407 from cardiovascular, and 835 from non-cancer and cardiovascular. Survival Curve was shown as Figure 1, determined by lifestyle score from without healthy behavior as zero up to all components have healthy behavior with score of 3. Table 1 shows the baseline characteristics of the study participants (n = 40708) according to lifestyle score. Individuals with greater healthy lifestyle score (HLS) were younger, more likely to be men, obese, educated and had a higher energy intake, BMI and waist circumference compared with those with the lowest score. Cigarette smoking and alcohol use were highly common among those in the lowest HLS than subjects with the highest HLS. Distribution of Turkmen participants was higher among those with the lowest HLS than those with the highest HLS.

Multivariable-adjusted relative risks of mortality from all causes, cardiovascular disease, and cancer during the 8.08 years of follow-up according to the HLS are provided in Table 2. Adherence to healthy lifestyle was associated with a lower risk of mortality from all causes, cardiovascular disease and cancer. After controlling for a wide range of confounders, including BMI, those with the highest score of HLS, had 32% lower risk of mortality from all causes (RR: 0.68; 95% CI:0.54, 0.86), 47% lower risk of mortality from cardiovascular disease (0.53; 95% CI: 0.37, 0.77), and 18% lower risk of mortality from cancer (0.82; 95% CI: 0.53; 1.26). When participants that recruited in the first two years of their follow up (n = 604) were excluded in our sensitivity analysis, the protective association between healthy lifestyle score and all causes mortality became weaker (0.72; 95% CI: 0.55, 0.94). The inverse association for mortality from cardiovascular did not alter much (0.55; 95% CI: 0.36, 0.84), whereas the association with cancer mortality became non-significant (0.92; 95% CI: 0.58, 1.48). Results are presented in Table 3.

Multivariable-adjusted RRs for mortality across categories of HLS, separately by gender, are indicated in Table 4. We found an inverse strong association between adherence to healthy lifestyle and mortality from all causes and cardiovascular disease in both sexes. After controlling for potential confounders including BMI, men with the greatest HLS were 32% less likely to have mortality

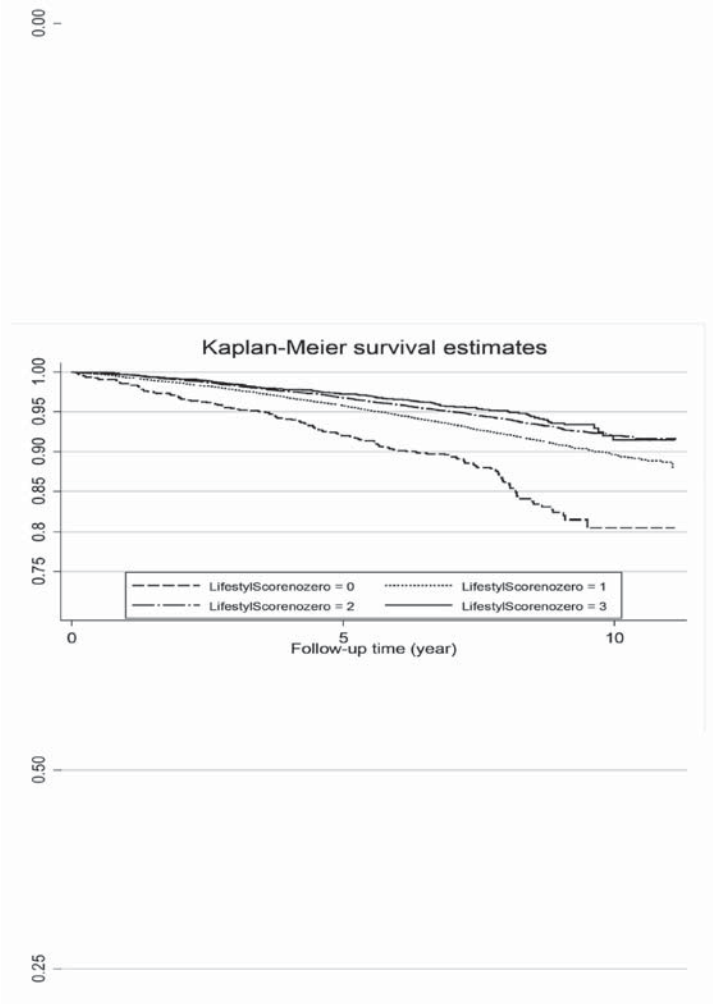

Figure 1. Survival Curve determined by lifestyle score from without healthy behavior as zero up to all components have healthy behavior with score of 3.

Table 1. Baseline characteristics of study (n = 40708) according to lifestyle score*

Characteristics	Lifestyle score				*P*-Value
	0 (n = 1776)	1 (n = 21558)	2 (n = 15494)	3 (n = 1880)	
Continuous					
Age, years	51.9 (8.7)	52.0 (9.0)	51.1 (8.7)	49.5 (7.4)	< 0.001
Energy intake, Kcal	2190(511)	2041 (535)	2349 (551)	2501(571)	< 0.001
BMI, Kg/m^2	24.2 (4.1)	26.9 (5.2)	27.5 (5.8)	25.9(4.2)	< 0.001
Waist circumference, Cm	91.9 (12.0)	95.5 (13.0)	96.9 (12.7)	94.2 (12.0)	< 0.001
Waist to hip ratio	0.94 (0.07)	0.96 (0.08)	0.96 (0.08)	0.95 (0.07)	< 0.001
Wealth Score	13.6 (7.7)	13.6 (7.8)	15.8 (9.0)	12.5 (7.2)	< 0.001
Categorical					
Male	1633 (91.9)	7382 (34.2)	6910 (44.6)	1479 (78.7)	< 0.001
Turkmen (ethnicity)	1575 (88.7)	18095 (83.9)	10198 (65.8)	905 (48.1)	< 0.001
Rural dweller	1527 (86.0)	18253 (84.7)	11164 (72.1)	1607 (85.5)	< 0.001
Illiterate	917 (51.6)	16533 (76.7)	9522 (61.5)	1076 (57.2)	< 0.001
Alcohol drinker	226 (12.7)	584 (2.7)	469 (3.0)	47 (2.5)	< 0.001
Opium user	966 (54.4)	3175 (14.7)	1784 (11.5)	272 (14.5)	< 0.001
Current smoker	1776 (100.0)	2037 (9.4)	510 (3.3)	0 (0.0)	< 0.001
Married	1694 (95.4)	18717 (86.8)	13893 (89.7)	1796 (95.5)	< 0.001
BMI > 30 Kg/m^2	166 (9.3)	5445 (25.3)	4484 (28.9)	320 (17.0)	< 0.001
Physical activity > 30 minute	0 (0.0)	553 (2.6)	2725 (17.6)	1880 (100.0)	< 0.001

Values are mean (SD) for continuous variables and number (%) for categorical variables.

Table 2. Risk of mortality during 8.08 years of follow-up according to lifestyle score

Outcome	Lifestyle score				*P*-Trend
	0 (n = 1776)	1 (n = 21558)	2 (n = 15494)	3 (n = 1880)	
All-cause mortality					
Cases	234 (13.2%)	1730 (8.0%)	973 (6.3%)	102 (5.4%)	
Person years	1099	8219	4743	499	
Unadjusted	1.00	0.59 (0.52 0.68)	0.45 (0.39 0.52)	0.40 (0.32 0.51)	< 0.001
Model 1	1.00	0.69 (0.60 0.79)	0.55 (0.47 0.63)	0.53 (0.42 0.67)	< 0.001
Model 2	1.00	0.83 (0.72 0.96)	0.72 (0.62 0.84)	0.68 (0.54 0.86)	0.002
Model 3	1.00	0.82 (0.72 0.56)	0.72 (0.62 0.83)	0.68 (0.54 0.86)	0.002
Cardiovascular mortality					
Cases	110 (6.2%)	795 (3.7%)	464 (3.0%)	38 (2.0%)	
Person years	470	3704	2231	179	
Unadjusted	1.00	0.58 (0.48 0.71)	0.46 (0.37 0.57)	0.32 (0.22 0.46)	< 0.001
Model 1	1.00	0.65 (0.53 0.79)	0.54 (0.44 0.66)	0.43 (0.30 0.62)	< 0.001
Model 2	1.00	0.76 (0.62 0.95)	0.69 (0.55 0.86)	0.54 (0.37 0.78)	< 0.001
Model 3	1.00	0.76 (0.61 0.93)	0.67 (0.54 0.84)	0.53 (0.37 0.77)	< 0.001
Cancer mortality					
Cases	60 (3.4%)	464 (2.2%)	239 (1.5%)	34 (1.8%)	
Person years	289	2156	1186	176	
Unadjusted	1.00	0.62 (0.47 0.81)	0.43 (0.33 0.57)	0.52 (0.34 0.79)	< 0.001
Model 1	1.00	0.72 (0.54 0.95)	0.52 (0.38 0.69)	0.67 (0.44 1.02)	< 0.001
Model 2	1.00	0.80 (0.61 1.06)	0.64 (0.47 0.86)	0.81 (0.52 1.25)	0.04
Model 3	1.00	0.81 (0.61 1.07)	0.66 (0.48 0.88)	0.82 (0.53 1.26)	0.04

Model 1: adjusted for age and gender; Model 2: additionally adjusted for resident area, ethnicity, socioeconomic status (Wealth Score), marital status, education, alcohol consumption, and opium use; Model 3: Further controlled for BMI (obese and non-obese)

Table 3. Sensitivity analysis, Risk of mortality during 6.09 years of follow-up according to the lifestyle score

Outcome	Lifestyle score				*P*-Trend
	0 (n = 1722)	1 (n = 21222)	2 (n = 15300)	3 (n = 1860)	
All-cause mortality					
Cases	185	1444	825	85	
Person years	1050	7919	4577	478	
Unadjusted	1.00	0.62 (0.54–0.73)	0.48 (0.41–0.56)	0.42 (0.33–0.55)	< 0.001
Model 1	1.00	0.72 (0.62–0.84)	0.58 (0.49–0.68)	0.56 (0.43–0.72)	< 0.001
Model 2	1.00	0.86 (0.73–1.01)	0.76 (0.64–0.90)	0.72 (0.55–0.94)	< 0.001
Model 3	1.00	0.86 (0.74–1.01)	0.76 (0.64–0.90)	0.72 (0.55–0.94)	< 0.001
Cardiovascular mortality					
Cases	84	662	388	30	
Person years	445	3565	2146	166	
Unadjusted	1.00	0.63 (0.50–0.79)	0.49 (0.39–0.63)	0.33 (0.22–0.50)	< 0.001
Model 1	1.00	0.70 (0.55–0.88)	0.57 (0.45–0.73)	0.44 (0.29–0.67)	< 0.001
Model 2	1.00	0.82 (0.65–1.05)	0.74 (0.58–0.95)	0.56 (0.36–0.85)	0.002
Model 3	1.00	0.81 (0.64–1.03)	0.72 (0.56–0.93)	0.55 (0.36–0.84)	0.001
Cancer mortality					
Cases	47	385	207	31	
Person years	272	2071	1153	173	
Unadjusted	1.00	0.65 (0.48–0.88)	0.48 (0.35–0.65)	0.61 (0.39–0.96)	< 0.001
Model 1	1.00	0.76 (0.56–1.04)	0.56 (0.41–0.78)	0.77 (0.49–1.22)	0.001
Model 2	1.00	0.83 (0.60–1.14)	0.69 (0.49–0.96)	0.91 (0.57–1.46)	0.07
Model 3	1.00	0.83 (0.61–1.15)	0.70 (0.58–1.48)	0.92 (0.58–1.48)	0.09

Model 1: adjusted for age and gender; Model 2: additionally adjusted for resident area, ethnicity, socioeconomic status (Wealth Score), marital status, education, alcohol consumption, opium use; Model 3: Further controlled for BMI (obese and non-obese)

Table 4. Risk of mortality during 8.08 years of follow–up according to the lifestyle score by Gender

Outcome	Men (17404)					Women (23304)				
	Lifestyle score				*P*–Trend	Lifestyle score				*P*–Trend
	0 (n = 1633)	1 (n = 7382)	2 (n = 6910)	3 (n = 1479)		0 (n = 143)	1 (n = 14176)	2 (n = 8584)	3 (n = 401)	
All–cause mortality										
Cases	202	875	608	86		32	855	365	16	
Person years	927	4123	2960	421		171	4096	1784	78	
Unadjusted	1.00	0.95 (0.82–1.11)	0.69 (0.59–0.80)	0.46 (0.36–0.59)	< 0.001	1.00	0.25 (0.17–0.35)	0.17 (0.12–0.24)	0.17 (0.09–0.32)	< 0.001
Model 1	1.00	0.67 (0.66–0.89)	0.63 (0.53–0.73)	0.54 (0.42–0.69)	< 0.001	1.00	0.29 (0.20–0.41)	0.21 (0.15–0.31)	0.35 (0.19–0.63)	< 0.001
Model 2	1.00	0.88 (0.76–1.00)	0.79 (0.67–0.93)	0.68 (0.52–0.88)	< 0.001	1.00	0.44 (0.31–0.63)	0.36 (0.25–0.53)	0.49 (0.27–0.91)	< 0.001
Model 3	1.00	0.88 (0.75–1.00)	0.79 (0.67–0.93)	0.68 (0.52–0.88)	< 0.001	1.00	0.44 (0.31–0.63)	0.36 (0.25–0.53)	0.49 (0.27–0.91)	< 0.001
Cardiovascular mortality										
Cases	96	389	280	32		14	406	184	6	
Person years	394	1796	1318	146		77	1908	913	33	
Unadjusted	1.00	0.89 (0.12–1.11)	0.67 (0.53–0.84)	0.35 (0.24–0.54)	< 0.001	1.00	0.27 (0.16–0.46)	0.19 (0.11–0.33)	0.15 (0.06–0.38)	< 0.001
Model 1	1.00	0.71 (0.57–0.89)	0.60 (0.48–0.76)	0.42 (0.29–0.64)	< 0.001	1.00	0.32 (0.19–0.54)	0.26 (0.15–0.44)	0.33 (0.13–0.85)	0.001
Model 2	1.00	0.79 (0.64–1.01)	0.73 (0.57–0.93)	0.52 (0.34–0.78)	0.001	1.00	0.49 (0.29–0.85)	0.44 (0.25–0.77)	0.45 (0.17–0.94)	0.01
Model 3	1.00	0.79 (0.63–0.98)	0.71 (0.56–0.91)	0.51 (0.34–0.77)	0.001	1.00	0.49 (0.28–0.85)	0.44 (0.25–0.76)	0.45 (0.17–0.93)	0.01
Cancer mortality										
Cases	51	239	133	29		9	225	106	5	
Person years	245	1113	671	156		44	1043	515	21	
Unadjusted	1.00	1.03 (0.76–1.39)	0.59 (0.43–0.82)	0.61 (0.39–0.96)	< 0.001	1.00	0.23 (0.12–0.45)	0.17 (0.09–0.57)	0.19 (0.06–0.57)	0.001
Model 1	1.00	0.84 (0.62–1.13)	0.54 (0.39–0.75)	0.72 (0.45–1.83)	< 0.001	1.00	0.26 (0.14–0.51)	0.21 (0.11–0.42)	0.34 (0.11–0.99)	0.02
Model 2	1.00	0.95 (0.70–1.29)	0.69 (0.49–0.96)	0.87 (0.55–1.40)	0.02	1.00	0.28 (0.14–0.56)	0.25 (0.12–0.51)	0.40 (0.13–1.22)	0.10
Model 3	1.00	0.96 (0.71–1.31)	0.70 (0.50–0.98)	0.88 (0.55–1.42)	0.03	1.00	0.29 (0.14–0.58)	0.26 (0.13–0.53)	0.41 (0.14–1.25)	0.16

Model 1: adjusted for age and gender; Model 2: additionally adjusted for resident area, ethnicity, socioeconomic status (Wealth Score), marital status, education, alcohol consumption, and opium use; Model 3: Further controlled for BMI (obese and non-obese)

Table 5. Risk of mortality during 8.08 years of follow–up according to the lifestyle score by BMI

Outcome	BMI less than 30					BMI more than 30				
	Lifestyle score				*P*–Trend	Lifestyle score				*P*–Trend
	0 (n = 1610)	1 (n = 16113)	2 (n = 11010)	3 (n = 1560)		0 (n=166)	1 (n = 5445)	2 (n = 4484)	3 (n = 320)	
All–cause mortality										
Cases	217	1409	762	87		17	321	211	15	
Person years	1034	6631	3715	412		65	1589	1029	87	
Unadjusted	1.00	0.64 (0.55–0.73)	0.49 (0.42–0.57)	0.41 (0.32–0.52)	< 0.001	1.00	0.55 (0.34–0.90)	0.42 (0.26–0.69)	0.44 (0.22–0.88)	< 0.001
Model 1	1.00	0.68 (0.59–0.79)	0.55 (0.47–0.64)	0.52 (0.40–0.67)	< 0.001	1.00	0.78 (0.48–1.29)	0.60 (0.36–0.99)	0.65 (0.33–1.32)	0.002
Model 2	1.00	0.82 (0.71–0.95)	0.71 (0.60–0.83)	0.66 (0.51–0.86)	< 0.001	1.00	0.84 (0.51–1.38)	0.72 (0.43–1.19)	0.76 (0.38–1.54)	0.08
Cardiovascular mortality										
Cases	100	642	353	31		10	153	111	7	
Person years	432	2958	1680	139		38	745	551	41	
Unadjusted	1.00	0.63 (0.51–0.78)	0.49 (0.39–0.62)	0.31 (0.21–0.47)	< 0.001	1.00	0.45 (0.24–0.85)	0.38 (0.20–0.72)	0.35 (0.13–0.92)	0.03
Model 1	1.00	0.64 (0.51–0.79)	0.53 (0.42–0.66)	0.41 (0.27–0.61)	< 0.001	1.00	0.67 (0.34–1.28)	0.56 (0.29–1.08)	0.52 (0.20–1.38)	0.06
Model 2	1.00	0.75 (0.60–0.94)	0.67 (0.53–0.85)	0.51 (0.34–0.77)	< 0.001	1.00	0.73 (0.38–1.42)	0.68 (0.35–1.32)	0.64 (0.24–1.69)	0.30
Cancer mortality										
Cases	57	384	181	28		3	80	58	6	
Person years	277	1756	893	141		12	400	294	35	
Unadjusted	1.00	0.65 (0.49–0.57)	0.45 (0.33–0.60)	0.49 (0.32–0.78)	< 0.001	1.00	0.78 (0.25–2.46)	0.66 (0.21–2.10)	1.00 (0.25–4.00)	0.54
Model 1	1.00	0.71 (0.53–0.95)	0.49 (0.36–0.67)	0.62 (0.39–0.87)	< 0.001	1.00	0.86 (0.26–2.87)	0.86 (0.26–2.78)	1.40 (0.35–5.70)	0.74
Model 2	1.00	0.79 (0.60–0.98)	0.61 (0.45–0.84)	0.71 (0.47–0.97)	0.003	1.00	0.92 (0.28–3.02)	0.92 (0.28–3.02)	1.42 (0.35–5.70)	0.87

Model 1: adjusted for age and gender; Model 2: additionally adjusted for resident area, ethnicity, socioeconomic status (Wealth Score), marital status, education, alcohol consumption, and opium use

Table 6. Hazard ratio (95% confidence intervals) of all cause, cardiovascular, and cancer mortality during 8.08 years follow-up

Variable		Death from any cause (Relative risk)	Cardiovascular death (Relative risk)	Cancer death (Relative risk)
Ever *V* never smoking	Unadjusted	1.62 (1.47–1.78)	1.64 (1.43–1.90)	1.50 (1.23–1.82)
	Adjusted	1.29 (1.15–1.43)	1.43 (1.22–1.68)	1.27 (1.10–1.58)
BMI ≥ 25 *V* 18.5–24.9	Unadjusted	1.54 (1.43–1.67)	1.24 (1.12–1.38)	1.81 (1.57–2.10)
	Adjusted	1.10 (1.00–1.12)	1.31 (1.17–1.46)	1.42 (1.22–1.71)
Healthy diet score in lower three fifth *V* upper two fifth	Unadjusted	1.19 (1.11–1.28)	1.11 (1.00–1.23)	1.34 (1.16–1.55)
	Adjusted	1.10 (1.02–1.20)	1.02 (0.91–1.14)	1.17 (1.06–1.37)
Physical activity < 30 min/day *V* ≥ 30 min/day	Unadjusted	1.36 (1.20–1.53)	1.75 (1.44–2.14)	1.14 (0.92–1.42)
	Adjusted	1.52 (1.34–1.73)	2.00 (1.59–2.54)	1.26 (1.02–1.50)
Age ≥ 50 *V* Age < 50	Unadjusted	4.37 (4.01–4.77)	5.03 (4.40–5.75)	4.15 (3.51–4.90)
	Adjusted	3.49 (3.18–3.82)	4.03 (3.50–4.63)	3.50 (2.93–4.17)

Relative risks adjusted for all potential risk factors (Gender, Age, resident area, marital status, socioeconomic status (Wealth Score), ethnicity, education level, alcohol, smoking, opium user, AHEI, and physical activity)

from all causes (RR: 0.68; 95% CI: 0.52, 0.88) and 49% lower risk of mortality from cardiovascular disease (0.51; 95% CI: 0.37, 0.71). The corresponding figures in women were 0.49 (95% CI: 0.27, 0.91) and 0.45 (95% CI: 0.17, 0.93), respectively. There was no significant association between adherence to healthy lifestyle and mortality from cancer in either gender.

Stratified analysis by BMI status revealed an inverse significant association between adherence to healthy lifestyle and mortality from all causes (in non-obese subjects: 0.66; 95% CI: 0.51, 0.86 and in obese participants: 0.76; 95% CI: 0.38, 1.54) and cardiovascular disease (in non-obese subjects: 0.51; 95% CI: 0.34, 0.77 and in obese participants: 0.64; 95% CI: 0.24, 1.69) among obese and non-obese participants (Table 5). The association between HLS and mortality from cancer was significant in non-obese participants (0.71; 95% CI: 0.47, 0.97), but it was not significant in obese subjects (1.42; 95% CI: 0.35, 5.70). Table 6 shows the multivariate adjusted relative risk for the high risk compared with the low risk stratified of lifestyle factors. Cigarette smoking could increase death from cancer more than other causes after adjusting for all potential confounders. Cardiovascular death was more common among obese individuals versus thin people. Healthy diet was more effective in cancer death in comparison to non-healthy diet. The high physical activity level was more effective in cardiovascular death compared to low physical activity.

Discussion

In the current study, during 8.08 years of follow among 40708 middle age Iranian men and women, an inverse association was found between adherence to healthy lifestyle and risk of mortality. Based on our knowledge, this is the first study examining the association between combined healthy lifestyle factors and mortality in a setting of a developing country. It must be kept in mind that opium and Hookah are risk factors for mortality. However, the lifestyle does not include these variables in its worldwide definition, we preferred not to include these variables in our definition of lifestyle to make our findings comparable with other investigations. When lifestyle score, by including hookah and opium usewas re-defined, our findings did not change significantly.

With rising age of the population and increased number of elderly people, identifying the cause of death is very important. Several studies have assessed contributing factors to mortality worldwide, among them individual lifestyle related factors have been assessed extensively. However, limited data are available linking a combination of lifestyle behaviors to mortality, in particular in developing nations. We found that healthy lifestyle was associated with a lower risk of mortality. In the framework of Nurses' Health Study, Van Den Brandt, et al. found that adherence to the healthy lifestyle was inversely related to the risk of mortality in either gender. When the least-healthy lifestyle was compared to the healthiest lifestyle, the HRs of 4.07 and 2.61 for mortality were found in women and men, respectively.[27] In addition, Van Dom, et al. found that the relative risks of mortality among those with five lifestyle score compared with zero lifestyle score were 3.26 for cancer mortality, 8.17 for cardiovascular mortality, and 4.31 for all-cause mortality.[2] In an EPIC Potsdam study, Ford, et al. demonstrated that healthy lifestyle was associated with reduced risk of chronic diseases. The hazard ratio for developing a chronic disease decreased progressively as the number of healthy lifestyle factures increased. Participants with all 4 lifestyle-related factors at baseline had a 78% lower risk of developing a persistent disease (diabetes, 93%; myocardial infarction, 81%; stroke, 50%; and cancer, 36%) than participants without a healthy factor.[11] The same findings were also reported from National Health and Nutrition Examination Survey III Mortality Study; where the number of low-risk behaviors was inversely related to the mortality risk. Compared with contributors who had no low-risk behaviors, those who had all 4, resulted in reduced all-cause mortality (adjusted hazard ratio 0.37) and mortality from malignant neoplasm's (AHR = 0.34), major cardiovascular disease (AHR = 0.35), and other causes (AHR = 0.43).[8] Hamer, et al. in National Diet and Nutrition Survey indicateda linear inverse association between the healthy behavior and mortality risk. Furthermore, they found that all of the individual healthy behaviors were associated with a lower risk of mortality.[12] Healthy lifestyle was also associated with a lower risk of mortality in China. Findings from Shanghai Women's Health study showed that a healthier lifestyle pattern was associated with reductions in all-cause and cause-specific mortality among lifetime non-smoking and non-drinking women. Adjusted hazard ratios reduced with an increasing number of healthy lifestyle factors for mortality. Compared to women with

a score of zero, hazard ratios for women with four to five factors were 0.57 for total mortality, 0.29 for CVD mortality, and 0.76 for cancer mortality.[5] In Japan Collaborative Cohort (JACC) Study, the investigators reported an inverse association between a combination of 6 healthy lifestyle factors and all-cause mortality. In a recent meta-analysis based on previous publications, it was shown that adherence to a healthy lifestyle was associated with a 66% reduction in the risk of all-cause mortality.[7] As it is clear from the above-mentioned studies, all came from non-developing countries. Our findings are the first report of the same associations from a Middle Eastern population. In addition, we controlled for a wide range of confounders, most have not been taken into account in earlier studies.[1] Another difference between our study and previous ones is considering the whole diet in the current study. Earlier publications have only used fruit and vegetable intake as a measure of healthy eating in the construction of lifestyle score.[5,9] Furthermore, despite the shorter duration of follow up in the current study compared with other studies, we found a strong inverse relationship indicating the greater importance of lifestyle in Middle Eastern nations.

When we stratified the analyses by gender and BMI status, the same associations in the whole population were reached, except for cancer mortality, where we did not find any significant association with healthy lifestyle in women and obese people. This might be explained by the small number of mortality cases. However, earlier investigations have shown the harmful effects of obesity on mortality. Some studies have demonstrated that healthy lifestyle habits were associated with a significant decrease in mortality regardless of baseline BMI status. Overall, examining the association between healthy lifestyle and mortality in different categories of BMI needs further investigations.[28,29]

Healthy lifestyle might influence risk of mortality through several mechanisms. Physical activity as a component of healthy lifestyle is associated with beneficial changes in the metabolic profile. An improved metabolic profile might reduce the prevalence of the metabolic syndrome and cardiovascular disease through which it can influence the risk of mortality.[30] Reductions in cancer mortality may come from reduced fat stores, increased energy expenditure, changes in sex hormone levels, improved immune function, reductions in levels of insulin and insulin-like growth factors.[30] Cigarette smoking contains at least 50 carcinogens,[31] and a lifetime of not smoking would lead to substantially less exposure to tobacco carcinogens by reducing smoking-related cancers (i.e., lung & bronchus, esophagus, pancreas, bladder, kidney, trachea, larynx, oral cavity, etc). Overall, a combination of these lifestyle factors may contribute to reducing most common cancer and total cancer events.[32,33]

This study has several strengths. This is the first report from developing countries in which the association of combined healthy lifestyle with mortality was assessed. The study was done in both rural and urban areas, in either gender and the response rate was relatively high. The prospective design of the study minimizes differential misclassification of participants in terms of exposure. Large sample size and adjustment for several potential confounders, in particular opium consumption, is strength of the study. The use of validated methods for assessment of exposure and outcome must also be taken into account. However, several limitations should also be considered. As in all observational studies, despite careful attention to the issue of confounder adjustment, it is possible that confounding by other unmeasured or insufficiently controlled risk factors explain the associations. Due to the use of a FFQ for dietary assessment, some degrees of measurement error in dietary intake are inevitable. However, the FFQ we used was previously validated in the same population. The short follow-up period of the study might also be considered as a further limitation.

In conclusion, we found that a combination of lifestyle-related behaviors was inversely associated with the risk of mortality in the setting of a developing country. The findings of the current study would provide a strong support for the promotion of healthy lifestylesby government and the health related organizations in developing countries.

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Conflicts of interest

Authors declared no potential conflicts of interest.

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References

1. Loef M, Walach H.The combined effects of healthy lifestyle behaviors on all cause mortality: a systematic review and meta–analysis. *Prev Med*. 2012; 55: 163 – 170.
2. van Dam RM, Li T, Spiegelman D, Franco OH, Hu FB. Combined impact of lifestyle factors on mortality: prospective cohort study in US women. *BMJ*. 2008; 16: 337
3. Odegaard AO, Koh WP, Gross MD, Yuan JM, Pereira MA. Combined lifestyle factors and cardiovascular disease mortality in Chinese men and women: The Singapore Chinese health study. *Circulation*. 2011; 124: 2847 – 2854.
4. Lee CD, Sui X, Hooker SP, Hebert JR, Blair SN. Combined impact of lifestyle factors on cancer mortality in men. *Annals of Epidemiology*. 2011; 21: 749 – 754.
5. Nechuta SJ, Shu XO, Li HL, Yang G, Xiang YB, Cai H, et al. Combined impact of lifestyle–related factors on total and cause–specific mortality among Chinese women: prospective cohort study. *PLoS Med*. 2010; 7: 1000339.
6. Yun JE, Won S, Kimm H, Jee SH. Effects of a combined lifestyle score on 10–year mortality in Korean men and women: A prospective cohort study. *BMC Public Health*. 2012; 12: 1471 – 2458.

7. Tamakoshi A, Tamakoshi K, Lin Y, Yagyu K, Kikuchi S. Healthy lifestyle and preventable death: Findings from the Japan Collaborative Cohort (JACC) Study. *Prev Med*. 2009; 48: 486 – 492.
8. Ford ES, Zhao G, Tsai J, Li C. Low–risk lifestyle behaviors and all–cause mortality: findings from the National Health and Nutrition Examination Survey III Mortality Study. *Am J Public Health*. 2011; 101: 1922 – 1929.
9. Khaw KT, Wareham N, Bingham S, Welch A, Luben R, Day N. Combined impact of health behaviours and mortality in men and women: the EPIC–Norfolk prospective population study. *PLoS Med*. 2008; 5: 0050012.
10. Larsson SC, Akesson A, Wolk A. Healthy diet and lifestyle and risk of stroke in a prospective cohort of women. *Neurology*. 2014; 83: 1699 – 1704.
11. Ford ES, Bergmann MM, Kroger J, Schienkiewitz A, Weikert C, Boeing H. Healthy living is the best revenge: findings from the European Prospective Investigation Into Cancer and Nutrition–Potsdam study. *Arch Intern Med*. 2009; 169: 1355 – 1362.
12. Hamer M, Bates CJ, Mishra GD. Multiple health behaviors and mortality risk in older adults. *J Am Geriatr Soc*. 2011; 59(2): 370 – 372.
13. Pourshams A, Khademi H, Malekshah AF, Islami F, Nouraei M, Sadjadi AR, et al. Cohort profile: the Golestan Cohort Study—a prospective study of oesophageal cancer in northern Iran. *International Journal of Epidemiology*. 2010; 39: 52 – 59.
14. Pourshams A, Saadatian–Elahi M, Nouraie M, Malekshah AF, Rakhshani N, Salahi R, et al. Golestan cohort study of oesophageal cancer: Feasibility and first results. *Br J Cancer*. 2005; 92: 176 – 181.
15. Azar M, Sarkisian E. Food composition table of Iran. Tehran: National Nutrition and Food Research Institute, Shaheed Beheshti University. 1980; 65.
16. United States Department of Agriculture, National Nutrient Database for Standard Reference, Release 25.
17. Malekshah A, Kimiagar M, Saadatian–Elahi M, Pourshams A, Nouraie M, Goglani G, et al. Validity and reliability of a new food frequency questionnaire compared to 24 h recalls and biochemical measurements: pilot phase of Golestan cohort study of esophageal cancer. *Eur J Clin Nutr.* 2006; 60: 971 – 977.
18. Malekshah AF, Kimiagar M, Pourshams A, Yazdani J, Kaiedi Majd S, Goglani G, et al. Vitamin deficiency in Golestan Province, northern Iran: A high–risk area for esophageal cancer. *Arch Iran Med*. 2010; 13: 391.
19. Islami F, Malekshah AF, Kimiagar M, Pourshams A, Wakefield J, Goglani G, et al. Patterns of food and nutrient consumption in northern Iran, a high–risk area for esophageal cancer. *Nutrition and Cancer*. 2009; 61: 475 – 483.
20. Khademi H, Malekzadeh R, Pourshams A, Jafari E, Salahi R, Semnani S, et al. Opium use and mortality in Golestan Cohort Study: prospective cohort study of 50 000 adults in Iran. *BMJ: British Medical Journal*. 2012; 344: 344
21. Malekzadeh MM, Khademi H, Pourshams A, Etemadi A, Poustchi H, Bagheri M, et al. Opium use and risk of mortality from digestive diseases: a prospective cohort study. *Am J Gastroenterol*. 2013; 108: 1757 – 1765.
22. McCullough ML, Willett WC. Evaluating adherence to recommended diets in adults: the Alternate Healthy Eating Index. *Public Health Nutr*. 2006; 9: 152 – 157.
23. McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, et al. Diet quality and major chronic disease risk in men and women: Moving toward improved dietary guidance. *Am J Clin Nutr*. 2002; 76: 1261 – 1271.
24. Esmaillzadeh A, Azadbakht L. Legume consumption is inversely associated with serum concentrations of adhesion molecules and inflammatory biomarkers among Iranian women. *J Nutr*. 2012; 142: 334 – 339.
25. Willett WC. Nutritional Epidemiology: Oxford University Press. 2012.
26. Islami F, Kamangar F, Nasrollahzadeh D, Aghcheli K, Sotoudeh M, Abedi-Ardekani B, et al. Socio–economic status and oesophageal cancer: results from a population–based case–control study in a high–risk area. *International Journal of Epidemiology*. 2009; 38: 978 – 988.
27. van den Brandt PA. The impact of a Mediterranean diet and healthy lifestyle on premature mortality in men and women. *Am J Clin Nutr*. 2011; 94: 913 – 920.
28. Matheson EM, King DE, Everett CJ. Healthy lifestyle habits and mortality in overweight and obese individuals. *J Am Board Fam Med*. 2012; 25: 9 – 15.
29. Li K, Husing A, Kaaks R. Lifestyle risk factors and residual life expectancy at age 40: A German cohort study. *BMC Med*. 2014; 12: 1741 – 7015.
30. Samitz G, Egger M, Zwahlen M. Domains of physical activity and all–cause mortality: systematic review and dose–response meta–analysis of cohort studies. *Int J Epidemiol*. 2011; 40: 1382 – 1400.
31. Phillips DH, Hewer A. DNA adducts in human urinary bladder and other tissues. *Environ Health Perspect.* 1993; 99: 45 – 49.
32. Mons U, Muezzinler A, Gellert C, Schottker B, Abnet CC, Bobak M, et al. Impact of smoking and smoking cessation on cardiovascular events and mortality among older adults: Meta–analysis of individual participant data from prospective cohort studies of the CHANCES consortium. *BMJ*. 2015; 20: 350
33. Gellert C, Schottker B, Brenner H. Smoking and all–cause mortality in older people: systematic review and meta–analysis. *Arch Intern Med*. 2012; 172: 837 – 844.