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CLINICAL RESEARCH
Prevention and epidemiology

Lower carbohydrate diets and all-cause and cause-specific mortality: a population-based cohort study and pooling of prospective studies

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Aims Little is known about the long-term association between low-carbohydrate diets (LCDs) and mortality. We evaluated the link between LCD and overall or cause-specific mortality using both individual data and pooled prospective studies.

Methods and results Data on diets from the National Health and Nutrition Examination Survey (NHANES; 1999–2010) were analysed. Multivariable Cox proportional hazards were applied to determine the hazard ratios and 95% confidence intervals (CIs) for mortality for each quartile of the LCD score, with the lowest quartile (Q1—with the highest carbohydrates intake) used as reference. We used adjusted Cox regression to determine the risk ratio (RR) and 95% CI, as well as random effects models and generic inverse variance methods to synthesize quantitative and pooled data, followed by a leave-one-out method for sensitivity analysis. Overall, 24 825 participants from NHANES study were included (mean follow-up 6.4 years). After adjustment, participants with the lowest carbohydrates intake (quartile 4 of LCD) had the highest risk of overall (32%), cardiovascular disease (CVD) (50%), cerebrovascular (51%), and cancer (36%) mortality. In the same model, the association between LCD and overall mortality was stronger in the non-obese (48%) than in the obese (19%) participants. Findings on pooled data of nine prospective cohort studies with 462 934 participants (mean follow-up 16.1 years) indicated a positive association between LCD and overall (RR 1.22, 95% CI 1.06–1.39, $P < 0.001$, $I^2 = 8.6$), CVD (RR 1.13, 95% CI 1.02–1.24, $P < 0.001$, $I^2 = 11.2$), and cancer mortality (RR 1.08, 95% CI 1.01–1.14, $P = 0.02$, $I^2 = 10.3$). These findings were robust in sensitivity analyses.

Conclusion Our study suggests a potentially unfavourable association of LCD with overall and cause-specific mortality, based on both new analyses of an established cohort and by pooling previous cohort studies. Given the nature of the study, causality cannot be proven; we cannot rule out residual bias. Nevertheless, further studies are needed to extend these important findings, which if confirmed, may suggest a need to rethink recommendations for LCD in clinical practice.

Keywords Low-carbohydrate diets • Cardiovascular • Mortality • Cancer

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Introduction

The pandemic of obesity is a major health issue worldwide and obesity is a predisposing factor for several chronic diseases, including cardiovascular disease (CVD), hypertension, Type 2 diabetes (T2DM), and cancer.[1,2] Cardiovascular disease represents the main global cause of death, currently accounting for >17.3 million deaths annually; this figure is predicted to rise to >23.6 million by 2030.[3] Cardiovascular disease was the leading cause of death in the USA in 2015, accounting for 864 000 deaths.[4]

There is little controversy over the benefit of eating a well-balanced diet and keeping active on cardiovascular (CV) health, as demonstrated in several large cohort studies.[3,5–7] However, different types of diet remain controversial and in particular low-carbohydrate diets (LCDs), which have become popular in recent years.[8] The effectiveness of diets low in carbohydrate and high in protein and fat to promote weight loss and reduce the cardiometabolic risk was reported in systematic reviews and meta-analyses.[9,10] However, long-term safety of consuming LCDs remains controversial.[11] Some studies have suggested that LCDs may increase the risk of CVD, overall and cancer morbidity and mortality.[8,12–16] Two European cohorts reported that a LCD-high protein (LC/HP) diet was associated with a significantly higher overall and cause-specific mortality.[12,13] A meta-analysis of observational cohort studies on the association of LCD with overall and CVD mortality, suggested that LCD was related to a significantly higher risk of all-cause death without any changes in CVD mortality and incidence.[17] However, this conclusion was tentative, because the authors reported a high chance of heterogeneity and publication bias.[17] They also reported that they might not have had enough power to evaluate the association of LCD with CVD mortality.[17] Some other studies,[8,11] published after this meta-analysis, have reported controversial and sometime opposite results in different populations. Nakamura *et al.*[11] found a lower risk of CVD mortality in Japanese individuals with a higher score of LCD, while they observed no association between LCD score and all-cause death. Another study that involved US women reported null results for the association of LCD with both overall and CVD mortality.[14] Clearly, randomized trials of LCDs on mortality are not easily feasible because of the difficulty in maintaining compliance and follow-up over several years. Furthermore, there are no studies available evaluating the link between LCDs and stroke risk.

Therefore, given the incompleteness of evidence, the controversial role of LCDs and the public health importance of the issue, we prospectively examined the relation between LCD and all-cause and cause-specific (CVD, stroke, and cancer) mortality in a large and nationally representative US cohort. We also aimed to contextualize these results by performing a comprehensive systematic review and meta-analysis to examine associations between LCDs and all-cause and cause specific mortality (CVD, stroke, and cancer) by using findings from prospective cohort studies.

Methods

NHANES population

This was a prospective cohort study using data from the US National Health and Nutrition Examination Survey (NHANES). The National Center for Health Statistics (NCHS) Research Ethics Review Board approved the underlying protocol and written informed consent was obtained from all participants. The current study is based on analysis of data from 2-year NHANES survey cycles between 1999 and 2010, restricted to participants aged ≥20 years. Details on the NHANES Laboratory/Medical Technologists Procedures and Anthropometry Procedures are described elsewhere.[18,19]

Dietary intake was assessed *via* 24 h recall obtained by a trained interviewer, with the use of a computer-assisted dietary interview system, i.e. the United States Department of Agriculture Automated Multiple-Pass Method (AMPM).[20,21] In brief, the type and quantity of all foods and beverages consumed 2 days before the dietary interview (from midnight to midnight) were collected with the use of the AMPM. The AMPM was designed to enhance complete and accurate data collection, while reducing respondent burden.[21,22]

Mortality

The de-identified and anonymized data of NHANES 1999–2010 participants were linked to longitudinal Medicare and mortality data using the NHANES assigned sequence number. Mortality follow-up data are available from the date of survey participation until 31 December 2011. We assessed all-cause mortality, as well as mortality due to heart diseases (I00-I09, I11, I13, I20-I51), cancer (C00-C97), and cerebrovascular disease (I60-I69). The cause of death was determined using the 10th revision of the International Classification of Diseases (ICD-10).

Low-carbohydrate diet score

We estimated the energy-adjusted intakes of protein, fat, and carbohydrates (all diet sources of carbohydrates were investigated) for each individual, using the residual method of Willet.[23,24] This method allows the evaluation of the effect of an energy-generating nutrient by using a simple regression of that nutrient on energy intake to calculate the residual. The use of energy-adjusted residuals was necessary because all energy-generating nutrients are generally strongly positively associated with total energy intake.[23,24] The carbohydrate categories were scored from 10 (lowest intake) to 0 (highest intake), whereas protein and fat categories were scored from 0 (lowest intake) to 10 (highest intake). Ranks were added to create a total score with a maximum value of 30, which represented the highest intake of total protein and total fat and the lowest intake of carbohydrate. We studied the scores for high protein and fat and low carbohydrate intake separately. Moreover, we also added them together to create a composite low carbohydrate-high protein-fat score (LC/HP).

Statistical analysis

Analyses were conducted according to the guidelines set by the Centers for Disease Control and Prevention for analysis of the NHANES dataset, accounting for the masked variance and using their suggested weighting methodology.[25] Continuous and categorical demographic variables were compared across quartiles of LCD score using analysis of variance (ANOVA) and χ^2 tests, respectively.

Multivariable Cox proportional hazards were applied to determine the hazard ratios (HRs) and 95% confidence intervals (CIs) of mortality for each quartile of the LCD score, with the lowest quartile (Q1) always used as reference. To derive the HR and 95% CI we had two different models: *Model 1* adjusted for age, sex, race, education, marital status, poverty to income ratio, total energy intake, physical activity, smoking, and alcohol consumption; and *Model 2* adjustment for Model 1 plus body mass index (BMI), waist circumference, hypertension, serum cholesterol and diabetes. A two-sided $P < 0.05$ was used to characterise significant results.

Data were analysed using SPSS® complex sample module version 22.0 (IBM Corp, Armonk, NY, USA).

Systematic review, meta-analysis

Literature search and study selection

This meta-analysis was designed, conducted, and reported according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines[26] (MOOSE checklist is provided in Supplementary material online, *Table S1*). The primary exposure of interest was LCD, whereas the primary outcome of interest was risk for overall and cause-specific mortality subsequent to LCD. Prospective cohort studies published up to August 2018 (without language restriction) were searched using PubMed, Embase, and Scopus database; the query syntax of searching is shown in the Supplementary material online, *Table S2*. This was complemented by hand searches of the reference list of eligible articles, and email correspondences with authors for additional data where relevant.

After excluding duplicates and based on titles and abstracts, we excluded studies on animals, with participants aged <18 years at baseline, populations with prior coronary heart disease, diabetes, or any other chronic diseases. Eligible studies were selected by using predefined inclusion criteria of prospective cohort studies, healthy populations and original articles on the association of LCD, and all-cause and cause specific mortality. In addition, supplementary hand searching of reference lists of previous reviews or meta-analyses was conducted. Of 19 eligible full articles, nine articles (eight based on literature search + NHANES results) met the inclusion criteria (Supplementary material online, *Figure S1*).

Study selection

Study selection started with the removal of duplicates, followed by screening of titles and abstracts by two reviewers (M.M. and M.B.). To avoid bias, they were blinded to the names, qualifications, or institutional affiliations of the study authors. The agreement between the reviewers was excellent (Kappa index 0.91; $P < 0.001$). Disagreements were resolved between reviewers prior to selected articles being retrieved (a flow chart is available in Supplementary material online, *Figure S1*). We included studies if they met all the following criteria: (i) the studies of interest were carbohydrates intake measured with the LC score; (ii) the studies were population-based cohort studies and reported overall and cause-specific mortality data; (iii) risk ratio (RR), HR, or odds ratio (OR) estimates with 95% CI adjusted for multivariable factors were available or could be calculated. Studies were excluded according to the following criteria: (i) reviews, letters, or comments; (ii) not population-based cohort studies; (iii) RR, HR, or OR estimates with 95% CI were not available or could not be calculated. Narrative reviews, comments, opinion papers, editorials, letters, or any other publications lacking primary data and/or explicit method descriptions, were also excluded.

Data extraction and management

Full text of studies meeting the inclusion criteria was retrieved and screened to determine eligibility by two reviewers (M.M. and M.B.). The study quality assessment was performed according to the Newcastle–Ottawa Scale (NOS, Supplementary material online, *Table S3*).[27] By evaluation of selection, comparability and outcome, the rating system scores studies from 0 (highest degree of bias) to 9 (lowest degree of bias). Additionally, we investigated the funding sources of all of the eligible studies. Following assessment of methodological quality, two reviewers (M.M. and M.B.) extracted data using a purpose-designed data extraction form and independently summarized what they consider to be the most important results from each study. These summaries were compared and any differences of opinion resolved by discussion and consultation with a third reviewer (N.K.). Any further calculations on study data considered necessary, was conducted by the first reviewer and checked by the second reviewer. Information extracted from each eligible study included the following items: author, year and references, country, study name, men (%), age, follow-up time (years), number of cases, number of subjects, parameter, outcome, and main confounders (Supplementary material online, *Table S4*).

Data synthesis and statistical analyses

For studies that reported results from different multivariable-adjusted models, the model with the most confounding factors was extracted for the meta-analysis. The random-effect model was applied to calculate pooled RRs, 95% CI, and *P*-values for heterogeneity. RRs comparing the highest score category with the lowest category were combined across studies to generate the summary associations. The extent of heterogeneity across studies was examined using the I^2 test[28–30] and I^2 >50% together with $P < 0.05$ indicated significant heterogeneity.[28–30]

Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, Begg's rank correlation, and Egger's weighted regression tests. The Duval and Tweedie trim method was used to adjust the analysis for the effects of publication bias.[31] The meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V3 software (Biostat, NJ, USA).[32]

Results

NHANES findings

Overall, 24 825 participants were included, with a mean age of 47.6 years, comprising 48.6% men and 51.4% women. The demographic characteristics of the participants are shown in *Table 1*. There was a significant, but not linear, age difference between quartiles of LCD ($P < 0.001$). From the first to the third quartile of LCD, women were the majority, while the highest quartile of LCD had more males (males: 56.4% vs. females: 43.6%, $P < 0.001$). The majority of the Mexican-Americans were in the highest quartile of LCD (19.9%), whereas the highest percentage of Non-Hispanic Black was in the lowest quartile of LCD (23.5%, $P < 0.001$). With regard to marital status, there was a significant difference across the quartiles of LCD ($P < 0.001$). The majority of the participants with '*less than high school*' level of education were in the highest quartile of LCD (Q1: 27.4 vs. Q4: 36.9).

During the follow-up period of 144 months, 3432 total deaths were recorded, including 827 cancer deaths, 709 heart disease deaths, and 228 cerebrovascular disease deaths. The distributions of overall and cause-specific mortality across quartiles of LCD are shown in *Table 1*.

Results from multivariable Cox regression models for risk of death across quartiles of LCD and LC/HP are shown in *Table 2*. Regarding LCD, participants in the top quartile (Q4) had the highest risk of overall mortality [Q2: 1.11 (1.02–1.96), Q3: 1.23 (1.11–1.43), Q4: 1.42 (1.27–1.96), $P < 0.001$, *Table 2*]. After adjustment for additional factors, there was still a significant positive association between LCD and overall mortality, i.e. participants in the top quartile (Q4) had a 32% higher chance of overall mortality [Q2: 1.09 (1.02–1.64), Q3: 1.19 (1.09–1.82), Q4: 1.32 (1.14–2.01), $P < 0.001$, *Table 2*, *Take home*

Table 1 Characteristics of the study participants by quartiles of low carbohydrate diet score

	Quartiles of low carbohydrate diet score				*P*-value
Median LCD score **Macronutrient amount (g/day; calories/day; %energy[a])**	**Q1 (*n* = 6206)** **12** **Carbohydrates: 367 (1468; 66%)** **Protein: 77 (308),** **Fat: 73 (657)**	**Q2 (*n* = 6205)** **15** **Carbohydrates: 245 (980; 57%)** **Protein: 69 (276),** **Fat: 65 (585)**	**Q3 (*n* = 6205)** **18** **Carbohydrates: 205 (820; 49%)** **Protein: 72 (288),** **Fat: 70 (630)**	**Q4 (*n* = 6209)** **21** **Carbohydrates: 214 (856, 39%)** **Protein: 103 (412),** **Fat: 105 (945)**	
Age (years), mean ± SEM	45.1 ± 0.2	48.2 ± 0.1	49.4 ± 0.2	47.2 ± 0.2	<0.001
Sex					
Men (%)	43.7	41.4	46.7	56.4	<0.001
Women (%)	56.3	58.6	53.3	43.6	
Race/Ethnicity					
Mexican-American (%)	17.4	19.4	19.2	19.9	<0.001
Non-Hispanic White (%)	48.4	46.1	47.6	46.6	
Non-Hispanic Black (%)	23.5	20.8	20.3	19.2	
Marital status					
Married (%)	52.3	47.6	53.5	51.8	<0.001
Widowed (%)	7.9	9.5	10.4	9.0	
Divorced (%)	11.4	8.6	9.8	10.1	
Education status					
Less than high school (%)	27.4	29.5	34.1	39.6	<0.001
Completed high school (%)	28.4	21.3	20.9	24.3	
More than high school (%)	44.2	49.2	45.0	36.1	
Mortality status					
Total mortality (*n*)	756	749	831	1096	<0.001
Cancer mortality (*n*)	165	189	222	251	<0.001
Heart disease mortality (*n*)	140	153	176	240	<0.001
Cerebrovascular disease mortality (*n*)	44	49	63	72	<0.001
Poverty to income ratio (*n*)	2.5 ± 0.03	2.3 ± 0.02	2.6 ± 0.02	2.6 ± 0.03	<0.001
Physical activity (%)	24.4	26.6	22.7	21.3	<0.001
Alcohol consumption (g/day)	3.2 ± 0.2	5.6 ± 0.2	9.8 ± 0.2	16.2 ± 0.2	<0.001
Smoking (%)	22.1	23.2	27.4	27.3	<0.001
Polyunsaturated fatty acids (g/day)	22.3 ± 0.2	16.2 ± 0.1	17.4 ± 0.1	13.6 ± 0.1	<0.001
Saturated fatty acids (g/day)	31.2 ± 0.4	24.3 ± 0.1	27.9 ± 0.2	22.7 ± 0.3	<0.001
Fibre intake (g/day)	17.1 ± 0.2	15.8 ± 0.1	15.4 ± 0.1	14.9 ± 0.2	<0.001
Body mass index (kg/m^2)	28.4 ± 0.1	28.8 ± 0.08	28.9 ± 0.08	28.7 ± 0.1	<0.001
Serum total cholesterol (mg/dL)	194.3 ± 0.9	196.2 ± 0.8	197.5 ± 0.9	197.6 ± 0.9	<0.001
Serum creatinine (SE)[b] (mg/dL)	0.89 (0.01)	0.89 (0.04)	0.90 (0.04)	0.93 (0.01)	<0.05
Systolic blood pressure (mmHg)	120.2 ± 0.4	123.3 ± 0.2	122.2 ± 0.2	124.5 ± 0.4	<0.001
Diastolic blood pressure (mmHg)	68.5 ± 0.3	68.6 ± 0.2	68.9 ± 0.2	69.9 ± 0.3	<0.001
Diabetes (%)	7.3	10.4	11.6	11.4	<0.001

Groups across the quartiles were compared by either χ^2 or analysis of variance.
[a]Refers only to carbohydrates.
[b]Data are presented as means (standard errors, SE).

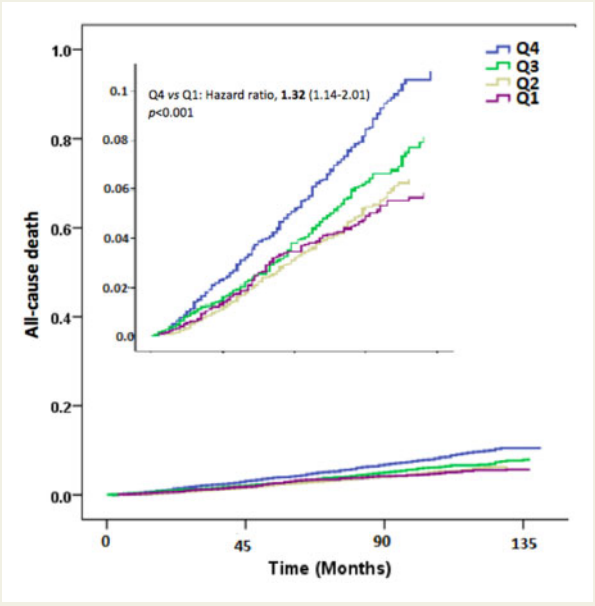
figure]. We also found a positive association between CVD and cerebrovascular mortality across increasing quartiles of LCD. This association remained significant after adjustment for additional confounding factors ($P < 0.001$ for all comparisons, *Table 2*)—in the second model, participants in the top quartile (Q4) of LCD had 50 and 51% higher risk of CVD and cerebrovascular mortality, respectively, compared with the first reference quartile (Q1).

A significant and positive association was found between LCD and the risk of cancer mortality in both Model 1 (52%) and Model 2 (36%); Model 1 = Q2: 1.12 (1.08–1.16), Q3: 1.19 (1.04–1.52), Q4: 1.52 (1.23–2.12), Model 2 = Q2: 1.09 (0.99–1.16), Q3: 1.13 (1.00–1.28), Q4: 1.36 (1.09–1.83), both $P < 0.001$, *Table 2*]. We also observed a positive and significant link between LC/HP diets and overall and cause-specific mortality (CVD, cerebrovascular, and

Table 2 Multivariable-adjusted hazard ratios (95% CIs) for mortality across quarters of low carbohydrate diet score and low carbohydrate-high protein diet score

		Quartiles of low carbohydrate diet				Quartiles of low carbohydrate-high protein diet			
		Q2 ($n = 6109$)	Q3 ($n = 6118$)	Q4 ($n = 6124$)	*P*-trend	Q2 ($n = 6275$)	Q3 ($n = 6277$)	Q4 ($n = 6279$)	*P*-trend
Macronutrient amount (g/day; calories/day)		**Carbohydrates: 245 (980), Protein: 69 (276), Fat: 65 (585)**	**Carbohydrates: 205 (820), Protein: 72 (288), Fat: 70 (630)**	**Carbohydrates: 214 (856), Protein: 103 (412), Fat: 105 (945)**		**Carbohydrates: 273 (1092), Protein: 69 (276), Fat: 73 (657)**	**Carbohydrates: 238 (952), Protein: 91 (364), Fat: 81 (729)**	**Carbohydrates: 217 (868), Protein: 111 (444), Fat: 67 (603)**	
No of deaths		749	831	1096		847	901	1048	
Total mortality	Model 1	1.11 (1.02–1.96)	1.23 (1.11–1.43)	1.42 (1.27–1.96)	<0.001	1.18 (1.09–1.30)	1.25 (1.12–1.43)	1.34 (1.28–1.56)	<0.001
	Model 2	1.09 (1.02–1.64)	1.19 (1.09–1.82)	1.32 (1.14–2.01)	<0.001	1.11 (1.02–1.33)	1.16 (1.08–1.46)	1.21 (1.04–1.39)	<0.001
Cancer mortality	Model 1	1.12 (1.08–1.16)	1.19 (1.04–1.42)	1.52 (1.23–1.51)	<0.001	1.18 (1.09–1.24)	1.26 (1.19–1.32)	1.37 (1.27–1.43)	<0.001
	Model 2	1.09 (0.99–1.16)	1.13 (1.00–1.28)	1.35 (1.06–1.69)	<0.001	1.11 (0.98–1.42)	1.19 (0.99–1.33)	1.22 (1.02–1.48)	<0.001
Coronary heart disease mortality	Model 1	1.22 (1.13–1.43)	1.39 (1.25–1.62)	1.65 (1.53–1.95)	<0.001	1.15 (1.04–1.26)	1.30 (1.20–1.39)	1.55 (1.50–1.61)	<0.001
	Model 2	1.19 (0.98–1.36)	1.30 (1.02–1.92)	1.51 (1.19–1.91)	<0.001	1.14 (0.97–1.22)	1.22 (0.94–1.36)	1.44 (1.02–2.09)	<0.001
Cerebrovascular disease mortality	Model 1	1.22 (1.14–1.34)	1.36 (1.19–1.73)	1.68 (1.38–1.95)	<0.001	1.30 (1.21–1.40)	1.42 (1.38–1.46)	1.56 (1.42–1.60)	<0.001
	Model 2	1.18 (1.00–1.64)	1.22 (1.04–2.10)	1.50 (1.12–2.31)	<0.001	1.18 (0.99–1.42)	1.25 (1.00–1.51)	1.41 (1.09–1.72)	<0.001

Model 1: Adjusted for age, gender, race, education, marital status, poverty to income ratio, total energy intake, physical activity, smoking, and alcohol consumption; *Model 2*: Adjusted for age, sex, gender, education, marital status, poverty to income ratio, total energy intake, physical activity, smoking, alcohol consumption, body mass index and waist circumference, hypertension, serum total cholesterol and diabetes. Q1, considered as reference.



Take home figure Probability of all-cause death by carbohydrate intake.

cancer) for both the partially (Model 1) and comprehensively (Model 2) adjusted model ($P < 0.001$ for all comparisons, *Table 2*, *Figure 1*).

By applying on our second model, we also examined the link between overall mortality and LCD for obese (BMI ≥30 kg/m^2) and non-obese (BMI <30 kg/m^2) participants; this link was stronger in the non-obese than in the obese participants [obese = Q2: 1.02 (1.01–1.09), Q3: 1.11 (1.03–1.23), Q4: 1.19 (1.11–2.25) vs. non-obese = Q2: 1.13 (1.07–1.19), Q3: 1.25 (1.11–1.76), Q4: 1.48 (1.37–2.01), *P-interaction* <0.001]. Furthermore, we performed an analysis by categorizing our participants to two age groups (age ≥55 vs. <55 years). The link between LCD and overall mortality was stronger for older participants [younger = Q2: 1.08 (1.02–1.012), Q3: 1.09 (1.03–1.20), Q4: 1.17 (1.10–2.01) vs. older = Q2: 1.19 (1.10–1.30), Q3: 1.29 (1.13–1.63), Q4: 1.52 (1.41–1.79), *P-interaction* <0.001].

Meta-analysis and systematic review

Overviews of the key characteristics of the nine prospective cohort studies are shown in Supplementary material online, *Table S4*. A total of 462 934 participants, with 45 609 mortality cases were included in the analysis. A total of five studies presented sex-specific results; two studies were in men and two in women. The duration of follow-up ranged from 4.9 to 29 years, with a mean follow-up of 16.1 years. The results of the quality assessment are shown in the Supplementary material online, *Table S3*, with five studies scoring 8 and four scoring 9.

Low-carbohydrate diet and mortality

There was a significant positive association between LCD and overall mortality (RR 1.22, 1.06–1.39, $P < 0.001$, $n = 8$ studies, *Figure 2A*), with minimal heterogeneity ($I^2 = 8.6$, $P = 0.912$). Furthermore, a significant association between LCD score and CVD mortality was observed (RR 1.13, 1.02–1.24, $P < 0.001$, $n = 6$ studies, *Figure 2B*), again with minimal evidence of heterogeneity ($I^2 = 11.2$, $p = 0.849$). Similar results were observed for LCD score and risk of cancer mortality (RR 1.08, 1.01–1.14, $P = 0.02$, $n = 3$ studies, *Figure 2C*), with minimal heterogeneity ($I^2 = 10.3$, $P = 0.902$).

Low-carbohydrate/high protein diet mortality

There was a significant association between LC/HP and overall mortality [RR 1.16, 1.07–1.26, $P < 0.001$, $n = 5$ studies, (no heterogeneity, $I^2 = 17.6$, $P = 0.825$), Supplementary material online, *Figure S2*], as well as a positive correlation between LC/HP and CVD mortality (RR 1.35, 1.07–1.69, $P < 0.001$, $n = 5$ studies, Supplementary material online, *Figure S3*), with minimal evidence of heterogeneity ($I^2 = 21.5$, $P = 0.736$). In contrast, a significant trend between LC/HP and cancer mortality was observed (RR 1.03, 0.99–1.07, $P = 0.084$, $n = 3$ studies, Supplementary material online, *Figure S4*), but with modest of heterogeneity, ($I^2 = 57.3$, $P = 0.036$).

Sensitivity analysis

In leave-one-out sensitivity analyses, the pooled effect estimates remained similar for the association of both LCD and LC/HP with overall mortality (RR 1.21, 1.06–1.39 and RR 1.16, 1.07–1.26, respectively).

Publication bias

Visual inspection of the funnel plot symmetry suggested no publication bias for the comparison of LCD and overall mortality (Supplementary material online, *Figure S5*). Egger's linear regression also supported the absence of publication bias (intercept = 1.77, 95% CI = 2.35–5.89, $P = 0.320$). Furthermore, Begg's rank correlation test (Kendall's Tau with continuity correction =0.285, $z = 0.901$, $P = 0.367$) was not indicative for publication bias. After adjustment of the effect size for potential publication bias using the 'trim and fill' correction, two potentially missing studies were imputed in the funnel plot (RR 1.21, 1.06–1.39 changed to RR 1.20, 1.06–1.36) (Supplementary material online, *Figure S6*). The 'fail-safe N' test showed that 32 studies would be needed to bring the weighted mean difference down to a non-significant ($P > 0.05$) value.

Discussion

Short-term LCD is useful to improve body weight, blood pressure, gluco-lipid parameters, and liver steatosis[33–35] and consequently many health professionals recommend it; however, its long-term safety has not yet been clearly defined.[36] To evaluate the association of LCD on overall and cause-specific mortality, we analysed a large and nationally representative sample of US adults. We also pooled prospective studies that have attempted to answer the same question. Our findings support a potentially adverse association of LCD and LC/HP diet with overall mortality, CVD, cerebrovascular, and cancer mortality. These results appeared robust to adjustment for potential confounding factors. Of interest, our findings also suggested a positive association between long-term LCD and the risk of stroke mortality. Findings from pooled studies were in line with our original analyses. Based on these results we might try to answer the question on the definition of LCD that might increase the long-term mortality risk (median LCD score =

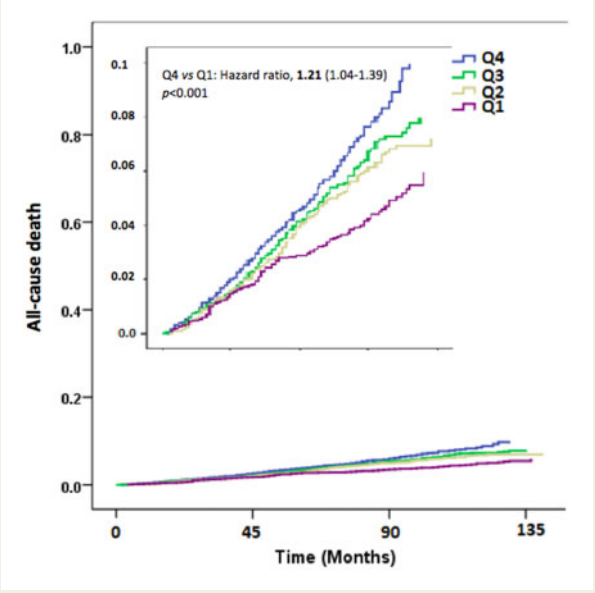


Figure 1 Probability of all-cause death by carbohydrate and protein intake.

21, which corresponds to 39% of energy and 214 g of carbohydrates/day), as well as on the definition of long-term LCD based on survival curves (*Figure 1*) for overall mortality (comparing Q4 to Q1–Q3 the risk increases still over 2 years, and for Q3 vs. Q1–Q2, over 5.5 years of LCD intake). We accept that other definitions of LCD require that intakes are <20% or <150 g/day of carbohydrate but even so, we believe our results are of value to the wider question of diets patterns and long term-risks, especially as few can sustain such low carbohydrate intakes for long periods of time.

Our results are somewhat in accordance with other studies reporting a positive link between LCD and overall, CVD and cancer mortality.[8,12–16] One of abovementioned, a Swedish study (12-year follow-up) in women using an overall score with a scoring algorithm similar to ours, reported that the LC/HP score was associated with CVD death only among women aged 40–49 years at baseline, but not among younger women (HR = 1.21 for each 10% increment in score).[12] However, this study reported no clear association between LC/HP diet and all-cause or cancer mortality.[12] Using the same calculation as ours, the LC/HP score was associated with a significantly increased risk of all-cause death (HR = 1.08 for each 10% increment) among Greek participants (22 944 healthy adults) of the European Prospective Investigation Cancer and Nutrition (EPIC) study during 10 years of follow-up.[13] In the EPIC study, similar associations were observed for CVD and cancer mortality.[13] A meta-analysis of cohort studies reported that LCDs were associated with a significantly higher risk of overall mortality, with a neutral association for CVD mortality and morbidity.[17] Fung *et al.*[14] studied 85 168 women (aged 34–59 years) and 44 548 men (aged 40–75 years) and found that the overall LCD score was associated with a modestly higher overall, CVD and cancer mortality. A study by Li *et al.*[8] in 2258 women from the Nurses' Health Study and 1840 men from the Health Professional Follow-Up Study, reported no apparent health benefit from a greater adherence to an LCD.

In contrast, a study among Asian populations with 29 years of follow-up (9200 participants, mean age = 51 years) found a beneficial association of a LCD on CVD and overall mortality.[11] The differences in the results of the aforementioned study in Japanese individuals[11] and those of previous studies (as well as the current study) carried out in Western countries, could be at least partly explained by the fact that people living in Japan consume a higher amount of carbohydrate (about 60% of the overall energy was from carbohydrates).[11]

The biology that underlies the positive association between LCDs and all-cause death is not fully elucidated yet. Some potential mechanisms may mediate the unfavourable impact of LCD on health, i.e. the reduced intake of fibre and fruits and the increased intake of protein from animal sources, cholesterol, and saturated fat,[37–39] all of which are risk factors for mortality and CVD.[40] It is postulated that differences in dietary bioactive components such as specific free fatty acids, protein, fibre, minerals, vitamins, and phytochemicals are involved.[14] Increased cancer risk has also been reported in relation to the intake of animal proteins[14] and the consumption of red and processed meat.[41] Further, it has been proposed that vegetables, fruits, cereals, and legumes, which have been found in several studies to be core components of healthy dietary patterns,[42] many of which contain important sources of fibre, are important sources of carbohydrates, so that reduced intake of these food groups is likely to have adverse effects on CV health. Moreover, several studies have reported that meat consumption or high intake of protein from animal sources may essentially increase the risk of CVD,[14] whereas high fibre diets appear to be associated with better long-term outcomes.[43]

Discussing the issue of the effect of carbohydrates on outcomes it is important to emphasize the potential differences of influence of refined and non-refined carbohydrates intake. We would like to highlight the latest results of two big studies carried out in the USA based on the data of 74 341 women in the Nurses' Health Study (1984–2010; mean follow-up of 26 years) and 43 744 men in the Health Professionals Follow-Up Study (1986–2010; follow-up up to 24 years). The authors reported an intake of 4.3 and 5.8 g/day of dietary whole grains in the lowest quartile and 35.6 and 52.6 g/day for the subjects in highest quartile, respectively.[44] This is a huge portion of the daily intake. They indicated that higher whole grain consumption was associated with lower total (by 9%) and CVD mortality (by 15%), independently of other dietary and lifestyle factors.[44] In the recent analysis of the prospective urban rural epidemiology (PURE) study up to 70% of energy intake was due to refined carbohydrates.[45] Of note, the results of the PURE study[45] (n = 135 335, aged 35–70 years in 18 countries with a median follow-up of 7.4 years) showed that a higher carbohydrate intake was associated with an increased risk of total mortality [highest vs. lowest category: HR 1.28 (95% CI 1.12–1.46)] but not with the risk of CVD mortality. The reason for increasing chance of mortality would be probably due to high consumption of simple sugar in their population (e.g. in developing countries), or that higher carbohydrate and low protein intakes are more markers of poverty in low and middle-income countries.[45] In this context, it seems to be critical to call for more studies in the field to take care of the covariates, which are important for each country due to their own nature of the diet and lifestyle.

In the present analysis, the association between LCD and mortality appeared somewhat greater in the non-obese than in the obese

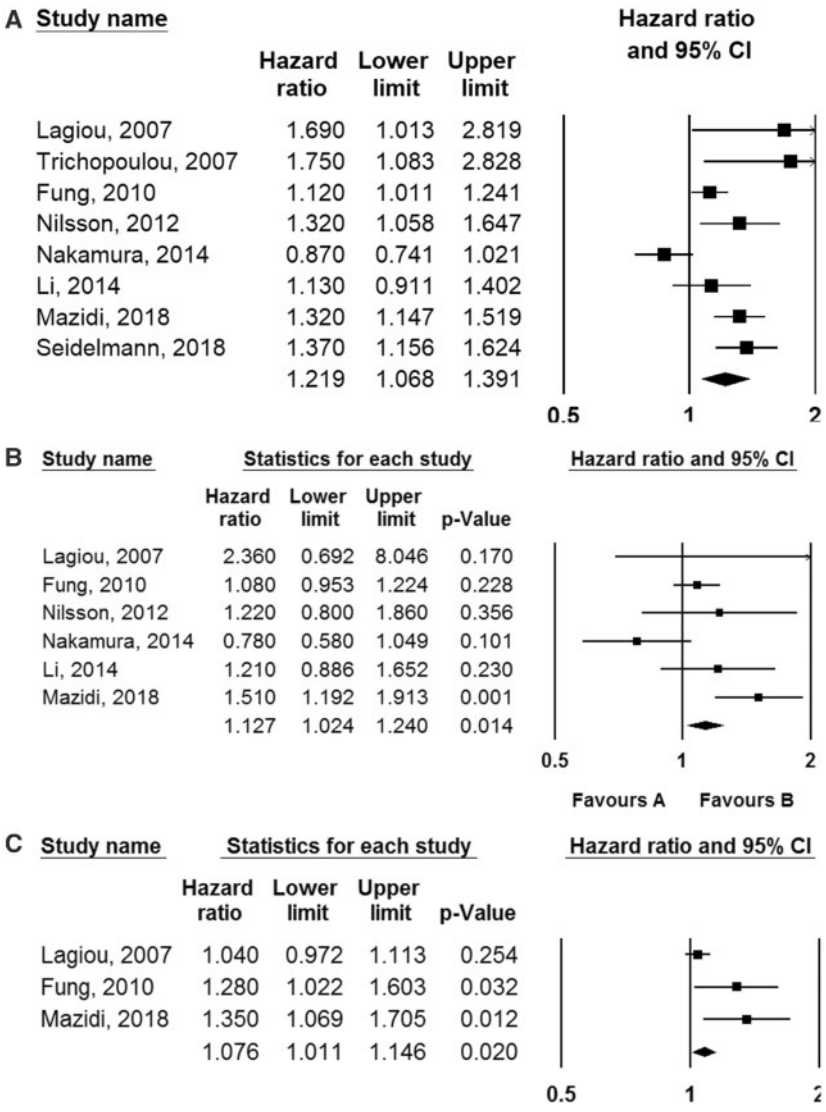


Figure 2 Forest plot of low-carbohydrate diet and risk of overall (A) and cause-specific [cardiovascular disease (B) and cancer (C)] mortality.

participants. In this context, the Mediterranean diet was reported to prevent overweight and obesity as well as reduce the risk of overall and CVD mortality, especially for non-obese individuals.[46] It could be suggested that diet components may mediate the link between adiposity and mortality.[47] These associations should be investigated in future studies. However, it is also possible that reverse causality is relevant to these findings given that findings were more apparent in older and leaner individuals and these are the group that would be losing more weight unintentionally.

The strength of the present study is that the analysis was mainly based on long-term large population-based data originating from several nations (varied ethnicity) and was performed using a random-effect model method. The heterogeneity of the results of the component studies was low, suggesting that each result was consistent and most variation was attributable to chance alone. The availability of detailed data on covariates allowed us to better control for confounding. However, as with other observational studies, some degree of measurement error in reporting dietary and other lifestyle characteristics is inevitable. NHANES data was collected (24 h dietary intake) by well-trained study personnel in a clinical setting, allowing to better report health data. However, we had no information on the dietary intake of the participants during the Follow-up which might have had an impact on the final results. Also, recall bias cannot be overcome as in such similar studies. Finally, as with many other observational study, reverse causality or residual confounding may potentially explain some findings. In future studies, it would be useful to carefully record the medication history as a potential confounding factor. We have taken into account the wide range of confounders (some of them were not available, including information on heart failure) in our analysis, which lessen, but do not exclude, the chance of residual confounding. Estimates of dietary intake are subject to day-to-day variability, so a single day of information may provide a

relatively imprecise estimate of usual intake. This might add to overall variability, thereby imprecision.[48]

In conclusion, despite the usual limitations of observational data (and so causality impossible to determine), our study highlights an unfavourable association of LCD with overall and cause specific mortality, based on both individual data and pooling previous cohort studies. Given the fact that long-term LCDs may well be associated with greater long-term harm, it might be important to consider whether we should routinely recommend such diets in clinical practice for weight loss until further higher quality studies evaluate this issue in greater detail. Rather, other dietary patterns that have been associated with both short-term weight loss and long-term benefits (such as low fat diets, with plentiful fibre) may be preferable.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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The material presented in this manuscript is original and has not been submitted for publication elsewhere. The results of this analysis were presented during a Press Conference on 27 August and as a presentation on 28 August 2018 during the *European Society of Cardiology* Congress in Munich, Germany.

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Conflict of interest: N.K. has given talks, attended conferences, and participated in trials sponsored by Amgen, Angelini, Astra Zeneca, Boehringer Ingelheim, MSD, Novartis, Novo Nordisk, Sanofi, and WinMedica. D.P.M. has given talks and attended conferences sponsored by MSD, AstraZeneca and Libytec; M.B. speakers bureau: Abbott/Mylan, Abbott Vascular, Actavis, Akcea, Amgen, Biofarm, KRKA, MSD, Sanofi-Aventis, Servier and Valeant; consultant to Abbott Vascular, Akcea, Amgen, Daichii Sankyo, Esperion, Lilly, MSD, Resverlogix, Sanofi-Aventis; Grants from Sanofi and Valeant; N.S. has consulted for or been on speakers bureau for: Amgen, Astra Zeneca, Boehringer Ingelheim, Eli-Lilly, Novo Nordisk, Napp, and Sanofi; he has received grant support from Boehringer Ingelheim. M.M. has no conflict of interest to declare.

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