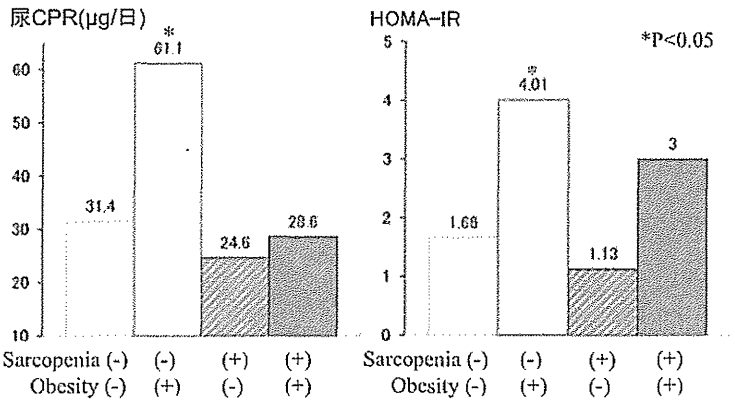

図1　Sarcopenic obesity とインスリン分泌，およびインスリン抵抗性

両者は炎症，インスリン抵抗性，低栄養，活動性低下といった共通の成因があり，これらが互いに影響し合い，悪循環を形成する．肥満による活動性低下はサルコペニアをきたし，さらに肥満をもたらす．

炎症は，肥満とサルコペニアの両者の成因に大きく関与する．65歳以上の871名を対象としたCHIANTY研究では，肥満と握力低下で定義したsarcopenic obesityの患者の血清CRP，IL-6，可溶性IL-6受容体が高値であると報告した[5]．脂肪細胞にマクロファージが浸潤するとIL-6やTNF-αなどの炎症性サイトカインが産生され，これらが筋肉に作用し，筋量や筋力の減少をもたらすことが考えられる．炎症サイトカインは脂肪量と正の相関，筋肉量と負の相関を示すという報告もある[6]．

上記の我々の患者でもRSMIと血清IL-6は負の相関を示した（$r=-0.622$，$P<0.05$）．この結果はIL-6高値が高齢者の筋力や移動能力の低下を予測するという報告と一致する[7]．機序としては，IL-6による筋肉でのIGF-1の同化作用の阻害，IL-6によるインスリン抵抗性亢進などが考えられる[8]．

インスリン抵抗性もsarcopenic obesityの重要な特徴の一つである．肥満や2型糖尿病患者で筋肉内に脂肪が蓄積するとインスリン抵抗性が惹起される．さらに，この筋肉でのインスリン抵抗性は筋力低下をもたらすことが考えられる．実際，高齢女性において握力の低下はインスリン抵抗性と関連したという報告がある[9]．高齢2型糖尿病患者では糖尿病がない人と比べて，下肢の筋力低下がおこりやすいが[10]，この糖尿病患者の下肢筋力低下もインスリン抵抗性の指標のHOMA-IRと関連した[11]．逆に，高齢糖尿病でもレジスタンス運動を行うと，除脂肪量が増加し，インスリン抵抗性が改善し，ADLが向上する[12]．

図1に示すように，上記の我々の患者でインスリン抵抗性の指標としてHOMA-IRを見ると，HOMA-IRは単なる肥満のみの患者で高値を示し，サルコペニアのみの患者では低値となり，sarcopenic obesityの患者で高値を示した（図1）．尿Cペプチドは単なる肥満のみで高値を示したことより，sarcopenic obesityではインスリン分泌がやや低下傾向があるのにインスリン抵抗性があるという状態であると考えられる．しかしながら，このsarcopenic obesityによるインスリン抵抗性は，高血糖とは関連を認めず，血糖コントロールには影響を及ぼしているとは考えにくい．

Sarcopenic obesityの予後

サルコペニアと肥満は身体機能低下の危険因子であるので，両者が重なったSarcopenic obesityは身体機能のADL低下，歩行障害，転倒などの悪影響を及ぼす．New Mexico Aging Process Studyの8年間の追跡調査では，sarcopenic obesityは対照と比較して年齢，性，活動度，高血圧，関節炎を調整しても，2.63倍IADLの低下をきたした[13]．また，New Mexico Elder Health Surveyでは，sarcopenic obesityの患者は対照と比べて要介護状態は男性で8.7倍，女性で12.0倍，歩行障害は男性で4.4倍，女性は5.5倍，転倒は男性は3.3倍，女性は2.1倍おこしやすく，そのリスクは単なる肥満やサルコペニアよりも高かった[1]．EPIDOS研究では，sarcopenic obesityは階段の昇降が対照と比べて約2倍障害されており，単なるサルコペニアでは身体機能の障害はなかった[3]．しかし，サルコペニアを筋肉量で定義した2つの断面調査では，sarcopenic obesityと身体機能との関連はなかったという報告もある[6]．

上記のわれわれの患者をサルコペニアと肥満の有無で4群に分けて比較検討した．表1に示すようにサルコペニアのみ群とsarcopenic obesity群では高齢でRSMIや

表1　研究の対象

	Obesity（−）Sarcopenia（−）(n=26)	Obesity（+）Sarcopenia（−）(n=22)	Obesity（−）Sarcopenia（+）(n=32)	Obesity（+）Sarcopenia（+）(n=16)
年齢（歳）	73.6±7.9	70.7±8.0	77.6±8.1***	78.3±6.3***
女性（%）	57.7	68.2	62.5	62.5
罹病年数	20±12	11±9*	20±11	10±6*
BMI	22.6±1.8	28.9±2.7***	20.1±2.3***	24.4±2.0*
体脂肪（%）	19.7±5.4	38.0±6.4*	20.4±8.1	34.0±4.8*
RSMI	6.7±0.7	6.8±0.8	5.3±0.5*	5.5±0.6*
大腿骨頸部 BMD	0.75±0.16	0.80±0.12	0.66±0.14*	0.69±0.13
HbA1c（%）	8.2±1.7	8.6±1.9	8.1±1.6	7.8±1.5

*P<0.05，**P<0.01，***P<0.001 vs Obesity（−），Sarcopenia（−）群

表2　サルコペニアと sarcopenic obesity の相違点

	RSMI (Sarcopenia)	Lean mass/Fat mass (Sarcopenic obesity)
年齢	r=−0.418***	n.s.
骨密度（大腿骨頸部）	r=0.526***	n.s.
血清アルブミン	r=0.218*	n.s.
Hb	r=0.268*	r=−0.240*
尿 CPR	r=0.377***	r=−0.342**
HOMA-IR	n.s.	r=−0.459**
SBP	r=−0.243*	n.s.
DBP	n.s.	r=−0.354*
MMSE	r=0.230（p=0.06）	r=−0.238（p=0.05）

*P<0.05，**P<0.01，***P<0.001，r：Spearman の相関係数

骨密度も低い傾向が見られた．肥満群，sarcopenic obesity 群とも罹病年数も短く，BMI，体脂肪量が大きかった．しかし，排泄と入浴の障害はサルコペニアのみ群と sarcopenic obesity群とで同様であり（それぞれ 38.1% vs 35.7，38.1% vs 42.9%）階段昇降の障害の頻度のみ，sarcopenic obesity の群が大きい（36.8% vs 61.5%，P<0.05）という結果が得られた．

一方，筋力で定義した sarcopenic obesityの方がより機能予後との関連が出やすい．Finish health 2000 Survey では握力の低下と肥満が重なるとより歩行障害をおこした[13]．CHIANTY 研究では多変量解析で他の交絡因子を調整すると，筋肉量ではなくて，歩行速度が死亡の予知因子となった[14]．

Sarcopenic obesity と心血管障害

Sarcopenic obesityはインスリン抵抗性が強く，心血管疾患のリスクとなることが考えられるが，心血管疾患との関連を示した論文はほとんどない．NHANES III 研究では Sarcopenic Obesityはインスリン抵抗性増加や慢性の高血糖と関連を示し，65 歳未満でこの関連が強かった[15]．Korean Longitudinal Study on Health and Aging では appendicular skeletal mass/体重でサルコペニアを定義し，肥満を CT における内臓脂肪面積 100 cm^2 以上と定義すると，Sarcopenic obesityは単なる肥満やサルコペニアと比べてメタボリックシンドロームのリスクが高いと報告している[16]．高齢者 3,366 名の 8 年間の追跡調査では，ウエスト周囲径と筋力で定義した Sarcopenic obesityは心血管疾患のリスクが単なる肥満やサルコペニアと比べて高く，1.23 倍のリスクがあると報告している[17]．

サルコペニアと sarcopenic obesityとの相違

DXA 法による RSMI をサルコペニアの指標，lean mass/fat mass を sarcopenic obesity の指標として，年齢，骨密度，血清アルブミン，Hb，尿 CPR，HOMA-IR，収縮期血圧，拡張期血圧，HbA1c，中性脂肪値との Spearman の相関を見て，比較検討を加えてみた．表 2 に示すように RSMI は年齢，骨密度，血清 Alb，Hb，尿 CPR，収縮期血圧との有意の相関があり，サルコペニアは加齢とともに増え，骨密度低下，低栄養，収縮期高血圧と関連し，インスリン欠乏とも関連が見られ，一連の加齢現象の一つと考えられた．一方，lean mass/fat mass は

HOMA-IR，尿CPR，Hb，拡張期血圧と負の相関を示した．即ち，sarcopenic obesityは年齢とは関連せず，インスリン抵抗性，高インスリン血症，多血症，拡張期高血圧と関連し，肥満の病気としての特徴を大きく反映している．

サルコペニア，sarcopenic obesityと転倒について

外来糖尿病患者63名（年齢：78.4±6.0歳，男15例，女性48例）を対象にsarcopenic obesityと過去1年間の転倒回数，身体能力との関連を検討した．対象のBMIは23.2±2.9，罹病年数は17±8年，HbA1cは6.9±0.9%，治療法は食事のみ11名，経口薬45名，インスリン7例であった．Sarcopenic obesityの指標であるlean mass/fat massは握力（r＝0.255，P＜0.05）や開眼片足時間（r＝0.291，P＜0.05）と相関を示したが，転倒回数，老研式活動能力指標とは有意の関連を認めなかった．筋量を示すRSMIは握力（r＝0.530，P＜0.01）とは相関を示したが，転倒の回数，老研式活動能力指標とは相関を示さなかった．これに対してUp & Goテストの時間は転倒回数（r＝0.247，P＜0.001），老研式活動能力指標（r＝−0.439，P＜0.001）と有意の相関を示した．この結果はsarcopenic obesityや単なる筋肉量よりも筋肉の機能である身体能力の方が転倒，IADLの障害との関連が大きいことを示している．最近，サルコペニアの定義に6m歩行時間，椅子立ち上がり時間，立位バランス，Up & Goテスト，握力などの筋肉の機能（身体能力）も含めることが提唱されている[4]．したがって，sarcopenic obesityも筋肉の機能を考慮した定義によって見直す必要があり，機能的予後，転倒，死亡との関連があるか否かを再検討すべきである．

COI等：本論文に関連して，開示すべきCOIはなし．

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OBSERVATIONS

Sitagliptin Successfully Ameliorates Glycemic Control in Werner Syndrome With Diabetes

Werner syndrome (WS) is an autosomal recessive disorder caused by a mutation in the *WRN* gene, and it is considered to be a representative type of progeroid syndrome (1). Patients with WS often exhibit insulin resistance, which is associated with the accumulation of visceral fat and disadipocytokinemia. We and others have previously reported that pioglitazone, a peroxisome proliferator–activated receptor γ ligand, improved glycemic control and insulin sensitivity with normalization of disadipocytokine levels in patients with WS (2,3).

Here we describe a diabetic subject with WS that had good glycemic control with pioglitazone initially but worsened because of abdominal obesity and increasing visceral fat area. Sitagliptin, an inhibitor of dipeptidyl peptidase-4, was then administered, which resulted in successful improvement of glycemic control.

A 58-year-old Japanese woman with WS was admitted to our hospital with poor glycemic control. At the first visit to our hospital at 46 years of age, she exhibited graying and loss of hair, short stature, a hoarse voice, refractory skin ulcers, bilateral juvenile cataracts, dyslipidemia, and diabetes. The diagnosis of WS was confirmed by genomic DNA analysis. At that time, her height was 1.46 m, weight was 36 kg, and BMI was 15.1 kg/m^2. Her visceral fat area was 111 cm^2 (normal range, <100 cm^2 for Japanese). She was prescribed 15 mg pioglitazone daily, which resulted in stable glycemic control. Her glycated hemoglobin (HbA_{1c}) level was maintained at ~6.9% for 12 years. However, she gradually gained weight and visceral fat area (191 cm^2), which worsened her glycemic control. At the present admission, continuous glucose monitoring system (CGMS) was performed, and postprandial hyperglycemia was observed. Therefore, a 50-mg daily dose of sitagliptin was added to the pioglitazone regimen. Her laboratory parameters before and after sitagliptin administration for 6 months were as follows: fasting glucose, 122 and 110 mg/dL; 2-h postprandial glucose, 162 and 129 mg/dL; fasting C-peptide, 2.81 and 3.32 mg/dL; 2-h postprandial C-peptide, 13.99 and 11.5 mg/dL; HbA_{1c}, 7.5 and 6.5%; and mean ± SD of glucose levels detected by CGMS, 163.2 ± 32.0 and 117.1 ± 20.6 mg/dL, respectively. CGMS confirmed that sitagliptin effectively suppressed postprandial hyperglycemia.

Although patients with WS are insulin resistant, it was suggested that only those who have impaired insulin secretion develop overt diabetes (4). We were unable to observe an improvement in 2-h postprandial C-peptide levels after sitagliptin administration; nevertheless, sitaglipitin may have improved early insulin secretion in response to meals. Furthermore, sitagliptin reportedly suppresses glucagon secretion. Because hyperglucagonemia has been observed in patients with WS (5), sitagliptin may ameliorate glycemic controls at least in part via correction of dysglucagonemia.

In conclusion, we demonstrated that a single dose of sitagliptin was well tolerated in a patient with WS and diabetes, resulting in a significant improvement in glycemic control. Sitagliptin may represent an alternative choice for treatment of diabetes in patients with WS. Further studies on the use of dipeptidyl peptidase-4 inhibitor in WS with diabetes will confirm our findings.

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Brief report

Atorvastatin ameliorates podocyte injury in patients with type 2 diabetes complicated with dyslipidemia

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ABSTRACT

We examined the effects of atorvastatin on urinary podocyte excretion. Thirteen patients with type 2 diabetes receiving 2.5 mg of rosuvastatin were recruited and the medication was switched to 10 mg of atorvastatin for a 24-week period. With the switch to atorvastatin, the urinary excretion of podocytes was significantly reduced.

1. Introduction

A number of clinical studies have indicated that the 3-hydroxyl-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitor (statin) has favorable effects on renal function by controlling dyslipidemia [1–3]. Furthermore, it has been reported that various statins have a different impact on renal function, especially on diabetic nephropathy (DN) [4].

The detachment of podocytes from the glomerular basement membrane and podocyte loss in the urine are associated with glomerulosclerosis progression [5]. Therefore, monitoring urinary podocytes could be clinically useful [6].

With this in mind, we examined the effect of atorvastatin, a lipophilic statin, on urinary podocytes when rosuvastatin, a hydrophilic statin was switched for atorvastatin.

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2. Methods and research design

2.1. Subjects

Thirteen patients with type 2 diabetes (10 men and 3 women) were recruited in this study (Table 1). All patients had received 2.5 mg rosuvastatin for at least 3 months. We only recruited patients with normo- to microalbminuria and excluded those with macroalbuminuria, hematuria, and collagen diseases. We also recruited patients who were positive for the excretion of urinary podocytes.

2.2. Study design

Statin therapy of diabetic patients positive for the excretion of urinary podocytes was switched from 2.5 mg of rosuvastatin to 10 mg of atorvastatin for a 24-week period, and the effects on urinary podocyte excretion evaluated before and after switching the statin. The present study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the ethical committee of the School of Medicine, Chiba University prior to its inception. All patients understood the study aims and methods and provided written, informed consent.

2.3. Clinical parameters of blood and urine

Venous blood samples and urine samples were collected at baseline and at the end of the intervention in the morning after a 12-h fast. Fresh samples of first-voided morning urine were collected and urinary podocytes were measured using the U-podocyte test (at Mitsubishi Chemical Medicine) as described previously [7].

Table 1 – Basic characteristics of patients.

Number (men/women)	13 (10/3)
Age (years)	61.3 ± 11.9
Systolic blood pressure (mmHg)	122.4 ± 16.9
Diastolic blood pressure (mmHg)	71.2 ± 1.0
Fasting blood glucose (mg/dl)	158.1 ± 64.4
HbA1c (mmol/mol)	53.7 ± 11.2
HbA1c (%)	7.06 ± 1.04
Serum creatinine (mg/dl)	0.82 ± 0.18
Estimated GFR (ml/min/1.73 m^2)	70.1 ± 19.2
Urinary albumin (μg/g Cre)	28.6 ± 21.4
Uric acid (mg/dl)	5.4 ± 1.6
LDL-C (mg/dl)	97.5 ± 21.9
HDL-C (mg/dl)	51.5 ± 12.1
TG (mg/dl)	169.2 ± 73.4
ARBs (%)	46.2
Sulfonylurea (%)	38.5
αGI (%)	46.1
Metformin (%)	61.5
Thiazolidine (%)	38.5
DPPIV-I (%)	38.6
Insulin (%)	30.7

LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, ARB: angiotensin II type 1 receptor blocker, αGI: alpha glucosidase inhibitor, DPPIV-I: dipeptidyl peptidase IV inhibitor.

2.4. Statistical analysis

Values are indicated as mean ± standard deviation (SD). A paired t-test was used and statistical analyses were performed using SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Atorvastatin significantly reduces urinary podocyte excretion

As shown in Fig. 1, urinary podocytes were undetected in 10/13 (77%) of patients after a 24-week period of atorvastatin administration. During the study period, there was no change in treatment for diabetes mellitus or hypertension, and there was no difference in lipid levels, glucose metabolism, or blood pressures before or after the statin switch (Table 2). The mean values of the oxidative stress marker, urinary F(2)-isoprostane, tended to decrease without reaching significance. Also, there were no adverse effects and no significant differences in urinary albumin excretion (Table 2) and estimated glomerular filtration rate (eGFR) (Fig. 1) before and after the statin switch.

4. Discussion

In this study in people with type 2 diabetes and urinary podocyte excretion, switching from rosuvastatin to atorvastatin significantly reduced urinary podocyte loss.

A recent meta-analysis has shown that statin therapies can reduce proteinuria and benefit kidney function [1] however the effect varies between statins. For instance, high-dose atorvastatin significantly reduced proteinuria but did not affect renal function, whereas rosuvastatin was associated with a significant decline in renal function and had no effect on proteinuria in patients with type 2 diabetes and moderate

Table 2 – Clinical parameters of patients before and after switching medication to atorvastatin from rosuvastatin.

	Before	After 6 months	p-Value
Urinary podocytes (cells/ml)	0.3 ± 0.2	0.07 ± 0.16	0.002
Estimated GFR (ml/min/1.73 m^2)	70.1 ± 19.2	69.5 ± 20.2	0.94
Urinary albumin (μg/g Cre)	28.6 ± 21.4	30 ± 29.2	0.89
LDL-C (mg/dl)	97.5 ± 21.9	95.4 ± 29.0	0.84
HDL-C (mg/dl)	51.2 ± 10.2	51.5 ± 12.1	0.94
Triglyceride (mg/dl)	169.2 ± 73.4	153.2 ± 55.1	0.53
F(2)-isoprostane (pg/ml)	449.1 ± 175.8	408.5 ± 117.8	0.53
FBS (mg/dl)	158.1 ± 64.5	136.6 ± 44.3	0.33
HbA1c (mmol/mol)	53.7 ± 11.2	51.0 ± 7.7	0.24
HbA1c (%)	7.06 ± 1.04	6.4 ± 0.71	0.5
Systolic blood pressure (mmHg)	122.4 ± 16.9	126.2 ± 18.4	0.59
Diastolic blood pressure (mmHg)	71.2 ± 14.0	70.0 ± 13.5	0.83

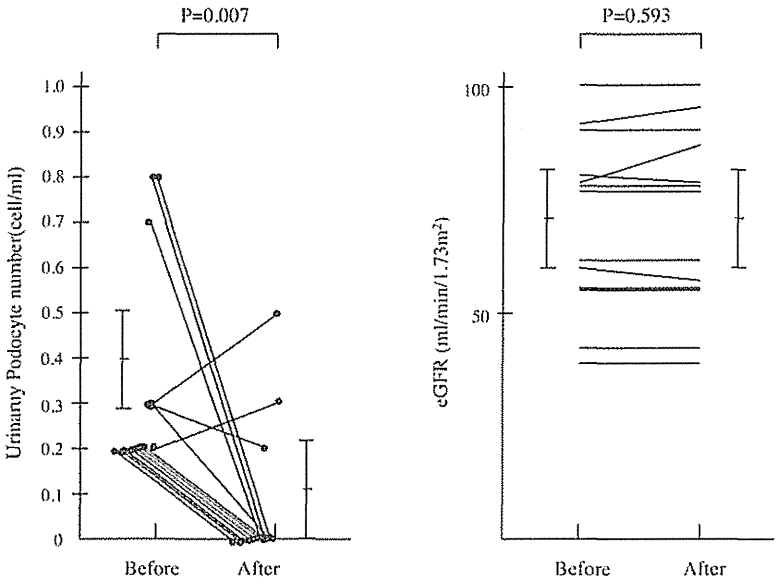

Fig. 1 – Effects of atorvastatin on urinary podocytes and estimated GFR Patients with urinary podocytes had their medications switched from 2.5 mg of rosuvastatin to 10 mg of atorvastatin for 24 weeks. The effects on urinary podocytes and eGFR were evaluated before and after switching the statins.

proteinuria [4]. Wu et al. reported that both atorvastatin and rosuvastatin improved glomerular filtration rate, whereas atorvastatin seemed to be more effective in reducing proteinuria in a meta-analysis of 16 clinical trials [8]. Our results also showed that atorvastatin seemed to be more protective for podocyte loss than rosuvastatin.

The role of podocytes in DN development has been thoroughly investigated and detection of urinary podocytes is a good marker for progression of several kidney diseases such as glomerulonephritis, IgA nephritis, focal glomerular sclerosis, and DN [6,7,9–11].

A protective role of statins on glomerular podocytes has also been demonstrated. Shibata et al. reported that fluvastatin suppressed puromycin aminonucleoside-induced activation of RhoA and actin cytoskeleton reorganization [12].

The renoprotective effects of statins are related to the pleiotropic effects beyond low density lipoprotein cholesterol (LDL-C) lowering effects. Statins vary differently in their rate of absorption, amount of protein binding, degree of renal excretion, metabolism and hydrophobicity. Small angle X-ray diffraction shows that statins distribute differently within cellular membranes because of minor variations in their chemistry, leading to differences in their efficacy and metabolism. Hydrophilic properties of rosuvastatin prevent access to the hydrophobic membrane hydrocarbon core. By contrast, atorvastatin intercalates into the upper hydrocarbon core of the membrane lipid bilayer adjacent to the glycerol backbone [13]. This membrane location may influence certain intracellular signaling processes in podocytes, particularly in membrane bound small GTPase which is important for podocyte cytoskeleton rearrangement [12].

Atorvastatin is a potent statin for lowering LDL-C and induces anti-oxidative stress [14]. Anti-oxidant therapy has been shown to reduce or retard damaging effects of ROS and diabetic complications [15,16]. Therefore, we examined levels of F(2)-isoprostane, a marker of oxidative stress, before and after switching statin medications, but were unable to detect any significant differences.

Although the occurrence of urinary podocytes was reduced by switching rosuvastatin to atorvastatin, we were not able to detect a significant difference in renal function such as eGFR and ACR. Because the metabolic parameters were already well controlled before switching the statins, not enough time may have passed, to detect a difference in renal function.

In conclusion, our results show that atorvastatin may protect people with type 2 diabetes from podocyte injury in addition to offering benefits of LDL-C lowering. However, large-scale population-based randomized controlled trials for longer periods of time are necessary to define the benefits of atorvastatin use in the patients with urinary podocyte excretion.

Conflict of interest

The authors declare that they have no conflict of interest.

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Preliminary communication

A tomato-rich diet is related to depressive symptoms among an elderly population aged 70 years and over: A population-based, cross-sectional analysis

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ABSTRACT

Background: Enhanced oxidative stress or defective anti-oxidant defenses are related to the pathogenesis of depressive symptoms. Lycopene is the most powerful antioxidant amongst the carotenoids. The aim of this study was to investigate the relationship between different vegetables, including tomatoes/tomato products (a major source of lycopene), and depressive symptoms in a community-based elderly population.

Methods: We analyzed a cross-sectional survey including 986 community-dwelling elderly Japanese individuals aged 70 years and older. Dietary intake was assessed using a valid self-administered diet-history questionnaire, and depressive symptoms were evaluated using the 30-item Geriatric Depression Scale with 2 cut-off points: 11 (mild and severe) and 14 (severe) or use of anti-depressive agents.

Results: The prevalence of mild and severe and severe depressive symptoms was 34.9% and 20.2%, respectively. After adjustments for potentially confounding factors, the odds ratios of having mild and severe depressive symptoms by increasing levels of tomatoes/tomato products were 1.00, 0.54, and 0.48 (p for trend <0.01). Similar relationships were also observed in the case of severe depressive symptoms. In contrast, no relationship was observed between intake of other kinds of vegetables and depressive symptoms.

Limitations: This is a cross-sectional study, and not for making a clinical diagnosis of depressive episodes.

Conclusions: This study demonstrated that a tomato-rich diet is independently related to lower prevalence of depressive symptoms. These results suggest that a tomato-rich diet may have a beneficial effect on the prevention of depressive symptoms. Further studies are needed to confirm these findings.

1. Introduction

For several decades, the health burden of stress-related diseases, including depressive symptoms and anxiety disorders, has been rapidly increasing. The presence of depressive symptoms in later life is recognized as a public health problem. Depressive symptoms contribute a significant independent risk for the onset of coronary disease (Wulsin and Singal, 2003), and disease susceptibility (Zorrilla et al., 2001). Depressive symptoms also worsens the outcomes of many medical disorders, promotes disability and increases mortality (Alexopoulos, 2005).

Several studies have suggested that enhanced oxidative stress or defective antioxidant defenses may be related to affective disorder or the pathogenesis of depressive symptoms (Bilici et al., 2001; Khanzode et al., 2003; Ozcan et al., 2004; Srivastava et al., 2002;

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Tsuboi et al., 2004). A longitudinal study in elderly residents showed preventive effects of vitamin E, a dietetically anti-oxidative compound, on the progression of depressive symptoms in male participants (Shibata et al., 1999). On the other hand, lycopene, a carotenoid antioxidant, is the most powerful antioxidant amongst carotenoids and there is no evidence of toxic effects (Heber and Lu, 2002). In vitro study of singlet oxygen quenching action, lycopene was shown to be 100 times more efficient than vitamin E (Atessahin et al., 2005). Thus, we hypothesized that a tomato-rich diet, a major source of lycopene (tomatoes and tomato-based sauces, juices, and ketchup account for more than 85% of the dietary intake of lycopene for most people (Rao and Rao, 2007)) may have a potentially beneficial effect on the prevention of depressive symptoms. However, to our knowledge, only a few studies have investigated the relationship between tomato/lycopene and depressive symptoms (Tsuboi et al., 2004). Moreover, no studies have fully investigated the relationship between a tomato-rich diet and depressive symptoms in a community-dwelling elderly population.

Because vegetables are good sources of antioxidant phytochemicals that mitigate the damaging effect of oxidative stress, we designed a cross-sectional study to compare the relationship between intake of several vegetables and tomato products with depressive symptoms in community-dwelling elderly participants aged ≥ 70 years.

2. Methods

2.1. Study participants

The Tsurugaya Project included subjects aged 70 years and older who were living in the Tsurugaya area of Sendai, one of the major cities in the Tohoku area of Japan. The data were obtained in 2002 from 1178 individuals giving their informed consent for data analysis. A detailed description of the methods has been published elsewhere (Niu et al., 2005a). The protocol of this study was approved by the Institutional Review Board of the Tohoku University Graduate School of Medicine.

In this study, depressive symptoms were assessed with the aid of the Geriatric Depressive symptoms Scale (GDS) (Brink et al., 1982). Of the 1178 subjects, 1169 completed the GDS. We also excluded those subjects whose did not have any information on diet ($n=94$). Furthermore, those who reported a history of cancer ($n=89$) and cognitive dysfunction (Mini Mental State Examination [MMSE] Score (Folstein et al., 1975) <18) ($n=17$) were also excluded. As a result of these exclusions, the final study population included 986 subjects.

2.2. Assessment of depressive symptoms

Depressive symptoms were assessed according to the Japanese version (Niino et al., 1991) of the 30-item GDS using 2 cut-off points (GDS score, ≥ 11 or 14) or the use of anti-depressive agents, indicating relatively mild to severe depressive symptoms or severe depressive symptoms (Brink et al., 1982).

2.3. Assessment of dietary intake

A brief self-administered diet history questionnaire (BDHQ) included 75 food items with specified serving sizes that were described by natural portions or standard weight and volume measures of the servings commonly consumed in this study population. For each food item, participants indicated their mean frequency of consumption over the past year, in terms of the specified serving size by checking 1 of the 7 frequency categories ranging from "almost never" to "2 or more times/d". The question of tomatoes/tomato products included some commonly eaten tomato foods such as tomato, tomato ketchup, stewed tomato, or tomato stew. According with BDHQ, Other kinds of vegetables were divided into four categories as follow: (1) Green-leaf vegetables, (2) Cabbage and Chinese cabbage, (3) Carrot, onion, burdock, lotus root and pumpkin, (4) Japanese white radish (daikon) and turnips. The mean daily intake of nutrients was calculated using an *ad hoc* computer program developed to analyze the questionnaire. The Japanese food composition tables, 4th edition, and the other sources (Sakai et al., 1995) were used as the nutrient database. The reproducibility and validity of the BDHQs have been described in detail elsewhere (Sasaki, 2005).

2.4. Assessment of other variables

Anthropometrics (height, body weight) were recorded using a standardized protocol. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Blood pressure (BP) was measured at home using an HEM747IC device (Omron Life Science Co. Ltd, Tokyo, Japan), which uses the cuff-oscillometric method to generate a digital display of systolic and diastolic pressures. The mean of 15.6 ± 10.5 (mean $\pm$ SD) BP measurements was used as the BP value. Participants who did not measure home BP for at least 3 days were treated as having missing information on hypertension. Hypertension was defined as a home systolic BP ≥ 135 mm Hg or a home diastolic BP ≥ 85 mm Hg or the use of antihypertensive agents (Chobanian et al., 2003).

Blood samples were drawn from the antecubital vein of the seated subject with minimal tourniquet use. Specimens were collected in siliconized vacuum glass tubes containing sodium fluoride for blood glucose, and no additives for lipids analyses. Blood glucose concentrations were measured using enzymatic methods (Shino-Test, Tokyo, Japan). Diabetes was defined as a casual blood glucose concentration of ≥ 200 mg/dL or the current use of antidiabetic medication.

Sociodemographic variables including gender, age, educational level, and perceived social support (PSS) were also assessed. Educational level attained was assessed by determining age at completion of schooling and was divided into 2 categories: ≤ 12 or >12 years. PSS was evaluated on the basis of responses ("yes" or "no") to the following 5 questions: "Do you have someone to whom you can talk when you are in trouble?" (PSS1); "Do you have someone to whom you can talk when your physical condition is not good?" (PSS2); "Do you have someone who can help you with daily housework" (PSS3); "Do you have someone who can take you to hospital when you do not feel well?" (PSS4); and "Do you have someone who can take care of you when you are ill in bed?" (PSS5). These questions were extracted from a previous study regarding social support and elderly depressive symptoms in a rural community (Muraoka et al., 1996). A single summed score was calculated based on the PSS 1–5. The lack of PSS was defined as PSS score=0.

Health-related variables assessed included history of physical illness, pain, cognitive function, instrumental activities of daily living (IADL), and current use of medication. History of physical illness was evaluated on the basis of responses ("yes" or "no") to questions. Pain within the previous 4 weeks was assessed by the question, "Have you had any pain recently? If so, how intensely do you feel such pain?" Possible answers were "no pain," "very mild pain," "mild pain," "moderate pain," and "severe pain." A subject who reported "mild" to "severe" pain was considered to have pain. Cognitive function was assessed on the basis of the MMSE and was classified into 2 categories: 18–23 and ≥ 24. IADLs were assessed using the Rouken–Shiki scale (Koyano et al., 1987) and a cut-off point of 10/11 was used to determine

impairment in IADL. The drug information was confirmed by a well-trained pharmacist.

Information on smoking status and drinking status were obtained from the questionnaire survey. Physical activity (PA) was assessed first by a self-reported single-item question on whether the participant undertook any PA during the past year. If yes, questions were asked about the frequency and duration of walking, brisk walking, and sports. PA was then classified into 3 categories, based on frequency and duration: (1) "High," at least 3–4 times per week for at least 30 min each time; (2) "Low," reporting some activity in the past year, but not enough to meet high levels; and (3) "None," no PA. Furthermore, PA was classified into 6 levels based on the above 3 categories and the type of physical activity, such as walking, brisk walking, and sports: (1) "Level 1," no walking, no brisk walking, no sports; (2) "Level 2," low walking, no brisk walking, no sports; (3) "Level 3," high walking, no brisk walking, no sports; (4) "Level 4," any walking, low brisk walking, no sports; (5) "Level 5," any walking, high brisk walking, no sports; (6) "Level 6," any walking, any brisk walking, low or high sports. Detailed information has been provided in previous reports (Niu et al., 2005b). Finally, subjects were divided into 2 categories: $\leq$ level 3 or $>$ level 3.

2.5. Statistical analysis

Descriptive data are presented as mean (95% confidence interval [95% CI]) or percentages. Depressive symptoms were used as the dependent variable and the tomato/tomato product and other vegetable intake level as the independent variable. Multiple logistic regression analysis was used to examine the relationship of tomato/tomato product and other vegetable intake with depressive symptoms after adjustment for age, sex, BMI, hypertension, diabetes, history of cardiovascular diseases, smoking and drinking habits, physical activity, cognitive status, impaired IADL, self-reported body pain, educational level, living alone, marital status, lack of PSS, total energy intake, and intake of all kinds of fruits (tertiles), green tea (tertiles) (Niu et al., 2009), and mutual other kinds of vegetables. The odds ratios (ORs) and 95% CIs for depressive symptoms for increasing tomato/tomato product and other vegetable intake levels, with the lowest level as the reference, were also calculated using multiple logistic regression analysis. Interactions between tomato/tomato product and other vegetable intake levels and confounders of depressive symptoms were tested by the addition of cross-product terms to the regression model. A significant difference was defined as $p < 0.05$. All statistical analyses were performed using a Statistical Analysis System 9.1 edition for Windows (SAS Institute Inc., Cary, NC, USA.).

3. Results

Among 986 subjects who were available to be analyzed, 34.9% and 20.2% were classified as having mild and severe and severe depressive symptoms, respectively.

Age- and sex-adjusted participant characteristics according to tomato/tomato product status are presented in Table 1. The proportion of male, current smoker, lower educational level, and widowed or divorced status were significantly lower across the tomatoes/tomato products groups (p for trend ≤ 0.03). The proportion of subjects who were married was significantly higher across the tomatoes and tomato products groups (p for trend $= 0.04$). Mean total energy intake was significantly higher across the tomatoes/tomato products groups (p for trend < 0.0001). The mean GDS score was significantly lower across the tomatoes/tomato products groups (p for trend < 0.0001). Otherwise, no significant difference

Table 1
Age- and sex-adjusted characteristics according to categories of tomato/tomato product consumption.

	Tomatoes/tomato products consumption			p for trend
	$\leq$ 1 time (wk)	2–6 times (wk)	$\geq$ 1 time (d)	
No.	139	325	522	–
Age (year)	75.5 (74.7–76.3)	75.9 (75.4–76.4)	76.1 (75.7–76.5)	0.45
Sex (male)	49.6	48.3	36.2	< 0.001
BMI (kg/m^2)	23.5 (23.0–24.1)	23.8 (23.5–24.2)	23.9 (23.6–24.2)	0.42
Diabetes	7.9	9.2	9.4	0.70
Hypertension	71.2	68.6	69.0	0.62
History of CVD	14.4	16.0	14.8	0.80
Smoking status	–	–	–	–
Current smoker	25.9	12.9	9.8	< 0.001
Ex-smoker	26.6	35.1	25.9	0.41
Drinking status	–	–	–	–
Current drinker	44.6	44.3	35.8	0.32
Ex-drinker	9.4	12.0	12.3	0.17
PA ($>$ level 3)	38.1	37.9	37.9	0.60
Self-reported total number of physical illness (≥ 2)	63.3	68.6	69.9	0.26
Cognitive ability (18 $\leq$ MMSE < 24)	5.8	9.9	7.1	0.61
Impaired IADL	12.2	14.2	10.5	0.19
Self-rated health (yes)	79.1	82.5	81.8	0.44
Body pain (yes)	70.5	81.2	77.4	0.23
Lack of PSS (total score=0)	15.1	14.8	13.4	0.34
Educational level ($\leq$ 12 years)	79.1	71.1	67.8	< 0.001
Living alone (yes)	28.1	21.5	25.1	0.13
Marital status married	59.0	63.4	60.5	0.04
Widowed or divorced	37.4	33.2	34.9	0.03
Total energy intake (kcal/d)	1841.7 (1768.4–1915)	1976.7 (1928.8–2024.6)	2084.4 (2045.8–2123)	< 0.0001
GDS	10.9 (10.1–11.8)	9.1 (8.5–9.7)	8.4 (7.9–8.8)	< 0.0001

BMI, body mass index; CVD, cardiovascular diseases; PA, physical activity; PSS, perceived social support; MMSE, Mini Mental State Examination; IADL, Instrumental Activity of Daily Living; GDS, Geriatric Depression Scale.
Variables are presented as mean (95% confidence interval).

Table 2
Adjusted association between consumption of tomatoes/tomato products and other kinds of vegetables and depressive symptoms [a].

Odds ratio (95% confidence interval)	Tomato and tomato product consumption			p for trend[b]
	≤1 time (wk)	2–6 times (wk)	≥1 time (d)	
Tomatoes and tomato products	–	–	–	–
No. of participants	139	325	522	–
No. of mild and severe depressive symptoms, defined as GDS of ≥11 or use of antidepressants	70	111	163	–
Crude	1.00	0.51 (0.34–0.77)	0.45 (0.31–0.66)	<0.001
Age- and sex-adjusted	1.00	0.49 (0.33–0.74)	0.40 (0.27–0.59)	<0.0001
Multiple adjusted [c]	1.00	0.54 (0.35–0.85)	0.48 (0.31–0.75)	<0.01
Green-leaf vegetables	–	–	–	–
No. of participants	188	523	275	–
No. of mild and severe depressive symptoms, defined as GDS of ≥11 or use of antidepressants	80	179	85	–
Crude	1.00	0.70 (0.50–0.99)	0.60 (0.41–0.89)	0.01
Age- and sex-adjusted	1.00	0.69 (0.49–0.97)	0.58 (0.39–0.85)	<0.01
Multiple adjusted [c]	1.00	0.78 (0.51–1.19)	0.72 (0.45–1.15)	0.19
Cabbage and Chinese cabbage	–	–	–	–
No. of participants	200	605	181	–
No. of mild and severe depressive symptoms, defined as GDS of ≥11 or use of antidepressants	78	203	63	–
Crude	1.00	0.79 (0.57–1.10)	0.84 (0.55–1.27)	0.37
Age- and sex-adjusted	1.00	0.78 (0.56–1.09)	0.79 (0.51–1.20)	0.24
Multiple adjusted [c]	1.00	1.07 (0.71–1.64)	1.46 (0.85–2.50)	0.18
Carrot, onion, burdock, lotus root and pumpkin	–	–	–	–
No. of participants	102	556	328	–
No. of mild and severe depressive symptoms, defined as GDS of ≥11 or use of antidepressants	42	199	103	–
Crude	1.00	0.83 (0.60–1.15)	0.61 (0.41–0.92)	0.02
Age- and sex-adjusted	1.00	0.78 (0.56–1.09)	0.56 (0.37–0.85)	<0.01
Multiple adjusted [c]	1.00	1.31 (0.77–2.27)	1.34 (0.74–2.45)	0.44
Japanese white radish (daikon) and turnips	–	–	–	–
No. of participants	265	519	202	–
No. of mild and severe depressive symptoms, defined as GDS of ≥11 or use of antidepressants	105	178	61	–
Crude	1.00	0.80 (0.59–1.08)	0.66 (0.45–0.97)	0.03
Age- and sex-adjusted	1.00	0.78 (0.57–1.06)	0.61 (0.41–0.90)	0.01
Multiple adjusted [c]	1.00	0.94 (0.65–1.37)	0.70 (0.43–1.13)	0.17

[a] GDS, Geriatric Depression Scale.
[b] Obtained by using multiple logistic regression analysis.
[c] Adjusted for age, sex, BMI, hypertension, diabetes, history of cardiovascular disease, smoking and drinking habits, physical activity, cognitive status, impaired instrumental activities of daily living (IADL), self-reported body pain, educational level, living alone, marital status, lack of perceived social support (PSS), total energy intake, all kinds of fruits, green tea, and mutual other kinds of vegetables.

was observed among tomatoes/tomato products groups (p for trend ≥0.13).

Table 2 shows the adjusted relationship between tomatoes/tomato products and other kinds of vegetables and mild and severe depressive symptoms. The ORs for mild and severe depressive symptoms decreased across the levels of tomato/tomato product intake. Age- and sex-adjusted ORs (95% CI) for depressive symptoms across tomato/tomato product intake levels were 1.00, 0.49 (0.33–0.74), and 0.40 (0.27–0.59) (p for trend <0.0001). These results were unchanged when adjusted for multiple confounding factors. Similar relationships were also observed when males and females were analyzed separately (p for interaction=0.08). Of the other covariants, smoking/drinking status and educational level were related with depressive symptoms. The tests for interactions between the categories of tomato/tomato product intake and these potential confounders in the final models were not found to be significant. Furthermore, because depressive status is also related to unhealthy eating habits and appetite (Andreasson et al., 2007; Cassano and Fava, 2002), a sensitivity analysis was added to assess the relationship between tomatoes/tomato products and depressive symptoms, excluding those who had very low (under 2.5%) or high (upper 2.5%) energy intake. However, this exclusion did not change the above results. Similar results were also observed when a cut-off of ≥14 or the use of antidepressants was used to indicate severe depressive symptoms. In the final model, the ORs (95% CI) for severe depressive symptoms across tomato/tomato product intake levels were 1.00, 0.64 (0.39–1.08), and 0.60 (0.37–0.99). In contrast to tomato/tomato product intake, no relationship was observed between intake of other kinds of vegetables and the prevalence of depressive symptoms (Table 2). Similar results were also observed when a cut-off of ≥14 or the use of antidepressants was used to indicate severe depressive symptoms (data not shown).

4. Discussion

This study examined the relationship between the intake of various vegetables, including tomatoes/tomato products, a main source of lycopene, and depressive symptoms among a community-dwelling elderly population aged 70 years and over. These results suggest that a high intake of tomatoes/tomato products was independently related to a lower prevalence of depressive symptoms. In contrast to tomato/tomato product intake, no relationship was observed between intake of other kinds of vegetables and depressive symptoms.

In this study, we have hypothesized that the intake of tomatoes/tomato products may have a potentially beneficial effect on the prevention of depressive symptoms. Although several studies have investigated the relationship between dietary antioxidant nutrients, such as folic acid and vitamin E, and depressive symptoms, few studies have reported the relationship between intake of tomatoes/tomato products and depressive symptoms (Alpert et al., 2000; Maes et al., 2000; Miyake et al., 2006; Shibata et al., 1999; Tsuboi et al., 2004). Only one study has assessed the correlations between serum lycopene and depressive score, in subjects consisting of 66 healthy female volunteers aged 38–70 years (Tsuboi et al., 2004). However, in that study, many confounding factors were not considered and the results have not suggested a significant correlation between lycopene and depressive score. In this larger community-based population study we adjusted for a considerable number of confounding factors. The current results suggest that high tomato/tomato product intake levels are independently related to a lower prevalence of depressive symptoms. Moreover, we also conducted a stratified analysis for sex. Similar relationships were also observed when males and females were analyzed separately.

Lycopene is the red-colored carotenoid predominantly found in tomatoes, but in few other fruits or vegetables (Bramley, 2000). Lycopene has the strongest antioxidant activity of various common carotenoids (Di Mascio et al., 1989). Oxidative stress may accelerate aging and increase the risk of chronic diseases, such as coronary heart disease, cancer, and rheumatoid arthritis; dietary intake of tomatoes/tomato products containing lycopene have been shown to be related to decreased risk of these chronic medical illnesses (De Pablo et al., 2007; Heber and Lu, 2002). Since these chronic medical illnesses are also related to the occurrence of depressive symptoms, particularly in elderly people, the presence or degree of these chronic medical illnesses may be a potential mechanism linking intake of tomatoes and tomato products to depressive symptoms. Furthermore, since enhanced oxidative stress or defective antioxidant defenses may be related to depressive symptoms, lycopene may directly link tomato and tomato product intake to depressive symptoms because of their anti-oxidative effect. Further study is needed to confirm these findings.

In the present study, lycopene concentration from tomatoes/tomato products was not calculated. In fact, food frequency questionnaires generally used in epidemiological studies vary greatly in their usefulness in estimating the true variation in lycopene intake among individuals. A review indicated that dietary intake of tomato/lycopene is difficult to quantify precisely for several reasons: different food habits, inaccurate estimation of dietary intake, the quality of the food database used, and variation of lycopene concentration within a given food (Porrini and Riso, 2005). Moreover, since lycopene is predominantly found in tomato and tomato-based products (at least 85%) (Bramley, 2000), but only in a few other fruits or vegetables (e.g., watermelon, pink grapefruit, guava, and papaya), the frequency of eating tomatoes/tomato products was used to assess the relationship between tomatoes/lycopene and depressive symptoms in this study.

This study had several limitations. First, the GDS has been designed for measuring the intensity of depressive symptoms and not for making a clinical diagnosis of depressive episodes. Therefore, a larger sample population using a standardized comprehensive structured diagnostic interview should be studied to confirm the effect of depressive symptoms on functional decline. Second, because this study was a cross-sectional study, we could not conclude that lower tomato and tomato product intake increased the occurrence of depressive symptoms or that depressive symptoms lead to a decline in tomato/tomato product intake. Therefore, a prospective study or trial should be undertaken to confirm the relationship between tomato/tomato product intake and depressive symptoms. Moreover, although we adjusted for a considerable number of confounding factors, we cannot exclude the possibility that depressive symptoms are affected by other dietary habits correlated with habitual dietary intake of tomatoes/tomato products. Therefore, an intervention study is necessary to establish a causal relationship between tomato/tomato product intake and depressive symptoms.

In conclusion, this study demonstrated that the intake level of tomatoes/tomato products, as measured by a self-administered questionnaire, is independently related to a lower prevalence of depressive symptoms in a community-dwelling older population. These results suggest that a tomato-rich diet may have a beneficial effect on the prevention of depressive symptoms. Further studies are needed to confirm these findings.

Role of funding source

Nothing declared.

Conflict of interest

All the authors have no conflicts of interest exists to disclose.

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PLOS ONE

Low-Carbohydrate Diets and All-Cause Mortality: A Systematic Review and Meta-Analysis of Observational Studies

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Abstract

Objective: Low-carbohydrate diets and their combination with high-protein diets have been gaining widespread popularity to control weight. In addition to weight loss, they may have favorable short-term effects on the risk factors of cardiovascular disease (CVD). Our objective was to elucidate their long-term effects on mortality and CVD incidence.

Data sources: MEDLINE, EMBASE, ISI Web of Science, Cochrane Library, and ClinicalTrials.gov for relevant articles published as of September 2012. Cohort studies of at least one year's follow-up period were included.

Review methods: Identified articles were systematically reviewed and those with pertinent data were selected for meta-analysis. Pooled risk ratios (RRs) with 95% confidence intervals (CIs) for all-cause mortality, CVD mortality and CVD incidence were calculated using the random-effects model with inverse-variance weighting.

Results: We included 17 studies for a systematic review, followed by a meta-analysis using pertinent data. Of the 272,216 people in 4 cohort studies using the low-carbohydrate score, 15,981 (5.9%) cases of death from all-cause were reported. The risk of all-cause mortality among those with high low-carbohydrate score was significantly elevated: the pooled RR (95% CI) was 1.31 (1.07–1.59). A total of 3,214 (1.3%) cases of CVD death among 249,272 subjects in 3 cohort studies and 5,081 (2.3%) incident CVD cases among 220,691 people in different 4 cohort studies were reported. The risks of CVD mortality and incidence were not statistically increased: the pooled RRs (95% CIs) were 1.10 (0.98–1.24) and 0.98 (0.78–1.24), respectively. Analyses using low-carbohydrate/high-protein score yielded similar results.

Conclusion: Low-carbohydrate diets were associated with a significantly higher risk of all-cause mortality and they were not significantly associated with a risk of CVD mortality and incidence. However, this analysis is based on limited observational studies and large-scale trials on the complex interactions between low-carbohydrate diets and long-term outcomes are needed.

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Introduction

A growing body of evidence has suggested that low-carbohydrate diets and their combination with high-protein diets are effective in weight loss. [1–3] In addition, they reportedly ameliorate the risk factors of cardiovascular disease (CVD) in the short term, [4–6] which would decrease incident CVD and mortality. However, recent cohort studies did not support this hypothesis [7–12] and their long-term health benefit and risk remain controversial. In fact, low-carbohydrate diets tend to result in reduced intake of fiber and fruits, and increased intake of protein from animal sources, cholesterol and saturated fat, all of which are risk factors for mortality and CVD. [13,14].

In light of the worldwide obesity epidemic and the widespread popularity of low-carbohydrate diets, explorations of their long-term health outcome are of clinical importance for the control of weight. Moreover, they are crucial in the areas of public health, since a modest increase in the risk of morbidity and mortality [15] translates into a substantial social burden. These circumstances prompted us to investigate, with greater precision, the effects of low-carbohydrate diets on mortality and CVD incidence by scrutinizing pertinent original reports and combining their data in an attempt to obtain meaningful clues for the evaluation of benefit and harm associated with dietary modification.

Methods

Search

Searches of MEDLINE, EMBASE, ISI Web of Science, Cochrane Library, and ClinicalTrials.gov from their inception until September 12, 2012, were performed. Studies evaluating the risks of mortality or CVD incidence among subjects with low-carbohydrate intake, compared with those with high-carbohydrate intake, were identified using a combination of the following keywords: 'low-carbohydrate diet' or 'carbohydrate-restricted diet', and 'mortality' or 'survival', and 'cardiovascular disease'. The reference lists of the pertinent articles were also inspected.

Selection

We assessed all the identified studies on the effects of low-carbohydrate diets on mortality and CVD risk based on original data analyses to determine their eligibility for inclusion in a qualitative analysis. The inclusion criteria in the meta-analysis were as follows: a published full-text report, randomized controlled trials (RCTs) or observational studies of at least one year's follow-up period, reporting relative risks, i.e. hazard ratios (HRs), risk ratios (RRs), or odds ratios with confidence intervals (CIs), adjusted for at least three of the following possible major confounders for CVD and death: age, gender, obesity, smoking status, diabetes, hypertension, hypercholesterolemia, prior history of CVD, and family history of CVD. Studies in which the low-carbohydrate/high-protein (LC/HP) score was utilized to evaluate the carbohydrate intake were also included.

Validity and Quality Assessment

To ascertain the validity of the eligible studies, the quality of each report was appraised in reference to the CONSORT statement [16] and the STROBE statement [17] as appropriate. The quality of the studies that were included in the meta-analysis were further evaluated using Newcastle-Ottawa Scale [18] with a score of 5 or less (out of 8) indicating a high risk of bias.

Data Abstraction

We reviewed each full-text report to determine its eligibility and extracted and tabulated all the relevant data independently. The extracted data included the characteristics of the subjects (including age, gender, and region), study design, published year, follow-up period, outcomes and the methods used for risk estimation. Any disagreement was resolved by a consensus among the investigators.

Quantitative Data Synthesis

If more than one study was published for the same cohort with identical endpoints, the report containing the most comprehensive information on the population was included to avoid overlapping populations. The reports were summarized both qualitatively and quantitatively.

In the computation of the low-carbohydrate diet score, percentages of energy from protein and carbohydrate were divided into deciles. [7] For carbohydrate, the lowest decile received 10 points and the highest received 0 points, inversely. We pooled the relative risk in the highest score (lowest-carbohydrate intake) group with the lowest score (highest-carbohydrate intake) group as a referent. If an original article classified diets by the carbohydrate intake amount rather than the proportion to the total energy intake, the inverse relative risk for the lowest intake group was calculated with the highest intake group as a referent. If a relative risk was given per score in the original study, the relative risk in the highest score (lowest-carbohydrate intake) group was estimated by calculating the relative risk per score to the ninth power with the lowest score (highest-carbohydrate intake) group as a referent. Sensitive analysis was done using a composite LC/HP score. For protein, participants in the highest decile received 10 points, participants in the ninth decile received 9 points, and so forth. The protein and carbohydrate scores were then summed to create the composite LC/HP score (ranging from 2 to 20), which simultaneously assessed the position of each participant in terms of protein and carbohydrate intake. [9] Thus, a participant with a score of 2 was one with very high consumption of carbohydrates and very low consumption of proteins, whereas a participant with a score of 20 was one with very low consumption of carbohydrates and very high consumption of proteins. We pooled the relative risks similarly.

In the meta-analysis, each adjusted relative risk with low-carbohydrate intake was combined and the pooled RR with a 95% CI was calculated using the random-effects model with inverse-variance weighting. If a study separately reported relative risks for men and women, an overall estimate for the study was calculated from the two relative risks using the fixed-effects model with inverse-variance weighting and these single estimates were used in the subgroup analysis evaluating the individual contribution of the gender. [19] The results based on the LC/HP score were pooled separately. Heterogeneity among the studies was evaluated using I^2 statistics. RevMan (version 5.1) was used for these calculations. All the procedures were in accordance with the guidelines for the meta-analysis of observational studies in epidemiology [20] and the PRISMA statement [21].

Results

Search Results

A total of 492 articles were identified during our search; of these, 18 were assessed with respect to their eligibility for inclusion in our review, which was aimed at determining the influence of low-carbohydrate diets on mortality and CVD incidence (**Fig. 1**). No RCTs were identified. One article [22] was excluded from the systematic review because of population overlapping. Out of these 18 articles, a total of 17 cohort studies [7–12,14,23–32] were included in the systematic review and meta-analysis.

Table 1 shows the characteristics of each included study according to the published year. The 17 selected articles included in the systematic review were moderately heterogeneous in terms of population demographics, carbohydrate intake parameter, and the assessment of confounding factors. The population sample size in these studies ranged from 647 to 129,716. The majority of the articles were published from Sweden and the United States (US).

The adjustment factors and the risk of bias among the studies are summarized in **Table 2** and **Table S1**, respectively. Major confounding factors such as total energy intake were not stated in two studies. [23,32] Few inspected any updates of the carbohydrate intake over the follow-up period. Protein source was added to analysis in 3 studies. [7,9,30] The risk of bias among the researches involved in the meta-analysis was low.

Qualitative Summary

The all of the studies included in our analysis were methodologically good in quality. Regression coefficients of the multiple logistic model were provided in two articles [23,24] and CI was not estimable in another report. [32] Five articles analyzed the risk by diet quality without quantifying carbohydrate intake. [14,25–28] These 8 articles were not included in the subsequent meta-analysis. Most of the studies included in the systematic review were conducted in the US and European countries and their follow-up

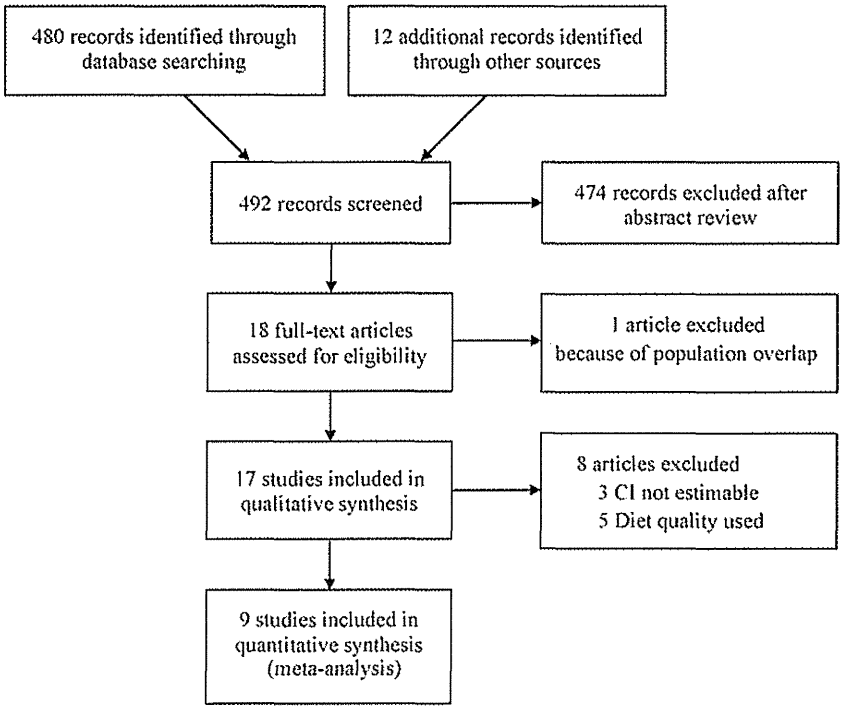

Figure 1. Flow diagram of study selection.
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durations were long enough for the outcomes to occur. Although the majority of the enrolled subjects were middle-aged and free of such chronic comorbidities as diabetes and coronary heart disease, healthcare professionals dominated in the US cohorts, who may not truly represented the average population in the community.

All-cause mortality was assessed in 7 reports. Four cohort studies using the low-carbohydrate score [7,10,11,32] and two using the LC-HP score [11,12] showed a significant increase associated with low-carbohydrate diets (relative risk range 1.12–25.0). One diet quality study suggested 0.27 shorter years of life in 10 years, which was statistically significant. [28] Only two out of five studies demonstrated a significantly elevated risk of CVD mortality (relative risk range 2.17–3.52) evaluated by the LC-HP score. [11,12] One article showed a significantly elevated risk of CVD incidence estimated by the low-carbohydrate score and the LC-HP score (relative risk range 1.42–1.55), [9] whereas three diet quality researches suggested a significantly increased risk of incident CVD (relative risk range 1.30–1.56). [14,26,27] Neither of the studies that calculated regression coefficients showed a significant correlation between low-carbohydrate diets and CVD. [23,24] Some studies suggested that low-carbohydrate diets might increase the risk of mortality and CVD in animal-based dietary patterns whereas they might decrease the risk in plant-based diets. [7,9,30].

The estimates in all the other analyses using either score were non-significant and none of these studies revealed that low-carbohydrate diets were associated with a significantly decreased risk of these outcomes.

Quantitative Summary (Meta-analysis)

A total of 9 articles that provided sufficient information using the low-carbohydrate score and/or the LC-HP score were included in the meta-analysis (**Fig. 1**). All the ascertainment of diagnosis was based on the valid registries but only a few specified the diagnostic criteria for CVD. [12,29,30] The follow-up rate was more than about 90% in each study. Carbohydrate intake was assessed by the residual method in 5 studies [8–12] and by the density method in 4 studies. [7,29–31] Of the 272,216 people in 4 cohort studies using the low-carbohydrate score, 15,981 (5.9%) cases of death from all-cause were reported. **Fig. 2** illustrates the significantly increased risk of all-cause mortality among those adherent to low-carbohydrate diets: the pooled RR (95% CI) 1.31 (1.07–1.59); $p = 0.007$; $I^2 = 53\%$ ($p = 0.09$). Analysis using the LC/HP score yielded a similar significant increase in the risk of all-cause mortality: RR 1.30 (1.01–1.68); $p = 0.04$; $I^2 = 65\%$ ($p = 0.04$). A dose-response was observed in 2 analyses. [7,12] Since heterogeneity among reports in the all-cause mortality using the low-carbohydrate score was statistically significant, we conducted a subgroup analysis according to the possible predictors. The pooled RRs of the studies conducted in Europe [10–12] and the United States [7] (RR 1.42 [1.18–1.72] vs 1.12 [1.01–1.24]) were both significantly elevated; and the diet assessment method (residual method [10–12] or density method [7]) coincided with these regions; the studies with follow-up length shorter than 10 years [10,12] were associated with a statistically high RR while those with follow-up length longer than 10 years [7,11] were not (RR 1.40 [1.12–1.74] vs 1.27 [0.88–1.84]); The pooled RR for men [7,10] was statistically elevated while that for women [7,9,10] was not (RR 1.19 [1.08–1.31] vs 1.34 [0.96–1.87]). We were unable to perform a subgroup analysis according to the body-mass index because the mean values were not stated or estimable in the majority of the reports.

A total of 3,214 (1.3%) cases of CVD death among 249,272 subjects in 3 cohort studies and 5,081 (2.3%) incident CVD cases among 220,691 women in different 4 cohort studies were reported. As summarized in **Fig. 3** and **Fig. 4**, the RRs of CVD mortality

Table 1. Study characteristics.

Source	Country, region/cohort	Follow-up, yr	N (women, %)	Age, yr	Diabetes, %	Coronary heart disease, %	Outcome, n
Garcia-Palmieri, 1980* [23]	USA, Puerto Rico	6	8218 (0)	45–64	NR	0	Myocardial infarction or coronary heart disease death 286
McGee, 1984* [24]	USA, Japanese ancestry	10	7088 (0)	45–68	NR	0	Coronary heart disease 456
McCullough, 2000* [25]	USA, NHS	12	67272 (100)	45–64	0	0	All CVD 1427
McCullough, 2000* [26]	USA, HPFS	8	51529 (0)	40–75	0	0	All CVD 1092
McCullough, 2002* [14]	USA,	8–12					
	a. NHS		a. 67271 (100)	a. 30–55	0	0	a. All CVD 1365
	b. HPFS		b. 38615 (0)	b. 40–75	0	0	b. All CVD 1092
Fung, 2001* [27]	USA, NHS	12	69017 (100)	38–63	0	0	Coronary heart disease 821
Diehr, 2003* [28]	USA, US Cardiovascular Health Study	10	5888 (58)	73	11	25	Coronary heart disease 2179
Oh, 2005 [29]	USA, NHS	18	78779 (100)	30–55	0	0	All stroke 1020
							Ischemic stroke 515
							Hemorrhagic stroke 279
Halton, 2006 [30]	USA, NHS	20	82802 (100)	30–55	0	0	Coronary heart disease 1994
Beulens, 2007 [31]	Netherland, Prospect-EPIC	Mean 9	15714 (100)	49–70	0	0	All CVD 799
							Coronary heart disease 556
							Stroke 243
Lagiou, 2007 [11]	Sweden, Scandinavian Women's Lifestyle and Health Cohort	Mean 12	42237 (100)	30–49	0	0	All-cause death 588
							CVD death 75
Massimino, 2007* [32]	Brazil, Japanese-Brazilians	8	647 (52)	Mean 63.5	20	NR	All-cause death 71
Trichopoulou, 2007 [12]	Greece, EPIC	Mean 4.9	22944 (59)	Adults	0	0	All-cause death 455
							CVD death 193
Fung, 2010 [7]	USA,						
	a. NHS	a. 26	a. 85168 (100)	a. 34–59	a. 0	a. 0	a. All-cause death 12555
							CVD death 2458
	b. HPFS	b. 20	b. 44548 (0)	b. 40–75	b. 0	b. 0	b. All-cause death 8678
							CVD death 2746
Sjögren, 2010 [8]	Sweden, Uppsala	Mean 10.1	924 (0)	Mean 71	0	0	All-cause death 215
							CVD death 88
Lagiou, 2012 [9]	Sweden, Uppsala Longitudinal Study of Adult Men cohort	Mean 15.7	43396 (100)	30–49	NR	0	All CVD 1268
							Ischemic heart disease 701

Table 1. Cont.

Source	Country, region/cohort	Follow-up, yr	N (women, %)	Age, yr	Diabetes, %	Coronary heart disease, %	Outcome, n
							Ischemic stroke 294
							Hemorrhagic stroke 70
							Subarachnoid hemorrhage 121
							Peripheral arterial disease 82
Nilsson, 2012 [10]	Sweden, Västerbotten Intervention Program	Median 10	77319 (51)	Median 49	3	NR	All-cause death 2383
							CVD death 681

NR: not reported, CVD: cardiovascular disease, LCHP: low-carbohydrate/high-protein,
*not included in meta-analysis, NHS: Nurses' Health Study, HPFS: Health Professionals Follow-up Study, EPIC: European Prospective Investigation into Cancer and Nutrition.
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and incidence were not statistically significant: RR 1.10 (0.98–1.24); p = 0.12; I^2 = 0% (p = 0.41), RR 0.98 (0.78–1.24); p = 0.87; I^2 = 53% (p = 0.09), respectively. The RR in CVD mortality using the LC/HP score was not statistically significant, either: RR 1.53 (0.88–2.67); p = 0.13; I^2 = 61% (p = 0.05). There was only one study on CVD incidence using the LC/HP score, which showed a significantly elevated risk. [9] There was a positive dose-response in 2 analyses. [7,9].

Discussion

Our systematic review and meta-analyses of worldwide reports suggested that low-carbohydrate diets were associated with a significantly higher risk of all-cause mortality in the long run. They also suggested that low-carbohydrate diets might not be protective or harmful in terms of CVD mortality and incidence. These findings support the hypothesis that the short-term benefits of low-carbohydrate diets for weight loss are potentially irrelevant. [13] In light of the fact that the number of people with obesity is exponentially increasing worldwide and obesity is one of the leading risk factors of mortality, [15] our findings have substantial clinical and public implications on a global scale and point to the need for the further investigation of the long-term health effects of low-carbohydrate diets and other nutritional factors.

The strength of our present study is that the analysis was mainly based on long-term large population-based data originating from multiple nations and was performed with a high level of precision and this is the first meta-analysis, to our best knowledge, on the health effects of low-carbohydrate diets. The included data were good in quality and apparently had power enough to detect the differences in the risk of these outcomes. The outcome ascertainment tools were valid, and each result was adjusted for multiple confounders and the significantly increased pooled RRs for all-cause mortality were robust in that the RRs based on both of the methods were almost identical and statistically significant. Heterogeneity of the results of the component studies was modest: low heterogeneity suggests that the each result was consistent and most variation was attributable to chance alone, and the large I^2 values in some analyses indicated that the range of the plausible risk estimates was wide, generally because of the diversity of study design, population backgrounds and ethnicities. The subgroup analysis suggested that the possible major source of heterogeneity was the region or the nutrition assessment method in addition to the publication bias. The main dietary source of protein and the obesity prevalence differ across countries [33]. The length of follow-up and the gender were possibly other sources of heterogeneity but these hypotheses cannot be statistically tested in light of the scarcity of data.

Evidence has been accumulating to suggest that low-carbohydrate diets and their combination with high-protein diets are effective in weight loss [1–3] and may have favorable short-term effects on the risk markers of CVD. [4–6] Low-carbohydrate diets may be nutritionally safe and valid insofar as the carbohydrates are simple and refined, and the main source of the protein is plants. Despite these facts, our study did not find a cardiovascular benefit and supports their potential long-term health harm when such nutritional quality is not considered. Low-carbohydrate diets tend to result in reduced intake of fiber and fruits, and increased intake of protein from animal sources, cholesterol and saturated fat, [27,30,34] all of which are risk factors for mortality and CVD. [13,14] It is postulated that differences in dietary bioactive components such as specific free fatty acids, protein, fiber, minerals, vitamins and phytochemicals are involved. [7] Subgroup analyses suggested that low-carbohydrate diets might increase the

Table 2. Methodological assessments of the included studies.

Source	Parameter	Outcome measures	Referent	Comparator	Adjustment factors
Garcia-Palmieri, 1980* [23]	Carbohydrate intake	Coefficient			Alcohol, systolic blood pressure, cholesterol, cigarettes smoked, and blood glucose
McGee, 1984* [24]	Carbohydrate intake	Coefficient			Energy intake, blood pressure, serum cholesterol, cigarettes smoked per day, body weight (in pounds), and physical activity index
McCullough, 2000* [25]	Healthy eating index-f	Relative risk	Quintile 5	Quintile 1	Age (5-y categories), smoking (never, past, 1–14 cigarettes/d, 15–24 cigarettes/d, or ≥25 cigarettes/d), time period, body mass index (quintiles), alcohol intake (7 categories), physical activity (6 categories of metabolic equivalents), history of hypertension or hypercholesterolemia at baseline, total energy intake (quintiles), postmenopausal status, postmenopausal hormone use, multivitamin and vitamin E supplement use
McCullough, 2000* [26]	Healthy eating index-f	Relative risk	Quintile 5	Quintile 1	Age (5-y categories), body mass index (quintiles), smoking (never, past, 1–14 cigarettes/d, 15–24 cigarettes/d, ≥25 cigarettes/d), alcohol intake (7 categories), physical activity (6 categories), total energy intake (quintiles), time period, multivitamin use, vitamin E use, and diagnosis of hypercholesterolemia and hypertension at baseline
McCullough, 2002* [14]	Recommended Food Score	Relative risk	Quintile 5	Quintile 1	Age (5-y categories), smoking (never, past, 1–14 cigarettes/d, 15–24 cigarettes/d, >25 cigarettes/d), time period, body mass index (quintiles), physical activity (6 categories of metabolic equivalents), total energy intake (quintiles), history of hypertension or hypercholesterolemia at baseline, vitamin E and multivitamin supplement, and for women, postmenopausal hormone use
Fung, 2001* [27]	Prudent pattern/ Western pattern	Relative risk	Quintiles 4,5/1	Quintiles 1/4,5	Age, period, smoking, body mass index, hormone replacement therapy, aspirin use, caloric intake, family history, history of hypertension, multivitamin and vitamin E use, and physical activity
Diehr, 2003* [28]	Diet quality	Years of life in 10 yr, CVD incidence	Healthy diet	Unhealthy diet (high fat, low fiber, low carbohydrate, high protein, high calorie)	Demographics, health, behaviors, and baseline health variables
Oh, 2005 [29]	Carbohydrate intake	Relative risk	Quintile 5	Quintile 1	Age (5-year categories), body mass index (five categories), smoking (never, past, current 1–14, 15–24, ≥25 cigarettes/day), alcohol intake (four categories), parental history of myocardial infarction, history of hypertension, hypercholesterolemia, and diabetes, menopausal status and postmenopausal hormone use, aspirin use (five categories), multivitamin use, vitamin E supplement use, physical activity (hours/week, five categories), energy, cereal fiber (quintiles), saturated fat, monounsaturated fat, polyunsaturated fat, trans-fat, and omega-3 fatty acids (quintiles)
Halton, 2006 [30]	Low carbohydrate score	Relative risk	Decile 1	Decile 10	Age (in 5-year categories), body-mass index (<22.0, 22.0 to 22.9, 23.0 to 23.9, 24.0 to 24.9, 25.0 to 27.9, 28.0 to 29.9, 30.0 to 31.9, 32.0 to 33.9, 34.0 to 39.9, or ≥40.0), smoking status (never, past, or current [1 to 14, 15 to 24, or ≥25 cigarettes a day]), postmenopausal hormone use (never, current use, or past use), hours of physical activity per week (<1, 1 to 2, 2 to 4, 4 to 7, or >7), alcohol intake (0, <5 g per day, 5 to 14 g per day, or ≥15 g per day), number of times aspirin was used per week (<1, 1 to 2, 3 to 6,7 to 14, or ≥15), use of multivitamins (yes or no), use of vitamin E supplement (yes or no), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), parental history of myocardial infarction (yes or no), and total calories
Beulens, 2007 [31]	Carbohydrate intake	HR	Quartile 4	Quartile 1	Age, hypertension, cholesterolemia, smoking (never/past/current smoking of 1 to 10, 11 to 20, and ≥20 cigarettes), body mass index, mean systolic blood pressure, total physical activity, menopausal status (pre or post), hormone replacement therapy use, oral contraceptives use, alcohol intake (≤10, 11 to 25, 26 to 50, ≥50 g/day energy-adjusted), total energy intake (in quintiles) and energy-adjusted intake of vitamin E, protein, dietary fiber, folate, saturated fat, and poly- and monounsaturated fat
Lagiou, 2007 [11]	Low carbohydrate score	HR	Per decreasing tenth of carbohydrate intake		Height (cm, continuously), body mass index (<25, 25–29.99 and 30 kg m2, categorically), smoking status (never smokers, former smokers of <10 cigarettes, former smokers of 10–14 cigarettes, former smokers of 15–19 cigarettes, former smokers of 20 or more cigarettes, current smokers of <10 cigarettes, current smokers of 10–14 cigarettes, current smokers of 15–19 cigarettes, current smokers of 20 or more cigarettes, categorically), physical activity [from 1 (low) to 5 (high), categorically], education (0–10, 11–13 and 14 or more years in school, categorically), energy intake (per 1000 kJ day), continuously), saturated lipid intake (per 10 g, continuously) and alcohol intake (<5, 5–25 or >25 g day, categorically).