



## Early gene–diet interaction between glucokinase regulatory protein (GCKR) polymorphism, vegetable and fish intakes in modulating triglyceride levels in healthy adolescents

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Received 13 February 2015; received in revised form 29 June 2015; accepted 29 June 2015

Available online ■ ■ ■

### KEYWORDS

Glucokinase  
regulatory protein;  
Vegetable intake;  
Fish intake;  
Triglyceride;  
Chinese;  
Adolescents

**Abstract** *Background and aims:* The benefits of dietary vegetable and fish consumptions on improving glucose and lipid metabolism have been well established. Recently, the T-allele of a common genetic variant rs780094 at *glucokinase regulatory protein* (GCKR) was reported to be associated with elevated triglyceride (TG) levels but reduced fasting plasma glucose (FPG) and type 2 diabetes risk. However, the dietary modulation on genetic risk is not clearly understood. *Methods and results:* A cohort of 2095 Chinese adolescents (mean age  $15.6 \pm 2.0$  years, 45.3% male) recruited from a population-based school survey for cardiovascular risk factor assessment, with dietary data including weekly vegetable and fish consumptions as well as clinical data were genotyped for the GCKR rs780094 polymorphism. In the linear regression analysis with adjustment for sex, age, body mass index, and socioeconomic status (school banding, paternal and maternal education levels), the frequency of vegetable intake per week was inversely associated with FPG ( $P = 0.044$ ). Individuals with low fish intake generally had elevated TG levels but reduced TC, HDL-C and LDL-C ( $0.006 < P < 0.029$ ). We also observed significant associations of the minor T-allele of GCKR rs780094 with decreased FPG ( $P = 0.013$ ) and increased TG levels ( $P = 2.7 \times 10^{-8}$ ). There were significant gene–diet interactions between rs780094 and vegetable consumption ( $P_{\text{interaction}} = 0.009$ ), and between rs780094 and fish consumption ( $P_{\text{interaction}} = 0.031$ ) in modulating TG levels. The T-allele of GCKR locus was associated with higher TG levels amongst individuals with  $\geq 7$  vegetable meals per week ( $P = 6.4 \times 10^{-9}$ ), and among individuals with  $< 7$  fish meals per week ( $P = 0.020$  and  $7.0 \times 10^{-7}$  for 4–6 and  $\leq 3$  meals per week, respectively). High intake of vegetable exerted a reduction in TG levels only among CC genotype carriers (Ptrend = 0.020), while high intake of fish was associated with reduced TG levels only among TT genotype carriers (Ptrend = 0.026).

*Conclusions:* In summary, our data indicated that the favorable associations of higher vegetable and fish intakes on TG levels are dependent on the genetic background of an individual. In particular, at-risk TT-genotype carriers of the GCKR variant may derive more benefits from a high fish intake, while the CC-genotype carriers may find further benefits from a high consumption of vegetable.

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## Introduction

Cardiometabolic risk factors which contribute to the risks of developing cardiovascular disease (CVD) and type 2 diabetes (T2D) are under the control of both genetic and environmental factors such as diet and lifestyle. Prior to pharmacological interventions, dietary modification coupled with physical activity, has been suggested to be a clinically effective and cost-effective interventional strategy for diabetes [1]. Dietary intake, for example, increased fish and vegetable intake may be surrogate markers of healthy lifestyle in general and their benefits on diminishing the risks of CVD and T2D, reducing plasma glucose levels, serum triglyceride (TG) and cholesterol levels have also been studied extensively [2–5]. It has been proposed that n-3 fatty acids found in fish, particularly in dark and oily species, as well as substances rich in vegetables such as fiber, carotenoids, vitamin C and magnesium might explain part of these associations [5–10]. Nevertheless, there is accumulating evidence showing that the general recommendations for a healthy diet may not benefit all individuals to the same extent [11], suggesting the existence of dietary modulation on genetic risk (i.e. gene–diet interactions) for T2D, CVD and the related cardiometabolic traits.

Glucokinase (GCK) regulates glucose storage and disposal in the liver for maintaining glucose homeostasis. The glucokinase regulatory protein (GCKR) regulates hepatic GCK activity competitively with respect to the substrate glucose, the phosphate esters fructose 6- and fructose 1-phosphate [12,13], which compete with each other for binding, and enhance or inhibit the action of the regulatory protein, respectively [14–16]. The common genetic variant rs780094, located near the *glucokinase regulatory protein* (GCKR) gene, was identified from genome-wide association studies of plasma triglyceride (TG) levels and has been robustly replicated in multiple populations [17,18]. Intriguingly, subsequent analyses have showed that the minor T-allele of GCKR rs780094 polymorphism which increases TG levels were associated with lower glucose and insulin concentrations, as well as better insulin sensitivity measured by homeostasis model assessment of insulin resistance (HOMA-IR) in different populations [19]. These paradoxical observations were further supported by *in vitro* analyses. A functional SNP rs1260326 (Pro446Leu) at GCKR locus were suggested to increase hepatic GCK activity [20], which could lead to both higher TG and lower glucose levels through the stimulation of lipogenic genes by the glycolytic pathway [21]. Recently, a meta-analysis of 14 cohorts studied the interactions of dietary whole-grain intake with fasting glucose and insulin related genetic loci in European population [22]. Among the individuals carrying the insulin-raising allele of GCKR rs780094 variant, higher intake of whole-grain was associated with a smaller reduction in fasting insulin concentration.

To date, only limited and inconsistent data are available concerning the roles of dietary intakes in modulating the genetic effects of the GCKR locus on cardiometabolic traits.

Given the physiological role of GCKR as well as the potential beneficial effects of vegetable and fish consumptions in glucose and lipid metabolisms, we therefore set out to evaluate the association between cardiometabolic traits and 1) frequency of vegetable and fish consumption; 2) GCKR rs780094 variant; and 3) the interactions of GCKR rs780094 variant and dietary intakes of vegetable and fish in 2095 Chinese adolescents.

## Methods

### Subjects

The study design, ascertainment, inclusion criteria, and phenotyping of the participants have been described [23]. Briefly, 2113 Chinese adolescents were randomly recruited from a population-based school survey for cardiovascular risk factor assessment. All subjects were Hong Kong residents of southern Han Chinese ancestry. The survey consisted of administration of questionnaire, clinical examination and biochemical assessments. This study was approved by the Clinical Research Ethics Committee of The Chinese University of Hong Kong. Written informed consent was obtained from parents of all adolescents.

All participants were asked to report the socioeconomic status (SES) including school banding, paternal and maternal education levels, as well as the weekly frequencies of vegetable and fish consumptions through a structured questionnaire. We excluded 18 (0.85%) individuals with missing information on the questions regarding the dietary habits. Finally, 2095 individuals (mean  $\pm$  SD age  $15.6 \pm 2.0$  years, 45.3% male) were included in the subsequent analyses.

### Outcomes

All participants were examined in the morning after an overnight fast. Anthropometric parameters including body weight and height, waist circumference (WC) and blood pressure were measured. Body mass index (BMI) was calculated as weight (kg) divided by squared height ( $m^2$ ). Fasting blood samples were collected for measurement of fasting plasma glucose (FPG), insulin (FPI) and serum lipids (total cholesterol [TC], triglycerides [TG], HDL-cholesterol [HDL-C] and LDL-cholesterol [LDL-C]). FPG was measured by a hexokinase method (Hitachi 911, analyzer Boehringer Mannheim, Mannheim, Germany). TC and TG were assayed enzymatically with commercial reagents (Baker Instruments Corporation, Allentown, Pennsylvania, USA) on a Cobas Mira analyzer (Hoffmann-La Roche and Co, Basle, Switzerland). HDL-C and its subfractions were determined after fractional precipitation with dextran sulphate-MgCl<sub>2</sub>. LDL-C was calculated using Friedewald's formula [24]. Urinary iodine and selenium, commonly used but not specific biomarkers for fish consumption [25–30], were collected in acid-washed bottles and stored at  $-80^\circ C$  before measurement by inductively-coupled plasma mass spectrometry (ICP-MS 7500c, Agilent Technologies, USA) [31].

## Exposures

To measure the dietary vegetable and fish intakes, we asked the questions as “the frequency of vegetable intake per week” and “the frequency of fish intake per week”, and three choices were provided as “3 times or less”, “4–6 times” and “7 times or more” per week.

DNA was extracted from fasting blood samples. The *GCKR* rs780094 polymorphism was genotyped on genomic DNA using the Sequenom MassARRAY platform (Sequenom, San Diego, CA) either at the McGill University and Genome Quebec Innovation Centre or the Genome Research Centre at University of Hong Kong. The overall genotype call rate was 97.4%, and concordance rate based on 144 blind duplicate comparisons was 99.3%. The SNP was not significantly deviated from Hardy–Weinberg equilibrium (HWE) ( $P = 0.1425$ ) assessed by the exact test of PLINK [32].

## Covariates

In Hong Kong, the secondary schools are divided into three “bands” (band 1, 2, and 3 mean better, average and lower grading of the school) and the students enrolled into different school bands according to their academic achievements in primary schools. Parental education was divided into no education, primary school, middle school, high school and university level.

## Statistical analysis

Continuous data with normal and non-normal distributions are presented as mean  $\pm$  SD or geometric mean (95% CI), respectively. Urinary selenium and iodine concentration adjusted for creatinine, FPI and serum TG level was log-transformed due to skewed distributions. The cardiometabolic traits were winsorized by replacing extreme values with four SDs from the mean. Less than 0.24% of data were replaced.

Associations testing for trends between dietary habit and cardiometabolic traits, as well as *GCKR* rs780094 genotype and cardiometabolic traits were analyzed by linear regression with and without adjustments for sex, age, SES (school banding, paternal and maternal education levels) and/or BMI as appropriated. The variables for weekly dietary habits were coded as 0, 1 and 2 for  $\geq 7$  times, 4–6 times and  $\leq 3$  times per week, respectively. Only additive genetic model for *GCKR* rs780094 variant was assumed. The gene–diet interactions were assessed by linear regression including three main effects, two interaction (product) terms and covariates in the same model (i.e. Trait = Vegetable intake + Fish intake + *GCKR* rs780094 + Vegetable intake  $\times$  *GCKR* rs780094 + Fish intake  $\times$  *GCKR* rs780094 + Covariates). In the interaction model, we adjusted for sex, age, SES and BMI.

All statistical analyses were performed using Statistical Package for Social Sciences for Windows v.18 (SPSS, Chicago, IL, USA). A two-tailed  $p$  value  $< 0.05$  was considered statistically significant. Multiple testing of phenotypic

traits was corrected by a sharper Bonferroni procedure suggested by Hochberg [33].

## Results

### Cohort descriptions

The demographic, clinical and biochemical characteristics of all participants are summarized in Table 1. Among 2095 adolescents, 55.9% had  $\geq 7$  meals including vegetables per week, 35.1% had 4–6 meals and 8.9% had  $\leq 3$  meals, while 13.2% had  $\geq 7$  meals including fish per week, 43.0% had 4–6 meals and 43.7% had  $\leq 3$  meals. In this cohort, the minor allele frequency of the genetic variant *GCKR* rs780094 was 0.46. Moreover, we found that the self-reported fish intake was significantly associated with measured urinary selenium ( $P = 3.2 \times 10^{-9}$ ) and urinary iodine ( $P = 0.001$ ) in the expected directions (Table 2), providing evidence that the self-reported fish intake is a valid measure of dietary intake.

### Associations of vegetable and fish consumptions as well as *GCKR* rs780094 with cardiometabolic traits

The frequency of vegetable consumption per week was inversely associated with FPG ( $P = 0.006$ ), i.e. individuals with low weekly vegetable intake generally had higher FPG than those with high intake (Table 2). When we stratified the adolescents by the weekly fish consumption, individuals with low fish intake generally had elevated TG levels ( $P = 0.012$ ) but lower TC ( $P = 0.008$ ), HDL-C ( $P = 0.003$ ) and LDL-C ( $P = 0.021$ ) (Table 2). Adjustments for sex, age, BMI and SES (school banding, paternal and maternal education levels) resulted in attenuated associations between vegetable intakes and FPG, as well as fish intake and lipid profile, but significance remained ( $0.006 < P_{\text{adjusted}} < 0.044$ ) (Table 2). Moreover, the minor T-allele of *GCKR* rs780094 was significantly associated with higher TG levels ( $P = 2.7 \times 10^{-8}$ ), and lower FPG ( $P = 0.013$ ) in the linear regression analysis after adjusting for sex, age, BMI and SES (Table 3). We did not observe any association for obesity traits, FPI and blood pressure with dietary intakes (Supplementary Table 2) and *GCKR* rs780094 polymorphism (Supplementary Table 3).

### Gene–diet interaction on fasting plasma glucose and triglyceride levels

Based on the observed associations and the biological roles of dietary vegetable and fish intakes and *GCKR* rs780094 genetic variant in lipid and glucose metabolisms, we hypothesized possible gene–diet interaction and examined the associations of *GCKR* locus with FPG and TG levels, stratified by food consumptions (Table 4). In the whole cohort, significant gene–diet interactions were found between rs780094 and vegetable consumption ( $P_{\text{interaction}} = 0.009$ ), and between rs780094 and fish consumption ( $P_{\text{interaction}} = 0.031$ ) in associations with TG levels. In particular, the TT-genotype carriers who took  $\leq 3$

**Table 1** Demographic, clinical and biochemical characteristics of Chinese adolescents.

Phenotypes	Values
<i>n</i>	2095
Sex (Male %)	45.30%
Age (years)	15.57 ± 2.04
Urinary selenium concentration adjusted for creatinine (μg/mmol)	0.046 (0.045–0.047)
Urinary iodine concentration adjusted for creatinine (μg/mmol)	0.036 (0.035–0.037)
Dietary habits	
Weekly vegetable consumption (%)	
≥7 times	55.94%
4–6 times	35.08%
≤3 times	8.88%
Weekly fish consumption (%)	
≥7 times	13.22%
4–6 times	43.01%
≤3 times	43.63%
Socioeconomic status	
School banding (%)	
1	45.06%
2	20.67%
3	34.27%
Paternal education levels (%)	
No education	2.20%
Primary school	28.69%
Middle school	34.03%
High school	26.06%
University level	7.35%
Maternal education levels (%)	
No education	5.01%
Primary school	30.98%
Middle school	32.84%
High school	26.44%
University level	3.87%
Obesity traits	
Body mass index (kg/m <sup>2</sup> )	19.93 ± 3.40
Waist circumference (cm)	
Male	71.29 ± 8.56
Female	65.70 ± 6.71
Glucose-related traits	
Fasting plasma glucose (mmol/l)	4.70 ± 0.35
Fasting plasma insulin (pmol/l)	47.04 (46.15–47.94)
Blood pressure	
Systolic blood pressure (mm Hg)	116.90 ± 12.64
Diastolic blood pressure (mm Hg)	72.50 ± 9.26
Lipids profile	
Total cholesterol (mmol/l)	4.18 ± 0.70
Triglycerides (mmol/l)	0.76 (0.75–0.77)
HDL-cholesterol (mmol/l)	1.60 ± 0.31
LDL-cholesterol (mmol/l)	2.20 ± 0.60
Allele frequencies	
GCKR rs780094 (C/T)	54.20%/45.80%

Data are presented as *n*, percentage, mean ± SD or geometric mean (95% CI).

fish meals weekly had the highest TG level while the CC-genotype carriers with ≥7 vegetable meals weekly had the lowest TG level. Amongst individuals with ≥7 vegetable meals per week, each copy of the rs780094 T-allele was strongly associated with a 0.101 ([S.E.], 0.017) mmol/l increase in TG levels ( $P = 6.4 \times 10^{-9}$ ). However, we observed no evidence of association with TG levels in individuals with <7 vegetable meals per week. On the other hand, each copy of the rs780094 T-allele was associated

with 0.045 ([S.E.], 0.019) mmol/l and 0.101 ([S.E.], 0.020) mmol/l increases in TG levels among individuals with 4–6 ( $P = 0.020$ ) and ≤3 ( $P = 7.0 \times 10^{-7}$ ) fish meals per week, respectively. No significant association with TG levels was observed in individuals with ≥7 fish meals per week. To investigate the protective effect of healthy dietary intake on TG levels, we next analyzed the association between food intakes and TG levels among different rs780094 genotype carriers (Table 4). We found that higher vegetable intake exerted a reduction in TG levels in a dose-dependent manner only among CC genotype carriers ( $P_{trend} = 0.020$ ), while higher fish consumption was associated with reduced TG levels only among TT genotype carriers ( $P_{trend} = 0.026$ ). Furthermore, there was no gene–diet interaction for FPG (data not shown). The total variance of TG level explained by this interaction model is 9.5%.

## Discussion

The present study investigated the potential interaction between the common variant rs780094 in *GCKR* and weekly vegetable and fish consumptions on cardiometabolic traits in total 2095 Chinese adolescents. This is one of few studies focusing on Chinese adolescents, in whom the prevalence of metabolic syndrome and obesity has been rapidly increasing [23]. We have previously reported that the minor T-allele of *GCKR* rs780094 was associated with higher triglyceride levels but not associated with FPG in 583 Chinese adults and 1061 Chinese adolescents [18]. In this study, the *GCKR* variant was genotyped in an additional 1034 adolescents and was concordantly associated with increased TG levels and decreased FPG in a total of 2095 adolescents.

Consistent with earlier studies [5,34], we have revealed a significant association between dietary vegetable intake with FPG, as well as dietary fish intake with lipid profile. The protective effects of the high consumption of vegetables in T2D, CVD and cardiometabolic traits including glucose and lipid traits have been reported in many studies [2–5], with consumption of 400 g fruit and vegetable per day recommended by WHO as important components of a healthy balanced diet (<http://www.who.int/dietphysicalactivity/fruit/en/>). In addition, evidence accumulated from epidemiological studies illustrated the lipid-lowering effects of fish consumption in different populations. An earlier study in Greenland Eskimos [35] and a more recent study from South India [34] reported that lower TG levels was found in fish eating as compared to non-fish eating people. Interestingly, they have also demonstrated that the level of cholesterol was significantly lower in fish eating group, in contrast with our findings of elevated TC and LDL levels in adolescents with higher fish consumption. This discrepancy may be due to the different age groups (adolescents versus adults) and other dietary habits (i.e. fish type, vegetable intake and cooking method) of the participants.

To our knowledge, this is the first report on the interaction between *GCKR* rs780094 polymorphism, vegetable



**Table 2** Associations of weekly vegetable and fish consumptions with fasting plasma glucose and lipid traits in Chinese adolescents.

	Weekly vegetable consumption			Trend test		Weekly fish consumption			Trend test	
	≥7 times	4–6 times	≤3 times	P		≥7 times	4–6 times	≤3 times	P	<i>P</i> <sub>adjusted</sub>
%	56.00%	35.12%	8.89%	—		13.23%	43.05%	43.67%	—	
Sex (Male %)	41.38% (485)	48.84% (359)	55.91% (104)	$6.0 \times 10^{-5}$		43.68% (121)	42.62% (384)	48.47% (443)	0.037	
Age (years)	15.72 ± 2.06	15.46 ± 2.00	15.12 ± 1.98	$3.2 \times 10^{-5}$		15.80 ± 2.00	15.56 ± 2.07	15.52 ± 2.02	0.076	
Urinary selenium concentration adjusted for creatinine (μg/mmol)	—	—	—	—		0.051 (0.048–0.054)	0.048 (0.046–0.049)	0.044 (0.043–0.045)	$3.2 \times 10^{-9}$	
Urinary iodine concentration adjusted for creatinine (μg/mmol)	—	—	—	—		0.038 (0.035–0.041)	0.037 (0.036–0.039)	0.034 (0.033–0.036)	0.001	
Fasting plasma glucose (mmol/l)	4.69 ± 0.35	4.72 ± 0.35	4.75 ± 0.40	0.006 (0.030) <sup>a</sup>	0.044	4.68 ± 0.34	4.70 ± 0.36	4.71 ± 0.35	0.248	0.522
Total cholesterol (mmol/l)	4.17 ± 0.70	4.19 ± 0.70	4.19 ± 0.68	0.527	0.152	4.22 ± 0.67	4.22 ± 0.69	4.12 ± 0.71	0.008 (0.016) <sup>a</sup>	0.015
Triglycerides (mmol/l)	0.75 (0.74–0.77)	0.76 (0.74–0.78)	0.80 (0.76–0.85)	0.126	0.269	0.72 (0.69–0.76)	0.75 (0.74–0.77)	0.77 (0.75–0.79)	0.012 (0.036) <sup>a</sup>	0.023
HDL-cholesterol (mmol/l)	1.61 ± 0.31	1.60 ± 0.31	1.60 ± 0.34	0.545	0.493	1.62 ± 0.32	1.63 ± 0.31	1.58 ± 0.31	0.003 (0.012) <sup>a</sup>	0.006 (0.030) <sup>a</sup>
LDL-cholesterol (mmol/l)	2.19 ± 0.60	2.21 ± 0.60	2.19 ± 0.58	0.627	0.365	2.24 ± 0.61	2.22 ± 0.60	2.16 ± 0.59	0.021	0.029

Data are presented as percentage, *n*, mean ± SD or geometric mean (95% CI). *P* values adjusted for sex, age, socioeconomic status (school banding, paternal and maternal education levels) and/or body mass index.

<sup>a</sup> Association remained statistically significant after multiple testing and the corrected *P*-values were shown in the brackets.

and fish consumption on TG level. Our data indicated that the genetic effect of *GCKR* variant was modulated through changes of both dietary vegetable and fish intakes. In particular, at-risk TT-genotype carriers of the *GCKR* variant may derive more benefits from a high fish intake, while the CC-genotype carriers may find further benefits from a high consumption of vegetable. There are now increasing reports on the modulating effects of gene–diet interactions on metabolic traits [22,36–39]. While several studies have reported interaction between *GCKR* variants and dietary intake with glucose and insulin related traits [22,37], hitherto, there was no similar report on lipid levels. In support of our findings, Sotos-Prieto et al. found that adherence to the Mediterranean diet (including a moderate to high intake of fish) modified the effects of *GCKR* polymorphism on TG levels, though they failed to detect any interaction between fish/shellfish intake and *GCKR* variant with TG levels [38].

One possible explanation for the observed interaction effect is that the common dietary chemicals (i.e. nutrients) influence the balance between health and disease by directly or indirectly altering the gene expression and/or epigenetic processes (e.g. DNA methylation) of an individual [40–43]. Recent studies suggested that the triglyceride-lowering benefit of fish was attributed to the n-3 fatty acids, possibly by reducing hepatic production and increasing fractional clearance rates of TG [6,8]. Moreover, n-3 fatty acids were also found to affect gene expression by regulating two groups of transcription factors including sterol regulatory element binding proteins and peroxisome proliferator activated receptors, which are important for modulating the expression of genes controlling both systemic and tissue specific lipid homeostasis [44]. Therefore, the lack of dietary chemicals such as n-3 fatty acids in fish, or the beneficial effects of fiber, carotenoids, vitamin C and magnesium in vegetables [5,7,9,10] may preferentially change the expression of *GCKR* or its target *GCK* genes or variants, which in turn may elevate the hepatic TG levels. To identify different regulatory patterns based on diet and genotypes of *GCKR*, additional research including laboratory animal studies with highly controlled genotypes and environmental conditions, and well-powered human studies controlled and monitored for dietary intakes will be required.

Our study had some limitations. First, the cross-sectional study design and recall biases precluded assessment of causality. The correlation we noted between fish intake and urinary iodine and selenium provided validation of the self-reported fish intake [25–29], but we cannot validate the data of self-reported vegetable intake. Second, the results were restricted to adolescents aged 12–20 years with limited generalizability. Third, we did not collect the information for physical activity and the categorization of the variables for dietary intake was not based on any current recommendation. Both factors may confound the observed associations in the current study. Fourth, only limited food-consumption data were available and the food-related questions have not been validated by an independent method. We did not use a

**Table 3** Associations of *GCKR* rs780094 polymorphism with fasting plasma glucose and lipid traits in Chinese adolescents.

	<i>GCKR</i> rs780094			Trend test
	CC	CT	TT	$P_{adjusted}$
%	28.52%	51.18%	20.30%	—
Sex (Male %)	46.92% (244)	45.34% (423)	45.41% (168)	—
Age (years)	15.42 ± 1.99	15.59 ± 2.06	15.57 ± 2.02	—
Fasting plasma glucose (mmol/l)	4.73 ± 0.37	4.69 ± 0.33	4.67 ± 0.35	0.013 (0.050) <sup>a</sup>
Total cholesterol (mmol/l)	4.19 ± 0.74	4.17 ± 0.71	4.21 ± 0.68	0.991
Triglycerides (mmol/l)	0.72 (0.69–0.74)	0.77 (0.75–0.79)	0.83 (0.79–0.87)	$2.7 \times 10^{-8}$ ( $1.4 \times 10^{-7}$ ) <sup>a</sup>
HDL-cholesterol (mmol/l)	1.61 ± 0.30	1.59 ± 0.32	1.58 ± 0.31	0.082
LDL-cholesterol (mmol/l)	2.24 ± 0.64	2.19 ± 0.59	2.22 ± 0.61	0.459

Data are presented as percentage, *n*, mean ± SD or geometric mean (95% CI). *P* values adjusted for sex, age, socioeconomic status (school banding, paternal and maternal education levels) and/or body mass index.

<sup>a</sup> Association remained statistically significant after multiple testing and the corrected *P*-values were shown in the brackets.

**Table 4** Distribution of triglyceride levels stratified by vegetable/fish consumption frequencies and *GCKR* rs780094 polymorphism in Chinese adolescents.

Frequency	<i>GCKR</i> rs780094			$P_{trend}$
	CC	CT	TT	
Weekly vegetable consumption				
≥7 times	0.68 (0.65–0.72) <i>n</i> = 280	0.77 (0.75–0.8) <i>n</i> = 516	0.83 (0.79–0.88) <i>n</i> = 207	$6.4 \times 10^{-9}$
4–6 times	0.72 (0.67–0.76) <i>n</i> = 184	0.76 (0.72–0.79) <i>n</i> = 328	0.74 (0.68–0.79) <i>n</i> = 129	0.1913
≤3 times	0.77 (0.69–0.86) <i>n</i> = 52	0.76 (0.7–0.83) <i>n</i> = 84	0.86 (0.74–0.99) <i>n</i> = 28	0.2925
$P_{trend}$	0.0202	0.5636	0.2076	$P_{interaction} = 0.0088$
Weekly fish consumption				
≥7 times	0.69 (0.62–0.77) <i>n</i> = 64	0.75 (0.69–0.8) <i>n</i> = 123	0.73 (0.65–0.82) <i>n</i> = 52	0.0798
4–6 times	0.76 (0.72–0.81) <i>n</i> = 224	0.76 (0.72–0.79) <i>n</i> = 399	0.83 (0.77–0.89) <i>n</i> = 158	0.0202
≤3 times	0.72 (0.68–0.76) <i>n</i> = 228	0.79 (0.76–0.83) <i>n</i> = 406	0.87 (0.81–0.93) <i>n</i> = 153	$7.0 \times 10^{-7}$
$P_{trend}$	0.9120	0.0721	0.0262	$P_{interaction} = 0.0314$

Data are expressed as mmol/l and presented as *n* and geometric mean (95% CI). *P* values were obtained by linear regression adjusted for sex, age, body mass index and socioeconomic status (school banding, paternal and maternal education levels).

validated questionnaire (e.g. a food frequency questionnaire) to assess the diet information such as the energy intakes from fat, protein and carbohydrates, as well as the quality and quantity of food consumption for detailed analysis.

Finally, because this is the first study to explore the interaction between *GCKR* locus and dietary vegetable and fish consumptions on cardiometabolic traits, the possibility of false-positive findings cannot be excluded. Thus, replication in other independent studies is required to confirm our findings.

In conclusion, our results suggested a protective association of healthy diet with hypertriglyceridemia, particularly in TT-genotype carriers of the *GCKR* variant who may derive more benefits from a high fish intake and in CC-genotype carriers who may find further benefits from a high consumption of vegetable. These findings highlight the potential beneficial effects of dietary treatment on cardio-metabolic traits which may motivate personalized dietary counselling in at-risk individuals. Pending

replication of these results, the early effects of lifestyle factors on lipid traits before adulthood reinforced the importance of promoting healthy lifestyles early, starting from the youth. Finally, a long term follow up of these adolescents are important to confirm these gene–diet interactions.

### Acknowledgment

We acknowledge support from the Hong Kong Foundation for Research and Development in Diabetes established under the auspices of The Chinese University of Hong Kong, the Hong Kong Governments Research Grant Committee Central Allocation Scheme (CUHK 1/04C), Research Grants Council Earmarked Research Grants (CUHK4055/01M, CUHK4724/07M, CU471713), and the National Institutes of Health Grant NIH-RFA DK-085545-01 (from the National Institute of Diabetes and Digestive and Kidney Diseases). We are grateful to all study participants. We thank all medical and nursing staff of the CUHK-PWH-IDF

Centre of Education, notably Ms. Cherry Chiu and her team, for their commitment and professionalism. We also thank the Genome Institution at Quebec and the University of Hong Kong for help with genotyping, as well as the Chinese University of Hong Kong Information Technology Services Centre for providing computing resources.

## Appendix A. Supplementary material

Supplementary material related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2015.06.011>

## References

- Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001;344:1343–50.
- Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2003;23:e20–30.
- Panagiotakos DB, Zimbenakis A, Boutziouka V, Economou M, Kourlaba G, Toutouzias P, et al. Long-term fish intake is associated with better lipid profile, arterial blood pressure, and blood glucose levels in elderly people from Mediterranean islands (MEDIS epidemiological study). *Med Sci Monit* 2007;13:CR307–12.
- Carter P, Gray LJ, Troughton J, Khunti K, Davies MJ. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *BMJ* 2010;341:c4229.
- Carter P, Gray LJ, Talbot D, Morris DH, Khunti K, Davies MJ. Fruit and vegetable intake and the association with glucose parameters: a cross-sectional analysis of the Let's Prevent Diabetes Study. *Eur J Clin Nutr* 2013;67:12–7.
- Vericel E, Calzada C, Chapuy P, Lagarde M. The influence of low intake of n-3 fatty acids on platelets in elderly people. *Atherosclerosis* 1999;147:187–92.
- Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med* 2000;342:1392–8.
- Despres JP, Lemieux I, Robins SJ. Role of fibric acid derivatives in the management of risk factors for coronary heart disease. *Drugs* 2004;64:2177–98.
- Song Y, Manson JE, Buring JE, Liu S. Dietary magnesium intake in relation to plasma insulin levels and risk of type 2 diabetes in women. *Diabetes Care* 2004;27:59–65.
- Coyne T, Ibiebele TI, Baade PD, Dobson A, McClintock C, Dunn S, et al. Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. *Am J Clin Nutr* 2005;82:685–93.
- Djouss L, Gaziano JM, Buring JE, Lee IM. Dietary omega-3 fatty acids and fish consumption and risk of type 2 diabetes. *Am J Clin Nutr* 2011;93:143–50.
- Matschinsky FM. Glucokinase as glucose sensor and metabolic signal generator in pancreatic beta-cells and hepatocytes. *Diabetes* 1990;39:647–52.
- Matschinsky FM. Regulation of pancreatic beta-cell glucokinase: from basics to therapeutics. *Diabetes* 2002;51(Suppl. 3):S394–404.
- Vandercammen A, Detheux M, Van Schaftingen E. Binding of sorbitol 6-phosphate and of fructose 1-phosphate to the regulatory protein of liver glucokinase. *Biochem J* 1992;286(Pt 1):253–6.
- Veiga-da-Cunha M, Van Schaftingen E. Identification of fructose 6-phosphate- and fructose 1-phosphate-binding residues in the regulatory protein of glucokinase. *J Biol Chem* 2002;277:8466–73.
- Brocklehurst KJ, Davies RA, Agius L. Differences in regulatory properties between human and rat glucokinase regulatory protein. *Biochem J* 2004;378:693–7.
- Saxena R, Voight BF, Lyssenko V, Burt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 2007;316:1331–6.
- Tam CH, Ma RC, So WY, Wang Y, Lam VK, Germer S, et al. Interaction effect of genetic polymorphisms in glucokinase (GCK) and glucokinase regulatory protein (GCKR) on metabolic traits in healthy Chinese adults and adolescents. *Diabetes* 2009;58:765–9.
- Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 2010;42:105–16.
- Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orholm-Melander M, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet* 2009;18:4081–8.
- Stoeckman AK, Ma L, Towle HC. Mlx is the functional heteromeric partner of the carbohydrate response element-binding protein in glucose regulation of lipogenic enzyme genes. *J Biol Chem* 2004;279:15662–9.
- Nettleton JA, McKeown NM, Kanoni S, Lemaitre RN, Hivert MF, Ngwa J, et al. Interactions of dietary whole-grain intake with fasting glucose- and insulin-related genetic loci in individuals of European descent: a meta-analysis of 14 cohort studies. *Diabetes Care* 2010;33:2684–91.
- Ozaki R, Qiao Q, Wong GW, Chan MH, So WY, Tong PC, et al. Overweight, family history of diabetes and attending schools of lower academic grading are independent predictors for metabolic syndrome in Hong Kong Chinese adolescents. *Arch Dis Child* 2007;92:224–8.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- Sanz Alaejos M, Diaz Romero C. Urinary selenium concentrations. *Clin Chem* 1993;39:2040–52.
- Rasmussen LB, Ovesen L, Bulow I, Jorgensen T, Knudsen N, Laurberg P, et al. Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. *Br J Nutr* 2002;87:61–9.
- Remer T, Fonteyn N, Alexy U, Berkemeyer S. Longitudinal examination of 24-h urinary iodine excretion in schoolchildren as a sensitive, hydration status-independent research tool for studying iodine status. *Am J Clin Nutr* 2006;83:639–46.
- Brantsaeter AL, Haugen M, Thomassen Y, Ellingsen DG, Ydersbond TA, Hagve TA, et al. Exploration of biomarkers for total fish intake in pregnant Norwegian women. *Public Health Nutr* 2010;13:54–62.
- Birgisdottir BE, Knutsen HK, Haugen M, Gjelstad IM, Jenssen MT, Ellingsen DG, et al. Essential and toxic element concentrations in blood and urine and their associations with diet: results from a Norwegian population study including high-consumers of seafood and game. *Sci Total Environ* 2013;463–464:836–44.
- Miklavcic A, Casetta A, Snoj Tratnik J, Mazej D, Krsnik M, Mariuz M, et al. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. *Environ Res* 2013;120:7–17.
- Chan IH, Kong AP, Leung TF, Tsui TK, Cheung RC, Osaki R, et al. Cadmium and lead in Hong Kong school children. *Pathology* 2012;44:626–31.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–75.
- Hochberg Y. A Sharper bonferroni procedure for multiple tests of significance. *Biometrika* 1988;75:800–2.
- Bulliyya G. Fish intake and blood lipids in fish eating vs non-fish eating communities of coastal south India. *Clin Nutr* 2000;19:165–70.
- Bang HO, Dyerberg J, Nielsen AB. Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. *Lancet* 1971;1:1143–5.
- Fisher E, Boeing H, Fritsche A, Doering F, Joost HG, Schulze MB. Whole-grain consumption and transcription factor-7-like 2 (TCF7L2) rs7903146: gene-diet interaction in modulating type 2 diabetes risk. *Br J Nutr* 2009;101:478–81.
- Perez-Martinez P, Delgado-Lista J, Garcia-Rios A, Mc Monagle J, Gulseth HL, Ordovas JM, et al. Glucokinase regulatory protein

- genetic variant interacts with omega-3 PUFA to influence insulin resistance and inflammation in metabolic syndrome. *PLoS One* 2011;6:e20555.
- [38] Sotos-Prieto M, Guillen M, Vicente Sorli J, Portoles O, Guillem-Saiz P, Ignacio Gonzalez J, et al. Relevant associations of the glucokinase regulatory protein/glucokinase gene variation with TAG concentrations in a high-cardiovascular risk population: modulation by the Mediterranean diet. *Br J Nutr* 2013;109: 193–201.
- [39] Qi Q, Chu AY, Kang JH, Huang J, Rose LM, Jensen MK, et al. Fried food consumption, genetic risk, and body mass index: gene-diet interaction analysis in three US cohort studies. *BMJ* 2014;348: g1610.
- [40] Kaput J. Diet-disease gene interactions. *Nutrition* 2004;20:26–31.
- [41] Qi L. Gene-diet interactions in complex disease: current findings and relevance for public health. *Curr Nutr Rep* 2012;1:222–7.
- [42] Phillips CM. Nutrigenetics and metabolic disease: current status and implications for personalised nutrition. *Nutrients* 2013;5:32–57.
- [43] Keating ST, El-Osta A. Epigenetics and metabolism. *Circ Res* 2015; 116:715–36.
- [44] Deckelbaum RJ, Worgall TS, Seo T. n-3 fatty acids and gene expression. *Am J Clin Nutr* 2006;83:1520S–5S.