

Dietary Cholesterol Increases the Risk whereas PUFAs Reduce the Risk of Active Tuberculosis in Singapore Chinese^{1,2}

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Abstract

Background: Experimental studies suggest that cholesterol enhances the intracellular survival of *Mycobacterium tuberculosis*, whereas marine ω -3 (n-3) and ω -6 (n-6) fatty acids (FAs) may modulate responses to *M. tuberculosis* in macrophage and animal models. However, there are no epidemiologic data from prospective studies of the relation between dietary cholesterol and FAs and the risk of developing active tuberculosis.

Objective: We aimed to investigate the relation between dietary intake of cholesterol and FAs and the risk of active tuberculosis in a prospective cohort in Singapore.

Methods: We analyzed data from the Singapore Chinese Health Study, a cohort of 63,257 Chinese men and women aged 45–74 y recruited between 1993 and 1998. Dietary intake of cholesterol and FAs was determined with the use of a validated food-frequency questionnaire. Incident cases of active tuberculosis were identified via linkage with the nationwide tuberculosis registry. Analysis was performed with the use of Cox proportional hazards models.

Results: As of 31 December 2013, 1136 incident cases of active tuberculosis were identified. Dietary cholesterol was positively associated with an increased risk of active tuberculosis in a dose-dependent manner. Compared with the lowest intake quartile, the HR was 1.22 (95% CI: 1.00, 1.47) for the highest quartile (P -trend = 0.04). Conversely, dietary marine n-3 and n-6 FAs were associated with a reduced risk of active tuberculosis in a dose-dependent manner. Compared with the lowest quartile, the HR for the highest intake quartile was 0.77 (95% CI: 0.62, 0.95) for marine n-3 FAs (P -trend = 0.01) and 0.82 (95% CI: 0.68, 0.98) for n-6 FAs (P -trend = 0.03). There was no association with saturated, monounsaturated, or plant-based n-3 FA intake.

Conclusion: Dietary intake of cholesterol may increase the risk of active tuberculosis, whereas marine n-3 and n-6 FAs may reduce the risk of active tuberculosis in the Chinese population. *J Nutr* 2016;146:1093–100.

Keywords: tuberculosis, diet, cholesterol, fatty acids, PUFA, epidemiology

Introduction

Tuberculosis is caused by infection with *Mycobacterium tuberculosis* (Mtb), and it is one of the leading causes of mortality in the world (1). Although some infected individuals can completely eliminate the Mtb bacillus from their body, most infected individuals continue to carry Mtb in a clinically latent state (2, 3). In individuals who carry the latent infection, it has been estimated that in ~5%, the infection progresses from a latent form to active disease within 2 y after infection, and an additional 5%

have the active disease at some later point in their lives (4, 5). It is estimated that one-third of the world's population is infected latently with Mtb (6), and they form a large reservoir for disease reactivation; hence, understanding the factors that affect active status and reactivation of tuberculosis is essential in the control of active tuberculosis.

Cholesterol is an integral component of the plasma membrane, and it has been reported to affect intracellular survival of Mtb via various mechanisms. The presence of cholesterol is essential for effective internalization of Mtb by macrophages (7). In addition, cholesterol mediates phagosomal association of coronin, a coat protein that prevents phagosome-lysosome fusion after internalization and hence allows Mtb to survive within the phagosome in the macrophage (7, 8). The tuberculosis granuloma is formed to facilitate immune-mediated containment

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of the infection, and it can proceed either to localized sterilization of the infection or to localized caseation and necrosis that culminates in the progression to active disease (9). The caseum of the granuloma has been found to be rich in cholesterol (10), which is an essential source of energy for *Mtb* to sustain a persistent infection (11).

PUFAs such as n-3 and n-6 also have been reported to affect host immunity by serving as precursors of the synthesis of inflammatory mediators (12, 13). Generally, lipid mediators produced from marine n-3 FAs, such as EPA and DHA, are anti-inflammatory, whereas lipid mediators produced from n-6 FAs are proinflammatory (14). Because of the anti-inflammatory property of marine n-3 FAs, it has been speculated that they impair host resistance to tuberculosis, because robust immune responses are essential in controlling bacterial infection (15). The reduced capability of controlling *Mtb* growth associated with EPA/DHA has been demonstrated in laboratory studies with the use of mouse macrophages (16, 17), as well as in guinea pig models (15, 18, 19). However, a study conducted with the use of mouse models reported increased pathogen killing in mice fed an EPA/DHA-enriched diet (16). The number of studies investigating the association between n-6 FAs and tuberculosis are limited, with no conclusive results (16, 18, 19). The inconsistency across the different studies investigating the association between PUFAs and tuberculosis highlights the inadequacy of extrapolating data from one study model to another, and it would be highly unreliable to predict the effect of dietary FAs on tuberculosis resistance in human populations based on macrophage or animal models.

In this study, we examined the associations between dietary intake of cholesterol and FAs and the risk of active tuberculosis by using prospective data from the Singapore Chinese Health Study, a population-based cohort of middle-aged and elderly Chinese men and women in Singapore. Participants of this cohort went through periods during which tuberculosis was highly prevalent in the country a few decades ago, and those who had acquired latent tuberculosis infection in those early years would be at risk of disease reactivation at older ages (20).

Methods

Study population. The design of the Singapore Chinese Health Study has been described previously (21). Briefly, the cohort consisted of 63,257 Chinese men and women aged 45–74 y recruited between April 1993 and December 1998. Our study participants included citizens or permanent residents of Singapore residing in government-built public housing estates, which is a government housing facility to accommodate the majority (>80%) of the resident population in Singapore (22). During the recruitment period, 86% of the Singapore population lived in such public housing estates. We restricted the study participants to the 2 major dialect groups of Chinese in Singapore—the Hokkiens, who originated from the southern part of Fujian Province, and the Cantonese, who came from the central region of Guangdong Province (21). This study was approved by the institutional review board at the National University of Singapore, and all study participants gave informed consent.

Assessment of dietary exposure. At recruitment, an in-person interview was conducted in each participant's home with the use of a structured questionnaire to collect information that included demographics, weight, height, physical activity, tobacco use, alcohol consumption, and physician-diagnosed history of medical events such as diabetes and cancer. A 165-item semiquantitative FFQ specifically developed for and validated in this study population (21) was used to assess the participants' usual diet over the previous 12 mo. The FFQ has been validated subsequently with the use of two 24-h dietary recall interviews and repeat administration of the

FFQ in a subset of 810 cohort participants, and most mean pairs for energy and nutrients were within 10% deviation from each other by the 2 different methods (21). The 165 items listed in the questionnaire were consumed commonly in the study population and belonged to food categories such as rice and noodles, meats, seafood, dairy products, nuts and seeds, cooking fats and oils, vegetables, and fruits. For each of the food items, the study participants were asked about the portion size of the food consumed (small/medium/large) with the aid of photographs, as well as their intake frequency of the food item from 8 categories ranging from "never or hardly ever" to "2 or more times a day."

The dietary intake of each nutrient for each study participant was computed with the use of the Singapore Food Composition Database, created specifically for this study, which contains ~100 nutritional and non-nutritional values/100 g of the edible raw and cooked foods for each food item or mixed dish (21). In the entire cohort, the main dietary sources of cholesterol were red meat and poultry (21%), fish and seafood (20%), and eggs (22%). The main dietary sources of marine n-3 FAs (EPA/DHA) were fish and seafood, whereas the main dietary sources of plant n-3 FAs [α -linolenic acid (ALA)] were grains (21% of n-3 intake), cooking oils (11%), and legumes and soy (9%). Overall, marine-based n-3 FAs (EPA/DHA) accounted for ~36% of total n-3 intake in this population, and plant-based n-3 FAs (ALA) made up the remaining 64% of total n-3 intake. The main dietary sources of n-6 FAs were cooking fats and oil (40%), grain products (20%), and legumes (7%).

Case ascertainment and follow-up. Cases of active tuberculosis were identified via linkage analysis of the cohort database with the National Tuberculosis Notification Registry. Under the Infectious Diseases Act in Singapore, it is compulsory for doctors to report all suspected and confirmed cases of tuberculosis to the Ministry of Health within 72 h of starting tuberculosis treatment and/or laboratory-confirmed results. Most of the active tuberculosis cases in Singapore are diagnosed by passive case-finding, when patients present with symptoms such as persistent cough, blood-stained sputum, fever and chills, and night sweats. A chest radiograph will first be done before sputum smear and culture tests are conducted to confirm a case of active tuberculosis. All culture-positive tuberculosis patients in Singapore also are captured comprehensively in the National Tuberculosis Notification Registry via electronic linkage with the 2 mycobacterial laboratories in Singapore (23). Follow-up of the cohort was made by regular linkage to the Singapore Registry of Births and Deaths to update vital status of cohort members. As of 31 December 2013, only 52 participants were known to be lost to follow-up because of migration out of Singapore or for other reasons. This suggests that emigration by the participants was negligible and vital statistics at follow-up was virtually complete.

Statistical analysis. We excluded participants with a past history of active tuberculosis as recorded in the National Tuberculosis Notification Registry before recruitment from our analysis ($n = 3012$). Participants with a history of cancer at baseline ($n = 1807$) were also excluded, because their dietary habits may have changed after cancer diagnosis. We further excluded participants who reported an extreme energy intake (<700 or >3700 kcal/d for men and <600 or >3000 kcal/d for women; $n = 967$), with the final analyses including 57,471 participants.

Person-years of follow-up for each participant were calculated from the date of recruitment to the date of diagnosis of active tuberculosis, death, or loss to follow-up, or 31 December 2013, whichever occurred earlier. The student's *t* test (for continuous variables) and a chi-square test (for categorical variables) were used to compare the differences in distributions of characteristics between participants who developed active tuberculosis and participants who remained tuberculosis-free. The energy-adjusted intake of dietary nutrients were computed by using the residual method (24) to control for potential confounding by total energy intake and to remove extraneous variation from total energy intake. This effectively allowed us to examine the variation from the specific nutrient composition of the diet rather than the combination of dietary composition and total energy intake. The quartile levels of each energy-adjusted nutrient were derived from their respective distributions among all the study participants in the cohort.

Using Cox proportional hazards regression models, we examined the associations between dietary intake of cholesterol and major FAs and the risk of active tuberculosis, with adjustments for confounders that were established or potential risk factors of tuberculosis. The strength of a given association was measured by the HR and its corresponding 95% CI. The model was adjusted for age at recruitment (years), year of baseline interview (1993–1995 or 1996–1998), sex, dialect group (Hokkien or Cantonese), level of education (no formal education, primary school, or secondary school or higher), BMI (kg/m²), smoking status (never, former, or current), baseline history of diabetes (yes or no), tea intake (none, monthly, weekly, or daily), alcohol intake (none, monthly, weekly, or daily), energy-adjusted intake of protein (grams per day; quartiles), and total energy intake (kilocalories per day). These factors have been shown in the literature potentially to modulate tuberculosis risk (25–27). The highest level of education attained by the study participant was our surrogate for socioeconomic status in our analysis. The final model included additional adjustment for quartile intake of diet cholesterol (milligrams per day), marine n-3 FAs (grams per day) and n-6 FAs (grams per day). To examine linear trend, median intake values of each quartile were entered as a continuous variable in the model. There was no violation of Cox proportional hazards assumptions for our variables of interest.

All statistical analyses were conducted with the use of the SAS version 9.3 statistical software package. All *P* values quoted were 2-sided, and *P* < 0.05 was considered to be statistically significant.

Results

As of 31 December 2013, there were 1136 incident cases of active tuberculosis in our cohort, with a mean \pm SD follow-up duration of 8.6 ± 5.3 y for tuberculosis cases and 16.4 ± 4.5 y for nontuberculosis cases. The mean age at tuberculosis diagnosis was 68.3 ± 9.0 y. In this cohort, the age-specific incidence rates of active tuberculosis in men were 196 per 100,000 person-years for those aged 60–69 y and 353 per 100,000 person-years for those aged ≥ 70 y, whereas the age-specific incidence rates of active tuberculosis in women were 54 per 100,000 person-years for those aged 60–69 y and 78 per 100,000 person-years for those aged ≥ 70 y. The incidence rates of active tuberculosis, adjusted to the age structure of our cohort, were 219 per 100,000 person-years in men and 55 per 100,000 person-years in women.

Those with tuberculosis in our cohort were significantly older and had a lower BMI than the rest of the cohort (*P* < 0.001) (Table 1). Compared with nontuberculosis patients, a significantly greater proportion of tuberculosis patients were men (*P* < 0.001), and they were more likely to smoke cigarettes, have a higher alcohol intake, and have a baseline history of diabetes (*P* < 0.001) (Table 1). Tuberculosis patients in our cohort also had a lower level of education than those without tuberculosis (*P* < 0.001), reflecting the influence of socioeconomic status on tuberculosis.

In our cohort, dietary intake of cholesterol was found to be positively associated with risk of active tuberculosis (*P*-trend = 0.04), whereas dietary intake of PUFAs was found to be inversely associated with risk of active tuberculosis in a dose-dependent manner (Table 2). We separated PUFAs into n-3 and n-6 FAs, and found a decreased risk of active tuberculosis for both types of PUFAs. When we further stratified n-3 FAs into marine n-3 (EPA/DHA) and plant-based n-3 FAs (ALA), the inverse association between n-3 FAs and tuberculosis risk was observed only for marine n-3 FAs (EPA/DHA) (*P*-trend = 0.01), but not for plant-based n-3 FAs (ALA) (*P*-trend = 0.58). No associations with risk of active tuberculosis were observed for dietary intake of total FAs, SFAs, and MUFAs (Table 2).

The independent effect of dietary cholesterol and n-3 and n-6 FAs on the risk of active tuberculosis was examined by including all 3 of these variables in the same Cox regression model, and the results essentially remained unchanged. Compared with those in the lowest quartile of the respective nutrient intake, those in the highest quartile of diet cholesterol had a 22% increase in risk of active tuberculosis (HR: 1.22; 95% CI: 1.00, 1.47; *P*-trend = 0.04), whereas those in the highest quartile of diet marine n-3 FAs had a 23% decrease in risk (HR: 0.77; 95% CI: 0.62, 0.95; *P*-trend = 0.01), and those in the highest quartile of diet n-6 FAs had an 18% reduction in risk (HR: 0.82; 95% CI: 0.68, 0.98; *P*-trend = 0.03) (Table 3). We performed further sensitivity analyses by including other micronutrients, such as dietary iron, vitamin A, and vitamin D, as covariates, and the results remained essentially the same (data not shown).

Because some tuberculosis cases could have remained undiagnosed at the time of recruitment because of the lag period between onset and diagnosis of the disease, the observed associations could be biased by the changes in dietary intake due to the subclinical condition of active tuberculosis. To overcome this potential bias, we reanalyzed the data after excluding tuberculosis cases diagnosed ≤ 2 y postenrollment and noncases with <2 y of follow-up. The results were essentially the same (Table 3).

Although sex has been reported to influence lipoprotein metabolism, as well as the response of plasma lipids in relation to diet (28, 29), we did not observe any sex differences in the associations in our study (*P*-interaction between intake quartiles and sex > 0.12; data not shown). We also did not observe any differences in the associations with age (*P*-interaction between intake quartiles and age > 0.17; data not shown). Finally, we excluded 5084 participants with baseline diabetes because they may have altered dietary intake compared with the general population, and the results were essentially the same (data not shown).

Discussion

Dietary lipids, such as cholesterol and PUFAs, have been reported to modulate immune responses (30). Although the relation between dietary lipids in modulating tuberculosis risk has been investigated in a number of experimental studies conducted in vitro (11, 16, 17) and in animal models (15, 16), this has not been examined in human populations with the use of prospective data. In our study, we found a positive dose-dependent association between cholesterol intake and risk of active tuberculosis. We also found an inverse dose-dependent association between the intake of marine n-3 and n-6 FAs and the risk of active tuberculosis.

To our knowledge, this is the first cohort study that has comprehensively examined the association between specific types of dietary lipids and the risk of developing active tuberculosis. Even though Singapore currently has an intermediate tuberculosis incidence rate of ~ 40 per 100,000 population, our study cohort consisted of older residents of the country who likely resided in the country during periods in which tuberculosis was as high as ~ 300 per 100,000 population (31). Hence many of our participants could have acquired latent infection in those early years, when tuberculosis was far more rampant (20), making our cohort particularly suitable to study the risk factors associated with latent reactivation of tuberculosis in the elderly. Other strengths of our study include its prospective population-based design and the presumed lack of recall bias in exposure

TABLE 1 Baseline characteristics of cohort members who developed active tuberculosis and those who remained free of active tuberculosis, the Singapore Chinese Health Study, 1993–2013¹

Characteristics	Active tuberculosis cases (<i>n</i> = 1136)	Non-cases (<i>n</i> = 56,335)	<i>P</i>
Age at recruitment, y	59.2 ± 7.9	56.2 ± 8.0	<0.001
BMI, kg/m ²	22.2 ± 3.5	23.2 ± 3.2	<0.001
Sex			<0.001
M	835 (73.5)	24,081 (42.8)	
F	301 (26.5)	32,254 (57.3)	
Dialect group			0.007
Cantonese	483 (42.5)	26,216 (46.5)	
Hokkien	653 (57.5)	30,119 (53.5)	
Level of education			<0.001
No formal education	279 (24.6)	15,328 (27.2)	
Primary school (1–6 y)	612 (53.9)	24,792 (44.0)	
Secondary school and above	245 (21.6)	16,215 (28.8)	
Smoking status			<0.001
Never smoker	477 (42.0)	40,019 (71.0)	
Former smoker	159 (14.0)	5914 (10.5)	
Current smoker	500 (44.0)	10,402 (18.5)	
History of diabetes	161 (14.2)	4923 (8.7)	<0.001
Alcohol intake			<0.001
None	855 (75.3)	45,824 (81.3)	
Monthly	83 (7.3)	4123 (7.3)	
Weekly	109 (9.6)	4565 (8.1)	
Daily	89 (7.8)	1823 (3.2)	
Tea intake			0.35
None	479 (42.2)	23,152 (41.1)	
Monthly	141 (12.4)	6815 (12.1)	
Weekly	252 (22.2)	13,808 (24.5)	
Daily	264 (23.2)	12,560 (22.3)	
Total energy intake, kcal/d	1595 ± 541	1545 ± 519	0.002
Protein intake, ² g/d	58 ± 10	59 ± 10	<0.001

¹ Data are means ± SDs for continuous variables and *n* (%) for categorical variables.² Protein intake was adjusted for energy using the residual methods.

data, because they were obtained many years before diagnosis of tuberculosis. Our study also had a long follow-up and high participant response rate, and data were collected through a face-to-face interview with the use of a FFQ that was specifically created and validated for this population (21). We also were able to adjust for other lifestyle, medical, and dietary factors that could possibly modulate the risk of tuberculosis, such as tea drinking, smoking, alcohol intake, history of diabetes, BMI, and protein intake (25–27). By identifying active tuberculosis cases in the cohort via linkage with the nationwide tuberculosis registry in Singapore, where the law requires the compulsory notification of all confirmed tuberculosis cases, the ascertainment of all diagnosed tuberculosis cases was virtually complete in the present study, especially with a negligible rate of loss to follow-up. In this population-based cohort, the age-specific incidence rates for men and women in our cohort are similar to the mean age-specific incidence rates reported in the Singapore resident population over the past 20 y (31–33). We also observed an expected higher prevalence of established risk factors among tuberculosis cases, such as with smoking, leanness, lower level of education, and history of diabetes (27). The much higher incidence rate in men than in women also has been reported consistently in other populations (34).

Limitations of our study include the use of self-reported dietary intake, which is subject to measurement error. The computation

of dietary nutrient intake based on the food composition database also does not take into account the interindividual variability in nutrient metabolism. Because the participants could have been infected with tuberculosis years before the baseline interview was conducted, the dietary habits assessed through the FFQ may not represent their diet at the time of primary infection. The assessment of dietary intake was only conducted at baseline and may also change over time. However, because data were collected prospectively before disease onset, any subsequent dietary changes would lead to nondifferential misclassification and result in an underestimation of the true effect size of the relation between dietary cholesterol/FAs and the risk of active tuberculosis. Another limitation is the lack of information on the blood concentrations of cholesterol and FAs for our cohort members. Hence, we were unable to establish whether increased intake of these dietary lipids correlated with higher blood concentrations. We also did not have information on the use of statins for the management of hyperlipidemia in our cohort to know whether this could affect the findings with diet cholesterol. Furthermore, we lacked information on the participants' neighborhood of residence or occupation that could affect their risk of infection, or whether the tuberculosis cases were index cases or cases in contacts. However, because Singapore is a small city-state, difference in neighborhood is unlikely to be an important risk factor. Furthermore, dietary intake is unlikely to be significantly different

TABLE 2 Dietary intake of cholesterol and different categories of FAs in relation to risk of active tuberculosis, the Singapore Chinese Health Study, 1993–2013¹

Dietary intake	Energy-adjusted intake by quartiles				P-trend
	Q1	Q2	Q3	Q4	
Cholesterol					0.04
Median (IQR), mg/d	103 (77.3, 119)	150 (141, 158)	184 (175, 194)	241 (221, 280)	
n/person-years	14,398/233,586	14,436/235,750	14,318/233,923	14,319/229,304	
Cases, <i>n</i>	303	273	258	302	
HR (95% CI)	1.00	1.07 (0.89, 1.27)	1.10 (0.91, 1.33)	1.22 (1.01, 1.48)	
Total FAs					0.56
Median (IQR), g/d	33.2 (26.1, 36.4)	41.5 (40.0, 42.9)	47.0 (45.6, 48.4)	54.6 (51.9, 58.7)	
n/person-years	14,387/229,999	14,404/233,878	14,316/234,038	14,364/234,648	
Cases, <i>n</i>	386	267	249	234	
HR (95% CI)	1.00	0.85 (0.72, 1.01)	0.91 (0.75, 1.10)	0.94 (0.76, 1.16)	
SFAs					0.39
Median (IQR), g/d	10.7 (8.5, 11.9)	14.2 (13.5, 14.9)	16.8 (16.1, 17.5)	20.4 (19.2, 22.4)	
n/person-years	14,440/235,273	14,387/233,888	14,275/231,471	14,369/231,932	
Cases, <i>n</i>	338	297	237	264	
HR (95% CI)	1.00	1.08 (0.91, 1.27)	0.94 (0.78, 1.13)	1.13 (0.94, 1.36)	
MUFAs					0.44
Median (IQR), g/d	10.9 (8.7, 12)	13.9 (13.4, 14.4)	15.9 (15.4, 16.5)	18.8 (17.8, 20.4)	
n/person-years	14,427/232,652	14,417/234,146	14,294/233,017	14,333/232,748	
Cases, <i>n</i>	347	290	248	251	
HR (95% CI)	1.00	1.04 (0.88, 1.24)	1.02 (0.84, 1.23)	1.09 (0.89, 1.34)	
PUFAs					0.03
Median (IQR), g/d	5.8 (4.5, 6.4)	7.6 (7.2, 8.0)	9.1 (8.7, 9.7)	12.4 (11.2, 14.4)	
n/person-years	14,359/227,366	14,342/229,845	14,313/233,373	14,457/241,980	
Cases, <i>n</i>	391	292	233	220	
HR (95% CI)	1.00	0.93 (0.79, 1.09)	0.83 (0.69, 0.99)	0.83 (0.69, 0.99)	
Marine n–3 FAs					0.01
Median (IQR), g/d	0.16 (0.11, 0.19)	0.26 (0.24, 0.29)	0.35 (0.33, 0.37)	0.48 (0.44, 0.57)	
n/person-years	14,297/228,015	14,314/231,458	14,391/235,885	14,469/237,205	
Cases, <i>n</i>	332	298	262	244	
HR (95% CI)	1.00	0.99 (0.84, 1.17)	0.87 (0.72, 1.05)	0.77 (0.63, 0.96)	
Plant n–3 FAs					0.58
Median (IQR), g/d	0.38 (0.32, 0.42)	0.49 (0.47, 0.51)	0.57 (0.55, 0.59)	0.73 (0.66, 0.91)	
n/person-years	14,384/229,732	14,315/232,012	14,372/233,518	14,400/237,303	
Cases, <i>n</i>	381	271	253	231	
HR (95% CI)	1.00	0.94 (0.80, 1.11)	0.96 (0.81, 1.14)	0.95 (0.79, 1.13)	
n–6 FAs					0.05
Median (IQR), g/d	5.1 (3.9, 5.6)	6.7 (6.4, 7.0)	8.1 (7.7, 8.7)	11.3 (10.2, 13.1)	
n/person-years	14,353/227,617	14,363/229,928	14,305/232,916	14,450/242,104	
Cases, <i>n</i>	385	287	248	216	
HR (95% CI)	1.00	0.93 (0.79, 1.10)	0.90 (0.76, 1.07)	0.83 (0.69, 1.00)	

¹ Cox proportional hazards regression models were used to estimate HRs and 95% CIs and were adjusted for age at recruitment (years), year of baseline interview (1993–1995 or 1996–1998), sex, dialect group (Hokkien or Cantonese), education level (no formal education, primary school, or secondary school or higher), BMI (kg/m²), smoking status (never, former, or current), history of self-reported diabetes at baseline, tea intake (none, monthly, weekly, or daily), alcohol intake (none, monthly, weekly, or daily), energy-adjusted intake of protein (grams per day; quartiles), and total energy intake (kilocalories per day). Q, quartile.

by occupation or between contacts and noncontacts of tuberculosis patients for the latter to be important confounding factors in the observed diet–tuberculosis associations. Finally, because most of the tuberculosis cases in our cohort were likely to be cases of tuberculosis reactivation, our results may not be generalized to primary tuberculosis infection.

Increased cholesterol intake was found to be associated with a higher risk of active tuberculosis in our study, and this is in line with strong experimental evidence. Mtb possesses a molecule with epitopes structurally similar to the human cholesterol-specific receptor-C₁, and interactions between the receptor-C₁–like molecule of Mtb and cholesterol-rich domains of the plasma

membrane create a stable junction between Mtb and the plasma membrane before the bacteria can be effectively internalized (7, 8, 35). Furthermore, cholesterol associates with coronin, a protein that prevents lysosomal delivery of the phagosome (36). Hence cholesterol-mediated sequestration of Mtb results in a coronin-coated phagosome resistant to phagosome–lysosome fusion, allowing the mycobacteria to resist host eradication (7, 8). During later stages of infection, macrophages are activated by IFN- γ , and they restrict the growth of intracellular mycobacteria via nutrient deprivation (36). With the lack of access to the more commonly used sources of carbon, Mtb is able to import and catabolize host cholesterol for energy to achieve

TABLE 3 Dietary intake of cholesterol, marine n-3 FAs, and n-6 FAs in relation to risk of active tuberculosis, the Singapore Chinese Health Study, 1993–2013¹

	Energy-adjusted intake by quartiles				
	Q1	Q2	Q3	Q4	<i>P</i> -trend
Cholesterol					
Whole cohort					0.04
<i>n</i> /person-years	14,398/233,586	14,436/235,750	14,318/233,923	14,319/229,304	
Cases, <i>n</i>	303	273	258	302	
HR (95% CI)	1.00	1.07 (0.89, 1.27)	1.10 (0.90, 1.33)	1.22 (1.00, 1.47)	
>2 y of follow-up					0.05
<i>n</i> /person-years	14,149/205,013	14,182/207,111	14,066/205,513	14,060/200,904	
Cases, <i>n</i>	265	240	231	267	
HR (95% CI)	1.00	1.06 (0.88, 1.29)	1.11 (0.90, 1.36)	1.22 (0.99, 1.50)	
Marine n-3 FAs					
Whole cohort					0.01
<i>n</i> /person-years	14,297/228,015	14,314/231,458	14,391/235,885	14,469/237,205	
Cases, <i>n</i>	332	298	262	244	
HR (95% CI)	1.00	0.99 (0.84, 1.17)	0.87 (0.72, 1.05)	0.77 (0.62, 0.95)	
>2 y of follow-up					0.02
<i>n</i> /person-years	14,032/199,652	14,057/203,071	14,142/207,330	14,226/208,487	
Cases, <i>n</i>	287	262	238	216	
HR (95% CI)	1.00	1.00 (0.84, 1.20)	0.90 (0.73, 1.10)	0.76 (0.61, 0.96)	
n-6 FAs					
Whole cohort					0.03
<i>n</i> /person-years	14,353/227,617	14,363/229,928	14,305/232,916	14,450/242,104	
Cases, <i>n</i>	385	287	248	216	
HR (95% CI)	1.00	0.91 (0.77, 1.07)	0.88 (0.74, 1.05)	0.82 (0.68, 0.98)	
>2 y of follow-up					0.07
<i>n</i> /person-years	14,087/199,150	14,098/201,443	14,043/204,552	14,229/213,394	
Cases, <i>n</i>	331	260	217	195	
HR (95% CI)	1.00	0.97 (0.81, 1.15)	0.89 (0.74, 1.08)	0.84 (0.69, 1.03)	

¹ Cox proportional hazards regression models were used to estimate HRs and 95% CIs and were adjusted for age at recruitment (years), year of baseline interview (1993–1995 or 1996–1998), sex, dialect group (Hokkien or Cantonese), education level (no formal education, primary school, or secondary school or higher), BMI (kg/m²), smoking status (never, former, or current), history of self-reported diabetes at baseline, tea intake (none, monthly, weekly, or daily), alcohol intake (none, monthly, weekly, or daily), total energy intake (kilocalories per day), energy-adjusted intake of protein (grams per day; quartiles), cholesterol (milligrams per day; quartiles), marine n-3 FAs (grams per day; quartiles) and n-6 FAs (grams per day; quartiles). Q, quartile.

persistence (11). Hence, a diet rich in cholesterol may increase the availability of an energy source that is usable by *Mtb*, and thus ensure its growth and survival in activated macrophages. Furthermore, hypercholesterolemia may also increase tuberculosis susceptibility by impeding the priming of adaptive immune responses (37).

Contrary to the above strong experimental evidence that shows that cholesterol enhances intracellular survival of *Mtb* and perpetuates persistent infection, hypocholesterolemia was observed to be more common in tuberculosis patients than in healthy controls (38, 39). However, because these studies were conducted in patients with active tuberculosis, hypocholesterolemia could be a consequence of tuberculosis rather than a risk factor. Hence, these retrospective case-control studies could not be used to infer a temporal relation between hypocholesterolemia and risk of tuberculosis because of the possibility of reverse causality bias.

FAs from the diet are either incorporated into the plasma membrane or converted into biologically active lipid mediators to modulate immune functions in the body (40). In laboratory studies, mouse macrophages treated with EPA or DHA were found to have impaired IFN- γ -induced activation and were less effective in controlling intracellular mycobacteria (16, 17),

suggesting that a diet enriched in n-3 FAs may increase tuberculosis susceptibility. This effect has also been shown in guinea pig models (18, 19). Our results, although contradictory to these studies, were consistent with one study conducted with the use of mouse models, in which increased *Mtb* killing was observed in mice fed an EPA/DHA-enriched diet (16). Although marine n-3 FAs are usually associated with anti-inflammatory effects, there are also studies that have shown fish oil to enhance immune responses (41–43). Together, these studies suggest that the effect of marine n-3 FAs on risk of tuberculosis may differ in different organisms, and macrophage or animal models may not be reliable predictors of the expected outcome in human populations. Moreover, the dose of FAs administered in experimental settings may not be a realistic representation of the dietary intake applicable to population-based studies in humans. Hence, it is imperative for more studies to validate the effect of marine n-3 FAs in reducing the risk of active tuberculosis in human populations.

Studies investigating the effect of n-6 FAs on tuberculosis are limited, with no conclusive results. Studies that used guinea pig models did not report any difference in tuberculosis susceptibility with an n-6-enriched diet (18, 19), whereas another study reported n-6 FAs to be beneficial against mycobacteriosis in

vitro work that used mouse macrophages but detrimental in mouse models (16). Hence, the observation of a decreased risk of active tuberculosis with a higher intake of n-6 FAs in our study concurs with the proinflammatory property of n-6 FAs (14), as well as results from the study that used mouse macrophages (16).

In conclusion, we report in a prospective cohort study that increased dietary intake of cholesterol is associated with a higher risk of active tuberculosis, and this strongly supports current literature from experimental studies. An increased dietary intake of marine n-3 and n-6 FAs is associated with a reduced risk of active tuberculosis, although the underlying mechanisms by which these PUFAs influence host resistance remain unclear. Because the inflammatory processes associated with tuberculosis infection are highly complex, further investigations are required to fully understand the role that dietary lipids play in modulating host resistance to tuberculosis.

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