



<b>Title</b>	<b>Pro-inflammatory adipokines as predictors of incident cancers in a chinese cohort of low obesity prevalence in Hong Kong</b>
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<b>Citation</b>	<b>PLoS One, 2013, v. 8 n. 10, p. e78594</b>
<b>Issued Date</b>	<b>2013</b>
<b>URL</b>	<b><a href="http://hdl.handle.net/10722/193374">http://hdl.handle.net/10722/193374</a></b>
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# Pro-Inflammatory Adipokines as Predictors of Incident Cancers in a Chinese Cohort of Low Obesity Prevalence in Hong Kong

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## Abstract

**Background:** Cytokines released from adipose tissues induce chronic low-grade inflammation, which may enhance cancer development. We investigated whether indices of obesity and circulating adipokine levels could predict incident cancer risk.

**Materials and Methods:** This longitudinal community-based study included subjects from the Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS) study commenced in 1995-1996 (CRISP-1) with baseline assessments including indices of obesity. Subjects were reassessed in 2000-2004 (CRISPS-2) with measurement of serum levels of adipokines including interleukin-6 (IL-6), soluble tumor necrosis factor receptor 2 (sTNFR2; as a surrogate marker of tumor necrosis factor- $\alpha$  activity), leptin, lipocalin 2, adiponectin and adipocyte-fatty acid binding protein (A-FABP). Incident cancer cases were identified up to 31 December 2011.

**Results:** 205 of 2893 subjects recruited at CRISPS-1 had developed incident cancers. More of the subjects who developed cancers were obese (22.1 vs 16.1%) or had central obesity (36.6 vs 24.5%) according to Asian cut-offs. Waist circumference (adjusted HR 1.02 [1.00-1.03] per cm;  $p=0.013$ ), but not body mass index (adjusted HR 1.04 [1.00-1.08] per kg/m<sup>2</sup>;  $p=0.063$ ), was a significant independent predictor for incident cancers after adjustment for age, sex and smoking status. 99 of 1899 subjects reassessed at CRISPS-2 had developed cancers. Subjects who developed cancers had significantly higher level of hsCRP, IL-6, sTNFR2 and lipocalin 2. After adjustment for conventional risk factors, only IL-6 (HR 1.51, 95% CI 1.18-1.95) and sTNFR2 (HR 3.27, 95%CI 1.65-6.47) predicted cancer development.

**Conclusions:** Our data supported the increased risk of malignancy by chronic low grade inflammation related to central obesity.

**Citation:** Yeung C-Y, Tso AW-K, Xu A, Wang Y, Woo Y-C, et al. (2013) Pro-Inflammatory Adipokines as Predictors of Incident Cancers in a Chinese Cohort of Low Obesity Prevalence in Hong Kong. PLoS ONE 8(10): e78594. doi:10.1371/journal.pone.0078594

**Editor:** Olga Y Gorlova, Geisel School of Medicine at Dartmouth College, United States of America

**Received:** April 19, 2013; **Accepted:** September 20, 2013; **Published:** October 24, 2013

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**Funding:** This study was financially supported by funding from the Hong Kong Research Grants Council (<http://www.ugc.edu.hk/eng/rgc/index.htm>, reference number HKU780210M) and Health and Health Services Research Fund of Food and Health Bureau of Hong Kong (<http://www.fhb.gov.hk/grants/english/welcome/welcome.html>, HHSRF reference number 06070951). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors declare that co-authors Yu Wang and Aimin Xu are PLOS ONE Editorial Board members. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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## Introduction

The prevalence of obesity has increased dramatically in the last three decades globally[1]. In a World Health Organization report, over 400 million people were estimated to be obese in

2005, and by 2015, the figure will rise to over 700 million[2]. Cardiometabolic disorders related to obesity, including type 2 diabetes, hypertension, dyslipidemia, ischemic heart disease and stroke, cause premature mortality and pose a serious challenge to the modern health care system[1]. In recent years,

the association of obesity, in particular central obesity, with cancers has been increasingly recognized[3]. Obesity is associated with adipose tissue inflammation[4] and an increased production of the pro-inflammatory adipokines such as leptin, interleukin-6(IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), lipocalin 2 and adipocyte fatty acid binding protein (A-FABP) which have been shown to enhance tumorigenesis in different tumor cell line models and animal based studies[5,6,7,8]. On the other hand, adiponectin, an anti-inflammatory adipokines with reduced expression in obesity, may be protective against the development of cancers such as breast, endometrial and colorectal cancers[9]. In humans, prospective epidemiological studies have also reported the association of high levels of interleukin-6(IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and A-FABP[10,11,12,13], and low level of adiponectin[9], with cancer development. Whether the proinflammatory adipokines are associated with cancer development in the Chinese population, which has a lower prevalence of obesity and different body fat distribution when compared with Caucasians[14,15], remains unclear. In this study, the role of body adiposity and circulating levels of pro-inflammatory adipokines as predictors of incident cancers was addressed in a population-based cohort of Hong Kong Chinese.

## Materials and Methods

### Patients

**The Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISP) Cohort.** The Hong Kong Cardiovascular Risk Factor Prevalence Study Cohort (CRISP) is a population-based prospective study on Chinese, mostly originated from Southern China. The study commenced as a cross-sectional survey in 1995-1996 (CRISP1) [16] with subjects recruited from the general population through random selections of telephone numbers. After baseline assessment, subjects were invited for prospective follow-up to assess the development of major cardiovascular diseases and cancers with subsequent assessments carried out in 2000-2004 (CRISPS2)[17]. Subjects attended all assessments at the Queen Mary Hospital after an overnight fast. At each assessment, demographic data, medical and drug histories, smoking and family histories of cardiovascular diseases and diabetes were obtained using a standardized questionnaire. Subjects were characterized for anthropometric parameters as previously described [16,17]. At CRISPS-2, plasma and serum samples were taken for the measurement of biomarkers including adipokines. Diagnoses of cancers, based on ICD-9 (140-165,170-176,179-209,230-239) and date of diagnosis were verified from the Hong Kong Hospital Authority database, with cut-off date of 31 December 2011. The prospective relationship between incident cancers over 16 years and obesity was assessed using baseline anthropometric parameters measured in 1995-6 (CRISP1). On the other hand, adipokine levels measured in 2000-4 (CRISP2) were used as baseline data in examining the relationship between 9.5-year cancer incidence and obesity-related adipokines. This study was approved by the Institutional Review Board of the

University of Hong Kong / Hospital Authority Hong Kong West Cluster, and all participants gave written informed consent.

### Biochemical Measurements

Blood glucose, insulin and lipid levels were measured as described [17,18]. T2DM was defined as fasting glucose  $\geq 126$ mg/dl and/or 2-hour post-OGTT glucose  $\geq 200$ mg/dl or already on treatment. Dyslipidemia was defined as having one or more of the following criteria: 1) fasting triglyceride  $\geq 150$ mg/dl; 2) HDL-cholesterol  $< 50$ mg/dl in females and  $< 40$ mg/dl in males; 3) LDL-cholesterol  $> 130$ mg/dl and 4) already on lipid-lowering drugs. HOMA-IR (homeostasis model assessment index) was calculated as fasting glucose (in mmol/L) times fasting insulin (in  $\mu$ U/ml) divided by 22.5 [19]. High-sensitivity C-reactive protein (hsCRP) was measured with a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, GmbH, Mannheim, Germany) using anti-CRP mouse monoclonal antibodies coupled to latex microparticles. Adiponectin and lipocalin 2 were measured with in-house sandwich ELISA established in our laboratory [18,20]. The other adipokines were measured using commercially available ELISA assay kits- adipocyte-fatty acid binding protein(A-FABP) and leptin: BioVendor Laboratory Medicine, Inc., Modrice, Czech Republic; soluble tumor necrosis factor- $\alpha$  receptor-2 (sTNFR2), as a surrogate marker of TNF- $\alpha$  activity: R&D System, Inc., Minneapolis, USA; IL-6: Bender MedSystems GmbH, Vienna, Austria.

### Statistical Methods

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS Version 16.0; SPSS Inc, Chicago). Baseline variables were compared using chi-square, partial correlation or analysis of variance as appropriate. Variables were tested for normality using Kolmogorov-Smirnov test, and skewed variables were natural-logarithmically transformed before analysis. Biologically relevant variables were entered into Cox proportional hazard regression analyses by forced entry to identify the independent predictors for incident cancers.

## Results

### Part I. Obesity-related Parameters and Incident Cancers

2895 subjects (1412 men and 1483 women) were recruited at CRISPS-1. Two patients with known history of malignancy were excluded. After a median follow-up of 16.0 years (interquartile range 15.6-16.5 years), 205 patients had developed cancers (Table S1 in File S1) with an incidence rate of 4.62 per 1000 person-years. The commonest type of cancer was carcinoma of the lung (44 subjects), followed by colorectal cancer (36), breast cancer (20), prostate cancer (18) and cancers of the female reproductive tract (14). The median time for cancer development from baseline was 8.7 years (interquartile range 4.9 to 13.2 years). Table 1 shows that, compared to subjects with no incident cancer on follow up, those who developed cancers were more likely to be male ( $p=0.004$ ), older ( $p<0.001$ ), current or ex-smoker ( $p=0.001$ ),

have higher body mass index ( $p=0.002$ ), higher waist circumference ( $p<0.001$ , sex-adjusted), and hypertension (systolic blood pressure  $\geq 140$  mmHg and/or diastolic blood pressure  $\geq 90$  mmHg or on treatment[21];  $p<0.001$ ), type 2 diabetes ( $p<0.001$ ), and dyslipidemia ( $p=0.004$ ) at baseline (1995-6; CRISP1). There was no significant difference in HOMA-IR ( $p=0.119$ ), fasting insulin level ( $p=0.683$ ) and alcohol use ( $p=0.678$ ). More of the subjects who developed cancers were obese ( $p<0.001$ ) or had central obesity ( $p<0.001$ ) according to Asian cut-offs (BMI $\geq 27.5$  kg/m<sup>2</sup> for obesity and WC $\geq 90$ cm in male or 80cm in female for central obesity [14,22]). If the general cut-off for obesity (BMI $\geq 30$  kg/m<sup>2</sup>) was used, the prevalence of obesity was 8.3% and 6.1% ( $p=0.197$ ) for cancer and non-cancer group respectively. After adjustment for potential confounding factors, including age, sex, smoking status, WC (adjusted HR 1.02 [1.00-1.03] per cm increase in WC;  $p=0.013$ ), but not BMI (adjusted HR 1.04 [1.00-1.08] per kg/m<sup>2</sup> increase in BMI;  $p=0.063$ ), was found to be a significant independent predictor for incident cancers (Table 2, Model 1 and Model 2). WC remained as an independent predictor when diabetes status was also taken into consideration. (Model 3 of Table 2; adjusted HR 1.02[1.00-1.03] per cm increase in WC;  $p=0.019$ ). Analyses were repeated according to estrogen dependency of the tumor types (estrogen dependent tumors included carcinoma of lung, colon, breast, prostate and female reproductive tract[23], which comprised 132 of the 205 cancer cases). WC, but not BMI, had a borderline association with incident cancer after controlling for age, sex and smoking status with or without DM (adjusted HR 1.02 [1.00-1.04];  $p=0.067$  with DM or HR 1.03[1.00-1.04];  $p=0.066$  without DM in models).

## Part II. Adipokines and Incident Cancers

1944 subjects returned for CRISPS2 (2000-4) assessment. After excluding 45 subjects with known cancers or incomplete data at CRISP-2, a total of 1899 (882 male and 1017 female) subjects were included in the analysis. 99 had developed cancers after a median follow up interval of 9.5 years (interquartile range 8.8-10.3). Table 3 shows that, compared with subjects with no cancer, those who developed cancers were more likely to be male ( $p=0.007$ ), older ( $p<0.001$ ), current or ex-smoker ( $p=0.03$ ), have higher waist circumference ( $p=0.014$ ), and type 2 diabetes ( $p=0.003$ ) and dyslipidemia ( $p=0.044$ ) at baseline (2000-4; CRISP2). There was no difference in BMI ( $p=0.189$ ), HOMA-IR ( $p=0.143$ ), fasting insulin level ( $p=0.323$ ), proportion with hypertension ( $p=0.062$ ) and alcohol use ( $p=0.518$ ).

Baseline biomarkers in 2000-4 (CRP, IL-6, sTNFR2, leptin, lipocalin 2 and A-FABP) had significantly positive correlation with indices of obesity WC and BMI while adiponectin had significant negative correlation (Table 4). Table 5 shows that subjects who developed cancers had a higher level of baseline hsCRP ( $p<0.001$ ); IL-6 ( $p<0.001$ , sex-adjusted); sTNFR2 ( $p<0.001$ , sex-adjusted) and lipocalin 2 ( $p=0.027$ , sex-adjusted). There was no difference between the two groups in the level of adiponectin, leptin and A-FABP.

After adjustment for age, sex, and smoking status (Model 1 of Table 6), only baseline IL-6 and sTNFR2 remained to be

**Table 1.** Baseline clinical characteristics of CRISPS-1 (1995-6) cohort subjects with and without incident cancers.

	Incident cancer	No cancer	p-value
N	205	2688	--
Sex (% Male)	120 (58.5%)	1290 (48.0%)	<b>0.004</b>
Age (years)	56 $\pm$ 11.6	45 $\pm$ 12.5	<b>&lt;0.001</b>
Ever smoker (%)	73 (35.6%)	667 (24.9%)	<b>0.001</b>
Alcohol drinker (%)	73 (35.6%)	994 (37.1%)	0.678
Body Mass Index (kg/m <sup>2</sup> )	24.9 $\pm$ 3.96	24.1 $\pm$ 3.57	<b>0.002</b>
Obesity (%)	45 (22.1%)	432 (16.1%)	<b>0.015</b>
Waist Circumference (cm)			<b>&lt;0.001<sup>b</sup></b>
Male	86.6 $\pm$ 10.2	82.7 $\pm$ 9.45	
Female	78.6 $\pm$ 9.08	75.0 $\pm$ 9.35	
Central obesity (%)	75 (36.6%)	658 (24.5%)	<b>&lt;0.001</b>
Hypertension (%)	74 (36.1%)	449 (16.7%)	<b>&lt;0.001</b>
Diabetes (%)	36 (18.1%)	241 (9.4%)	<b>&lt;0.001</b>
HOMA-IR <sup>a, c</sup>	1.21 (0.79-2.11)	1.11 (0.72-1.79)	0.119
Fasting insulin (mIU/L)	5.10 (3.30-7.80)	4.90 (3.20-7.40)	0.683
Dyslipidemia (%)	151 (73.7%)	1707 (63.6%)	<b>0.004</b>

Data are expressed as mean $\pm$ SD unless stated otherwise; <sup>a</sup> log-transformed before analysis; <sup>b</sup> sex-adjusted p-value; <sup>c</sup> excluded 164 subjects with missing data; obesity was defined as BMI  $\geq 27.5$ kg/m<sup>2</sup>; central obesity was defined as WC $>90$  cm for men, $>80$ cm for women; ever smoker was defined as current or former smoker; alcohol drinker was defined as self-reported regular consumption of alcohol.

doi: 10.1371/journal.pone.0078594.t001

independent predictors for incident cancers with hazard ratio (HR) of 1.51 (95% CI 1.18-1.95,  $p=0.001$ ) per natural log unit increase in IL-6, and 3.27 (95% CI 1.65-6.47,  $p=0.001$ ) per natural log unit increase in sTNFR2, respectively. Similar conclusion was reached when WC and/or diabetes status were also included as covariates (Model 2, 3 and 4 of Table 6). Since tumors are possible sources for inflammatory cytokines like IL-6 and TNF- $\alpha$ , analyses were repeated after excluding 8 patients who developed cancers within one year from baseline, to reduce the effect of a preclinical malignancy. Similar results were obtained with baseline IL-6 and sTNFR2 remaining as independent predictors of incident cancers, with HR of 1.53 (1.18-2.00,  $p=0.002$ ) per natural log unit increase in IL-6, and 3.15 (1.53-6.47,  $p=0.002$ ) per natural log unit increase in sTNFR2, respectively.

## Discussion

There is now increasing evidence that obesity contributes to the development of many cancers, including cancers of colon, breast, liver, gall bladder, endometrium, kidney, prostate and pancreas [24,25]. A 5 kg/m<sup>2</sup> increase in body mass index (BMI) above normal was found to increase the risk of developing many types of cancers by 24 to 59% in both sexes [3]. Study in Asia-Pacific region also showed a 5-unit increase in BMI above 18.5 kg/m<sup>2</sup> raised all cancer-mortality by 1.09 times [26]. Although the prevalence of obesity has been shown to be lower in Chinese populations when compared with Western populations even when Asian-Pacific cut-offs for obesity are

**Table 2.** Cox Proportional hazard regression showing that waist circumference, but not body mass index, independently predicted cancer development.

Baseline parameters	Model 1		Model 2		Model 3	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Men	1.21 (0.86-1.69)	0.272	1.36 (0.98-1.89)	0.065	1.34 (0.97-1.86)	0.080
Age (years)	1.07 (1.06-1.08)	<b>&lt;0.001</b>	1.07 (1.06-1.08)	<b>&lt;0.001</b>	1.07 (1.06-1.08)	<b>&lt;0.001</b>
WC (cm)	1.02 (1.00-1.03)	<b>0.013</b>	--	--	1.02(1.00-1.03)	<b>0.019</b>
BMI (kg/m <sup>2</sup> )	--	--	1.04 (1.00-1.08)	0.063	--	--
Diabetes	--	--	--	--	1.20 (0.85-1.68)	0.300
Ever smoker	1.20 (0.86-1.68)	0.295	1.20 (0.86-1.68)	0.287	1.05 (0.96-1.15)	0.313

Ever smoker was defined as current or former smoker; WC, waist circumference; BMI, body mass index.

doi: 10.1371/journal.pone.0078594.t002

**Table 6.** Cox proportional hazard regression models showing baseline IL-6 and sTNFR2 independently predicted incident cancers.

Baseline parameters	Model 1 (Base Model <sup>b</sup> )		Model 2 (Base Model + WC)		Model 3 (Base Model + Diabetes)		Model 4 (Base Model + WC +Diabetes)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
CRP, per mg/L <sup>a</sup>	1.20 (0.99-1.44)	0.062	1.17 (0.96-1.42)	0.124	1.19 (0.98-1.43)	0.073	1.17 (0.96-1.42)	0.127
IL-6, per pg/ml <sup>a</sup>	1.51 (1.18-1.95)	<b>0.001</b>	1.49 (1.15-1.91)	<b>0.002</b>	1.52 (1.18-1.96)	<b>0.001</b>	1.50 (1.16-1.94)	<b>0.002</b>
sTNFR2, per ng/ml <sup>a</sup>	3.27 (1.65-6.47)	<b>0.001</b>	3.21 (1.61-6.39)	<b>0.001</b>	3.20 (1.62-6.29)	<b>0.001</b>	3.16 (1.59-6.25)	<b>0.001</b>
Lipocalin 2, per ng/ml	1.23 (0.79-1.93)	0.363	1.21 (0.77-1.90)	0.401	1.23 (0.78-1.93)	0.367	1.22 (0.77-1.91)	0.397

<sup>a</sup> Log-transformed before analysis; <sup>b</sup> Base Model adjusted for age, sex and smoking status.

doi: 10.1371/journal.pone.0078594.t006

adopted [15], a rising trend, along with the incidence and mortality of many cancers, is observed and is believed to be related to the westernization of lifestyle[27]. Moreover, body fat composition in Chinese is different from their Caucasian counterparts and Chinese tend to have more central obesity, and thus visceral fat, for a given BMI after adjustment for age and sex [14]. Since the circulating levels of the pro-inflammatory adipokines are higher in the portal vein than in the peripheral arteries in obese subjects [28], visceral fat may play a more important role in contributing to systemic inflammation than the subcutaneous fat. In this Chinese community cohort with a relative low prevalence of obesity (16.5% and 6.2% having BMI  $\geq 27.5$  kg/m<sup>2</sup> and BMI  $\geq 30$  kg/m<sup>2</sup> respectively), we demonstrated that waist circumference, an indicator of central adiposity, but not BMI which represents general obesity, was a significant independent predictor of incident cancer development, even after adjusting for the effect of age, the strongest risk factor in this cohort. This differential effect of central and general adiposity on cancer risk has also been demonstrated in other studies in the Asian-Pacific region. In a case-control study of relatively lean Chinese population with mean BMI 21.9 kg/m<sup>2</sup>, an increase in waist-hip ratio increased the risk of prostate cancer by 3 folds while the risk incurred by BMI was not significant[29]. Again, in a meta-analysis of 30 cohort studies in Asian-Pacific region, WC but not BMI, was associated with increased mortality from pancreatic cancer[30].

**Table 3.** Baseline characteristics of CRISPS-2 (2000-4) cohort subjects with and without incident cancers.

	Incident cancer	No cancer	p-value
N	99	1800	---
Sex (% Men)	59 (59.6)	823 (45.7)	<b>0.007</b>
Age (years)	61.2 $\pm$ 11.4	51.8 $\pm$ 11.8	<b>&lt;0.001</b>
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 4.06	24.1 $\pm$ 3.51	0.189
Waist circumference (cm)			<b>0.014<sup>b</sup></b>
Men	86.2 $\pm$ 9.50	84.2 $\pm$ 9.06	
Women	79.0 $\pm$ 9.22	76.2 $\pm$ 9.10	
Ever smoker (%)	35 (35.4)	459 (25.5)	<b>0.030</b>
Alcohol drinker (%)	33 (33.3)	656 (36.5)	0.518
HOMA-IR <sup>a, c</sup>	1.85 (1.28-2.89)	1.66 (1.15-2.49)	0.143
Fasting insulin (mIU/L)	7.40 (5.60-11.0)	7.30 (5.20-10.5)	0.323
Diabetes (%)	26 (26.3)	267 (15.0)	<b>0.003</b>
Hypertension (%)	35 (35.4)	482 (26.8)	0.062
Dyslipidemia (%)	70 (70.7)	1089 (60.6)	<b>0.044</b>

Data are expressed as mean $\pm$ SD unless stated otherwise; obesity was defined as BMI  $\geq 27.5$ kg/m<sup>2</sup>; central obesity was defined as WC>90 cm for men, >80cm for women; <sup>a</sup> log-transformed before analysis; <sup>b</sup> sex-adjusted p-value; <sup>c</sup> excluded n=99 subjects on diabetic treatment; Ever smoker was defined as current or former smoker; Alcohol drinker was defined as self-reported regular consumption of alcohol.

doi: 10.1371/journal.pone.0078594.t003

**Table 4.** Partial correlations between baseline obesity parameters and adipokines of CRISPS-2 (2000-4) cohort subjects.

Biomarkers	BMI		WC	
	Sex-adjusted r	p-value	Sex-adjusted r	p-value
CRP	0.38	<0.001	0.37	<0.001
IL-6	0.16	<0.001	0.17	<0.001
sTNFR2	0.11	<0.001	0.17	<0.001
Adiponectin	-0.33	<0.001	-0.30	<0.001
Leptin	0.60	<0.001	0.58	<0.001
Lipocalin 2	0.06	0.009	0.11	<0.001
A-FABP	0.43	<0.001	0.46	<0.001

All biomarkers were log-transformed before analysis.

doi: 10.1371/journal.pone.0078594.t004

**Table 5.** Baseline biomarkers of CRISPS-2 (2000-4) cohort subjects with and without incident cancers.

	Incident cancer	No cancer	p-value
N	99	1800	---
<i>Biomarkers</i>			
CRP (mg/L)	0.98 (0.50-1.99)	0.72 (0.33-1.55)	<b>0.001</b>
IL-6 (pg/ml)			<b>&lt;0.001</b>
Men	0.76 (0.53-1.06)	0.58 (0.36-0.89)	
Women	0.65 (0.38-0.98)	0.54 (0.36-0.81)	
sTNFR2 (ng/mL)			<b>&lt;0.001</b>
Men	2411.0 (1980.4-2752.6)	1990.4 (1700.1-2368.2)	
Women	2052.4 (1817.2-2787.0)	1815.2 (1562.3-2147.8)	
Adiponectin (mg/ml)			0.150
Men	5.88 (4.41-9.15)	5.50 (3.57-8.61)	
Women	7.90 (4.59-11.92)	7.82 (5.36-11.59)	
Leptin (ng/ml)			0.129
Men	5.50 (2.19-8.76)	4.53 (2.25-7.06)	
Women	13.66 (8.23-21.98)	11.40 (7.98-16.76)	
Lipocalin 2 (ng/ml)			<b>0.027</b>
Men	39.04 (30.14-51.73)	37.58 (30.41-47.53)	
Women	34.23 (26.64-57.18)	31.47 (25.32-40.18)	
A-FABP (ng/ml)			0.076
Men	20.90 (15.17-26.66)	19.15(14.09-25.90)	
Women	27.49 (19.14-41.33)	24.24 (17.41-33.36)	

Data are expressed as mean±SD unless stated otherwise; all biomarkers were log-transformed before analysis; all sex-adjusted p-value except for CRP.

doi: 10.1371/journal.pone.0078594.t005

Pro-inflammatory adipokines have been shown in vitro to play significant pathophysiological roles in different stages of tumorigenesis including cancer cell proliferation, angiogenesis and metastasis [6,31]. Apart from their direct effects, adipokines may also interact with sex hormones, sex-hormone binding globulin, insulin-like growth factor or the binding proteins to mediate the development of obesity-related cancers [32]. In this study, we demonstrated the significant correlations between indices of obesity and the baseline levels of pro-

inflammatory markers, among which IL-6 and sTNFR2 independently predicted incident cancer development.

IL-6 and TNF- $\alpha$  are key cytokines involved in inflammation and immunity [33,34]. Both of them had been shown in vitro to be the links between chronic inflammatory states and cancers, by promoting cell proliferation, enhancing angiogenesis and cancer cell metastasis[35,36]. Epidemiological data also supported the role of IL-6 and TNF- $\alpha$  in cancer promotion. In a nested case-control study of 1298 postmenopausal women in the United States, Ho GY et al. demonstrated that subjects with IL-6 in highest quartile had a relative risk of 1.41 of developing colorectal cancer though the association was insignificant after adjustment for baseline insulin level[10]. Heikkila K et al. also showed IL-6 was associated with increased lung and breast cancer risks in a meta-analysis of two prospective cohorts, the British Women’s Heart and Health study and the Caerphilly Cohort[11]. In addition, in a sub-group analysis of the Nurses’ Health Study, Chan et al. showed that the relative risk of development colorectal cancer was 1.67(95% CI 1.05-268, p=0.03) in participants with highest quartile of sTNFR-2 level, when compared with those in the lowest quartile[12]. While our study was the first prospective study examining the effects of the adipokines on cancer development in Chinese population, our findings on IL-6 and sTNFR-2 were in line with those of western cohorts.

Smoking, a well known carcinogenic risk factor, has been associated with level of both IL6 and TNF- $\alpha$ [37,38]. In the current study, smoking was more prevalent at baseline among those who subsequently developed cancers. In the multivariate analysis, however, smoking was not a significant independent risk factor of cancer development, possibly suggesting that the effect of smoking on cancer development is, at least in part, mediated by these proinflammatory cytokines.

The negative findings on adiponectin in this study was unexpected as this anti-inflammatory adipokine inhibited the growth of several breast cancer cell lines in vitro [39]. In epidemiological studies, hypoadiponectinemia had been shown to predict the development of breast, endometrial, colonic, renal cell carcinoma and many hematological malignancies [9]. The association between adiponectin and prostate or lung cancers, on the other hand, was less consistent [40,41]. This might explain why adiponectin did not emerge as a protective factor in our cohort as prostate and lung cancers constituted a significant proportion of our cancer cases.

This study has several limitations. Firstly, in view of the relatively small numbers of incident cancer cases, the findings of this study should be validated in studies involving larger sample size. Secondly, since baseline assessments were performed more than a decade ago and the list of adipokines is growing, not all adipokines were measured in this study. Thirdly, estradiol and its metabolites might be other important confounding factors which were not measured. Numerous studies have demonstrated the effects of estrogens on the development and progression of different cancers[23] and estrogen production in obesity state will be increased by peripheral aromatization in the adipose tissues. However, our subgroup analyses on obesity parameters and estrogen dependent cancers were limited by small numbers of events,

resulting in insignificant power to make a definitive conclusion. Nonetheless, levels of CRP, IL-6, sTNFR2 were found to be higher in subjects with low estrogen levels (post-menopausal females, defined as women aged older than 55, and male subjects) when compared to female subjects aged less than 55 with presumably higher estrogen levels (Table S2 in File S1). Previous studies have also reported a suppressive effect of estrogen on both adipokines in high levels, and at low levels, no effect on IL-6 but stimulatory effect on TNF- $\alpha$  secretion[42]. In obesity, the increased estrogen production by the adipose tissue would tend to lower the secretion of these adipokines and hence their effect on cancer development. Fourthly, other factors which may impact on cancer development such as family history of malignancy, lifestyle factors like dietary factors and physical activity, and personal history of chronic inflammatory diseases, were not included in the analyses. Furthermore, detailed data on concomitant medications which may impact on inflammation or adipokines levels, such as aspirin, statins, blockers of the renin-angiotensin system and thiazolidinediones, were not recorded. Finally, due to the limitation of an observational study design, our findings could only imply association but not causal relationship, though multiple molecular mechanisms have been identified to link cancer development with elevated cytokines in chronic inflammatory states.

## Conclusions

In conclusion, we have demonstrated in a population-based study that high waist circumference, and raised serum levels of the pro-inflammatory adipokines, IL-6 and TNFR2, can predict the development of cancers in a Chinese cohort with relatively low obesity prevalence. These associations are supportive of the role of chronic low grade inflammation, related to central obesity, in the development of cancers even though a causal

relationship cannot be concluded. When more epidemiological data become available and with a better understanding of the roles of these adipokines in tumorigenesis, risk models can be constructed to identify high risk patients for the development of malignancy so that appropriate measures may be employed to reduce their risks. On the other hand, in patients with established malignancy, the inflammatory cytokines level may act as novel prognostic factors. Furthermore, the inhibition of the inflammatory cytokines provides potential new strategies for the treatment of cancers or for sensitizing tumors to chemotherapeutic agents. While IL-6 and TNF-alpha receptor antagonists are now used clinically for the treatment of a wide range of rheumatological or hematological disorders, it will be of interest to follow-up on patients treated with such agents for any potential beneficial effects on cancer development.

## Supporting Information

**File S1. Supporting tables.**  
(DOCX)

## Acknowledgements

The authors thank medical staffs of Department of Health of Hong Kong for their assistance with data retrieval on life-death status and causes of death.

## Author Contributions

Conceived and designed the experiments: AWKT THL NMSW JW BMYC KSL. Analyzed the data: CYY AWKT THL CHYF KSL. Contributed reagents/materials/analysis tools: AX YW. Wrote the manuscript: CYY AWKT THL CHYF KSL. Data Collection: YCW SVL.

## References

- Hossain P, Kowar B, El Nahas M (2007) Obesity and diabetes in the developing world—a growing challenge. *N Engl J Med* 356: 213-215. doi:10.1056/NEJMp068177. PubMed: 17229948.
- World Health Organization, Office of Health Communications and Public Relations (2006) Obesity and overweight. Geneva: World Health Organization. p. 3. p.A paragraph return was deleted
- Rehman AG, Tyson M, Egger M, Heller RF, Zwahlen M (2008) Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 371: 569-578.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL et al. (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796-1808. doi:10.1172/JCI19246. PubMed: 14679176.
- Neurath MF, Finotto S (2011) IL-6 signaling in autoimmunity, chronic inflammation and inflammation-associated cancer. *Cytokine Growth Factor Rev* 22: 83-89. doi:10.1016/j.cytogfr.2011.02.003. PubMed: 21377916.
- Balkwill F (2006) TNF-alpha in promotion and progression of cancer. *Cancer Metastasis Rev* 25: 409-416. doi:10.1007/s10555-006-9005-3. PubMed: 16951987.
- Leung L, Radulovich N, Zhu CQ, Organ S, Bandarchi B et al. (2012) Lipocalin2 promotes invasion, tumorigenicity and gemcitabine resistance in pancreatic ductal adenocarcinoma. *PLOS ONE* 7: e46677. doi:10.1371/journal.pone.0046677. PubMed: 23056397.
- Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R et al. (2011) Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med* 17: 1498-1503. doi:10.1038/nm.2492. PubMed: 22037646.
- Dalamaga M, Diakopoulos KN, Mantzoros CS (2012) The role of adiponectin in cancer: a review of current evidence. *Endocr Rev* 33: 547-594. doi:10.1210/er.2011-1015. PubMed: 22547160.
- Ho GY, Wang T, Gunter MJ, Strickler HD, Cushman M et al. (2012) Adipokines linking obesity with colorectal cancer risk in postmenopausal women. *Cancer Res* 72: 3029-3037. doi:10.1158/0008-5472.CAN-11-2771. PubMed: 22511581.
- Heikkilä K, Ebrahim S, Lawlor DA (2007) A systematic review of the association between circulating concentrations of C reactive protein and cancer. *J Epidemiol Community Health* 61: 824-833. doi:10.1136/jech.2006.051292. PubMed: 17699539.
- Chan AT, Ogino S, Giovannucci EL, Fuchs CS (2011) Inflammatory markers are associated with risk of colorectal cancer and chemopreventive response to anti-inflammatory drugs. *Gastroenterology* 140: 799-808, quiz: 10.1053/j.gastro.2010.11.041. PubMed: 21115010.
- Hancke K, Grubeck D, Hauser N, Kreienberg R, Weiss JM (2010) Adipocyte fatty acid-binding protein as a novel prognostic factor in obese breast cancer patients. *Breast Cancer Res Treat* 119: 367-367. doi:10.1007/s10549-009-0577-9. PubMed: 19842034.
- WHO. expert consultation (2004) Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 363: 157-163
- Pacific Asia Cohort Studies Collaboration (2007) The burden of overweight and obesity in Pacific: The Asia Publishing House region. *Obes Rev* 8: 191-196
- Janus ED, Watt NM, Lam KS, Cockram CS, Siu ST et al. (2000) The prevalence of diabetes, association with cardiovascular risk factors and

- implications of diagnostic criteria (ADA 1997 and WHO 1998) in a 1996 community-based population study in Hong Kong Chinese. *Hong Kong Cardiovascular Risk Factor Steering Committee American Diabetes Association. Diabet Med* 17: 741-745.
17. Cheung BM, Wat NM, Man YB, Tam S, Thomas GN et al. (2007) Development of diabetes in Chinese with the metabolic syndrome: a 6-year prospective study. *Diabetes Care* 30: 1430-1436. doi:10.2337/dc06-1820. PubMed: 17337491.
  18. Chow WS, Cheung BM, Tso AW, Xu A, Wat NM et al. (2007) Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. *Hypertension* 49: 1455-1461. doi:10.1161/HYPERTENSIONAHA.107.086835. PubMed: 17452504.
  19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412-419. doi:10.1007/BF00280883. PubMed: 3899825.
  20. Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J et al. (2007) Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. *Clin Chem* 53: 34-41. PubMed: 17040956.
  21. Jones DW, Hall JE (2004) Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and evidence from new hypertension trials. *Hypertension* 43: 1-3. doi:10.1161/01.HYP.0000105111.06482.7E. PubMed: 14676222.
  22. Alberti KG, Zimmet P, Shaw J (2005) The metabolic syndrome—a new worldwide definition. *Lancet* 366: 1059-1062. doi:10.1016/S0140-6736(05)67402-8. PubMed: 16182882.
  23. Folkert EJ, Dowsett M (2010) Influence of sex hormones on cancer progression. *J Clin Oncol* 28: 4038-4044. doi:10.1200/JCO.2009.27.4290. PubMed: 20644089.
  24. McMillan DC, Sattar N, McArdle CS (2006) ABC of obesity. *Obesity and cancer. BMJ* 333: 1109-1111. doi:10.1136/bmj.39042.565035.BE1. PubMed: 17124223.
  25. Giovannucci E, Michaud D (2007) The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology* 132: 2208-2225. doi:10.1053/j.gastro.2007.03.050. PubMed: 17498513.
  26. Parr CL, Batty GD, Lam TH, Barzi F, Fang X et al. (2010) Body-mass index and cancer mortality in the Asia-Pacific Cohort Studies Collaboration: pooled analyses of 424,519 participants. *Lancet Oncol* 11: 741-752. doi:10.1016/S1470-2045(10)70141-8. PubMed: 20594911.
  27. Jemal A, Center MM, DeSantis C, Ward EM (2010) Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 19: 1893-1907. doi:10.1158/1055-9965.EPI-10-0437. PubMed: 20647400.
  28. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S (2007) Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes* 56: 1010-1013. doi:10.2337/db06-1656. PubMed: 17287468.
  29. Hsing AW, Deng J, Sesterhenn IA, Mostofi FK, Stanczyk FZ et al. (2000) Body size and prostate cancer: a population-based case-control study in China. *Cancer Epidemiol Biomarkers Prev* 9: 1335-1341. PubMed: 11142419.
  30. Ansary-Moghaddam A, Huxley R, Barzi F, Lawes C, Ohkubo T et al. (2006) The effect of modifiable risk factors on pancreatic cancer mortality in populations of the Asia-Pacific region. *Cancer Epidemiol Biomarkers Prev* 15: 2435-2440. doi:10.1158/1055-9965.EPI-06-0368. PubMed: 17164367.
  31. Huang SP, Wu MS, Shun CT, Wang HP, Lin MT et al. (2004) Interleukin-6 increases vascular endothelial growth factor and angiogenesis in gastric carcinoma. *J Biomed Sci* 11: 517-527. doi:10.1007/BF02256101. PubMed: 15153787.
  32. Tworoger SS, Mantzoros C, Hankinson SE (2007) Relationship of plasma adiponectin with sex hormone and insulin-like growth factor levels. *Obesity (Silver Spring)* 15: 2217-2224. doi:10.1038/oby.2007.263. PubMed: 17890489.
  33. Kishimoto T (2010) IL-6: from its discovery to clinical applications. *Int Immunol* 22: 347-352. doi:10.1093/intimm/dxq030. PubMed: 20410258.
  34. Locksley RM, Killeen N, Lenardo MJ (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104: 487-501. doi:10.1016/S0092-8674(01)00237-9. PubMed: 11239407.
  35. Grivnennikov S, Karin E, Terzic J, Mucida D, Yu GY et al. (2009) IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 15: 103-113. doi:10.1016/j.ccr.2009.01.001. PubMed: 19185845.
  36. Lin WW, Karin M (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest* 117: 1175-1183. doi:10.1172/JCI31537. PubMed: 17476347.
  37. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH (2000) Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 101: 1767-1772. doi:10.1161/01.CIR.101.15.1767. PubMed: 10769275.
  38. Fernandez-Real JM, Broch M, Vendrell J, Ricart W (2003) Smoking, fat mass and activation of the tumor necrosis factor-alpha pathway. *Int J Obes Relat Metab Disord* 27: 1552-1556. doi:10.1038/sj.jco.0802472. PubMed: 12975637.
  39. Grossmann ME, Nkhata KJ, Mizuno NK, Ray A, Cleary MP (2008) Effects of adiponectin on breast cancer cell growth and signaling. *Br J Cancer* 98: 370-379. doi:10.1038/sj.bjc.6604166. PubMed: 18182989.
  40. Baillargeon J, Platz EA, Rose DP, Pollock BH, Ankerst DP et al. (2006) Obesity, adipokines, and prostate cancer in a prospective population-based study. *Cancer Epidemiol Biomarkers Prev* 15: 1331-1335. doi:10.1158/1055-9965.EPI-06-0082. PubMed: 16835332.
  41. Petridou ET, Mitsiades N, Gialamas S, Angelopoulos M, Skalkidou A et al. (2007) Circulating adiponectin levels and expression of adiponectin receptors in relation to lung cancer: two case-control studies. *Oncology* 73: 261-269. doi:10.1159/000127424. PubMed: 18424891.
  42. Straub RH (2007) The complex role of estrogens in inflammation. *Endocr Rev* 28: 521-574. doi:10.1210/er.2007-0001. PubMed: 17640948.