

# Circulating levels of obesity-related markers and risk of renal cell carcinoma in the PLCO cancer screening trial

Linda M. Liao<sup>1</sup> · Jonathan N. Hofmann<sup>1</sup> · Eunyoung Cho<sup>2,3,4</sup> · Michael N. Pollak<sup>5</sup> · Wong-Ho Chow<sup>6</sup> · Mark P. Purdue<sup>1</sup>

## Abstract

**Purpose** Obesity is an established risk factor for renal cell carcinoma (RCC). It is unclear what biologic mechanisms underlie this association, although recent evidence suggests that the effects of circulating hormones such as insulin-like growth factors (IGF) and adipokines may play a role.

**Methods** To address this question, we conducted a nested case-control study of RCC (252 cases, 252 controls) within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial investigating associations with pre-diagnostic serum levels of total adiponectin, high-molecular-weight (HMW) adiponectin, IGF-1, IGF-binding protein-3 (IGFBP-3), and C-peptide. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated using conditional logistic regression.

**Results** After adjustment for potential confounders, non-significant associations with RCC were observed for total adiponectin (OR for highest vs. lowest quartile = 0.65, 95% CI 0.37–1.14;  $p_{\text{trend}} = 0.35$ ), HMW adiponectin (0.67, 0.38–1.17;  $p_{\text{trend}} = 0.36$ ), IGF-1 (1.35, 0.77–2.39;  $p_{\text{trend}} = 0.17$ ), IGFBP-3 (1.47, 0.83–2.62;  $p_{\text{trend}} = 0.53$ ), and C-peptide (1.52, 0.86–2.70;  $p_{\text{trend}} = 0.15$ ). In a joint analysis with body mass index (BMI,  $\text{kg}/\text{m}^2$ ), obese individuals (BMI  $\geq 30$ ) with above-median levels of IGFBP-3 had a significantly higher risk versus those with BMI  $< 25$  and below-median IGFBP-3 (OR 2.42, 1.11–5.26), whereas obese individuals with low IGFBP-3 did not (1.18, 0.53–2.64) ( $p_{\text{interaction}} = 0.35$ ).

**Conclusions** The results of this study, while not clearly supporting associations with these obesity-related hormones, suggest that the association between obesity and RCC may be partially modified through mechanisms related to elevated IGFBP-3.

**Keywords** Obesity · Kidney cancer · Biomarker · Cohort

✉ Linda M. Liao  
Linda.Liao@nih.gov

<sup>1</sup> Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Bethesda, MD 20892, USA

<sup>2</sup> Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

<sup>3</sup> Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA

<sup>4</sup> Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA

<sup>5</sup> Oncology Department, McGill University and Segal Cancer Centre, Jewish General Hospital, Montreal, QC, Canada

<sup>6</sup> MD Anderson Cancer Center, Houston, TX, USA

## Introduction

Kidney cancer is among the most commonly diagnosed cancers in men and women in the United States (U.S.), and the incidence of renal cell carcinoma (RCC), the most common form of kidney cancer, has risen in the U.S. over the past few decades [1]. Obesity is an established risk factor for RCC, but the specific biological mechanisms through which obesity acts to increase the risk of this malignancy are unclear [2]. Hypothesized mechanisms of obesity-related carcinogenesis include the following: altered expression of adipokines, hormones secreted by adipose tissue [3, 4]; increased insulin resistance and

chronic hyperinsulinemia; and alterations in circulating levels of hormones and related binding proteins in the insulin-like growth factor (IGF) pathway. Investigations of these obesity-related markers may provide insight into the mechanism underlying the association between obesity and RCC. To date, only one prospective study has been conducted; in an investigation within the alpha-tocopherol and beta-carotene (ATBC) Cancer Prevention Study, RCC cases had significantly lower pre-diagnosis serum concentrations of adiponectin and IGF-1 compared to controls, and near-significant elevated levels of IGFBP-3 [5, 6]. As the study participants consisted entirely of male Finnish smokers, the generalizability of these findings is unclear.

To better understand the relationships between these obesity-related biomarkers and RCC risk in a population of predominantly non-smoking men and women, we conducted a nested case-control study in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. In this study, we also investigated serum levels of C-peptide, a widely measured surrogate of insulin secretion.

## Materials and methods

### Study population

The PLCO study is a population-based multi-center randomized screening trial of approximately 155,000 predominantly white men and women aged 55–74 years from ten U.S. cities, recruited between 1993 and 2001 [7]. The methods for enrollment and specimen collection have been described in detail previously [8]. Half of the participants were randomized to the screening arm of the trial; these participants provided non-fasting blood samples at six annual medical examinations, including at study entry. Samples were processed and frozen within 2 h of collection and stored at  $-70^{\circ}\text{C}$ . The trial was approved by institutional review boards at the National Cancer Institute and the ten study centers, and all participants provided written informed consent.

After excluding study participants with a history of cancer at baseline and those with a prevalent RCC (diagnosed within one year of follow-up), we identified 252 incident RCC cases and 252 matched controls that provided a blood sample at the baseline visit. Incident RCC cases (ICD-0-3 C64.9) were ascertained by medical record review of suspected cancers reported through mailed annual study update questionnaires, reports from physicians and relatives, and linkage to the National Death Index (NDI) or local cancer registries. All cases were histopathologically confirmed RCC cases. Controls were selected from among PLCO cohort members and individually matched to cases (1:1 ratio) by age at baseline (5-year

categories), sex, race, date of phlebotomy (3-month categories), and study year of specimen collection. Based on a two-sided test with  $\alpha = 0.05$ , we had sufficient statistical power ( $\geq 80\%$ ) to detect a dose-response trend if the OR comparing the highest and lowest quartiles for a given marker was  $< 0.52$  for an inverse association (or  $\geq 1.95$  for a positive association).

### Laboratory methods

Serum concentrations of total adiponectin, HMW adiponectin, IGF-1, IGFBP-3, and C-peptide were measured in duplicate by enzyme-linked immunosorbent assay with reagents purchased from Millipore (St. Charles, MO). Samples were run in duplicate, with cases and their matched controls analyzed in the same batch. Sample concentrations are the average of duplicate measurements. The overall coefficients of variation for total adiponectin, HMW adiponectin, IGF-1, IGFBP-3, and C-peptide were 6.1, 7.0, 2.7, 3.3, and 4.6%, respectively.

### Statistical analyses

Differences between paired cases and controls were tested for statistical significance using the McNemar's or Bowker's test for categorical variables or the Wilcoxon signed-rank tests for continuous variables. Circulating levels of obesity-related markers were categorized into quartiles by cut-points determined by the distributions among controls. We computed odds ratios (OR) and 95% confidence intervals (95% CI) for the association between total adiponectin, HMW adiponectin, IGF-1, IGFBP-3, and C-peptide and RCC using conditional logistic regression models with adjustment for the following potential confounders: age at baseline, education level, cigarette smoking status, and history of hypertension. We also performed analyses with additional adjustment for history of diabetes and categories of body mass index (BMI;  $< 25\text{ kg/m}^2$ ,  $25\text{--}29\text{ kg/m}^2$ ,  $\geq 30\text{ kg/m}^2$ ). Tests for trend were calculated by modeling the median value within each category. In addition, we conducted a joint analysis with BMI, analyses stratified by sex and median follow-up time ( $\leq 7.5$  years,  $> 7.5$  years), and analyses restricted to clear cell RCC (ICD-O-2 8310 and 8312;  $n = 236$  cases) and ever smokers. All statistical tests in the analysis were two-sided and all analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC). A meta-analysis combining the total adiponectin, IGF-1 and IGFBP-3 results from this study and the previously conducted prospective studies was also conducted using a random effects model. The amount of total variation among studies due to heterogeneity was assessed using the  $I^2$  statistic. The meta-

analysis was conducted using STATA version 13.0 (Stat Corp., College Station, TX).

## Results

Some selected characteristics of study cases and controls are summarized in Table 1. Cases and controls had similar distributions of matching factors (Table 1). Compared to controls, cases were more likely to have a history of hypertension ( $p = 0.01$ ) and a higher BMI ( $p = 0.004$ ). Cases had lower levels of total and HMW adiponectin and higher levels of C-peptide, IGF-1, and IGFBP-3 than controls, but none of these were statistically significant ( $p > 0.05$ ). The correlations between analytes and with BMI are shown in Supplementary Table 1. Among controls, BMI was inversely correlated with adiponectin levels and positively correlated with C-peptide. Levels of total and HMW adiponectin were almost perfectly correlated, and a strong correlation between IGF-1 and IGFBP-3 was

also observed. Correlations of analytes were mostly similar and in the same direction among females versus males (Supplementary Table 1). The more notable differences were between BMI and C-peptide: 0.03 versus 0.39, BMI and IGF1:  $-0.18$  versus  $-0.01$ , and BMI and IGFBP-3:  $-0.12$  versus 0.02 (females vs. males, respectively).

Overall, the measured analytes were weakly associated with RCC risk, although not at a level of statistical significance; total adiponectin and HMW adiponectin were suggestive of an inverse association with RCC, while C-peptide, IGF-1, and IGFBP-3 were suggestive of a positive association with RCC (Table 2). Additional adjustment for history of diabetes and BMI did not change effect estimates appreciably. In sensitivity analyses, mutual adjustment of IGF-1 and IGFBP-3 in the same model did not change effect estimates for IGFBP-3 substantially, while effect estimates for IGF-1 attenuated (data not shown). The associations for the obesity-related markers varied somewhat between men and women, although tests of interaction by sex were not statistically significant.

**Table 1** Selected baseline characteristics of RCC cases and controls in the PLCO Screening Trial

| Characteristics                                  | Cases ( $n = 252$ )     | Controls ( $n = 252$ )  |
|--|-------------------------|-------------------------|
| Age (years)                                      |                         |                         |
| 55–59  | 70 (27.8)               | 70 (27.8)               |
| 60–64  | 90 (35.7)               | 92 (36.5)               |
| 65–69  | 59 (23.4)               | 58 (23.0)               |
| 70–74  | 33 (13.1)               | 32 (12.7)               |
| Sex  |                         |                         |
| Female   | 84 (33.3)               | 84 (33.3)               |
| Male   | 168 (66.7)              | 168 (66.7)              |
| Race   |                         |                         |
| White, non-Hispanic                              | 225 (89.3)              | 225 (89.3)              |
| Black, non-Hispanic                              | 13 (5.2)                | 13 (5.2)                |
| Hispanic   | 4 (1.6)                 | 4 (1.6)                 |
| Asian/Pacific Islander                           | 9 (3.6)                 | 9 (3.6)                 |
| American Indian                                  | 1 (0.4)                 | 1 (0.4)                 |
| Level of education                               |                         |                         |
| High school or less                              | 84 (33.3)               | 69 (27.4)               |
| Some college or other training after high school | 88 (34.9)               | 85 (33.7)               |
| College  | 80 (31.8)               | 98 (38.9)               |
| History of hypertension, yes                     | 118 (45.0)              | 81 (32.1)               |
| History of diabetes, yes                         | 29 (11.5)               | 20 (7.9)                |
| BMI ( $\text{kg}/\text{m}^2$ )                   | 28.0 (25.2–31.3)        | 26.7 (24.4–29.7)        |
| Adiponectin concentration (ng/mL)                | 7128.7 (4427.7–11223.8) | 7744.1 (5177.0–11591.4) |
| HMW adiponectin concentration (ng/mL)            | 4273.7 (2466.8–7108.3)  | 4694.3 (2925.9–7292.4)  |
| C-peptide concentration (ng/mL)                  | 2.10 (1.38–3.52)        | 1.97 (1.20–3.05)        |
| IGF-1 concentration (ng/mL)                      | 101.7 (72.4–136.1)      | 98.6 (70.6–124.6)       |
| IGFBP-3 concentration (ng/mL)                    | 2922.3 (2380.0–3423.3)  | 2779.7 (2254.3–3359.3)  |

$n$  (%) or median (interquartile range) are presented

Table 2 Adjusted ORs and 95% CIs for the risk of RCC and obesity-related biomarkers

|                                    | All subjects   |                  |                          |                          |                |                  | Females                  |                          |                | Males            |                          |                          |
|------------------------------------|----------------|------------------|--------------------------|--------------------------|----------------|------------------|--------------------------|--------------------------|----------------|------------------|--------------------------|--------------------------|
|                                    | Cases/controls |                  | Adjusted OR <sup>a</sup> | Adjusted OR <sup>b</sup> | Cases/controls |                  | Adjusted OR <sup>a</sup> | Adjusted OR <sup>b</sup> | Cases/controls |                  | Adjusted OR <sup>a</sup> | Adjusted OR <sup>b</sup> |
|                                    |                |                  |                          |                          |                |                  |                          |                          |                |                  |                          |                          |
| <b>Adiponectin<sup>c</sup></b>     |                |                  |                          |                          |                |                  |                          |                          |                |                  |                          |                          |
| Q1                                 | 81/63          | 1.00             | 1.00                     | 1.00                     | 14/10          | 1.00             | 1.00                     | 1.00                     | 67/53          | 1.00             | 1.00                     | 1.00                     |
| Q2                                 | 61/63          | 0.68 (0.38–1.19) | 0.68 (0.38–1.22)         | 0.68 (0.38–1.22)         | 18/21          | 0.80 (0.25–2.53) | 0.85 (0.25–2.94)         | 0.80 (0.34–1.29)         | 43/42          | 0.66 (0.34–1.29) | 0.61 (0.30–1.23)         | 0.61 (0.30–1.23)         |
| Q3                                 | 52/63          | 0.67 (0.39–1.16) | 0.72 (0.41–1.27)         | 0.72 (0.41–1.27)         | 21/16          | 1.27 (0.41–3.92) | 1.57 (0.47–5.24)         | 0.53 (0.27–1.02)         | 31/47          | 0.53 (0.27–1.02) | 0.52 (0.26–1.03)         | 0.52 (0.26–1.03)         |
| Q4                                 | 58/63          | 0.65 (0.37–1.14) | 0.75 (0.41–1.38)         | 0.75 (0.41–1.38)         | 31/37          | 0.73 (0.24–2.25) | 1.11 (0.33–3.79)         | 0.72 (0.37–1.44)         | 27/26          | 0.72 (0.37–1.44) | 0.73 (0.35–1.54)         | 0.73 (0.35–1.54)         |
| <i>p</i> continuous                |                | 0.35             | 0.79                     | 0.79                     |                | 0.92             | 0.21                     | 0.22                     |                | 0.22             | 0.29                     | 0.29                     |
| OR Per 1 SD increase               |                | 0.97 (0.80–1.18) | 1.05 (0.85–1.29)         | 1.05 (0.85–1.29)         |                | 1.08 (0.78–1.47) | 1.33 (0.91–1.93)         | 0.86 (0.65–1.13)         |                | 0.86 (0.65–1.13) | 0.87 (0.64–1.17)         | 0.87 (0.64–1.17)         |
| <b>HMW adiponectin<sup>c</sup></b> |                |                  |                          |                          |                |                  |                          |                          |                |                  |                          |                          |
| Q1                                 | 81/63          | 1.00             | 1.00                     | 1.00                     | 18/11          | 1.00             | 1.00                     | 1.00                     | 63/52          | 1.00             | 1.00                     | 1.00                     |
| Q2                                 | 61/63          | 0.73 (0.43–1.25) | 0.78 (0.45–1.37)         | 0.78 (0.45–1.37)         | 14/19          | 0.53 (0.15–1.89) | 0.78 (0.20–3.06)         | 0.87 (0.47–1.60)         | 47/44          | 0.87 (0.47–1.60) | 0.85 (0.45–1.60)         | 0.85 (0.45–1.60)         |
| Q3                                 | 52/63          | 0.67 (0.39–1.15) | 0.72 (0.41–1.26)         | 0.72 (0.41–1.26)         | 20/17          | 0.80 (0.25–2.55) | 1.07 (0.32–3.61)         | 0.62 (0.33–1.16)         | 32/46          | 0.62 (0.33–1.16) | 0.62 (0.32–1.20)         | 0.62 (0.32–1.20)         |
| Q4                                 | 58/63          | 0.67 (0.38–1.17) | 0.78 (0.42–1.45)         | 0.78 (0.42–1.45)         | 32/37          | 0.57 (0.18–1.77) | 0.97 (0.28–3.40)         | 0.77 (0.39–1.52)         | 26/26          | 0.77 (0.39–1.52) | 0.77 (0.36–1.63)         | 0.77 (0.36–1.63)         |
| <i>p</i> continuous                |                | 0.36             | 0.85                     | 0.85                     |                | 0.87             | 0.29                     | 0.31                     |                | 0.31             | 0.43                     | 0.43                     |
| OR Per 1 SD increase               |                | 0.98 (0.81–1.19) | 1.06 (0.86–1.31)         | 1.06 (0.86–1.31)         |                | 1.06 (0.77–1.46) | 1.32 (0.91–1.93)         | 0.88 (0.68–1.16)         |                | 0.88 (0.68–1.16) | 0.90 (0.67–1.21)         | 0.90 (0.67–1.21)         |
| <b>C-peptide<sup>c</sup></b>       |                |                  |                          |                          |                |                  |                          |                          |                |                  |                          |                          |
| Q1                                 | 43/63          | 1.00             | 1.00                     | 1.00                     | 18/20          | 1.00             | 1.00                     | 1.00                     | 25/43          | 1.00             | 1.00                     | 1.00                     |
| Q2                                 | 70/63          | 1.38 (0.80–2.37) | 1.35 (0.77–2.38)         | 1.35 (0.77–2.38)         | 23/23          | 0.96 (0.39–2.33) | 0.93 (0.36–2.39)         | 1.91 (0.94–3.91)         | 47/40          | 1.91 (0.94–3.91) | 2.09 (0.98–4.48)         | 2.09 (0.98–4.48)         |
| Q3                                 | 61/63          | 1.08 (0.62–1.89) | 0.88 (0.48–1.61)         | 0.88 (0.48–1.61)         | 20/25          | 0.70 (0.27–1.83) | 0.51 (0.18–1.45)         | 1.52 (0.74–3.12)         | 41/38          | 1.52 (0.74–3.12) | 1.39 (0.63–3.09)         | 1.39 (0.63–3.09)         |
| Q4                                 | 78/63          | 1.52 (0.86–2.70) | 1.24 (0.68–2.28)         | 1.24 (0.68–2.28)         | 23/16          | 1.59 (0.48–5.25) | 1.06 (0.30–3.76)         | 2.01 (0.98–4.13)         | 55/47          | 2.01 (0.98–4.13) | 1.91 (0.88–4.13)         | 1.91 (0.88–4.13)         |
| <i>p</i> continuous                |                | 0.15             | 0.45                     | 0.45                     |                | 0.62             | 0.86                     | 0.08                     |                | 0.08             | 0.14                     | 0.14                     |
| OR Per 1 SD increase               |                | 1.12 (0.93–1.34) | 1.08 (0.89–1.30)         | 1.08 (0.89–1.30)         |                | 1.08 (0.74–1.56) | 1.00 (0.70–1.43)         | 1.18 (0.94–1.48)         |                | 1.18 (0.94–1.48) | 1.17 (0.92–1.48)         | 1.17 (0.92–1.48)         |
| <b>IGF-1<sup>c</sup></b>           |                |                  |                          |                          |                |                  |                          |                          |                |                  |                          |                          |
| Q1                                 | 56/63          | 1.00             | 1.00                     | 1.00                     | 36/41          | 1.00             | 1.00                     | 1.00                     | 20/22          | 1.00             | 1.00                     | 1.00                     |
| Q2                                 | 65/63          | 1.26 (0.74–2.14) | 1.24 (0.72–2.15)         | 1.24 (0.72–2.15)         | 26/20          | 1.37 (0.61–3.07) | 1.57 (0.65–3.78)         | 1.14 (0.54–2.41)         | 39/43          | 1.14 (0.54–2.41) | 1.04 (0.48–2.26)         | 1.04 (0.48–2.26)         |
| Q3                                 | 57/63          | 1.08 (0.62–1.89) | 1.00 (0.56–1.78)         | 1.00 (0.56–1.78)         | 12/13          | 0.98 (0.36–2.70) | 1.19 (0.40–3.50)         | 1.02 (0.49–2.15)         | 45/50          | 1.02 (0.49–2.15) | 0.86 (0.40–1.88)         | 0.86 (0.40–1.88)         |
| Q4                                 | 74/63          | 1.35 (0.77–2.39) | 1.32 (0.73–2.36)         | 1.32 (0.73–2.36)         | 10/10          | 1.01 (0.32–3.18) | 1.11 (0.32–3.83)         | 1.37 (0.66–2.82)         | 64/53          | 1.37 (0.66–2.82) | 1.23 (0.58–2.62)         | 1.23 (0.58–2.62)         |
| <i>p</i> continuous                |                | 0.17             | 0.17                     | 0.17                     |                | 0.48             | 0.23                     | 0.29                     |                | 0.29             | 0.41                     | 0.41                     |
| OR per 1 SD increase               |                | 1.16 (0.94–1.43) | 1.16 (0.94–1.44)         | 1.16 (0.94–1.44)         |                | 1.14 (0.74–1.77) | 1.26 (0.78–2.03)         | 1.16 (0.91–1.48)         |                | 1.16 (0.91–1.48) | 1.13 (0.88–1.45)         | 1.13 (0.88–1.45)         |
| <b>IGFBP-3<sup>c</sup></b>         |                |                  |                          |                          |                |                  |                          |                          |                |                  |                          |                          |
| Q1                                 | 48/63          | 1.00             | 1.00                     | 1.00                     | 18/26          | 1.00             | 1.00                     | 1.00                     | 30/37          | 1.00             | 1.00                     | 1.00                     |
| Q2                                 | 59/63          | 1.33 (0.76–2.32) | 1.35 (0.77–2.39)         | 1.35 (0.77–2.39)         | 21/22          | 1.82 (0.65–5.10) | 2.63 (0.80–8.66)         | 1.14 (0.56–2.29)         | 38/41          | 1.14 (0.56–2.29) | 1.13 (0.55–2.30)         | 1.13 (0.55–2.30)         |
| Q3                                 | 76/63          | 1.68 (0.96–2.93) | 1.60 (0.91–2.81)         | 1.60 (0.91–2.81)         | 20/11          | 3.09 (0.97–9.89) | 3.98 (1.07–14.81)        | 1.32 (0.68–2.56)         | 56/52          | 1.32 (0.68–2.56) | 1.24 (0.63–2.42)         | 1.24 (0.63–2.42)         |
| Q4                                 | 69/63          | 1.47 (0.83–2.62) | 1.42 (0.79–2.57)         | 1.42 (0.79–2.57)         | 25/25          | 1.64 (0.54–5.02) | 2.32 (0.66–8.16)         | 1.33 (0.65–2.72)         | 44/38          | 1.33 (0.65–2.72) | 1.25 (0.60–2.61)         | 1.25 (0.60–2.61)         |

Table 2 continued

|                      | All subjects        |                          |                          | Females          |                          |                          | Males            |                          |                          |
|----------------------|---------------------|--------------------------|--------------------------|------------------|--------------------------|--------------------------|------------------|--------------------------|--------------------------|
|                      | Cases/controls      | Adjusted OR <sup>a</sup> | Adjusted OR <sup>b</sup> | Cases/controls   | Adjusted OR <sup>a</sup> | Adjusted OR <sup>b</sup> | Cases/controls   | Adjusted OR <sup>a</sup> | Adjusted OR <sup>b</sup> |
|                      | <i>p</i> continuous | 0.53                     | 0.63                     | 0.76             | 0.91                     | 0.76                     | 0.54             | 0.75                     | 0.54                     |
| OR per 1 SD increase | 1.07 (0.89–1.30)    | 1.06 (0.87–1.29)         | 1.11 (0.78–1.57)         | 1.03 (0.74–1.43) | 1.11 (0.78–1.57)         | 1.08 (0.84–1.38)         | 1.04 (0.80–1.36) | 1.08 (0.84–1.38)         | 1.04 (0.80–1.36)         |

<sup>a</sup> Adjusted for age, education, cigarette smoking status, and history of hypertension

<sup>b</sup> Adjusted model further adjusted for history of diabetes and body mass index (categorical)

<sup>c</sup> Quartile cut-points were assigned based on the distribution among all controls as follows: Adiponectin ng/ml, Q1 ≤5,177.0, Q2 >5,177.0–≤7,744.1, Q3 >7,744.1–≤11,591.4, Q4 >11,591.4; HMW adiponectin ng/ml Q1 ≤2,925.9, Q2 >2,925.9–≤4,694.3, Q3 >4,694.3–≤7,292.4, Q4 >7,292.4; C-peptide ng/ml Q1 ≤1.20, Q2 >1.20–≤1.97, Q3 >1.97–≤3.05, Q4 >3.05; IGF-1 ng/ml, Q1 ≤70.6, Q2 >70.6–≤98.6, Q3 >98.6–≤124.6, Q4 >124.6; IGF-1 ng/ml, Q1 ≤2,254.3, Q2 >2,254.3–≤2,779.7, Q3 >2,779.7–≤3,359.3, Q4 >3,359.3

Analyses restricted to clear cell RCC ( $n = 236$  cases) were not substantially different (Supplementary Table 2).

When the obesity-related markers and BMI were considered jointly, we observed suggestive evidence of effect modification for serum IGF-1 (Table 3); an increased risk of RCC was observed among obese individuals (BMI ≥30) with high levels of IGF-1 (OR 2.42, 95% CI 1.11–5.26), but not among obese individuals with low IGF-1 (OR 1.18, 95% CI 0.53–2.64). A test of interaction between BMI and IGF-1, however, was not statistically significant ( $p = 0.35$ ). We did not observe evidence of effect modification with BMI for other biomarkers (Supplementary Table 3).

We conducted analyses of BMI with adjustment for each biomarker separately to evaluate whether they may modify the established relationship between obesity and RCC. Adjustment for each biomarker did not seem to have a noticeable effect on the BMI association. A modest attenuation of the BMI association was observed when adjusting for either adiponectin measure, with the OR per 5 kg/m<sup>2</sup> increase changing from 1.19 (95% CI 0.97–1.47) to 1.14 (95% CI 0.91–1.43) (Supplementary Table 4). Sex-stratified results of these BMI models were comparable (data not shown). Stratified analyses by median follow-up time (≤7.5 years, >7.5 years), and analyses restricted to ever smokers did not reveal any evidence of differences in RCC risk across strata for any marker (data not shown).

We also conducted a meta-analysis combining our risk estimates for adiponectin, IGF-1, and IGF-1 with those from the previous ATBC study [5, 6]. In the meta-analysis, adiponectin levels were inversely associated with RCC at near statistical significance ( $p$  value = 0.06), with summary ORs for the quartiles of adiponectin from lowest to highest of 0.70 (95% CI 0.48–1.00), 0.81 (95% CI 0.57–1.16), and 0.58 (95% CI 0.39–0.85), respectively (Supplementary Table 5). Increasing quartiles of IGF-1 levels were associated with an increased risk of RCC with summary ORs of 1.40 (95% CI 0.87–2.24), 2.08

Table 3 Joint analysis between IGF-1 and BMI and risk of RCC

| BMI   | IGF-1 | Cases | Controls | OR (95% CI) <sup>a</sup> |
|-------|-------|-------|----------|--------------------------|
| <25   | Low   | 27    | 35       | 1.00                     |
| <25   | High  | 32    | 37       | 1.02 (0.46–2.24)         |
| 25–29 | Low   | 50    | 59       | 1.09 (0.54–2.19)         |
| 25–29 | High  | 54    | 59       | 1.23 (0.61–2.47)         |
| 30+   | Low   | 30    | 29       | 1.18 (0.53–2.64)         |
| 30+   | High  | 59    | 28       | 2.42 (1.11–5.26)         |

Low indicates < median value and High indicates ≥ median value for IGF-1

<sup>a</sup> Adjusted for age, education, cigarette smoking status, and history of hypertension

(1.24–3.48), and 1.78 (0.97–3.25), but did not demonstrate a significant trend ( $p$  value = 0.58). Evidence of significant heterogeneity in the IGF-1 estimates ( $I^2 > 50\%$ ) made those summary estimates difficult to interpret.

## Discussion

In this prospective study, we did not observe clear evidence of a relationship between circulating obesity-related biomarkers and RCC risk, although the weak associations we observed for adiponectin and IGFBP-3 are in the same direction as those from the previous ATBC investigation. We also observed suggestive evidence of effect modification, with a statistically significant association with RCC observed for obese individuals with high levels of IGFBP-3, but not for obese individuals with low IGFBP-3.

In conjunction with insulin, the IGF system is a complex network that regulates metabolism through the promotion and inhibition of cell growth. IGF-1 is a polypeptide hormone that stimulates growth and cell proliferation [3]. IGFBP-3 is the primary carrier of circulating IGF-1 [9]. IGFBP-3 influences the amount of free IGF-1 in circulation and regulates the activity of IGF-1 at target tissues [3]. Independent of IGF-1 bioavailability, IGFBP-3 is also thought to have pro-apoptotic properties through its interactions with several signaling pathways [9]. In relation to RCC, IGF-1 has been associated with increased normal kidney growth in both animal and human models, and may contribute to glomerular sclerosis [10]. Experimental studies have also demonstrated that IGF-1 and IGFBP-3 expression is increased among both clear cell renal tumors and renal cancer cell lines [11, 12]. IGF-1 has been associated with an increased risk of several cancers, including colorectal cancer, prostate cancer, and pre-menopausal breast cancer [13]. Higher concentrations of IGFBP-3 have been positively associated with pre-menopausal breast cancer only [13]. Risk of RCC in relation to pre-diagnostic serum levels of IGF-1 and IGFBP-3 was previously evaluated in a nested study within the ATBC cohort [6]. Contrary to expectations, IGF-1 was inversely associated with RCC risk; subjects with IGF-1 levels  $>113$  ng/mL were 59% less likely to develop RCC than those who had levels  $\leq 113$  ng/mL. The reason for this inverse association is unclear, but is inconsistent with experimental evidence [12]. They did not observe a clear trend with IGFBP-3 levels and RCC risk. In our study, higher levels of both IGF-1 and IGFBP-3 were suggestively associated with RCC risk, but we did not see evidence of a trend in risk across quartiles. When evaluated jointly with BMI, high levels of IGFBP-3 with high BMI were associated with a statistically significant twofold increased risk of RCC compared to those with low levels of both. A similar

pattern was observed in a joint analysis between IGF-1 and BMI, but findings did not reach statistical significance. An overall trend of increasing risk of RCC with higher levels of IGFBP-3 was observed in our meta-analysis of two studies. Taken together, our results suggest that elevated IGFBP-3 may play a role in RCC development, particularly among obese individuals.

Adiponectin is an insulin-sensitizing hormone produced exclusively by adipocytes with potentially anti-inflammatory, pro-apoptotic, and anti-angiogenic properties [4]. Adiponectin exists in several oligomeric forms. HMW adiponectin has been suggested to be the most biologically active form of adiponectin and evaluation of these specific multimers could provide a better indicator of effect than adiponectin [4, 14]. Circulating levels of adiponectin are reduced among obese individuals [15] and were inversely associated with BMI in our controls ( $r = -0.33$ ). Experimental studies have demonstrated that both adiponectin receptors, AdipoR1 and R2, are expressed in normal and renal tumor tissue but appear to be downregulated in tumor tissue compared to normal tissue [16, 17]. Previous epidemiologic case-control studies evaluating adiponectin levels and RCC have yielded mixed results, but interpretation of these associations is limited by the use of post-diagnostic samples [18, 19]. In the prior prospective investigation within the ATBC cohort, high total adiponectin levels were associated with a statistically significant reduced RCC risk (Q4 vs. Q1: OR 0.52, 95% CI 0.30–0.88) [5]. In our study, higher concentrations of adiponectin and HMW adiponectin were also suggestive of inverse associations with RCC, although the ORs were weaker with wide confidence limits. In our meta-analysis of the two studies, the summary association for adiponectin reached statistical significance with no between-study heterogeneity detected. Thus, the collective cohort evidence to date supports an association between low circulating adiponectin and increased risk of RCC. Additional prospective investigations are needed to further delineate this relationship.

A recent analysis of metastatic RCC patients demonstrated a positive association between excess body weight and a longer overall survival, which may be due to a decreased expression of the fatty acid synthase (*FASN*) gene in obese patients [20]. As adipose tissue is composed of fatty acids, the decreased expression of *FASN* is likely to influence the production of adipokines and other obesity-related markers in RCC patients and could be one of the mechanisms through which carcinogenesis occurs.

Our study has several strengths, including its prospective study design, use of pre-diagnostic serum samples, and inclusion of non-smokers and women. The prospective design reduces the potential impact of reverse causality on our observed associations. As is typical of most nested

case-control studies, we were limited to measurements from a single banked specimen from each participant, which may not accurately capture adipokine or IGF levels over time. Although our study participants provided non-fasting samples, adiponectin levels have been shown to be generally stable over time and remain similar across fasting status [21]. Waist-hip ratio and other anthropometric measurements were not available in our study, so we were limited in our evaluation of obesity to the BMI measure.

In conclusion, the results of this study, while not clearly supporting associations between these obesity-related biomarkers and RCC risk, are consistent with previously reported findings for adiponectin, and suggest an association with elevated IGFBP-3 among obese individuals. Confirmation of these findings in other prospective studies is needed.

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