Synergy of Sex Differences in Visceral Fat Measured with CT and Tumor Metabolism Helps Predict Overall Survival in Patients with Renal Cell Carcinoma¹

**Purpose:** To determine if sex differences in abdominal visceral fat composition, measured by using computed tomography (CT), and tumor glucose metabolism, measured by gene expression, can help predict outcomes in patients with clear cell renal cell carcinoma (RCC).

**Materials and Methods:** This retrospective cohort study included 222 patients with clear cell RCC from The Cancer Imaging Atlas. By using CT, body fat was segmented into subcutaneous fat and visceral fat areas (VFAs) and normalized to total fat to obtain the relative VFA (rVFA) and relative subcutaneous fat area. Multivariate Cox proportional hazard regression models were performed to identify effects of rVFA on sex-specific survival. Expression profiles for 39 glycolytic genes in tumors from these patients were obtained from The Cancer Genome Atlas to determine sex differences in metabolism and compared with rVFA. Key mutations in clear cell RCC were analyzed for association with rVFA and tumor glycolytic profiles.

**Results:** Women with rVFA greater than 30.9% had an increased risk of death (hazard ratio, 3.66 [95% confidence interval: 1.64, 8.19]) for women vs 1.13 ([95% confidence interval: 0.58, 2.18] for men, \(P = .028\)). Glycolytic gene expression stratified both men and women, and the combination of low rVFA and low glycolysis identified 19 women with excellent overall survival (\(P < .001\)). SETD2 and BAP1 mutations were uniquely enriched in female tumors with high glycolysis (\(P = .036\) and \(P = .001\), respectively). No significant differences were identified in tumor mutations between patients with high and low rVFA.

**Conclusion:** Sex differences in visceral fat and tumor glucose metabolism may provide a new risk-stratification system for patients with clear cell RCC.

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Demographic analyses have disclosed sex differences in numerous cancers with male-predominant incidences as well as mortality (1–5). One potential mechanism that may explain this phenomenon is metabolic differences between men and women. A sexual dimorphism in body fat distribution exists where men accumulate more abdominal visceral fat and premenopausal women accumulate more subcutaneous fat. Increased visceral fat contributes to insulin resistance, metabolic syndrome, and greater cardiovascular risk. In contrast, subcutaneous fat may counterbalance these negative effects, consistent with findings that premenopausal women are more protected against cardiovascular and metabolic derangements (6). Because conventional measurements of obesity using body mass index preclude evaluation of body fat distribution, abdominal imaging (eg, computed tomography [CT]) allows accessible and accurate measurement of visceral and subcutaneous fat and therefore is a potentially more robust biomarker for outcomes.

Increased visceral fat is associated with poorer outcomes for patients with colorectal and pancreatic cancers, as well as those with metastatic melanoma (7,8). Furthermore, increased visceral fat rather than body mass index alone may predict negative outcomes in patients with colorectal cancer (9,10). Outcomes in renal cell carcinoma (RCC) are less clear, as increased absolute visceral fat was protective in patients with advanced RCC undergoing therapy in one study (11), while it appeared to be detrimental in another (12). Meanwhile, relative visceral fat in one study showed no influence on survival outcomes in patients with RCC. However, these analyses did not examine sex-specific differences (13).

Sex differences in RCC exist. On the phenotypic level, men with these tumors present with larger tumor size, higher grade, and higher stage relative to women (14). On the molecular level, RCC exhibits robust molecular differences, including sex-specific mutation incidence and survival (15,16). Thus, sex differences in visceral fat of the patient may be linked to sex differences in the molecular composition of the tumor, specifically its metabolism.

Enhanced uptake of fluorodeoxyglucose positively correlates with poor outcomes in numerous cancers including RCC (17). Although sex differences in tumor fluorodeoxyglucose uptake have not been identified, there are developmental differences in metabolism characterized by enhanced male glycolysis (18–20). Thus, sex differences in the metabolism of the host may be linked with sex differences in the metabolic composition of the tumor.

The purpose of this study was to determine if sex differences in abdominal visceral fat composition (measured by CT) and tumor metabolism (measured by gene expression) could identify sex differences in outcomes in patients with clear cell RCC.

**Implications for Patient Care**

- CT measurement of relative visceral fat area can help stratify prognosis in female patients with renal cell carcinoma (RCC).
- Identification of radiographic predictors of prognosis in RCC patients at initial diagnosis can potentially guide therapeutic and surgical options.
- These findings further support the idea that imaging modalities that target metabolism may need to be interpreted differently based on sex and may require sex-specific treatment options.

**Materials and Methods**

**Software**

The license for the fat assessment tool was provided by Vital images, but the data for the study were obtained independently by the authors and are under their control for publication.

**Datasets**

The results published here are in whole or part based on data generated by The Cancer Genome Atlas (TCGA) Research Network (http://cancergenome.nih.gov/). We obtained multi-institutional CT imaging examinations from The Cancer Imaging Archive (TCIA) Kidney Renal Clear Cell Carcinoma (KIRC) database collected under respective institutional review boards and de-identified under Health Insurance Portability and Accountability Act spanning 2002–2013 (accessed on May 2016) (21,22). This retrospective cohort study included patients with presurgical baseline abdominal CT images. Four patients were excluded because the field of view of the associated CT examinations had colimated an estimated greater than 10% of the abdominal wall so as to render quantification erroneous. In total, 222 patients (143 male, 77 female) were used for imaging analysis.

Our strategy to identify prognostic sex differences in clear cell RCC using biomarkers for patient and tumor metabolism is described in Figure 1. Level 3 RNA-Seq gene expression data from all KIRC samples in TCGA were obtained from the National Cancer Institute Genomic Data Commons data portal and Broad GDAC Firehose data portal. The mutation information for the KIRC samples was obtained from...
the GDAC firehose Oncotated Calls MAF files. In total, transcriptome data were available for 345 men (two tissue samples were present for TCGA-DV-A4V0 = 346 male tissue samples) and 189 women, and mutation data were available for 294 men and 157 women with clinical information. Therefore, a subset of TCGA-KIRC patients with genomic and transcriptomic data had corresponding imaging data. Clinical-pathologic data for matched TCGA and TCIA samples were downloaded from the cBioPortal for cancer genomics (http://www.cbioportal.org/). For imaging, transcriptome, and genome analyses, all available patients were used for that particular analysis.

Body Fat Distribution Segmentation
Subcutaneous fat area (SFA) and visceral fat area (VFA) from CT examinations were obtained as previously described (23). Briefly, CT examinations in Digital Imaging and Communications in Medicine format were transferred to a workstation equipped with the Vitrea Fat Measurement Application (Vital Images, Minnetonka, Minn). This software application has been validated by using cryosection photographs and CT images from the National Institutes of Health Visible Human Project and has been published previously (23). SFAs and VFAs at the level of the umbilicus were mapped by using Hounsfield unit thresholds ranging between −150 and −50. Errors from software-defined areas were corrected manually and overseen by one of the authors, a subspecialized attending radiologist (V.M.M.) with 10 years of experience in interpreting abdominal CT scans. Total fat area (TFA) was summed from absolute SFA and VFA. Relative VFA (rVFA) and relative SFA (rSFA) was calculated as percentage of TFA (eg, rVFA = VFA/TFA). By using this method, rVFA and rSFA become complementary (rVFA + rSFA = 100%), and any analyses that investigate both of these normalized quantities are redundant. Because of this, we focused solely on rVFA for all subsequent analyses.

Statistical Analysis
Tumors were classified on the basis of the American Joint Committee on Cancer TNM classification. Because of low prevalence of Fuhrman grade 1 disease among this cohort (one of 222, 0.5%), Fuhrman grade was dichotomized for the purpose of analysis. Fuhrman grades 1 and 2 were considered low grade, and Fuhrman grades 3 and 4 were considered high grade. Similarly, stage was dichotomized given the low prevalence of stage II disease (17 of 222, 7.7%). Stages I and II were considered low stage, and stages III and IV were considered high stage. Race was also dichotomized into white and nonwhite groups.

Overall survival from TCGA database was analyzed by using the Kaplan-Meier method and differences were assessed by using the log-rank test. Patients lost to follow-up were defined by the American College of Surgeons Commission on Cancer as patients for whom updated follow-up information had not been collected within the past 15 months and all efforts to contact the patient had been exhausted (including reviewing the Social Security death index).

Continuous variables were reported as means (± standard deviation) and categorical variables were reported as proportions, unless otherwise specified. Comparisons between sexes for
categorical and continuous variables were performed by using Fisher exact test and Student t test, respectively. Correlations between SFA and VFA were assessed by using Pearson correlation coefficient (r). We used a biomarker cutoff optimization algorithm (Cut-off Finder; http://molpath.charite.de/cutoff/) to determine the optimal rVFA value required to maximally stratify male and female overall survival (24). If no optimal value was discovered, patients were stratified according to the median value.

To simultaneously assess the risk of multiple factors on overall survival, we conducted a Cox proportional hazards regression. The risk of stage, tumor size, age at diagnosis, and the interaction of high versus low rVFA with sex on survival was evaluated. Models were constructed in a backward stepwise method, and the final model selected had the lowest Akaike information criterion. The proportional hazards assumptions were verified by assessing the cumulative sum of the Martingale residuals.

Statistical analyses were performed by using Prism 5.04 (GraphPad Software, La Jolla, Calif) and SAS v9.4 (SAS Institute, Cary, NC) software. Two-tailed statistical tests were performed where applicable, and P < .05 was considered to indicate a statistically significant difference.

Transcriptome Analysis
Transcriptome analyses were based on a previously published method to identify sex differences in glycolytic gene expression (25). Gene expression values from 39 genes that characterize glucose uptake (SLC2A1, SLC2A2, SLC2A3, SLC2A4), glycolysis (HK1, HK2, HK3, GCK, GPI, PFKM, PFKL, PFKP, ALDOA, ALDOB, ALDOC, TPI1, GAPDH, GAPDHS, PKL1, PKG2, PGAM1, PGAM2, PGAM4, BPGM, ENO1, ENO2, ENO3, PKM2, PKLR, LDHAL6A, LDHAL6B, LDHA, LDHB, LDLIC, LDHID), and monocarboxylate (lactate and pyruvate) transport (SLC16A1, SLC16A3, SLC16A7, SLC16A8) were analyzed. RNA-Seq by expectation-maximization (RSEM) gene expression values were transformed to sex-specific Z scores. To identify a Z score for glycolytic gene expression that could maximally stratify men and/or women, the Z-score threshold using all 39 genes simultaneously was scaled from 0 to 4 in 0.25-unit increments. The log-rank test was used to calculate the P value indicating statistical significance of survival difference and derive the hazard ratio with 95% confidence interval. The Z-score threshold that maximized differences in both male and female survival (Z = 1) was selected for transcript-specific analyses. We assigned patients with overexpression of at least one of the genes associated with sex-specific decreased overall survival to a high-glycolysis group. All other patients were defined as those with low glycolysis.

RCC Mutation Enrichment
To investigate the enrichment of RCC mutations in glycolysis and rVFA groups, we investigated 10 common mutations in clear cell RCC that have been identified from large-scale sequencing projects. Moreover, a subset of these mutations have sex-specific enrichment in clear cell RCC (16). Fisher exact test was performed to identify significantly (P < .05) enriched mutations in the high-glycolysis or high-rVFA groups.

Results
Study Population Characteristics
The study population consisted of 145 male and 77 female patients who ranged in age from 33 to 88 years (mean age ± standard deviation, 60
Sex Differences in Body Fat Distribution

As expected, VFA was significantly greater in men (180.5 ± 87.5 vs 125.6 ± 69.0, P < .001), Fig E1a [online]) and SFA was significantly greater in women (300.9 ± 135.5 vs 259.0 ± 125.1, P = .022, Fig E1b). However, TFA was similar between men and women (439.4 ± 184.1 vs 426.5 ± 187.9, P = .621) (Fig E1c [online]).

Next, we hypothesized that visceral fat proportionally increases with subcutaneous fat as patients become more obese. This effect could confound subsequent analyses that investigate sex-specific effects of visceral or subcutaneous fat stores on outcomes. Because of the significant positive correlation (P < .001) between visceral and subcutaneous fat in both men and women (Fig E2a, E2b [online]), we focused investigation on relative fat stores, which may unmask sex-specific differences. As seen with VFA, rVFA was significantly higher in men compared with women (40.9% ± 11.65 in men vs 28.7% ± 10.59 in women, P < .001) (Fig 3a). Conversely, the rSFA was significantly higher in women compared with men (59.12% ± 11.65 in men vs 71.35% ± 10.59 in women, P < .001) (Fig 3b).

Sex-specific Differences in Overall Survival

The median overall survival was not significantly different between men and...
women ($P = .135$). However, given the known association between visceral fat and prognosis, we used a published algorithm (24) to identify a rVFA threshold that could maximally stratify overall survival in men and women. Unexpectedly, we identified that rVFA uniquely stratified women, but not men. Women with rVFA greater than 30.9% had a median overall survival of 40.4 months and those with rVFA less than 30.9% had a median overall survival that was greater than the observation period ($P < .001$). No rVFA could be identified that significantly stratified men with clear cell RCC, and therefore, the male median rVFA of 41.1% was selected to stratify this subpopulation ($P = .612$) (Fig 4).

**Sex-specific Risk Factors for Clear Cell RCC**

Premenopausal women typically accumulate more subcutaneous fat than men, suggesting that rVFA in women could increase as a function of age (6). Our data demonstrated that only women exhibited a significant positive correlation between rVFA and age (Fig E3a, E3b [online]). Using a multivariate Cox proportional hazard regression model accounting for age, stage, and tumor size, we identified that the interaction of sex and rVFA was statistically significant ($P = .028$) where women with a high rVFA had an increased risk of death when compared with women with low rVFA (hazard ratio, 3.66; 95% confidence interval: 1.64, 8.19). No increased risk of death was observed for high versus low rVFA in men (hazard ratio, 1.13; 95% confidence interval: 0.58, 2.18) (Table 2).

**Transcriptome Analysis of Clear Cell RCC Metabolism**

We investigated the potential interaction of rVFA and glycolytic gene expression in clear cell RCC tumors. Using a Z-score thresholding approach for the entire pathway, we identified that women exhibited significant stratification at 13 individual Z scores where men were significant at only four Z scores (Table E1 [online]). We discovered that a Z score of 1.0 maximized the survival differences in both men and women (female hazard ratio = 5.317, $P = .001$; male hazard ratio = 1.957, $P = .031$). For this reason, we chose this threshold to look at the effects of glycolytic expression on survival.

Next, we used this optimized Z-score threshold to identify which of the 39 glycolytic transcripts were driving the survival differences in men and women. The Z-score threshold of 1.0 identified a total of 13 genes ($SLC2A3$, HK3, PFKL, ALDOA, ALDOB, GAPDH, TPH1, PGK2, ENO2, LDHA16B, SLC16A1, SLC16A8, and SLC16A3) whose overexpression was associated...
with significantly decreased overall survival in women. The same threshold identified 10 genes in men. Eight of these genes (SLC2A1, HK1, HK3, PGK2, ENO2, ENO3, LDHAL6B, and SLC16A1) were associated with significantly decreased overall survival. Two genes (PFKP and PGAM4) were associated with significantly better overall survival (Fig E4a, Table E2 [online]).

The high-glycolysis group consisted of 106 women and 202 men. Both male and female high-glycolysis groups were associated with poor prognosis (Fig E4b [online]). Because of the poor outcomes identified in women with rVFA and glycolysis, we anticipated a positive correlation between the two in women. Unexpectedly, there were no significant differences in rVFA between high- and low-glycolysis groups in either men or women (Fig E5 [online]).

This suggested to us that rVFA and tumor glycolysis may operate independently. We plotted four permutations of high and low rVFA and high and low glycolysis groups in men and women. We discovered that this approach further stratified women, where a cohort of 19 patients with low rVFA and low glycolysis showed exceptional prognosis with zero deaths (P < .001) (Fig 5a). Interestingly, either high rVFA or high glycolysis resulted in equivalent median overall survival for women, and women with high rVFA and glycolysis performed somewhat worse (Fig 5a). Although men with low rVFA and low glycolysis did perform better than the rest of the groups (P = .026) (Fig 5b), these differences were driven more by the differences in glycolysis and were not as robust as in women.

**RCC Mutation Enrichment**

The presence of sex-specific mutation enrichment in RCC (16) prompted us to investigate the enrichment of RCC mutations in glycolysis and rVFA groups. We discovered that the SETD2 and BAP1 mutations were significantly enriched in female high-glycolysis samples (Fig E6, Table E3 [online]). When enrichment of mutations in the tumor as a function of the rVFA was analyzed, no significant differences were identified. However, the TP53 mutation approached significance in high-rVFA female patients (P = .063) as did BAP1 in high-rVFA male patients (P = .059) (Table E4 [online]).

**Discussion**

In this study, we propose a new sex-based stratification system that integrates the sex of the patient, visceral fat quantity, and tumor metabolism. The unique ability of our imaging strategy to risk-stratify women with clear cell RCC using a combination of visceral fat and tumor metabolism suggests a synergy between host and tumor metabolism that directly plays a role in outcomes independent of conventional assessments of prognosis (eg, grade and stage). It should be noted that sex differences in cancer metabolism are not just restricted to clear cell RCC, as a recent study identified sex differences in glioma glycolysis, and that sex differences may be identified with clinical imaging (25,26). This suggests that sex differences may not be isolated to a specific histology or tumor such as clear cell RCC and that imaging workflows that integrate patient sex and metabolism could be used to stratify patients without the need for histologic validation prior to decision making. We anticipate that these findings may encourage more investigation on the underlying mechanisms behind sex-specific differences in host and tumor metabolism that will not only be important in assessing prognosis at initial diagnosis, but potentially useful for following response to therapy. Moreover, the possibility that metabolism can stratify patients with clear cell RCC suggests the potential for dietary interventions and exercise to modulate metabolism, and thereby improve prognosis.

Our approach to visceral fat differs from others. A previous study demonstrated that greater absolute VFA and SFA are associated with a favorable overall survival in patients with clear cell RCC with the theory that absolute visceral adiposity may be a marker for nutritional status (11). However, absolute visceral fat quantity does not control for increased concomitant subcutaneous fat quantity that can be seen in more obese individuals, necessitating the need for normalized rVFA as a more robust biomarker for sex-specific outcomes. rVFA in another study showed no influence on survival outcomes in patients with clear cell RCC; however, the analysis combined men and women (13). Our study would arrive at the same conclusion had we not considered its interaction with sex.

To understand other sex-specific factors that may affect body fat distribution, we found that female age at diagnosis was an important factor. rVFA in men was unchanged with age, whereas it increased in older women.

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**Table 2**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hazard Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>3.66 (2.16, 6.23)</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Tumor size</td>
<td>0.88 (0.62, 1.24)</td>
<td>.456</td>
</tr>
<tr>
<td>Age</td>
<td>1.03 (1.01, 1.05)</td>
<td>.013*</td>
</tr>
<tr>
<td>Sex</td>
<td>1.19</td>
<td>.065</td>
</tr>
<tr>
<td>rVFA</td>
<td>1.21</td>
<td>.065</td>
</tr>
<tr>
<td>rVFA according to sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (high rVFA vs low rVFA)</td>
<td>3.66 (1.64, 8.19)</td>
<td>.028*</td>
</tr>
<tr>
<td>Male (high rVFA vs low rVFA)</td>
<td>1.13 (0.58, 2.18)</td>
<td>.210</td>
</tr>
</tbody>
</table>

Note.—Data in parentheses are the 95% CI. rVFA = relative visceral fat area.

* Significant (P < .05).
Although controversial in the context of RCC, there is renewed interest in the application of fluorodeoxyglucose PET as a stratification tool for RCC (17). Rapidly proliferating tumor cells require increased nutrient consumption in the form of glucose, as well as amino acids or lipids (29,30). Thus, it is possible that sex differences in nutrient stores could result in sex differences in how tumors acquire energy.

As with other investigations, the limitations of our study include the nonrandomized retrospective design and small sample size, which may lead to selection bias. In addition, thresholds were defined and evaluated on the same patient population. Thus, these results need to be validated in not only additional patients with clear cell RCC, but potentially other cancer types to determine the extent of sex differences in visceral fat on patient outcomes. In addition, we used gene expression of the glycolytic pathway as a surrogate marker for glucose metabolism; therefore, biochemical studies and fluorodeoxyglucose PET imaging are needed for further analysis. Lastly, as this study focused primarily on white patients, it will be difficult to determine the magnitude and direction of bias were we to extrapolate results to a non–white patient population with a lower incidence of obesity. Nevertheless, our study builds on a growing body of evidence demonstrating metabolic differences between men and women and its potential impact on cancer survival (25). Additional prospective analyses that investigate sex-specific differences in visceral fat, cancer metabolism, and its relation to overall survival in cancers including clear cell RCC are urgently needed.

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References


