

1 **Thick perirenal fat predicts the growth pattern of renal cell carcinoma**

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14 **Running Title:**

15 Perirenal fat distribution in renal cell carcinoma growth

16 **Key words: renal cell carcinoma; visceral fat; perirenal fat; outer location; inner**

17 **location**

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19

20 **Abstract**

21 Objective: To examine the relationship between the direction of renal cell carcinoma
22 growth and the visceral/perirenal fat volume.

23 Patients and Methods: We retrospectively reviewed computed tomography scans of 153
24 patients with stage 1 renal cell carcinoma who underwent radical or partial nephrectomy
25 in our hospital between January 2013 and July 2016. We calculated the
26 visceral/subcutaneous/perirenal fat volumes using SYNAPSE VINCENT®. Of the 60
27 patients, the perirenal fat was immunohistochemically stained for leptin, adiponectin,
28 COX-2 and UCP-1, and the association with outward tumor protrusion was evaluated.

29 Results: Of the 153 cases, 88 had confirmed outward expansion (57.5%), 110 were
30 classed as pT1a (52 and 58 with outer and inner expansion, respectively), 43 were classed
31 as pT1b (36 and 7 with outer and inner expansion, respectively; $P<0.0001$). Multivariate
32 logistic regression model showed a trend toward significance in pT1b (vs pT1a, [OR]
33 6.033, 95%CI=2.409-15.108, $P=0.0001$), perirenal fat percentage >1.0 (vs ≤ 1.0 , [OR]
34 2.596, 95%CI=1.205-5.591, $P=0.014$). as independent predictors for outer protrusion.

35 Immunohistochemical staining was positive for UCP-1 expression in 31 out of 41
36 outgrowth types (75.6%), and all 19 endogenous types (100%; $P=0.003$).

37 Conclusions: Renal cell carcinoma with thick perirenal fat correlates with an increased

38 likelihood of developing outward tumor protrusion; therefore, fat distribution may affect
39 the development of renal cell carcinoma.

40 Key words: renal cell carcinoma; visceral fat; perirenal fat; outer location; inner location

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44 **Introduction**

45 Renal cell carcinoma (RCC) consists of a heterogeneous group of cancers that are
46 derived from the nephron. Various histological and molecular RCC subtypes exist. The
47 international TNM staging system is used to classify RCC because it reflects patient
48 outcomes. The TNM staging system classifies RCC according to the size of the tumor
49 and the grade of extension. In recent years, partial nephrectomy has been the standard
50 surgical treatment for T1a tumors (<4 cm) and select T1b tumors (4–7 cm). Among these
51 tumor classes, the tumor location can be divided into inner or outer. The RCC location is
52 very important to surgeons because intraparenchymal tumors are relatively hard to
53 partially resect and have high perioperative complications [1]. Furthermore, slow-
54 growing RCCs tend to be outwardly located in comparison with rapidly growing types
55 [2]. Therefore, the tumor location may predict surgical complications and malignant

56 potential. However, to date, little attention has been paid to the mechanism of the pattern
57 of RCC growth.

58 There is lots of epidemiological evidence to suggest that long-standing obesity is one
59 of the primary causes of cancer; including cancers of the breast, colon, esophagus,
60 pancreas and kidney [3]. In the human body there are two types of adipose tissue: white
61 adipose tissue (WAT) and brown adipose tissue (BAT) [4]. WAT is important for energy
62 storage and releases hormones and cytokines that regulate metabolism and insulin
63 sensitivity [5-7]. In contrast, BAT is important for thermogenesis. BAT expresses
64 uncoupling protein 1 (UCP-1), which is rich in mitochondria and uncouples
65 mitochondrial respiration from ATP synthesis to facilitate heat production [5, 8]. BAT is
66 mainly located in thyroid, mediastinal, supraclavicular and perirenal tissues [8]. The
67 kidney is surrounding by perirenal fat; however, no studies have been done to investigate
68 the relationship between RCC and BAT. Perirenal fat is located in the retroperitoneum
69 and is a type of visceral fat [9]. Adipose tissue is generally considered as the storage site
70 of excess energy, although recently it has been revealed that adipocytes have endocrine
71 activity and producing hormones, inflammatory cytokines and adipocytokines [10].

72 A previous study found that prostate cancer (PCa) patients that had higher
73 periprostatic fat had more aggressive PCa [11]. Moreover, patients with metabolic

74 syndrome, including obesity, have poorer outcomes after radical prostatectomy [12], and
75 obesity is a risk factor for aggressive PCa [13]. For breast cancer patients, obesity is
76 associated with advanced disease at diagnosis and with a poor prognosis [14]. Also in
77 RCC patients, thickness and stranding of perirenal fat affect progression free survival of
78 clinically localized kidney cancer [15]. These results suggest that adipose tissue may also
79 affect RCC growth. In this study, we examined the association between adipose tissue,
80 especially around kidney, and the growth pattern of RCC in patients in our hospital who
81 underwent radical or partial nephrectomy.

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83

84 **Material and Methods**

85

86 Study population

87 After receiving approval of institutional review board, we retrospectively included 153
88 patients who were diagnosed with cT1 RCC and who underwent partial or radical
89 nephrectomy. All of the patients were treated at Kyushu University from January 2013
90 and July 2016. Patients who had hemodialysis were excluded.

91

92 Computed tomography measurement of fat volume

93 Computed tomography (CT) studies were performed using a 4-slice multidetector CT

94 scanner (Aquilion; Toshiba Medical Systems, Tokyo, Japan). Adipose tissue was

95 identified as the pixels ranging from -250 to -50 Hounsfield units. All imaging data were

96 transferred to a computer workstation for analysis of the total abdominal fat volume. The

97 visceral fat (VF) volume, subcutaneous fat (SF) volume, perirenal fat (PF) volume and

98 abdominal volume were calculated using SYNAPSE VINCENT® software (Fuji Film,

99 Tokyo, Japan). To calculate the visceral fat/subcutaneous fat ratio (V/S ratio), the VF

100 volume was divided by the SF volume. The PF volume was measured by marking the

101 area of adiposity on each CT image (Fig. 1A, B). To calculate the PF percentage, the PF

102 volume was divided by the abdominal volume. To minimize inter-observer variation,

103 adipose tissue assessments were carried out by the same examiner. When the tumor was

104 50% or more exophytic it was classified as an 'outer location' and if it was less than 50%

105 exophytic it was classified as an 'inner location'.

106

107 Immunohistochemistry

108 Immunohistochemistry was performed on 5- μ m thick adipose tissue sections taken

109 from partial or radical nephrectomy specimens which are attached with tumor. Primary

110 antibodies to leptin (dilution 1:100; SC-842, SANTA CRUZ, Dallas, TX, USA),
111 adiponectin (dilution 1:100; ab22554, Abcam, Cambridge, MA, USA), UCP-1 (dilution
112 1:500; U6382, Sigma, St Louis, MO, USA), or COX-2 (dilution 1:100; 160112, Cayman,
113 Ann Arbor, MI, USA) were applied, followed by a broad-spectrum secondary antibody
114 (Invitrogen, Carlsbad, CA, USA), as described previously [16].

115 For scoring leptin, adiponectin, UCP-1 and COX-2 expression, stained cells were
116 divided into three categories as follows: 0, negative; 1, positive; and 2, strong positive.
117 All stains were visually quantified by a single pathologist (T. A.) blinded to the sample
118 identity.

119 **Statistical analysis**
120 All statistical analyses were performed using JMP13 software (SAS Institute, Cary, NC,
121 USA). Univariate and multivariate analyses were performed using the logistic regression
122 model. Correlations between parameters were examined by χ^2 test. *P* values <0.05 were
123 considered as significant.

124

125 **Results**

126 **Clinical characteristics**
127 The clinical features of the patients are shown in Table 1. The median age at diagnosis

128 was 62 (34–83) years, and of the 153 patients, 112 (73.2%) were men and 41 (26.8%)
129 were women. Seventy-three patients (47.7%) had right side RCC and 80 (52.3%) had left
130 side RCC; 88 (57.5%) were outer and 65 (42.5%) were inner expansion; 132 (86.3%) had
131 clear cell RCC, 11 (7.2%) had papillary RCC and 9 (5.9%) had chromophobe RCC; 110
132 (71.9%) of the patients presented with pT1a tumors and 43 (28.1%) with pT1b; 23
133 (15.0%) of the patients had Fuhrman nuclear grade 1, 105 (68.6%) had grade 2, 24
134 (15.7%) had grade 3 and 1 (0.7%) had grade 4. The median body mass index (BMI) was
135 23.5 (16.0–36.5). The median V/S ratio was 1.00 (0.11–2.73) and the median perirenal
136 fat percentage was 1.01 (0.03–3.59).

137

138 Univariate and multivariate analyses between characteristics of patients and expansion
139 pattern

140 Table S1 summarizes the expansion pattern and characteristics of the patients. To
141 determine what are the important factors to grow outward, we performed univariate and
142 multivariate analyses. In the univariate analysis, gender (odds ratio [OR] 2.129, 95%
143 confidence interval (CI) 1.030-4.400, $P=0.041$), pT stage ([OR] 5.736, 95% CI 2.351-
144 13.995, $P=0.0001$) and PF percentage divided by 1.0 ([OR] 2.713, 95% CI 1.399-5.258,
145 $P=0.003$) were associated with expansion pattern (Table 2). In the multivariate analyses

146 showed that the pT stage ([OR] 6.033, 95% CI 2.409-15.108, $P=0.0001$) and PF

147 percentage ([OR] 2.596, 95% CI 1.205-5.591, $P=0.014$) are independent factors.

148

149 Leptin, adiponectin, COX-2 and UCP-1 expression in the perirenal fat of RCC patients

150 We immunohistochemically stained for leptin, adiponectin, COX-2 and UCP-1 in

151 samples from the 60 patients. Positive signals representing these proteins were

152 predominantly detected in adipocytes (Fig. 2), and their expression patterns are

153 summarized in Table 3. Leptin was detected in 3 (7.4%) of the 41 outer expansion samples

154 (3 [7.4%] 1+) and 2 (10.5%) of the 19 inner expansion samples (2 [10.5] 1+; $P=0.681$, 0

155 vs 1+/2+). Adiponectin was positive in all 41 (100%) of the outer expansion samples (3

156 [7.3%] 1+, 38 [92.7%] 2+) and all 19 (100%) of the inner expansion samples (1 [5.2%]

157 1+, 18 [94.8%] 2+; $P=0.762$, 0/1+ vs 2+). COX-2 was also positive in all 41 (100%) of

158 the outer expansion samples (41 [100%] 2+) and all 19 (100%) of the inner expansion

159 samples (1 [5.2%] 1+, 18 [94.8%] 2+; $P=0.126$, 0/1+ vs 2+). UCP-1 was positive in 31

160 (75.6%) of the 41 outer expansion samples (31 [75.6%] 1+) and all 19 (100%) of the inner

161 expansion samples (14 [73.7%] 1+, 5 [26.3%] 2+; $P=0.003$, 0 vs 1+/2+).

162

163 **Discussion**

164 The aim of this study was to determine whether body fat affect the pattern of RCC
165 expansion. Our starting hypothesis was that visceral/perirenal fat affects the growth
166 direction of RCC. Our results support this hypothesis and suggest that perirenal fat plays
167 an important role in the RCC growth pattern.

168 Around 80% of all body fat is located subcutaneously and 20% is located in visceral
169 areas [17]. The area of visceral fat increases with age, and accumulation of visceral fat
170 increases the risk of not only metabolic diseases [18], but also PCa in men [19]. The V/S
171 ratio is useful for classifying obesity into subgroups: generally, a V/S ratio of 0.4 or above
172 is considered as visceral obesity, and a V/S ratio below 0.4 is considered as subcutaneous
173 obesity [20, 21].

174 Table 2 showed that pT stage and PF percentage were also independent factors for
175 tumor location. When the tumor becomes larger, it is difficult to stay in the kidney
176 therefore it is reasonable that pT stage is one of the risk factor. Even in pT1a tumor, less
177 than 4cm, PF percentage was correlated with growth pattern (Table S2). Together, these
178 results suggest that perirenal fat can affect the growth direction of renal tumors even if
179 pT stage is the important factor.

180 In this study, a 50% or more exophytic mass was defined as ‘outer expansion’ because
181 a high level of outgrowth is often easy to resect. Our classification also corresponds with

182 the R.E.N.A.L nephrectomy score, a classification system based on RCC anatomy [22].

183 Anatomically, kidney cancer can grow into the renal parenchyma or into the

184 perirenal fat. The pressure inside the perirenal fat may be lower than that inside the renal

185 parenchyma; therefore, it may be easier for tumors to grow outside the kidney. This easier

186 growth may also influence the RCC shape and give rise to asymmetrical and/or non-cubic

187 tumors. However, most masses are symmetrical and cubic [22], and it has been reported

188 that RCCs with regular shapes localize to the outside of the kidney and grow more slowly

189 [2]. These studies suggest that the pressure around the RCC may not have a large

190 influence on the direction of growth. We found that perirenal fat may stimulate the RCC

191 to grow into the perirenal fat. Adipose tissue is recognized as the endocrine organ and

192 secretes not only adipocytokines but also biologically effective molecules such as vascular

193 endothelial growth factor, interleukin 6 (IL 6), and TNF α [23]. IL6 and TNF α can induce

194 inflammation and tumorigenesis [24, 25]. However, perirenal adipose tissue secretes

195 certainly these molecules are not definite in this study. Therefore, how the perirenal fat

196 induces the progression and/or carcinogenesis of RCC is unclear. Further studies are

197 required to explore the precise molecular mechanisms involved.

198 Correlations between adipocytokines, such as leptin and adiponectin, and the clinical

199 characteristics of RCC have been studied. The serum leptin concentration is associated

200 with RCC progression and invasion [26]. In contrast, serum adiponectin is inversely
201 associated with the incidence of RCC [27], and reduced serum adiponectin levels are
202 correlated with increased tumor size and metastasis [28]. The serum leptin concentration
203 is directly associated with BMI, while the adiponectin level is inversely associated with
204 BMI [10]. Leptin interacts with its receptor (ObR) and activates many signals, including
205 VEGF via hypoxia-inducible factor-1 α (HIF-1 α) and NF- κ B [29], and the janus
206 kinase/signal transducer and activator of transcription 3 (JAK/STAT3) [30]. In contrast,
207 adiponectin deficiency suppresses AMPK activation and, as a result, increases
208 angiogenesis in RCC cell lines [31]. These results suggest that adipocytokines play
209 important roles in RCC and may be useful therapeutic targets.

210 We immunohistochemically stained leptin, adiponectin, COX-2 and UCP-1 in
211 perirenal fat. The expression patterns of leptin and adiponectin did not correlate with the
212 growth pattern (Table 3), in contrast from the results of previous studies of serum levels.
213 In most of our cases, leptin was negative (55 of 60 cases) and adiponectin was positive
214 (60 of 60 cases), and there were few cases in which expression of both adipocytokines
215 were detected. More sensitive modalities will be required in future to quantify these
216 adipocytokines. We also examined COX-2 because obesity can cause adipose tissue
217 overgrowth and inflammation associated with tumorigenesis [32]. However, COX-2

218 expression also did not correlate with the growth pattern in our study. UCP-1 is a marker
219 of BAT mitochondria [8]. We found that UCP-1 expression was associated with inner-
220 type growth, suggesting that BAT prevents outwards RCC growth. TNF α , which are also
221 secreted by adipose tissue, inhibits UCP1 expression via extracellular-regulated kinases
222 (ERKs) [33], and inflammation also inhibited UCP1 in mice [34]. These results suggest
223 that low expression of UCP1 may reflect the inflammation around the kidney.
224 Furthermore, PTEN, tumor suppressor gene, affect metabolism and regulate *UCP1*
225 transcription [35], therefore UCP1 also reflect the expression of PTEN and as a result, it
226 may suppress RCC protrusion. Further studies are needed to clarify how UCP1 may
227 influence RCC expansion.

228 We propose that adipose tissue stimulates renal cancer in two ways: (i)
229 adipocytokines that are generated in visceral and/or perirenal fat reach the kidney through
230 the blood stream; or (ii), adipocytokines directly activate RCC through paracrine
231 mechanisms. In this study, we showed that RCCs in patients with thick perirenal fat tend
232 to grow outwardly, such that the RCCs protrude into areas that are rich in adipocytokines
233 and/or inflammatory cytokines. This suggests that paracrine mechanisms are more
234 important for RCC growth direction.

235 Although thick perirenal fat appears promising, the sample size is relatively small, and

236 this study gave us further hypothesis. We need to investigate the precise mechanisms of

237 adipocyte related signals and also the correlation with cancer progression.

238 In Conclusion, an increased perirenal fat percentage predicts the RCC growth pattern.

239 Adipose tissue, especially BAT may play an important role in RCC extension. Further

240 studies are needed to investigate the mechanisms involved.

241

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246

247 **Author contributions**

248 EK led the design, analysis, interpretation of data and writing the manuscript. FK and KI

249 performed immunohistochemistry and TA evaluated them. MS, KM, AT, JI, KT and ME

250 contributed to the interpretation of data and reviewing the manuscript.

251

252 **Conflict of Interest**

253 Each author declares no conflict of interest.

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339

340

341 **Figure legends**

342 **Figure 1**

343 Measurement of the fat area using 5-mm CT slices and SYNAPSE VINCENT® software.
344 (A) Perirenal adiposity measurement (left side, RCC is outer type). (B) Perirenal adiposity
345 measurement (right side, RCC is inner type).

346

347 **Figure 2**

348 Immunohistochemistry of adiponectin (A), COX-2 (B), leptin (C) and UCP-1 (D) in
349 perirenal fat (original magnification: 200 \times).

350

351 Table 1

352 Patient characteristics.

353

354 Table 2

355 Univariate and multivariate analyses between characteristics of patients and expansion

356 pattern.

357

358 Table 3

359 Expression of Leptin, Adiponectin, COX-2 and UCP-1 in perirenal fat.

360

361 Table S1

362 Correlations between expansion pattern and characteristics of patients.

363

364 Table S2

365 Correlations between expansion pattern and characteristics of pT1a patients.

Table 1. Patient characteristics (n=153).

variable	
Median age, Years (range)	62 (34-83)
Gender, n (%)	
male	112 (73.2%)
female	41 (26.8%)
Laterality, n (%)	
right	73 (47.7%)
left	80 (52.3%)
Expansion pattern, n(%)	
outer	88 (57.5%)
inner	65 (42.5%)
Histology, n (%)	
clear cell	132 (86.3%)
papillary	11 (7.2%)
chromophobe	9 (5.9%)
collecting duct	1 (0.6%)
pT stage, n (%)	
pT1a	110 (71.9%)
pT1b	43 (28.1%)
Fuhrman nuclear grade, n (%)	
1	23 (15.0%)
2	105 (68.6%)
3	24 (15.7%)
4	1(0.7%)
Median BMI, kg/m ² (range)	23.5 (16.0-36.5)
Median visceral fat, % (range)	33.0 (5.1-59.9)
Median subcutaneous fat, % (range)	15.7 (2.0-37.9)
Median body fat, % (range)	32.8 (5.0-54.8)
Median V/S ratio, ratio (range)	1.00 (0.11-2.73)
Median Perirenal fat, % (range)	1.01 (0.03-3.59)
Hypertension, n (%)	
Negative	76 (49.7%)
Positive	77 (50.3%)
Diabetes mellitus, n (%)	
Negative	137 (89.5%)
Positive	16 (10.5%)
Hyperlipidemia, n (%)	
Negative	121 (79.1%)
Positive	32 (20.9%)

V/S ratio: Visceral fat/ subcutaneous fat ratio

Body fat percentage: (Visceral fat + Subcutaneus fat)/abdominal volume

Perirenal fat percentage: perirenal fat/ abdominal volume

Table 2. Univariate and multivariate analyses between characteristics of patients and expansion pattern.

Variable	Univariate analysis			multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Gender (Male vs. Female)	2.129	1.030-4.400	0.041	1.420	0.601-3.357	0.423
Laterality (Right vs. Left)	0.899	0.473-1.709	0.746			
Historogy (clear vs. non-clear)	0.809	0.314-2.084	0.661			
pTstage (pT1b vs. pT1a)	5.736	2.351-13.995	0.0001	6.033	2.409-15.108	0.0001
BMI (>23.5 vs. ≤23.5)	1.146	0.601-2.184	0.677			
Body fat percentage (>32.8 vs. ≤32.8)	1.400	0.736-2.675	0.304			
Subcutaneous fat percentage (>16 vs. ≤16)	0.819	0.430-1.556	0.542			
visceral fat percentage (>33 vs. ≤33)	1.400	0.735-2.663	0.305			
V/S ratio (>1.0 vs. ≤1.0)	1.738	0.910-3.321	0.093			
PF percentage (>1.0 vs. ≤1.0)	2.713	1.399-5.258	0.003	2.596	1.205-5.591	0.014
Hypertension (positive vs. negative)	0.747	0.703-2.543	0.375			
Diabetes mellitus (positive vs. negative)	0.402	0.138-1.171	0.094			
Hyperlipidemia (positive vs. negative)	0.493	0.224-1.086	0.079			

clear: clear cell RCC, non-clear: papillary RCC and chromophobe RCC

pT stage: pathological T stage, T1a: <4 cm, T1b: 4–7 cm

V/S ratio: Visceral fat/ Subcutaneus fat ratio

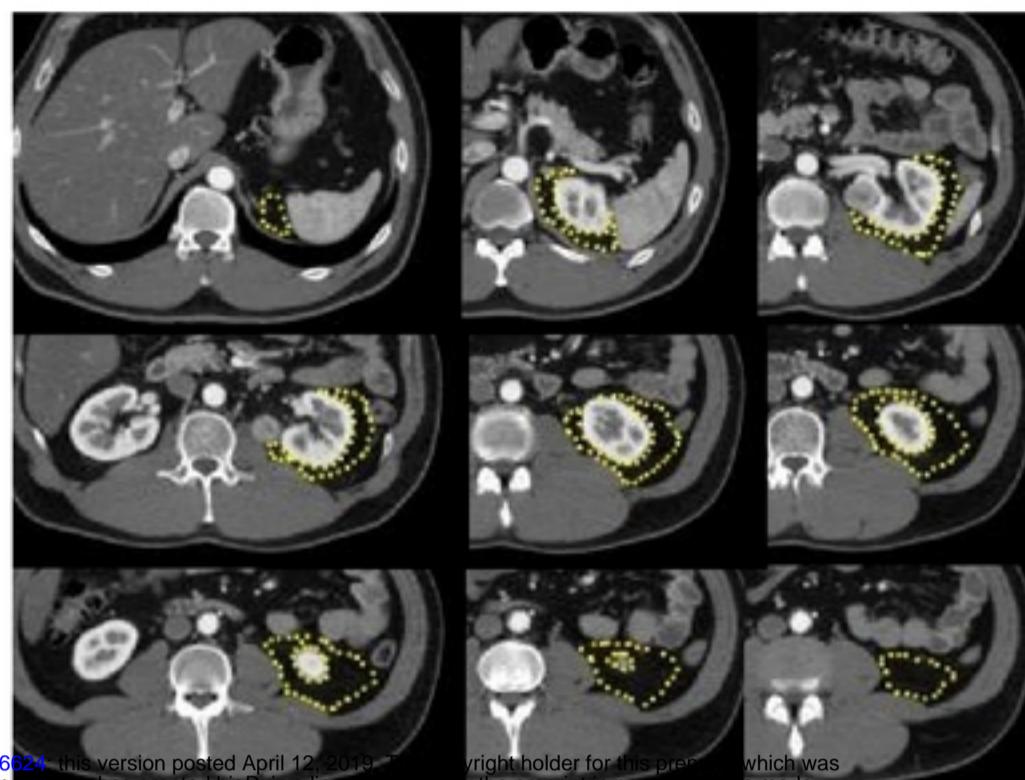
PF percentage: Perirenal fat/ abdominal fat volume percentage

Table 3. Expression of Leptin, Adiponectin, COX-2 and UCP-1 in perirenal fat.

n	Expression levels			<i>P</i> value	
	Negative		Positive	0 vs 1+/2+	0/1+ vs 2+
	0	1+	2+		
Leptin					
outer expansion	41	38 (92.6%)	3 (7.4%)	0 (0%)	0.681
inner expansion	19	17 (89.5%)	2 (10.5%)	0 (0%)	NA
Adiponectin					
outer expansion	41	0 (0%)	3 (7.3%)	38 (92.7%)	NA
inner expansion	19	0 (0%)	1 (5.2%)	18 (94.8%)	0.762
COX-2					
outer expansion	41	0 (0%)	0 (0%)	41 (100%)	NA
inner expansion	19	0 (0%)	1 (5.2%)	18 (94.8%)	0.126
UCP-1					
outer expansion	41	10 (24.4%)	31 (75.6%)	0 (0%)	0.003
inner expansion	19	0 (0%)	14 (73.7%)	5 (26.3%)	0.287

Figure 1 Kashiwagi et al.

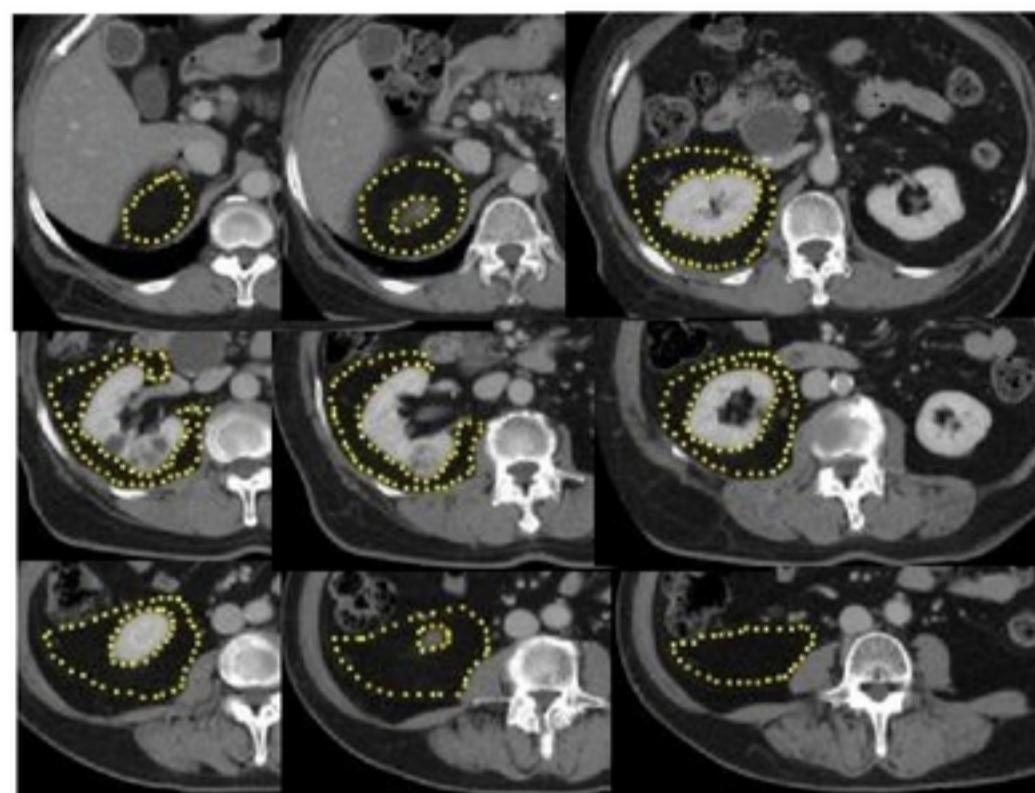
A



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perirenal adiposity measurement (left, outer type)

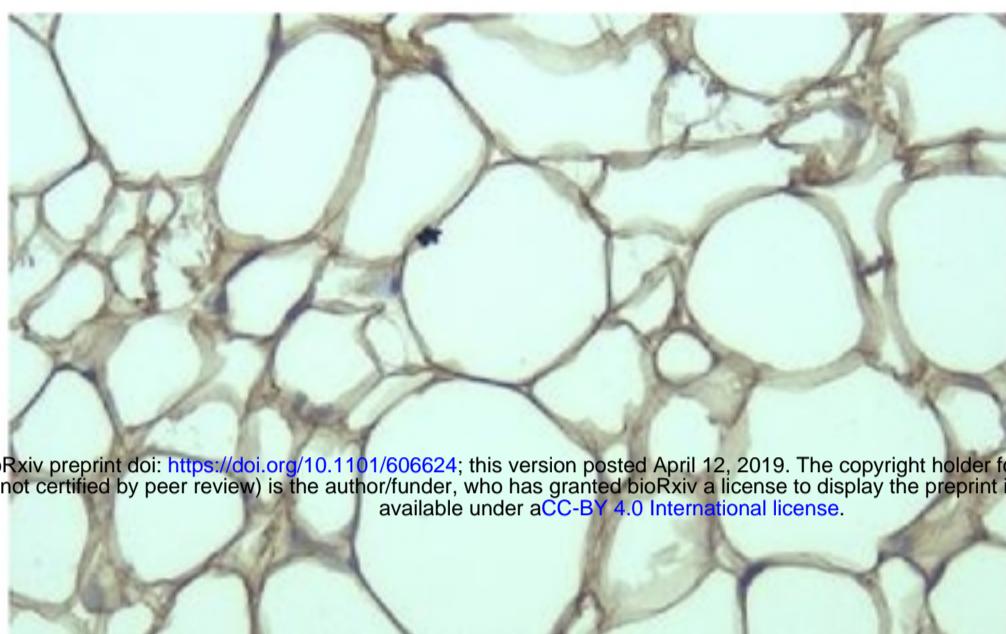
B



perirenal adiposity measurement (right, inner type)

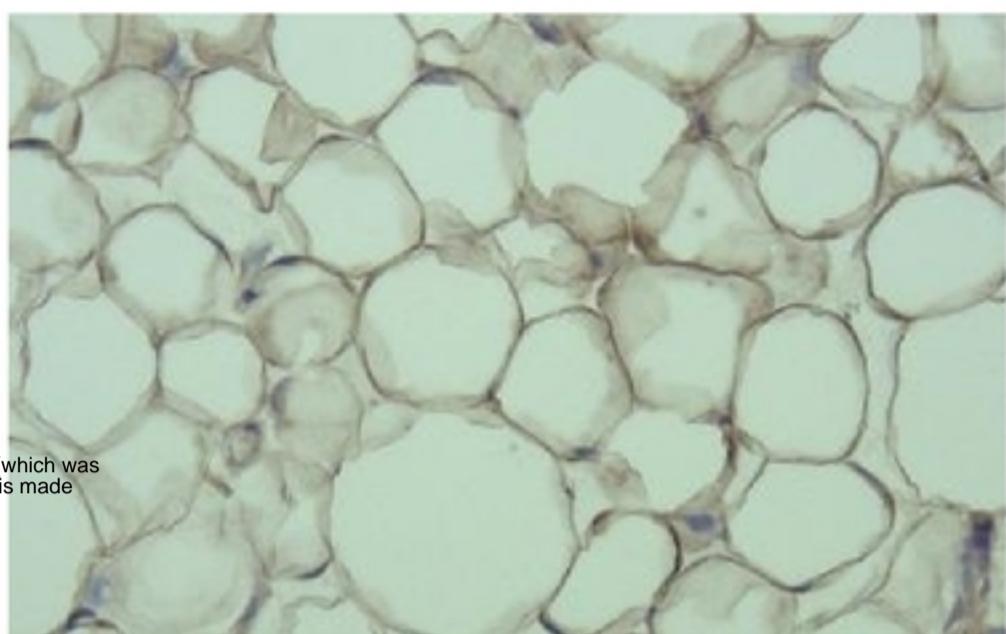
Figure 2 Kashiwagi et al.

A



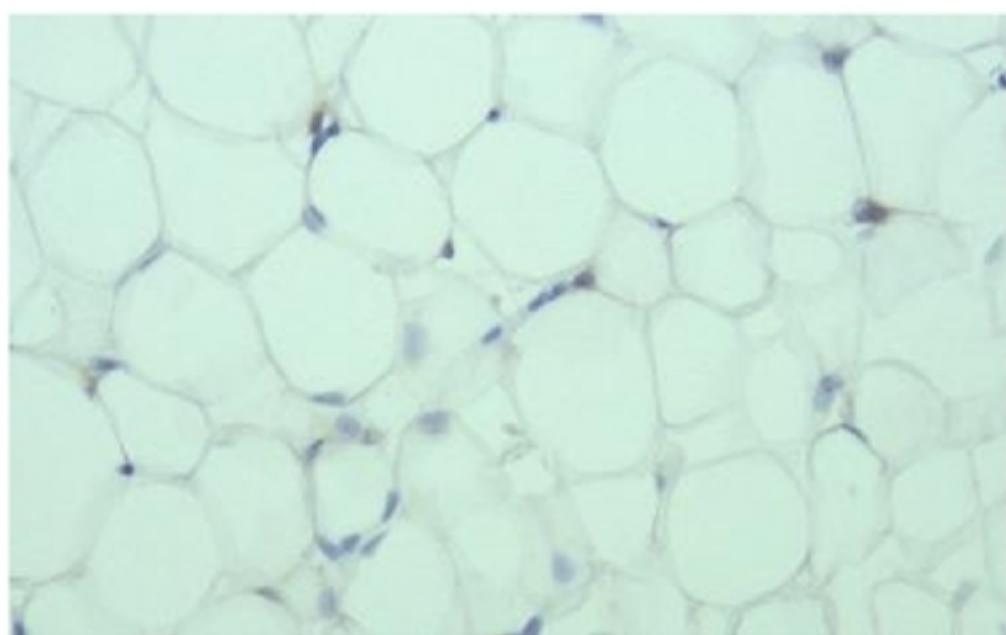
adiponectin

B



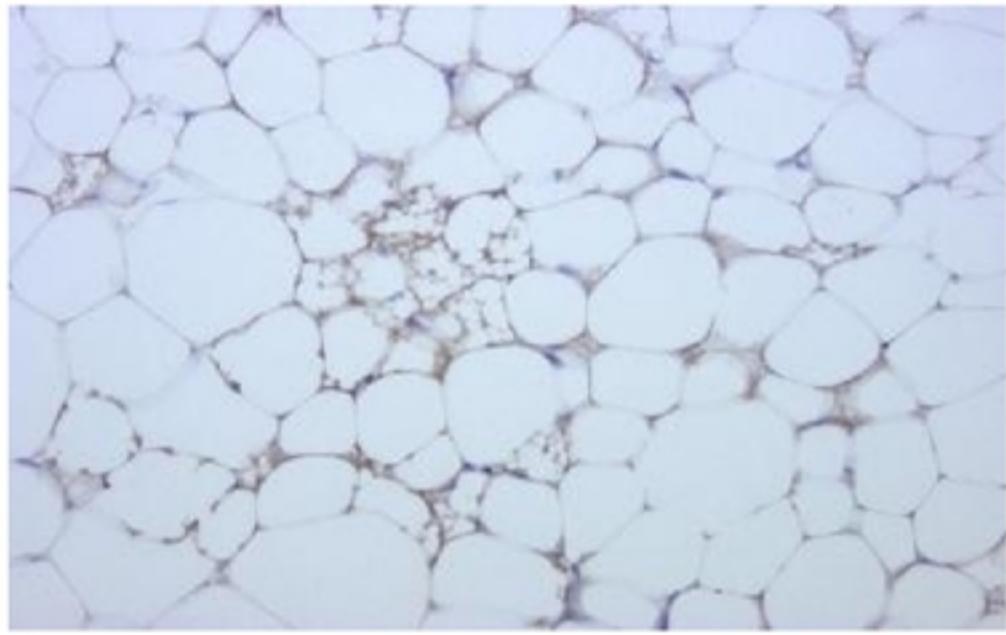
COX-2

C



leptin

D



UCP-1

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