

1 **Title:** Serum biochemistry suggests grey squirrels (*Sciurus carolinensis*) have poorer
2 physiological condition in urban settings
3

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22 **Abstract**

23 Human food waste in cities presents urban wildlife with predictable, easily accessible high-
24 calorie food sources, but this can be both beneficial and harmful for individual health. We
25 analyzed body condition and serum chemistry (electrolyte levels, markers of kidney and liver
26 function, protein, glucose, and cholesterol) in an urban and rural population of eastern grey
27 squirrels (*Sciurus carolinensis*) to assess whether proximity to the human food waste that is
28 associated with urban habitats had ill effects on health. We found no differences in body
29 condition between habitats and no evidence of malnutrition at either site. However, urban
30 squirrels had higher blood glucose, lower potassium, phosphorus, chloride, and albumin:globulin
31 ratios. These results align with previous findings of increased dietary sugar in cities, and suggest
32 that urban populations of grey squirrels are under greater environmental stress than rural
33 populations.

34

35 **Keywords:** grey squirrel, metabolic disorder, nutrition, serum chemistry, urbanization

36 **Introduction**

37 The distribution of resources available to animals is fundamentally altered in cities. Urban
38 vegetation and tree cover, important natural food sources for wildlife, are reduced and patchy.
39 Exotic species in urban plant communities may differ in phenology from native species, leading
40 to changes in diet and foraging behavior among urban fauna. Human food waste is a plentiful
41 new resource in cities which often allows animals to reach high population densities. Dietary
42 shifts and higher densities in stressful urban environments could cause animals to be in poor
43 physiological condition (1, 2). To explore how changing habitat quality associated with
44 urbanization affects animal physiology, we looked for signs of ill health in a successful urban
45 mammal, the eastern grey squirrel (*Sciurus carolinensis*).

46

47 In general, urban wildlife populations will lose access to natural food sources while gaining
48 access to new foods associated with human trash and supplemental feeding. Human foods found
49 as litter tend to be easily accessible, calorie-rich (3), and have lower nutritional value than
50 natural food sources (4). Human food subsidies are a double-edged sword: increased calorie
51 intake may positively influence body condition, survival, and reproductive success, however at
52 the same time its limited nutrient quality may make it detrimental to animals' overall health (5).
53 Alterations in food availability, predictability, and quality has the potential to induce major shifts
54 in nutritional status among urban wildlife (3).

55

56 Successful urban colonizers tend to be generalist, opportunistic omnivores that can take
57 advantage of human food waste, sometimes to the point of complete dependency (6). In such
58 species, urbanization leads to rapid changes in diet composition (2, 3, 6). Diets high in

59 carbohydrates and saturated fats are linked to increasing trends in obesity in humans (7), as well
60 as to elevated risk of cardiovascular disease, Type 2 diabetes and other metabolic disorders (8,
61 9). Similar metabolic conditions could also occur in non-human wild species (10–12). For
62 instance, urban crows have been shown to have elevated plasma cholesterol (13), and brown
63 bears with access to human food subsidies have higher carbohydrate and lower protein levels
64 than those in more natural habitats (14). Greater body mass in urban-dwelling individuals has
65 been reported in rats (15), foxes (16, 17), baboons (18), deer (19), and raccoons (20). Whether
66 these symptoms indicate poor health is uncertain. Urban raccoons were found to be heavier and
67 hyperglycemic, but leptin levels (which are higher in obese humans) were normal (20). Urban
68 baboons with access to human garbage exhibited symptoms consistent with metabolic disorder in
69 humans such as high body mass index, hyperglycemia, high leptin, and insulin resistance (18).
70 For the most part however, we do not know if the risk of developing metabolic disorders should
71 be a general concern among urban mammals (2).

72
73 We compared results from a suite of standard biochemical assays of blood samples collected
74 from urban and rural eastern grey squirrels. Serum biochemistry analysis is an effective way to
75 measure population-level health, as it is a good indicator of disease state, nutritional status, and
76 habitat quality (21). If we assume that rural individuals living in natural environments generally
77 have normal, approximately healthy blood biochemistries, then we can treat those results as a
78 baseline from which deviations in urban populations could point to health issues. Grey squirrels
79 are a successful urban species, abundant in cities both within and outside their natural range (22).
80 They are omnivorous with a wide dietary breadth, typically consisting of the nuts, flowers, and
81 buds of a variety of hardwood trees (such as oaks, hickory, and beech), fungi, insects, cultivated

82 crops, and from time to time, small animals and bones (23). Grey squirrel abundance is
83 positively associated with human food provisioning (24, 25). More anecdotally, grey squirrels
84 are frequently observed feeding on high calorie human food waste (26–29) and on birdseed at
85 feeders which is known to be less healthy than natural foods for birds (4). We measured body
86 condition, and used serum chemistry analyses to assess electrolyte levels, markers of kidney and
87 liver function, protein, glucose, and cholesterol, to get a general picture of population health in
88 urban and rural squirrels. We hypothesized that, due to differing food sources, urban squirrels
89 would show signs of ill health related to a poor-quality urban diet. Similar to previous studies of
90 urban mammals, we specifically expected to find higher glucose, cholesterol, and body condition
91 in urban squirrels.

92

93 **Methods**

94 *Sampling.* Grey squirrels were sampled from May – June 2019 in two sites with differing levels
95 of anthropogenic food. The ‘urban’ site is an approximately 10 ha park located on the University
96 of Manitoba campus in Winnipeg, Manitoba. Relative to natural forests it is sparsely treed, and is
97 adjacent to a river, a suburb, and bordered by major roads on two sides. This park experiences
98 high human foot traffic. Squirrels at this site have easy access to birdfeeders and human food
99 waste in trash cans, and litter. Our second site was a rural hardwood forest patch of
100 approximately 34 ha located in southern Manitoba (49°14'39"N, 98°00'47"W). The forest is
101 bordered by agricultural land and a road (at a distance of 47m), and has minimal anthropogenic
102 food sources.

103 Squirrels were trapped using Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, WI,
104 USA) baited with peanut butter, then restrained in a capture bag (30) without anesthetic. Sex,

105 approximate age (adult or juvenile), weight (grams), reproductive status (scrotal or non-scrotal
106 for males; lactating or non-lactating for females), and body length (centimeters) from the base of
107 the skull to the base of the tail were recorded for each individual. We implanted passive
108 integrated transponder (PIT) tags for later identification. In total, 20 individuals were trapped at
109 the urban location, and 10 in the rural location. We measured body condition by taking the
110 residuals from a linear regression of mass on body size (here, spine length). This method corrects
111 for the effects of an individual's structural size on its mass (31, 32). We computed body
112 condition independently for the sexes within each location. Juveniles were excluded from body
113 condition calculations (Table 1). Squirrels were released after capture. Our sampling protocol
114 was approved by the University of Manitoba animal care and use committee.

115 *Serum samples and tests.* Blood samples were collected from a subset of all individuals captured
116 at each site (Table 1). Samples were taken at the urban site between May 13-15, 2019, and the
117 rural site between June 7-14, 2019. Blood (≤ 1 mL) was drawn on-site from the femoral vein
118 using a syringe without anticoagulant and stored on ice until delivery to the Manitoba Veterinary
119 Diagnostics Services lab (department of Manitoba Agriculture Food and Rural Development,
120 Winnipeg, MB). Samples were processed within 12h of capture. Serum biochemistry profiles
121 were run for each sample, consisting of the following tests: sodium, potassium, chloride, urea,
122 creatinine, calcium, phosphorus, magnesium, amylase, lipase, alkaline phosphatase (ALKP),
123 gamma-glutamyl transferase (GGT), bilirubin, alanine aminotransferase (ALT), aspartate
124 aminotransferase (AST), creatine kinase, glucose, cholesterol, total protein, albumin, and
125 globulin. Some tests were omitted when sample volume was lacking; for this reason, we do not
126 report results for amylase, lipase, or creatine kinase. Sample sizes for each comparison are given
127 in Table 1.

128 *Statistical analysis.*— Boxplots were created in R version 3.6.1 (33). We first plotted and
129 visually inspected the data, then used principal components analysis (PCA) to visualize
130 groupings, if any. We then tested differences in serum variables between sites using non-
131 parametric Kruskal-Wallis tests due to small sample size and unbalanced groups. We found no
132 sex differences for serum measurements (Fig. 1, 2, 3), thus did not include sex as a factor in our
133 analyses.

134

135 **Results**

136 Mean body mass (urban: 678 ± 62.2 , rural: 653 ± 70.1 , mean \pm SD), and body condition (urban:
137 0.23 ± 47.8 , rural: 4.09 ± 43.4) for all adult individuals trapped between May-August were
138 similar between sites (Table 1). There were no detectable differences in body condition between
139 sexes (Table 1).

140 Urban squirrels had higher glucose levels than those from the rural site ($P < 0.01$, $X^2 = 8.70$; Fig.
141 1, Table 1), in line with our expectations. Cholesterol levels did not detectably differ by location
142 (Fig. 1, Table 1). Among serum ions, potassium ($P = 0.02$, $X^2 = 5.57$), phosphorus ($P < 0.01$, X^2
143 = 9.6), and chloride ($P = 0.01$, $X^2 = 5.95$) were lower in urban squirrels (Fig. 1, Table 1).

144 Sodium, calcium and magnesium levels were comparable at both sites (Fig. 1, Table 1).

145 ALT was lower at the urban site ($P = 0.05$, $X^2 = 3.73$; Fig. 2, Table 1). A majority of urban
146 samples were below the detection limit (3 U/L) (Fig. 2). We did not detect any differences in
147 other serum enzymes (ALKP, GGT, AST; Figure 2). Total bilirubin, creatinine, urea, total
148 protein levels, as well as albumin and globulin were similar at both sites (Figs. 2 and 3, Table 1).

149 The ratio of albumin to globulin (A:G ratio) was higher in rural squirrels ($P = 0.05$, $X^2 = 3.91$;
150 Fig. 3).

151 Seven principal components had eigenvalues greater than 1—these accounted for 91% of the
152 variation in response variables. PC1 (25.6% of total variation) captured the variation between
153 sites (Fig. 4, Table 2). Chloride, phosphorus, glucose and AST loaded most strongly on PC1
154 (Table 2).

155

156 **Discussion**

157 In general, serum parameters at both sites were within published ranges for grey squirrels (23,
158 34) and reference ranges for another member of *Sciuridae*, Prevost's squirrel (*Callosciurus*
159 *prevostii*) (35) (Figs. 1, 2, 3). We found no difference in body condition between sites, therefore
160 the chemistry values in the present study were not directly related to body condition as measured
161 here (size-corrected weight). Even if serum analytes are not correlated with body condition, we
162 can begin to understand some potential differences in habitat quality between our urban and rural
163 sites, and how this affects the nutritional status of these populations with these data.

164

165 We found elevated blood glucose in urban squirrels, supporting our expectation that individuals
166 with easy access to human food waste have higher sugar intake. This result was consistent with
167 previous findings in raccoons and baboons (18, 20), potentially reflecting a more general trend
168 among urban mammalian omnivores. In addition to increased blood sugar, chloride, phosphorus,
169 and potassium concentrations varied in ways that are consistent with metabolic disorders, which
170 in humans frequently co-occur with electrolyte abnormalities (36). However, these results might
171 reflect differing compositions of anthropogenic versus natural foods, rather than physiological

172 responses to a change in diet (37). Given similar body conditions between urban and rural sites,
173 and that glucose values were within previously published ranges, it is currently unclear if these
174 squirrels are at risk of developing metabolic disorders. Other results were inconsistent with
175 known symptoms of diabetes and metabolic disorders in humans. We found lower ALT in urban
176 populations, and no differences in other liver enzymes – human diabetic and pre-diabetic states
177 are characterized by high levels of ALT, AST, and GGT (38, 39). Due to their ecology and
178 physiology, grey squirrels may have mechanisms in place for regulating body mass and fat
179 accumulation which reduce the risk of metabolic disturbance while accumulating lipid reserves.
180 Grey squirrels are a seasonal fat-accumulating species that are relatively inactive during winter,
181 but do not hibernate (23). They have high metabolic rates and calorie requirements, and are
182 efficient users of available energy (40, 41). Peak annual mass is reached in autumn: during this
183 time squirrels are hyperphagic, and carry more adipose tissue (42, 43). Captive grey squirrels
184 also displayed voluntary reduction in food intake during winter periods (41). Thus, increased
185 access to supplemental food may help autumn weight gain in grey squirrels—similar to brown
186 bears, which are better able to optimize food intake to maximize weight gain prior to hibernation
187 when they have access to anthropogenic food (14, 44). These characteristics suggest that grey
188 squirrels may be pre-adapted to exploit transient food excesses, such as human food wastes, and
189 therefore are robust to major health consequences of urban diets.
190
191 We also note interesting patterns with regard to markers for serum protein. Serum albumin
192 reflects long-term protein status, while urea levels shift in response to short-term protein
193 availability (45). Creatinine is reduced when muscle mass decreases due to protein catabolism in
194 the body (45, 46). Although we did not find statistically detectable differences between

195 populations in creatinine and urea, in both cases they tended to be lower in urban populations.

196 Total protein was not different between sites and was on the high margin compared to previously

197 published intervals (23, 34), indicating that neither population was malnourished, at least with

198 respect to protein intake. Serum albumin was comparable in urban and rural squirrels, and

199 though globulin tended higher in urban squirrels, the difference was small. However, we found

200 significantly lower ratios of albumin to globulin (A:G ratios) in urban squirrels. Globulin is

201 expected to be increased after infection, and is also positively associated with inflammatory

202 responses and nutritional stress (47). Individuals in better physiological condition have higher

203 A:G ratios. This result is interesting but difficult to interpret at this stage, because inflammation

204 is typically the first physiological response to a stressor of any kind. Urbanization introduces

205 numerous stressors which might cause inflammation, such as pollution, increased disease

206 transmission, and dietary changes (48, 49). Obesity and metabolic disorders are also

207 characterized by a chronic pro-inflammatory state (50). Previous studies using transcriptomic

208 data report candidate genes associated with metabolism and immunity which are under selection

209 in cities, and greater expression of genes involved in the inflammatory response in urban

210 populations (51, 52). Our results align with general trends of greater physiological stress

211 associated with environmental stressors in urban populations, but the underlying causes remain

212 to be explored.

213

214 There are few published studies of serum biochemistry in grey squirrels, making inferences

215 about individual health difficult. Our values were well within reported ranges in Hoff et al. (34)

216 (Figs. 1, 2, 3). We note however, that those ranges were obtained from samples taken year-

217 round, and thus might obscure potential seasonal variation in analyte levels. Autumn

218 hyperphagia and rapid weight gain would be expected to elevate serum glucose and lipid markers
219 including cholesterol and albumin, which in mammals is involved in fatty acid transport via the
220 circulatory system (53). Seasonal variation in diet and food intake in grey squirrels (23) would
221 also drive seasonal differences in blood chemistry, making comparison of our values to annual
222 averages in serum parameters not ideal.

223

224 Our main findings—higher glucose, shifts in electrolyte balance, and lower A:G ratio—suggest
225 physiological responses to differing habitat quality between urban and rural sites. Taken
226 together, this is evidence that urban grey squirrels are less healthy than rural ones in our study
227 populations. We note that this was an exploratory analysis, and results should be interpreted with
228 caution. Sample sizes were small, and many of the variables examined here can vary based on a
229 number of factors such as age, individual health, time since last meal, and stress associated with
230 capture and handling. Here, all squirrels were baited, captured and handled in the same way, in
231 essence controlling for these effects on serum biochemistry. However, in future the effects of
232 stress and food intake on glucose might be minimized by measuring glycated serum proteins (as
233 in (20)), which is an indicator of circulating blood sugar integrated over a longer period of time
234 rather than instantaneous measurements of serum glucose concentrations. Of note, interpretation
235 of glycated serum proteins on needs to be done with caution because values will be a function of
236 integrated circulating carbohydrate levels and the relative turnover rates of circulating proteins.
237 Albumin is a predominant soluble extracellular protein in mammal blood and based on the half-
238 life of albumin (~ 17-20 days for humans, ~ 1.5-3 days for rats (54–57)) it is likely there is a
239 substantial interspecific size-scaling effect where the period of integration of circulating
240 carbohydrate levels based on glycated serum proteins will be much shorter for smaller species.

241 Future studies examining the risk of metabolic disorders in grey squirrels could additionally
242 measure circulating hormone levels, such as insulin, and urine glucose to encompass the main
243 symptoms used to diagnose metabolic disorders in other species (11, 12, 58). Furthermore,
244 pinpointing causes of inflammation would be informative for identifying specific features of
245 urbanization that have physiological effects on wildlife. Potential sources of inflammation could
246 be untangled using hematological tests to distinguish between infection (parasitic, viral, or
247 bacterial), or other chronic influences (e.g., diet, pollution). We note that we obtained blood
248 smears for urban individuals and a small subset of the rural individuals presented here ($n = 2$),
249 but due to lack of rural samples were unable to make a comparison. However, among those
250 individuals for which we did obtain samples, no blood parasites were detected.

251

252 The effects of food provisioning on individual health has important impacts on population and
253 evolutionary dynamics. Food subsidies are partly responsible for increased abundances and high
254 population densities seen in successful urban species like rats, white-tailed deer, raccoons, and
255 grey squirrels (25, 59). However, as our results demonstrate, greater abundance does not always
256 correspond to good overall health (20, 60, 61), meaning large urban populations may suffer
257 higher mortality. Food provisioning can have indirect consequences on survival by promoting
258 the spread of disease and parasites in denser populations (3, 62) which are weakened by poor
259 nutrition and stress. In this way, supplemental food may be an important selection pressure in
260 cities shaping evolution in successful urban species. Higher prey density in cities, in addition to
261 supplemental human food, also increases the frequency of human-wildlife interactions (44, 60,
262 61, 63). What this means for population demographics in the long-term—even for species which
263 do well in cities—is unclear. The consequences of human food waste reverberate throughout

264 levels of the urban ecosystem, and understanding these complex relationships is important for
265 both safety and controlling populations of synanthropic species in cities.

266

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275

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423 **Figure legends**

424 **Figure 1.** Boxplots for serum electrolytes. Circles are females, triangles are males. Reference
425 ranges are shown for analytes when available. Asterisks (*) indicate noteworthy differences
426 between urban and rural populations ($p < 0.05$).

427

428 **Figure 2.** Boxplots for creatinine, urea, and enzymatic markers for liver and kidney function.
429 Circles are females, triangles are males. Reference ranges are shown for analytes when available.
430 ALKP = Alkaline phosphatase; ALT = alanine aminotransferase[†]; AST = aspartate
431 aminotransferase; GGT = gamma-glutamyl transferase. Asterisks (*) indicate noteworthy
432 differences between urban and rural populations ($p < 0.05$). [†]Note: 5 of 6 urban samples had ALT
433 levels below the detectable limit (3 U/L).

434

435 **Figure 3.** Boxplots for serum protein, cholesterol, and glucose. Circles are females, triangles are
436 males. Reference ranges are shown for analytes when available. Asterisks (*) indicate noteworthy
437 differences between urban and rural populations ($p < 0.05$).

438

439 **Figure 4.** PCA biplot. PC1 explained 25.6%, and PC2 20.4% of the total variation in response
440 variables. Sites were separated along PC1.

441 **Table 1.** Body condition and serum parameters in urban and rural grey squirrels. Sample sizes
442 differ when individuals were excluded due to age (mass and body condition) or low serum
443 sample volume.

	Urban		Rural	
	<i>n</i>	Mean \pm SD	<i>n</i>	Mean \pm SD
Mass (g) (both sexes)	20	678 \pm 62.2	8	653 \pm 70.1
Body condition (female)	11	-4.21 \pm 49.6	5	3.79 \pm 55.5
Body condition (male)	9	5.66 \pm 47.9	3	4.58 \pm 20.9
Sodium (mmol/L)	6	145.17 \pm 2.48	9	146.22 \pm 2.91
Potassium (mmol/L)	6	5.87 \pm 2.20	9	9.32 \pm 2.61
Chloride (mmol/L)	6	113.50 \pm 1.76	9	116.78 \pm 2.33
Calcium (mmol/L)	6	2.29 \pm 0.13	8	2.20 \pm 0.10
Phosphorus (mmol/L)	6	1.83 \pm 0.37	8	3.25 \pm 0.54
Magnesium (mmol/L)	6	1.21 \pm 0.11	8	1.22 \pm 0.19
Urea (mmol/L)	6	8.10 \pm 2.75	9	9.71 \pm 2.13
Creatinine (μ mol/L)	6	46.17 \pm 3.82	9	54.56 \pm 15.74
ALKP (U/L)	6	608.00 \pm 269.94	8	859.00 \pm 378.97
GGT (U/L)	6	9.33 \pm 3.88	8	15.00 \pm 13.03
ALT (U/L)	6	4.83 \pm 4.49	8	17.63 \pm 18.01
AST (U/L)	6	143.00 \pm 67.91	8	120.13 \pm 100.60
Glucose (mmol/L)	6	7.80 \pm 1.80	9	4.71 \pm 1.33
Cholesterol (mmol/L)	6	5.40 \pm 0.88	8	4.80 \pm 0.94
Total protein (g/L)	6	70.67 \pm 8.64	9	68.67 \pm 8.26
Albumin (g/L)	6	37.67 \pm 4.68	8	38.50 \pm 6.63
Globulin (g/L)	6	33.00 \pm 4.43	8	28.75 \pm 2.96

444

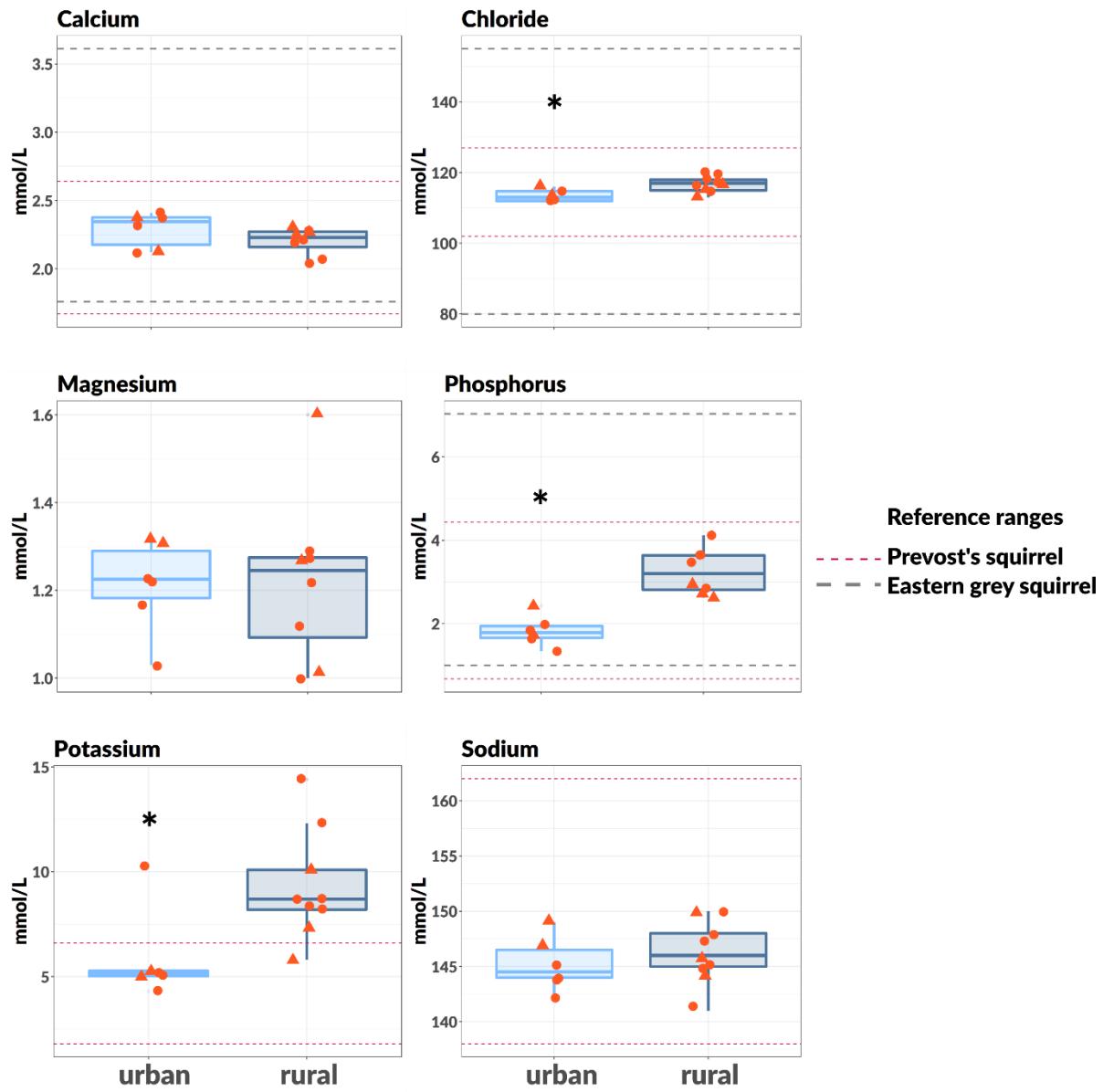
445 **Table 2.** Factor loadings on the first two principal components (PCs). Urban and rural sites were
446 separated along PC1.

	PC1	PC2
Sodium	0.156365	-0.27516
Potassium	0.277074	0.29981
Chloride	0.334378	-0.1606
Urea	0.164741	0.338645
Creatinine	0.096411	0.055769
Calcium	-0.29924	0.046322
Phosphorus	0.395276	0.08438
Magnesium	0.034263	0.180375
ALKP	0.228345	0.092981
GGT	0.200064	0.219328
ALT	0.256803	-0.23847
AST	0.344987	0.005621
Glucose	-0.379	0.008177
Cholesterol	-0.2204	0.170467
Total protein	-0.0298	0.465441
Albumin	0.069876	0.428948
Globulin	-0.15862	0.316649
Body condition	-0.05741	0.073088

447

448 **Figure 1**

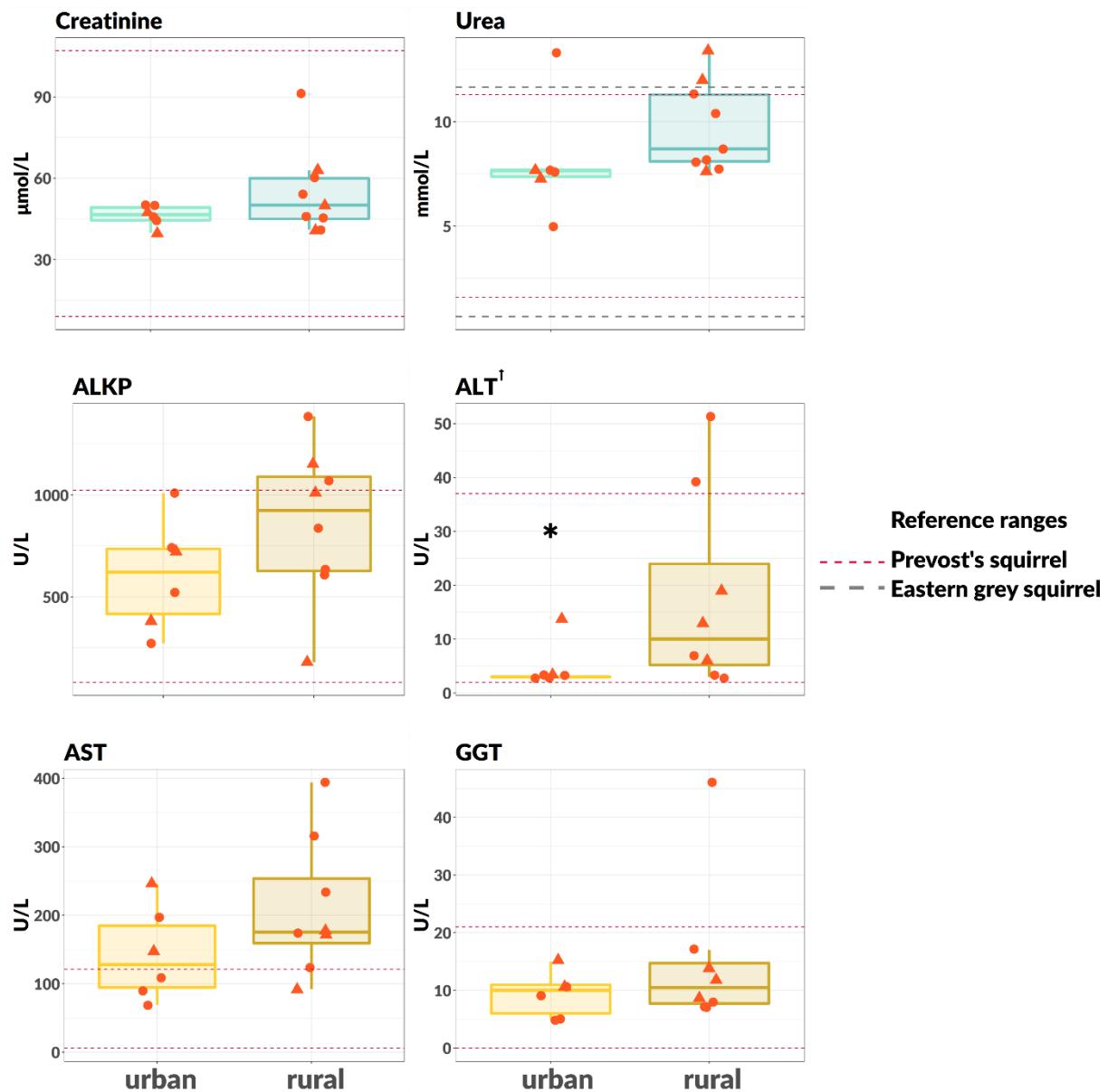
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451 **Figure 2**

452

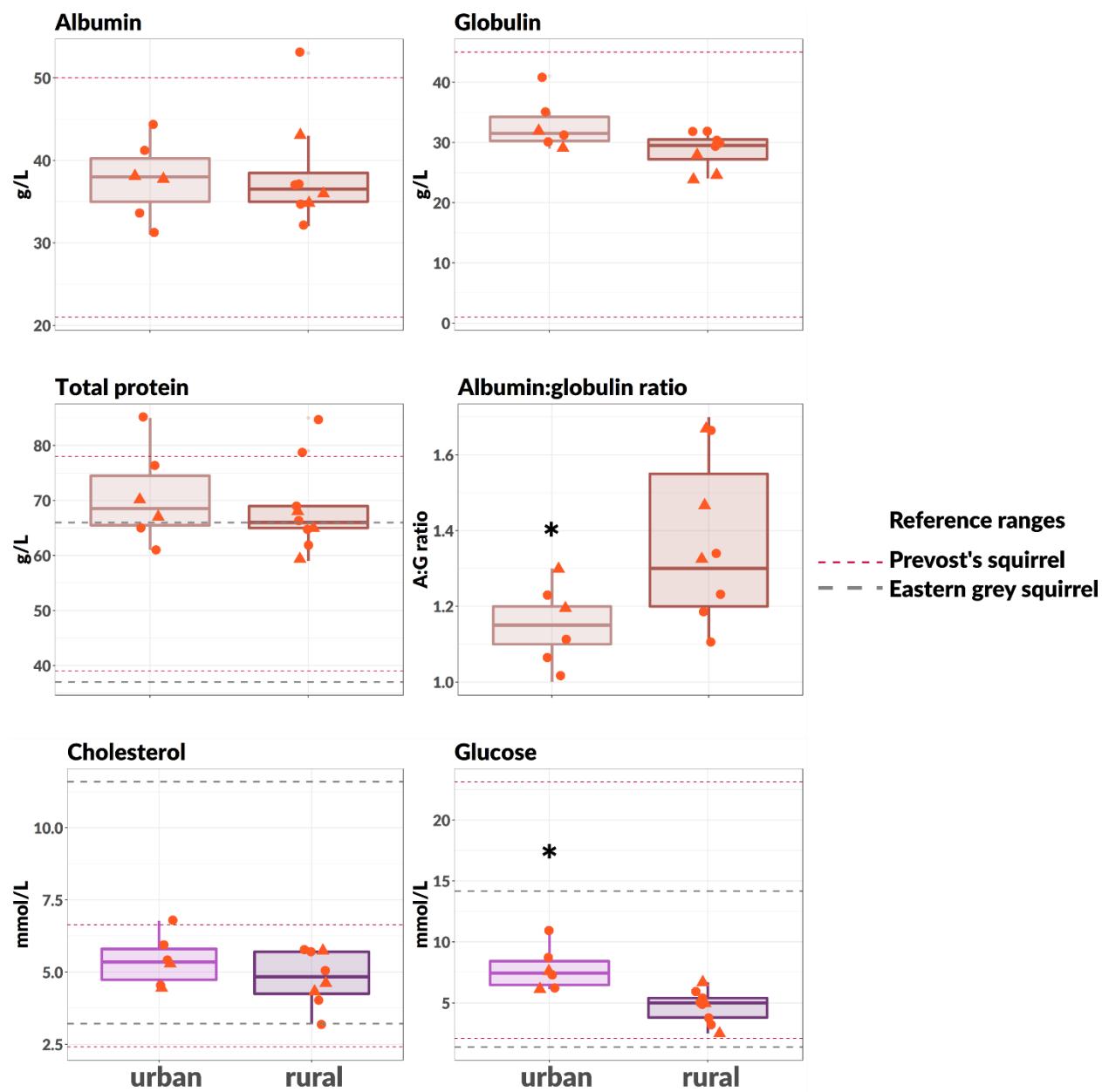


453

454

455 **Figure 3**

456



457

458

459

460 **Figure 4**

