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21 Research paper

22 **USE OF LISPRO INSULIN FOR TREATMENT OF DIABETIC KETOACIDOSIS IN CATS**

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Abstract

Objectives: The aim of this study was to evaluate the efficacy and safety of lispro insulin for the treatment of feline diabetic ketoacidosis (DKA). Times to resolution of hyperglycemia, ketosis and acidosis were compared between cats treated with continuous rate infusion (CRI) of lispro insulin and cats treated with CRI of regular insulin.

Methods: Client-owned cats with naturally occurring DKA, newly diagnosed with diabetes mellitus (DM) or already on treatment for DM, were included. The diagnosis of DKA involved the presence of at least two clinical signs consistent with DKA (e.g., polyuria/polydipsia, anorexia, severe lethargy, vomiting and dehydration), blood glucose concentration > 13.9 mmol/L (> 250 mg/dl), blood beta hydroxybutyrate (BHB) concentration > 2.5 mmol/L and venous pH < 7.3 or bicarbonate < 15 mEq/L.

Cats were treated with a standard protocol with IV CRI of regular insulin (Group R) or lispro insulin (Group L). The time to resolution of DKA was defined as the time interval from when the IV CRI of insulin began until marked hyperglycemia (BG > 13.9 mmol/L [> 250 mg/dL]), ketosis (BHB concentration > 1 mmol/L) and acidosis (venous pH < 7.3 and/or bicarbonate < 15 mEq/L) resolved.

Results: Eighteen DKA cases (nine per group) were enrolled into the study. There were no significant differences in the median time to resolution of three variables (hyperglycemia, ketosis and acidosis) between the two groups. Two cats in Group R developed hypoglycaemia during the CRI of insulin. One cat in Group L and three cats in Group R developed hypophosphataemia, which required phosphate supplementation.

Conclusions and relevance: Intravenous CRI of insulin lispro has few side effects and appears to be as effective as IV CRI regular insulin in the treatment of cats with DKA.

69   Diabetic ketoacidosis (DKA) is the most common complication of naturally occurring diabetes mellitus (DM)

70   and is characterized by a biochemical triad of hyperglycaemia, ketosis and acidosis.[1-5] Treatment of DKA

71   comprises intravenous (IV) fluid resuscitation, correction of acid/base and electrolyte derangements, insulin

72   therapy and targeted therapy for comorbid conditions.[5]

73   During DKA regular insulin is usually administered intramuscularly or intravenously in cats and dogs;[6] in

74   humans it is also injected subcutaneously.[7] Nevertheless, the dehydration and shock state of patients with DKA

75   leads to erratic and inconstant absorption of intramuscular and subcutaneous insulin.[7] For this reason, IV

76   infusion of regular insulin has been the mainstay of treatment of DKA as it causes a more predictable fall in

77   blood glucose and it allows for rapid adjustments.[8]

78   Insulin lispro is a genetically engineered analogue of human insulin in which proline at position B28 and lysine

79   at position B29 are inverted in their sequence reducing the formation of insulin dimers and hexamers. This

80   structural change ensures more rapid absorption and elimination from the subcutaneous injection site, resulting

81   in the rapid onset and a short duration of hypoglycaemic activity.[9,10] Furthermore, one study in human medicine

82   comparing the end-organ metabolic effects of intravenous lispro insulin, regular insulin and glulisine insulin

83   showed that all these insulins have similar effects on the suppression of endogenous glucose production, glucose

84   uptake and free fatty acid, glycerol and lactate levels.[11] The success of lispro insulin, as well as other insulin

85   analogues, has gradually reduced the use of regular insulin, as demonstrated by Eli Lilly's financial report.[12]

86   Assuming that the production of regular insulin may be discontinued, a valid alternative for treating DKA in

87   dogs and cats should be found. Two studies demonstrated that IV continuous rate infusion (CRI) of lispro and

88   aspart insulin is safe and appears to be as effective as IV CRI regular insulin for the treatment of canine

89   DKA.[13,14]

90   The aim of this study was to evaluate the efficacy and safety of lispro insulin for the treatment of feline DKA by

91   comparing the times to resolution of hyperglycaemia, ketosis and acidosis between cats treated with CRI of

92   lispro insulin and cats treated with CRI of regular insulin.

93

94   **Materials and methods**

95   Client-owned cats admitted to the University Veterinary Hospital of Bologna (Italy) between May 2009 and

96   March 2017 with naturally occurring DKA, either newly diagnosed with DM or with known DM, were

97   considered for inclusion. The diagnosis of DKA involved the presence of at least two clinical signs consistent

98   with DKA (e.g., polyuria/polydipsia, anorexia, severe lethargy, vomiting and dehydration), blood glucose

99   concentration > 13.9 mmol/L (> 250 mg/dl), blood beta hydroxybutyrate (BHB) concentration > 2.5 mmol/L[15]

100 and venous pH < 7.3 or bicarbonate < 15 mEq/L. Cats with DKA, admitted between May 2009 and February

101 2012, and treated with a protocol for insulin therapy adapted from a published protocol[16] using IV CRI of regular

102 insulin (Humulin R, Ely Lilly and Co, Indianapolis, IN) were used as part of the control group of this study.

103 From March 2012 to April 2014 cats with DKA admitted to the University Veterinary Hospital were treated with

104 the same insulin protocol but using lispro insulin (Humalog, Eli lilly and Co), until the number of cats was the

105 same in both groups. Between May 2014 and March 2017, cats admitted for DKA were alternately treated with

106 regular insulin or lispro insulin.

107 Cases were divided according to whether they received IV CRI of regular insulin (Group R) or IV CRI of lispro

108 insulin (Group L). Cats with multiple hospitalizations for DKA management during the study period were

109 included in the analyses with each hospitalization event treated as a separate case.

110 Cases were excluded from the study if they had unavailable or missing medical records and if they died or were

111 euthanized prior to administration of insulin therapy. The trial was approved by the Scientific Ethics Committee,

112 University of Bologna, Italy. Owners signed the written informed consent before enrollment in the study.

113 At the time of admission to the hospital, history, physical examination findings and results of blood gas analysis,

114 complete blood count (CBC), serum biochemistry profile, urinalysis and bacterial culture from urine collected

115 via cystocentesis were performed in each cat in order to confirm DKA and identify any concurrent disorder. An

116 abdominal ultrasound was performed in order to detect any abnormalities (e.g., acute pancreatitis, neoplasia).

117 Thoracic radiographs or other diagnostic tests were also performed according to the clinician's discretion.

118

119 ***Definitions of "resolution time" of hyperglycaemia, ketosis, acidosis and ketoacidosis, and of the time of***

120 ***subcutaneous insulin administration and length of hospitalization***

121 The "resolution time" for the variables hyperglycaemia, ketosis and acidosis was calculated starting from "time

122 zero", which was the time at which IV CRI of insulin treatment was initiated. The time to resolution of

123 pronounced hyperglycaemia was defined as the time interval between "time zero" and the time at which the

124 blood glucose concentration fell to < 13.9 mmol/L (< 250 mg/dl). The time to resolution of ketosis was defined

125 as the time interval between "time zero" until BHB was ≤ 1.0 mmol/L for two consecutive measurements 1 hour

126 apart. The time to resolution of acidosis was defined as the interval between "time zero" and the time at which

127 venous pH was ≥ 7.3 and/or bicarbonate ≥ 15 mEq/L. The time to resolution of ketoacidosis was defined as the

128 time interval between "time zero" and the time at which ketosis and acidosis had both resolved. The IV CRI of

129 insulin was stopped when ketosis and acidosis had resolved and the cat was eating well.

130 The time to subcutaneous (SC) insulin administration was defined as the time interval from the resolution of

131 ketoacidosis (when the transition from the intravenous to the subcutaneous insulin administration occurs) up to

132 the hospital discharge. The length of hospitalization (LOH) was defined as the time interval between "time zero"

133 and discharged from the hospital.

134

135 *Monitoring protocol*

136 Blood glucose was monitored hourly during the first 24 hours with a hand-held glucometer, previously validated

137 for cats (Optium Xceed, Optium Glucose Test Strips; Abbott Laboratories),[17] and then every 2-3 hours during

138 the entire time that the cat received an IV CRI of insulin. Blood BHB was measured every 4 hours using a

139 portable ketometer, previously validated for cats (Optium Xceed, Optium β-ketone Test Strips; Abbott

140 Laboratories)[17] until BHB was ≤ 1.0 mmol/L; in this case the BHB was measured 1 hour later and if a BHB ≤

141 1.0 mmol/L was confirmed then ketosis was deemed to be resolved. A blood gas analysis (including pH, base

142 excess, serum bicarbonate, sodium, potassium, ionized calcium and lactate) was performed with a point-of-care

143 analyser (Idexx VetStat, Idexx Laboratories, Italy) every 8 hours during the first 24 hours, and then every 12

144 hours until ketosis was resolved.

145   Insulin-induced hypoglycaemia was defined as a blood glucose concentration < 4.4 mmol/L (< 80 mg/dL);

146   hypokalaemia was defined as serum potassium < 3.6 mEq/L. Hypophosphataemia was defined as serum

147   phosphate < 0.5 mmol/L (< 1.5 mg/dL).

148

149   ***Treatment protocol***

150   Upon admission, all cats were treated with intravenous crystalloids (Ringer Lactate or Acetate or 0.9% NaCl)

151   prior to and while receiving insulin treatment. The initial rate of fluid administration was determined by the

152   attending clinician to meet the specific rehydration needs of each cat. Intravenous insulin CRI was initiated from

153   2 to 8 hours after fluid administration had been started, depending on clinician's perception that severe

154   dehydration had resolved. The insulin solution, which was administered in a separate line from the fluids,

155   consisted of 48 mL of 0.9% NaCl to which 1.1 units per kg body weight of lispro insulin or regular insulin were

156   added.[16] To saturate binding of insulin to the IV tubing, the insulin solution was allowed to stand in the line for

157   30 min and then run through the IV line.[18] At this point, the insulin solution was re-prepared and the infusion

158   was started. The initial insulin CRI rate was based on the patient's blood glucose concentration when the CRI

159   was started ("time zero") (Table 1).[6] The rate of insulin CRI was adjusted every 1-2 hours based on the patient's

160   blood glucose. Adjustments to the insulin CRI rate and the addition of dextrose were implemented at each

161   clinician's discretion based on previously published guidelines (Table 1).[6] Long-term insulin was initiated when

162   ketoacidosis was resolved and the cat was eating and appropriately hydrated.

163   Serum potassium concentration was corrected as previously described.[16] If serum phosphate concentration

164   decreased to < 0.5 mmol/L (< 1.5 mg/dL) it was corrected by administration of IV CRI of potassium phosphate

165   at a rate of 0.01-0.03 mmol phosphate/kg/h for 6 hours and then phosphatemia was re-evaluated.

166   Supplementation with potassium was taken into account when giving potassium phosphate for correction of

167   hypophosphataemia. Antimicrobials were administered to all cats for the duration of hospitalization; additional

168   drugs including gastroprotectants, antiemetics and analgesics were administered as deemed appropriate by the

169   attending clinician according to the clinical condition and concurrent disorders.

170

171 **Statistical methods**

172 Statistical analysis was performed using commercially available software (GraphPad Prism 5, GraphPad

173 Software Inc., San Diego, CA). Due to the small number of cases in each group, continuous variables were

174 considered to be nonparametric and descriptive statistics are reported as median (minimum, maximum). The

175 Mann Whitney U-test was used to compare variables between the two insulin groups. The Wilcoxon signed rank

176 test was used to compare changes from baseline of the continuous variables within each insulin group. A P-

177 value < 0.05 was considered significant. In order to compare the different variables between cats with newly

178 diagnosed DM and cats with known DM, regardless of the type of insulin used, Mann Whitney U-test was used.

179

180 **Results**

181 Twenty-four diabetic ketoacidosis cases were evaluated in the study period. Four cases were excluded because

182 cats died or were euthanized before initiating insulin CRI therapy, and two cases were excluded because of

183 incomplete medical records. A total of 18 cases in 15 cats were included in the study; one cat had three DKA

184 events (in two events received lispro insulin and in another one received regular insulin) and another cat had two

185 DKA events during the study period (in one received lispro insulin and in the other received regular insulin).

186 Nine cases were managed with lispro insulin (Group L) and nine cases were managed with regular insulin

187 (Group R). In fifteen cases cats were discharged from the hospital; one cat from Group L died and two cats from

188 Group R were euthanized; these three cats were newly diagnosed with DM.

189

190 *Symptoms, history, clinical signs and physical examination findings*

191 There was no significant difference between groups with regards to the median age, body weight, breed and

192 sexual status (neutered or intact) (Table 2). The median age among all 18 cases was 10.4 years (range, 7.7-16.5

193 years). The median body weight of all 18 cases was 4.5 kg (range, 2.6-8 kg) and their median body condition

194 score (BCS) was 6.5 (range, 2-8). All fifteen cats included in the study were European short hair cats except one

195 Persian cat in group R and one Birman cat in group L. Eight cats were neutered males, five were neutered

196 females, one was an intact male and one was an intact female.

197 In 10 cases cats were newly diagnosed with DM at the time of enrollment into the study. In 8 cases (7 cats), cats

198 had previously been diagnosed with DM (five in Group L and three in Group R), a median of 8 months (range,

199 1-12 months) prior to enrollment into the study, and they were all receiving glargine insulin (Lantus, 100 U/mL

200 glargine, Aventis Pharmaceuticals, Germany). Insulin dosage at the time of enrollment into the study was 0.5 U

201 twice daily in one cat, 1 U twice daily in four cats, 2 U twice daily in two cats and 2.5 U twice daily in one cat.

202 Clinical signs observed by the owner prior to admission into the hospital included lethargy (observed in 15 of 18,

203 83%), anorexia (15 of 18, 83%), polyuria and polydipsia (10 of 18, 56%), vomiting (6 of 18, 33%), weight loss

204 (5 of 18, 28%), asthenia (4 of 18, 22%) and diarrhoea (3 of 18, 17%). Medications administered to the cats at the

205 time of admission into the hospital included insulin (7 of 18), tylosin (2 of 18), methimazole (Tapazole®) (1 of

206 18), marbofloxacin (Aristos®) (1 of 18) and enrofloxacin (Baytril®) (1 of 18).

207 At the time of admission, the most common abnormalities included some degree of dehydration (observed in 17

208 of 18, 94%), dull or depressed mentation (17 of 18, 94%), hypothermia (8 of 18, 44%), overweight body

209 condition (7 of 18, 39%), underweight body condition (5 of 18, 28%), pale mucous membranes (5 of 18, 28%),

210 jaundice (4 of 18, 22%), muscle atrophy (3 of 18, 17%), heart murmur (2 of 18, 11%) and palpable thyroid

211 nodule (1 of 18, 6%).

212

213 *Clinicopathological findings*

214 At the time of admission into the hospital, median blood glucose concentration, BHB concentration, venous pH

215 and serum bicarbonate concentration did not differ significantly between the lispro insulin and regular insulin-

216 treated group (Table 2).

217 The median blood glucose concentration in Group L and Group R was 20.8 mmol/L (range, 12.4-35 mmol/L)

218 (374 mg/dL [224-630 mg/dL]) and 22.9 mmol/L (12.4-41.3 mmol/L) (413 mg/dL [224-744 mg/dL]),

219 respectively. At "time zero", the median blood glucose concentration was 22.5 mmol/L (11-27.8 mmol/L) (405

220 mg/dL [198-500 mg/dL]) and 21.7 mmol/L (13.4-27.8 mmol/L) (391 mg/dL [241-500 mg/dL]) in Group L and

221 Group R, respectively. No significant differences were detected in blood glucose concentration between the two

222 treatment groups at the time of admission ($P = 0.69$) or at "time zero" ($P = 0.86$). The rate of decrease in blood

223 glucose concentration was < 5.6 mmol/L/h (100 mg/dl/h) in all 18 cases during the entire study.

224 The median BHB concentration in Group L and Group R was 6.2 mmol/L (range, 3.7-8 mmol/L) and 7.2

225 mmol/L (4.9-8 mmol/L), respectively. At "time zero", the median BHB concentration was 5.4 mmol/L (4.2-7.8

226 mmol/L) and 7.2 mmol/L (4.7-8 mmol/L) in Group L and Group R respectively. No significant differences were

227 detected in BHB concentration between the two treatment groups at the time of admission ($P = 0.42$) or at "time

228 zero" ($P = 0.13$).

229 At the time of admission, there were also no significant differences between the two treatment groups with

230 respect to any of the biochemical parameters analysed (Table 2).

231 The median time interval between the time at which fluid therapy was initiated until "time zero" was 4 hours in

232 the lispro insulin group (range, 2-8 h) and 4.5 hours (range, 1-8 h) in the regular insulin group; there was no

233 significant difference between the two groups ($P = 0.62$).

234

235 *Adverse drug reactions*

236 No local or systemic adverse effects associated with IV insulin administration were noted in either group. Two

237 cats in Group R developed hypoglycaemia during the CRI of insulin (4.39 mmol/L [79 mg/dL] and 2.22 mol/L

238 [40m g/dL] respectively), but these cats did not show clinical signs compatible with hypoglycaemia. In all 18

239 cases, cats developed transient hypokalaemia during the study. Median minimum potassium concentrations did

240 not differ significantly between the lispro (2.8 mmol/L; range 2.2-3.7 mmol/L) and regular (2.6 mmol/L; range

241 2.2-3.5 mmol/L) insulin treatment groups ($P = 0.82$). One cat in Group L and three cats in Group R developed

242 hypophosphataemia which required supplementation during the study.

243

244 *Resolution time of hyperglycaemia, acidosis and ketosis, time of SC insulin administration and LOH*

245 Severe hyperglycemia resolved in all 18 cases, acidosis resolved in 15 cases (7 in Group L and 8 in Group R)

246 and ketosis resolved in 16 cases (8 in Group L and 8 in Group R). One cat in Group L died prior to resolution of

247 acidosis and ketosis. Acidosis did not resolve in one cat in Group L and one cat in Group R that had suffered an

248 acute kidney injury (AKI) at the time of admission; ketosis did not resolve in one other cat in Group R, possibly

249 due to the insulin-resistance secondary to a concurrent carcinoma.

250 There were no significant differences in the median time to resolution of three variables (hyperglycaemia,

251 ketosis and acidosis) between the two groups when evaluated separately; there was no significant difference in

252 the median time to resolution of ketoacidosis (Table 3).

253 The median times to resolution of severe hyperglycaemia in Group L and Group R were 8 hours (range, 0-25 h)

254 and 9 hours (range, 0-24 h), respectively (P = 0.72). The median time to resolution of ketosis was 29 hours

255 (range, 16-94 h) in Group L and 26.5 hours (range, 21-53 h) in Group R (P = 0.83). The median time to

256 resolution of acidosis in Group L and Group R was 8 hours (range, 8-32 h) and 20 hours (range, 8-48 h),

257 respectively (P = 0.26). The median time to resolution of ketoacidosis in Group L and Group R was 33 hours

258 (range, 16-94 h) and 28 hours (range, 21-53 h), respectively (P = 1).

259 There were no significant differences between newly diagnosed and previously diagnosed diabetic cats with

260 respect to median time to resolution of hyperglycaemia and ketosis (analysed separately), and ketoacidosis

261 (Table 4). However, the median time to resolution of acidosis in the newly diagnosed diabetics (12 h; range, 8-

262 24 h) was significantly shorter than in previously diagnosed diabetics (24 h; range, 8-48 h; P = 0.02).

263 Venous pH decreased during the first hours of treatment, before it began to rise, in five of 18 cases, (one case in

264 Group L and four cases in Group R). The lowest pH for these five cases was reached at a median of 8 hours

265 (range, 8-16 h) from the time at which fluid infusion had begun. The median lowest pH for the five cases in

266 which this initial decline occurred was 7.07 (range, 6.94-7.25), and did not differ significantly (P = 0.06) from

267 the pH of the same patients at admission (median 7.15; range 7.02-7.28). Also there was no significant

268 difference in length of hospitalization between cases in which pH decreased before it began to rise and cases in

269 which this did not happen.

270 The median time to administration of SC insulin, in the 15 cases that were discharged, did not differ significantly

271 between Group L (76 h; range, 34-168 h) and Group R (89 h; range, 48-244 h; P = 0.25). Likewise the median

272 duration of hospitalization for these 15 cases, did not differ significantly between Group L (110.5 h; range, 74-

273 268 h) and Group R (146 h; range, 94-294 h; P = 0.18) (Table 3).

274 No significant differences were found in median time to administration of SC insulin and in median duration of

275 hospitalization between newly diagnosed and previously diagnosed diabetic cats (Table 4).

276 The lispro insulin-treated cat that died had been hospitalized for 45 hours at the time of death; the two cats

277 treated with regular insulin that were euthanized had been hospitalized for 106 and 129 hours respectively at the

278 time of euthanasia.

279

280 *Evaluation for presence of concurrent disorders*

281 Based on the diagnostic protocol, concurrent disorders were identified in 11 cases (five in Group L and six in

282 Group R). In Group L, two cases had inflammatory bowel disease, one cat had pancreatitis, one cat had

283 concurrent pancreatitis, lipidosis and acute kidney injury and one cat had pulmonary neoplasia. In Group R, one

284 cat was diagnosed with a bacterial urinary tract infection based on urinary culture, one cat was diagnosed with

285 pancreatitis and inflammatory bowel disease, one cat had pancreatitis and polycystic kidney disease, one cat had

286 hyperthyroidism and herpesvirus infection, one cat had chronic kidney disease and one cat had a giant cell

287 tumor.

288 The diagnosis of pancreatitis was based on abdominal ultrasound (enlarged, irregular, hypoechoic pancreas

289 surrounded by hyperechoic mesentery, and mild-to-moderate ascites) and positivity to a feline pancreatic lipase

290 immunoreactivity (fPLI) test.

291

292 **Discussion**

293 Lispro insulin was developed to resolve the problems associated with the use of regular human insulin (peak of

294 activity reached too late, hypoglycaemic effect possibly lasting too long) by subcutaneous injection.[19] The major

295 difference between lispro insulin and regular insulin is the rate of their self-disassociation, which causes

296 differences in the rate of absorption from the injection site. However this difference may not exist with

297 intravenous administration. A study on rabbits showed that the hypoglycaemic response profiles after

298 intravenous administration of lispro insulin and regular human insulin were very similar in pattern and

299 confirmed that their biological activities are equivalent.[20]

The aim of this study was to evaluate the efficacy and safety of lispro insulin for the treatment of feline DKA.

The need to test a new insulin, which could provide an alternative to regular insulin, has arisen because insulin analogues are widely used for the management of DM and treatment of uncomplicated DKA in human medicine, although some patients with severe comorbidities still require intensive care and IV insulin administration.[8,21-24] It is possible that the production of regular insulin may be discontinued in the future, and since only regular insulin is currently indicated for the treatment of DKA with the constant low-dose intravenous insulin infusion technique in cats, a viable alternative to regular insulin will need to be found to manage these patients.

In a prospective randomized study, Sears et al. compared the efficacy and safety of IV CRI of lispro with that of regular insulin in a population of 12 dogs with DKA.[13] They observed comparable improvement in glycaemia, ketosis and acidosis between the two groups and the time to resolution of ketoacidosis was significantly shorter in the lispro insulin group, though the length of hospitalization did not differ significantly. They concluded that IV CRI of lispro insulin is safe and appears to be as effective as IV CRI of regular insulin for the treatment of canine DKA.

In our study on feline DKA, the time of resolution of hyperglycaemia, ketosis, acidosis and ketoacidosis was similar in cases treated with lispro insulin and those treated with regular insulin; also the time of SC insulin administration and the LOH did not differ significantly between the two groups. Nevertheless the time to resolution of acidosis and the LOH were both shorter in the group of cases treated with lispro insulin, although these differences were not significant. A number of variables, including concurrent disorders, may have contributed to these findings, and it seems reasonable to suppose that studying a larger group of cases in the future could reveal that each of these times is significantly shorter with lispro insulin treatment.

The median time to resolution of acidosis in the newly diagnosed diabetic cats was significantly shorter than in previously diagnosed diabetics; this result may reflect a different efficiency in the buffering system and a difference in acid-base status between newly and previously diagnosed diabetic cats.

In the first hours after the onset of insulin therapy, venous pH decreased before it began to rise in only one cat in Group L and in four cats in Group R; these results could be attributed to the more rapid action of lispro insulin compared to regular insulin. Furthermore, hyperglycaemia resolved in all 18 cases, acidosis resolved in 7/9 cases

326 in Group L and in 8/9 cases in Group L, and ketosis resolved in 8/9 cases in Group L and Group R. On the basis

327 of these results IV CRI of lispro insulin appears to be as effective as IV CRI of regular insulin for the treatment

328 of cats with DKA.

329 With regards the safety, on our results, side effects were seen less frequently in cases treated with lispro insulin

330 than in cases treated with regular insulin; in fact, in Group R in 2 occasions cats developed hypoglycaemia and

331 in 3 developed hypophosphataemia; while only one cat in Group L developed hypophosphataemia. Despite

332 supplementation, transient hypokalaemia occurred in all cats during the IV infusion of insulin, regardless of the

333 type of insulin. Although hypokalaemia that develops during DKA rarely becomes symptomatic, in our opinion

334 it would be more appropriate to use higher rates of supplementation than those normally reported in textbooks

335 during the first hours of insulin therapy; this was also suggested by Nelson, but only in those patients with

336 normal urinary production and if frequent assessments of kalaemia are possible.[5]

337 In this study there were no significant differences in blood glucose concentration or BHB concentration between

338 the two treatment groups at the time of admission or at "time zero", when insulin therapy began. On the contrary,

339 a decrease in blood glucose concentration during the first hours of fluid therapy has been reported in human

340 medicine and in some studies in veterinary medicine, and has been attributed to rehydration-induced renal

341 excretion of glucose, decreased concentrations of the counterregulatory hormones or improved perfusion and

342 delivery of endogenous insulin.[13-14,25-26] This reduction was not observed during our study, probably due to the

343 conservative fluid therapy, which was not too "aggressive". On that note, one of the current study's limitations is

344 the lack of standardized criteria for the evaluation of the degree of dehydration (determined subjectively) to

345 determine when to start insulin therapy.

346 Another important limitation is the small number of cases enrolled which influenced the power of statistics. It is

347 likely that some differences between groups were not detected because of this bias. Furthermore, some cats were

348 included more than once and this is also a possible bias; in fact it is possible that a cat responds in a similar

349 manner with repeated treatment compared with a different cat, or that a cat can be more severely affected with

350 subsequent visits because of progression of concurrent illness or even less severely affected because owners

351 recognized the signs earlier.

352　Finally, other study's limitations are the absence of randomization and the heterogeneity of the population with

353　regard to the presence of concurrent disorders. However, our population's characteristics were very similar to

354　those in other studies about feline DKA[27-31] suggesting that this small population is representative of cats with

355　spontaneous DKA.

356　In conclusion, the results demonstrate that IV CRI lispro insulin treatment did not show severe side effects in

357　cats of this study and appears to be as effective as IV CRI regular insulin treatment in managing cats with DKA.

358

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426

427   Table 1: Sliding scale for adjustment of IV CRI insulin treatment and dextrose supplementation for cats with diabetic ketoacidosis.

Blood glucose concentration (mmol/L) (mg/dL)	Fluids	Rate of administration of insulin solution (mL/h)*
>13.9 (>250)	0.9% NaCl or Ringer	2
11.1–13.9 (200-250)	2.5% dextrose[a]	1.5

8.4-11.0 (150-199)	2.5% dextrose[a]	1.5
5.6-8.3 (100-149)	5% dextrose[b]	1
<5.5 (<100)	5% dextrose[b]	Stop insulin infusion

428 * Insulin solution composed of 1.1 U/kg of regular or lispro insulin added to 48 mL of 0.9%NaCl.

429 [a] 2.5% dextrose composed of 25 mL dextrose 50% added to 475 mL of 0.9% NaCl or Ringer.

430 [b] 5% dextrose composed of 50 mL dextrose 50% added to 450 mL of 0.9% NaCl or Ringer.

431

432 Table 2: Baseline data and blood glucose and BHB concentration at "time zero" in cats with diabetic ketoacidosis treated with IV CRI of

433 lispro insulin (Group L) and treated with IV CRI of regular insulin (Group R).

	Group L	Group R	P-value
Age (years)	10.5 (8.25-14.2)	10.25 (7.75-16.5)	0.8
Body weight (kg)	4.8 (2.8-5.7)	4.2 (2.6-8)	0.27
Male : female	4 : 5	5 : 4	1
Spayed	5/9	3/9	
Castrated	4/9	4/9	
Female	0/9	1/9	
Male	0/9	1/9	
Blood glucose concentration (mmol/l) [RR 4.1-8.8 mmol/l]	20.8 (12.4-35)	22.9 (12.4-41.3)	0.69
Blood glucose concentration (mmol/l) at "time zero" [RR 4.1-8.8 mmol/l]	22.5 (11-27.8)	21.7 (13.4-27.8)	0.86
BHB concentration (mmol/l) [RR < 2.5 mmol/l]	6.2 (3.7-8)	7.2 (4.9-8)	0.42
BHB concentration (mmol/l) at "time zero" [RR < 2.5 mmol/l]	5.4 (4.2-7.8)	7.2 (4.7-8)	0.13
Serum bicarbonate (mmol/l) [RR 18-23.2 mmol/l]	12.9 (8.2-30.2)	12.1 (7.8-17.6)	0.89
Venous pH [RR < 7.3]	7.16 (7.02-7.24)	7.15 (7.06-7.28)	0.69
CO_2 (mmol/l) [RR 32.7-44.7 mmol/l]	29.8 (25.2-40.6)	37.6 (22.6-43.1)	0.3
Sodium (mmol/l) [RR 141-155 mmol/l]	148 (131-154)	148 (135-165)	0.51
Potassium (mmol/l) [RR 3.6-5.8 mmol/l]	3.3 (2.2-4.4)	3.9 (2.5-4.6)	0.4
Chloride (mmol/l) [RR 119-132 mmol/l]	116 (92-122)	109 (83-126)	0.59
Creatinine (µmol/l) [RR 70.7-159.1 µmol/l]	119 (80-273)	156 (93-254)	0.56
Phosphate (mmol/l) [RR 0.94-2.69 mmol/l]	1.4 (1.2-1.8)	1.6 (1.1-2.4)	0.42
Calcium (mmol/l) [RR 1.5-2.63 mmol/l]	2.38 (2-2.48)	2.38 (1.93-2.53)	1
Total protein (g/l) [RR 60-80 g/l]	74.4 (57.5-93.5)	77.1 (52.8-85.8)	1
Albumin (g/l) [RR 21-33 g/l]	32.2 (24.4-39.3)	31 (22.8-34.7)	0.54
AST (U/l) [RR 14-41 U/l]	154 (48-1849)	113 (18-291)	0.41
ALT (U/l) [RR 22-45 U/l]	216 (98-1478)	154 (34-237)	0.14
ALP (U/l) [RR 0-120 U/l]	76 (32-193)	40 (32-82)	0.06
GGT (U/l) [RR 0-3 U/l]	0.4 (0.1-1.2)	0.1 (0.1-2)	0.68
Total bilirubin (µmol/l) [RR 0-11.98 µmol/l]	4.4 (2.2-65.1)	9.6 (3.8-64.9)	0.31
Cholesterol (mmol/l) [RR 1.65-5.94 mmol/l]	5.9 (3.3-8.0)	8.5 (1.8-11.1)	0.18

434 Data are expressed as median; values in parentheses indicate range. RR = reference range for healthy cats.

435 Data were compared with Mann-Whitney U-tests.

437

438

439

440   Table 3: Time to resolution of hyperglycaemia, ketosis, acidosis and ketoacidosis, time of subcutaneous insulin administration and length

441   of hospitalization in cats with diabetic ketoacidosis treated with IV CRI of lispro insulin (Group L) and treated with IV CRI of regular

442   insulin (Group R).

	Group L	**Group R**	*P*-value
Resolution time of hyperglycaemia (h)	8 (0-25)	9 (0-24)	0.72
Resolution time of ketosis (h)	29 (16-94)	26.5 (21-53)	0.83
Resolution time of acidosis (h)	8 (8-32)	20 (8-48)	0.26
Resolution time of ketoacidosis (h)	33 (16-94)	28 (21-53)	1
Time of SC insulin administration (h)	76 (34-168)	89 (48-244)	0.25
Length of hospitalization (h)	110.5 (74-268)	146 (94-294)	0.18

443   Data are expressed as median; values in parentheses indicate range.

444

445   Table 4: Time to resolution of hyperglycaemia, ketosis, acidosis and ketoacidosis, time of subcutaneous insulin administration and length

446   of hospitalization in cats with diabetic ketoacidosis, comparing cats with newly diagnosed diabetes mellitus and cats with known diabetes

447   mellitus.

	Newly diagnosed DM	**Known DM**	*P*-value
Resolution time of hyperglyacemia (h)	9 (0-15)	10 (4-25)	0.37
Resolution time of ketosis (h)	25 (21-51)	40 (16-94)	0.96
Resolution time of acidosis (h)	12 (8-24)	24 (8-48)	**0.02**[*]
Resolution time of ketoacidosis (h)	26.5 (21-51)	42 (16-94)	0.48
Time of SC insulin administration (h)	70.5 (34-244)	87 (48-177)	0.3
Length of hospitalization (h)	97 (74-292)	137 (81-294)	0.19

448   Data are expressed as median; values in parentheses indicate range.