Comparative Medicine
Copyright 2024
by the American Association for Laboratory Animal Science

Vol 74, No 3
June 2024
Pages 179–185

# A Retrospective Analysis of Liraglutide (GLP-1 Agonist) Use in a Chinchilla (*Chinchilla lanigera*) Model of Auditory Blast Injury

**Deidra Marchi, MS, DVM,[1] Clarissa Cerepaka, MS, DVM,[1] Lori Garman, PhD,[2] and Wendy R Williams, MS, DVM[1,*]**

**Chinchillas are a relatively novel research model compared with other rodent species. They require special considerations when it comes to their husbandry and daily care. Chinchillas tend to be shy animals that are well adapted to masking clinical signs of illness. These characteristics can make them a difficult species to maintain in a research setting. The authors' institution has maintained chinchillas and established standardized daily animal care procedures for them. Chinchillas are most commonly used for auditory research. They are often used to study the mechanism of different induced auditory conditions or injuries as well as exploration for potential alleviating treatments. Often, tested therapeutics have demonstrated potentially beneficial effects but have not been applied in the specific condition or injury of interest. The development of new applications for therapeutics can lead to groundbreaking discoveries, but testing of new therapeutic applications is often initially performed in an animal model without knowing how the therapeutic will behave in the species. During testing, unexpected adverse events may manifest that require more focused monitoring and supportive care. This scenario occurred when adverse effects were observed in a chinchilla blast-injury model after receiving an acylated glucagon-like peptide-1 (GLP-1) receptor agonist. The study involved evaluation of this therapeutic over an extended amount of time after inducing a controlled pressurized blast-injury followed by multiple repeated hearing tests under anesthesia. Chinchillas enrolled in the study exhibited several clinical signs including weight loss, lethargy, labored breathing, neurologic abnormalities, decreased appetite or decreased fecal output, and otitis. Five primary abnormalities were reported on pathology: aspiration pneumonia, hepatic steatosis, right ventricular dilation, pancreatitis, and tubulointerstitial nephritis. Initially abnormal clinical signs, early mortality rates, and pathology were attributed to multiple anesthetic events. However, a retrospective analysis evaluating the association of different study variable exposures in a stratified comparison demonstrated that the early mortality rates were actually associated with the therapeutic drug given for the first time in this species. In this study, we describe the detailed findings of the retrospective analysis and explore different strategies that can be incorporated to maintain good animal welfare and decrease early animal loss.**

**Abbreviations and Acronyms:** CMH, Cochran–Mantel–Haenszel; GLP-1, glucagon-like peptide-1; OR, odds ratio.

**DOI:** 10.30802/AALAS-CM-23-000077

## Introduction

Chinchillas are a comparatively recent and specialized addition to the biomedical research community, only being established as a model for acoustic research in the late 1970s.[34] They have similarities to human auditory anatomy and arrangement with the benefit of large tympanic bullae. They are also easy to handle, have good reproductive success in a laboratory setting, and have long lifespans. Chinchillas historically have been used to study otitis media, noise-induced and chemical-induced hearing loss, atherosclerosis, cerebral vasculature, and reproduction.[10,11] Chinchillas are now predominantly used in acoustic and auditory research.[34] Chinchillas have been used for anatomic, physiologic, and behavioral studies in auditory science.[36] They have been used in hearing sensitivity and sound discrimination, auditory brainstem responses, otoacoustic emissions, awake auditory function, models for hearing loss, and pharmaceutical rescue and prevention studies.[33,36]

Chinchillas can prove challenging for investigators for many reasons. They are not as well characterized as other rodent models. There has been recent difficulty in their acquisition due to the limited number of stable vendors, leading to supply reductions. Wild South American chinchillas are considered endangered, by the International Union for the Conservation of Nature (IUCN) red list and listed in appendix I by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), largely due to habitat loss and decades of hunting for the fur trade industries.[7,11,19] Due to their endangered status, wild-caught chinchillas have not been used in modern research institutions. As they are a prey species that have adapted to hide illness and injury as a survival mechanism, it is difficult to reliably evaluate their health without prior training and experience. They have specialized gastrointestinal systems as hindgut fermenters, making their dietary and husbandry requirements demanding.[34] Chinchillas require a diet higher in fiber content than most of our other rodent species. They also require dust bathing to reduce oil buildup and to maintain a healthy coat. Chinchillas are particularly sensitive to developing heat stress

*Submitted: 08 Nov 2023. Revision requested: 22 Nov 2023. Accepted: 26 Mar 2024.*
*[1]Division of Comparative Medicine, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and [2]Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma*
**Corresponding author. Email: wendy-r-williams@ouhsc.edu*

with temperatures >90 °F and humidity >60%.[11] Anesthetic procedures are risky, with a documented anesthetic-related mortality risk of >3.25%, almost double the risk of other rodent species (1.75%).[6] Hypothermia, hypoglycemia, subclinical and clinical disease, compromised airway, and inexperience with this species are documented as increased risk factors for chinchillas during anesthesia.[32]

At the authors' institution chinchillas are often used as a model for acoustic and auditory research studies, specifically blast-related injury, and repair. In more recent years, a number of these studies have explored potential treatments to aid in the prevention, management, or amelioration of hearing loss and other auditory injuries. Among these potential therapeutics, some studies have focused on liraglutide, an acylated glucagon-like peptide-1 (GLP-1) receptor agonist. GLP-1 receptors are found in the upper gastrointestinal tract, pancreatic islets, enteric visceral afferent nerves, CNS, and cardiovascular system. In the CNS, GLP-1 receptors are widely distributed throughout the brain and densely expressed in hypothalamic nuclei involved in regulation of appetite, such as the paraventricular and arcuate nuclei.

Liraglutide is an FDA-approved, long-acting treatment for type 2 diabetes mellitus and obesity in humans.[26,27] By increasing intracellular cAMP, liraglutide stimulates the release of insulin from pancreatic β-cells when blood glucose levels are elevated, decreasing glucagon secretion in a glucose-dependent manner and delaying gastric emptying.[26] GLP-1 agonists also increase β-cell glucose sensitivity and β-cell mass.[22,23] GLP-1 agonists have demonstrated protective effects in the enteroendocrine system, cardiovascular system, hepatic system, and nervous system in humans.[3,5,13,16,17,35] Liraglutide administration in Alzheimer and dementia rodent models has shown promising protective effects in the brain, including increased neurogenesis along with improvements in learning and memory.[1,14,15,29] In a traumatic brain injury study performed in rats, liraglutide treatment reduced cerebral edema and cortical neuronal injury while preserving the blood–brain barrier.[13] These previous studies suggest that GLP-1 agonists could show promising effects to ameliorate damage associated with trauma-induced auditory impairment involving the CNS and peripheral nervous system.

A research group at our institution sought to explore the potential benefits of liraglutide treatments in chinchillas after an auditory blast injury. The use of liraglutide in chinchillas had never previously been described in the literature, and this institution had no previous experience using liraglutide in this species. The study design evaluating the effects of this GLP-1 agonists also involved frequent animal handling, multiple lengthy anesthetic events, a blast-induced hearing injury, and multiple auditory hearing tests. This research group began to experience an unusually high mortality rate. Because this research group had years of experience performing auditory research, with similar procedures, using this species with mortality rates <12%, the study design was reevaluated for refinement opportunities. The health status of the animals was evaluated, husbandry practices were evaluated, anesthesia protocols were evaluated, monitoring and blast-induced hearing procedures were evaluated, and the source and purity of the GLP-1 agonist were evaluated. Owing to the complexity of the experimental design and unexpected mortality rate, changes were made to the following: animal vendor, the GLP-1 agonist manufacturer, and the anesthetic protocol (before, during, and after). However, the mortality rate remained higher than expected. In addition to the modifications aimed at reducing early loss during the duration of this project by the veterinary staff, a retrospective case-control study was performed with the hypothesis that liraglutide treatment is associated with an increased mortality rate in chinchillas when compared with liraglutide-naive animals. Only the animal records generated by the Laboratory Animal Resources staff and veterinarians were used for this retrospective analysis. All recorded factors were considered as potential variables associated with this mortality rate, including husbandry practices, preexisting conditions, anesthetic complications, liraglutide treatments, and pneumatic pressure procedures. Variables consistently provided throughout the study, such as husbandry practices and pneumatic pressure procedures, were not evaluated as contributing factors to early death. Variables that differed between sets of animals, specifically sex, vendor, protocol, refinement, and liraglutide treatment, were evaluated for associations with early death and as confounding variables to the main effects. After animal records were collated, we also analyzed trends in clinical characteristics to describe enhanced animal monitoring strategies, pathology, and identification of appropriate endpoints.

We anticipate that our findings will offer insight to investigators and other veterinary teams to improve the wellbeing of chinchillas used for a variety of biomedical research models.

## Materials and Methods

**Animals and housing.** All chinchilla care and procedures were performed according to federal regulations and institutional policies and approved by the University of Oklahoma Institutional Animal Care and Use Committee. Animals were obtained from 2 US commercial vendors and were acclimated for a minimum of 72 h after arrival to the animal facility. The diet, caging, bedding, and enrichment provided were in accordance with the *Guide for the Care and Use of Laboratory Animals*.[18] Chinchillas received ad libitum chinchilla chow 5M01 (Mazuri, Purina Mills, St. Louis, MO), timothy hay (Bio-Serv, Flemington, NJ), and tap water via water bottles. Chinchillas were single housed in 2 different caging systems: stainless steel suspended rabbit bank caging system (Bussey Products, Chicago, IL) and ventilated microisolation cages (Allentown Nexgen™ guinea pig 1800) with aspen shavings (P.J. Murphy Forest Products Sani Chips, Montville, NJ). Bedding, feeders, cage lids, and cages were changed at least once weekly. Chinchillas were provided with dust baths (Blue Beauty dust, Napa, CA) at least once a week. Temperature and humidity were monitored and recorded daily. Chinchillas were housed in a climate-controlled room that maintained temperatures in the range of 17 to 25 °C (63 to 77 °F) and relative humidity kept between 30% and 60%. Fluorescent lighting was on an automatic schedule to provide a 12-h light/12-h dark cycle.

In accordance with the approved animal care and use protocol, veterinary care of sick or injured animals was permitted and animal care was provided by the veterinary staff. Animals were provided with appropriate supportive care (fluid therapy, assisted feeding, nutritional supplementation, heat support, and gastrointestinal motility treatment), diagnostic testing, antibiotics, and pain medication. Humane endpoints included animals that exhibited neurologic illness without improvement or had acute neurologic signs with evidence of pain/distress, and animals that exhibited evidence of lethargy/dullness, inappetence, or pain/distress refractory to treatment. In addition, any animal with a condition that was considered to carry a poor prognosis, as indicated by the veterinarian, was humanely euthanized in accordance with the AVMA Guidelines on Euthanasia.

**Background experimental design.** Animals ($n = 150$) received a series of anesthetic events to assess hearing function prior to and after blast injury. In addition, some animals received

liraglutide treatment to study the potential to prevent or restore blast-induced hearing loss.[20] The blast chamber was a custom-built piece of equipment that exposed the whole body of the animal from rostrum to tail to emulated blast waves. Further experimental details have not been included in this document at the request of the research group. The experimental details omitted from this document do not pertain to the intended objectives of this retrospective analysis.

**Retrospective analysis terms defined.** More than 300 animal records were reviewed. The inclusion criteria were animal records belonging to animal use protocols that administered liraglutide to the experimental treatment group and did not administer liraglutide to the control group. The inclusion criteria narrowed the sample for the retrospective analysis to the records of 150 animals. Data were analyzed in a retrospective case-control study of 150 adult chinchillas (>1 y of age). Demographic and clinical characteristics of these animals are reported in Table 1. Animal records used in this analysis span between the years 2017 and 2020.

For analysis, outcome was early death, defined as an animal that died or was euthanized prior to the scheduled experimental endpoint. The variable of interest was liraglutide and the covariables were sex, vendor, protocol, and refinement. Records used in this retrospective study met the following inclusion criteria: collected from 2017 to 2020, used chinchillas in one of the 2 experimental protocols, and had complete datasets for the variables analyzed (Table 2). Animal records that did not include information about the outcome, variable of interest, and one or more of the covariables were excluded from the retrospective analysis. Each variable was analyzed in a dichotomous manner.

Two formulations of liraglutide were used during this study (246.7 µg/kg/d SC, compounding pharmacy or Novo Nordisk, Plainsboro, NJ). Liraglutide treatment was defined as animals that received at least one dose of liraglutide treatment; those not given liraglutide treatment were defined as no treatment. Sex was defined as male or female. We sourced animals from 2 unique US vendors that are referred to as vendor A and vendor B. Protocol was defined as protocol 1 or protocol 2, describing 2 experimental animal protocols. Each experimental animal protocol followed a different anesthetic regimen. This resulted in 2 anesthetic regimens; however, the experimental design remained almost identical. The differences in anesthetic regimens included the following: animals in protocol 1 received an injectable anesthetic consisting of ketamine (25 to 40 mg/kg SC, Henry Schein, Dublin, OH) and xylazine (3 to 10 mg/kg SC, Akorn, Lake Forest, IL) for anesthetic events during 14 experimental days. Animals in protocol 2 received ketamine and xylazine as previously described for the first anesthetic event, then isoflurane (1% to 5%, Covetrus, Dublin, OH) delivered by face mask for each procedure thereafter. Protocol 2 was designed with experimental endpoints ranging between 7 and 28 d from the start of experimental manipulation.

Animals were euthanized using the previously described ketamine and xylazine doses followed by administration of Euthasol (390 mg of pentobarbital/50 mg of phenytoin sodium IC, Virbac, Fort Worth, TX) for tissue harvest. Anesthetic modifications were incorporated during the 4-y study period; however, refinement defined as the covariable was a standardized anesthetic protocol developed by the veterinarians that was instituted during a specific time of the most recent study protocol and included modifications to fasting periods, preanesthetic preparations, anesthetic doses, intraprocedural monitoring, and specific postprocedural supportive care. As part of the refinement, anesthetic monitoring was performed by qualified veterinary staff.

**Retrospective clinical abnormalities.** Data for abnormal clinical signs were collected from the 2017 through 2020 medical records ($n = 34$) and organized into 1 of 6 categories for analysis: neurologic abnormalities, labored breathing, lethargy, decreased appetite or decreased fecal output, weight loss (weight loss >25 g), and otitis. Due to the ambiguous presentation of clinical signs in this species and small number of clinical abnormalities captured in the medical records, these data are not included in the statistical analysis; however, they are included in results for completeness.

**Table 1.** Summary of cohort characteristics

| Category | *n* (%) |
|---|---|
| Age | |
| Adults >1 y | 150 (100) |
| Sex | |
| Female | 57 (38) |
| Male | 93 (62) |
| Vendor[a] | |
| A | 21 (14) |
| B | 129 (86) |
| Protocol[b] | |
| 1 | 58 (38.7) |
| 2 | 92 (61.3) |
| Refinement[c] | |
| No refinement | 103 (68.7) |
| Refinement | 47 (31.3) |
| Liraglutide | |
| No treatment | 24 (16) |
| Early death[d] | |
| Early death | 45 (30) |
| Nonearly death | 105 (70) |

The number and percentage of chinchillas are categorized by age, outcome (early death, nonearly death), variable of interest (liraglutide, no treatment), and covariables (sex, vendor, protocol, refinement).

[a]Animals came from 1 of 2 commercial breeders Moulton Chinchilla Ranch, MN (2017 to 2018) and Ryerson Chinchilla Ranch, OH (2018 to 2020).

[b]Protocol 1 used injectable anesthetic of ketamine/xylazine and was performed over 14 experimental days. Protocol 2 used ketamine/xylazine for the first anesthesia and then gas anesthetic for each procedure thereafter. Protocol 2 was designed with experimental endpoints at 7, 14, or 28 d.

[c]Refinement began in 2020 when the laboratory animal resource department developed a standard operating procedure for chinchilla anesthesia and provided direct anesthesia support during procedures.

[d]Early deaths were defined as an animal that died or was euthanized prior to the scheduled experimental endpoint.

**Table 2.** Variable of interest and covariables used in the retrospective study

| Year study was conducted | 2017 through 2020 | |
|---|---|---|
| Treated with liraglutide (variable of interest) | Yes | No |
| Sex (covariable) | Female | Male |
| Vendor (covariable) | Vendor A | Vendor B |
| Protocol (covariable) | Protocol 1 | Protocol 2 |
| Refinement (covariable) | Yes | No |

**Retrospective histopathology abnormalities.** Necropsy tissue collections were prepared and processed through the Histology Core Laboratories in the Department of Cell Biology at The University of Oklahoma Health Sciences Center. Histopathology was reviewed and interpreted by a board-certified veterinary pathologist. A limited number of histopathology examinations ($n = 16$) were performed on animals that received liraglutide treatment and experienced early death from protocol 2 that met the inclusion criteria, except one animal that was used for colony surveillance and to establish apparently normal, no exposure (liraglutide, pneumatic pressure) data for histopathologic comparison. This animal received one dose of ketamine and xylazine as well as isoflurane prior to euthanasia, as previously described. For the remaining 15 animals, each abnormality was counted by the major organ system, then percentages of the total abnormalities were calculated.

**Retrospective statistical analysis.** Statistical analysis was performed using SAS software, version 9.4 of the SAS System for UNIX (SAS Institute, Cary, NC). Descriptive statistics, odds ratios (ORs), a Fisher exact test, a Breslow–Day test, and a Cochran–Mantel–Haenszel (CMH) test were used to evaluate the independence, statistical association, covariable interaction, confounders, and significance of the outcome in relation to the variable of interest, respectively. Univariate analyses were performed before using the CMH test as a series of two-by-two tables, one per stratum, to determine whether the two-by-two effect persisted across the strata. Statistical significance was defined as a $P$ value ≤0.05.

For analysis, the outcome of early death was defined as early death or no early death. Liraglutide, sex, vendor, protocol, and refinement were examined as potential predictor variables. All of the subjects included in the analysis had complete datasets for the variables analyzed.

## Results

**Liraglutide treatment increases the risk of early death.** There was an unexpectedly high rate of early death (30%, 45/150) in the cohort of adult chinchillas (>1 y) over a 4-y period. As the cohort was heterogeneous in both demographic and experimental variables (Table 1), we first assessed whether sex, vendor, protocol, refinement, or liraglutide treatment was associated with early death. In total, 31.6% of female chinchillas experienced early death, and 29% of male chinchillas experienced early death; 15% of vendor A chinchillas experienced early death, and 32.6% of vendor B chinchillas experienced early death; 31% of protocol 1 chinchillas experienced early death, and 29.3% of protocol 2 chinchillas experienced early death; 23.4% of refinement chinchillas experienced early death, and 33% of no refinement chinchillas experienced early death; and 34.1% of liraglutide treatment chinchillas experienced early death, and 8.3% of no liraglutide treatment chinchillas experienced early death (Table 1, Figure 1). We found that the proportion of early death was roughly 4-fold higher in animals that had received liraglutide (43/126) compared with those that did not (2/24; OR = 5.69 [95% CI: 1.28 to 25.38]; Figure 1). No other variable was significantly associated with early death (Fisher exact $P$ values: 0.12 to 0.85).

We then assessed whether the association of liraglutide with early death persisted across strata of sex, vendor, protocol, and refinement by 2 methods. When each additional variable was controlled for by the CMH test (Table 3), the effect size of the association between liraglutide and early death remained both fairly constant (OR: 5.06 to 5.78) as well as statistically significant. In addition, risk of early death as a function of liraglutide treatment across strata of each covariant (sex, vendor, protocol, refinement) was homogeneous by the Breslow–Day test ($P > 0.05$). Our findings indicate that liraglutide treatment

**Table 3.** Risk of early death with liraglutide remains constant when adjusted for potential confounding variables

| | OR (95% CI) | $P$ value |
|---|---|---|
| Liraglutide alone | 5.69 (1.28–25.38) | 0.01* |
| Liraglutide controlling for sex | 5.71 (1.28–25.48) | 0.01* |
| Liraglutide controlling for vendor | 5.06 (1.15–22.37) | 0.01* |
| Liraglutide controlling for protocol | 5.78 (1.29–25.85) | 0.01* |
| Liraglutide controlling for refinement | 5.16 (1.17–22.7) | 0.01* |

When assessed by the Cochran–Mantel–Haenszel test, the strength of the association of liraglutide treatment with early death was comparable (OR: 5.06 to 5.78). OR, odds ratio.
*$P < 0.05$, indicating that the effect of liraglutide on risk of early death remained strong after adjusting for the variable listed.

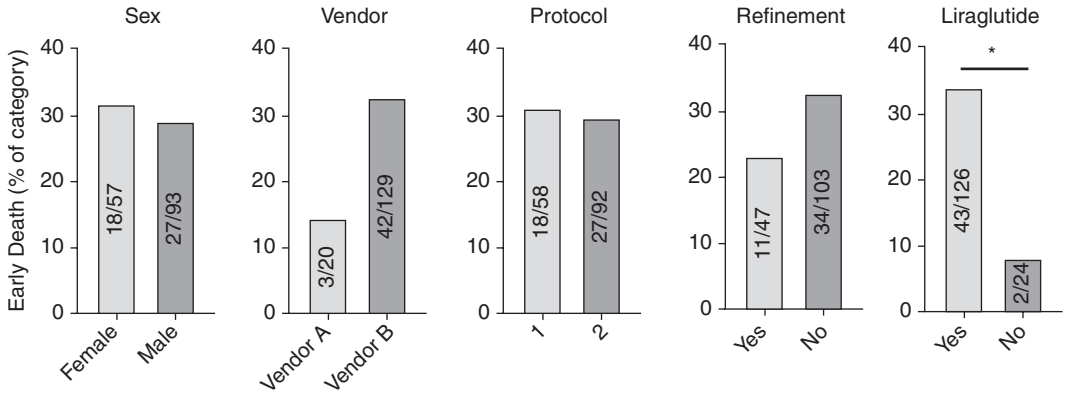


**Figure 1.** Liraglutide treatment is associated with early death. The proportion of animals that died early were compared across sex, vendor, protocol, intervention, and liraglutide treatment by a Fisher exact test. Animals that received liraglutide ($n = 126$) were more likely to die early ($n = 43$) than those that did not receive liraglutide (*, $P = 0.01$ by Fisher exact test). No other variables were associated with early death. The number of animals that experienced early death as a fraction of all animals in each group are indicated within bars. Refinement was defined as a standardized anesthetic protocol developed by the veterinarians that was instituted during a specific time of the most recent study protocol and included modifications to fasting periods, preanesthetic preparations, anesthetic doses, intraprocedural monitoring, and specific postprocedural supportive care. As part of the refinement, anesthetic monitoring was performed by qualified veterinary staff.

increases the risk of early death regardless of other demographic and experimental variables evaluated in this retrospective study.

**Clinical and histopathology findings.** Of the detailed clinical medical records collected from 2017 to 2020, there were 34 of 150 animals with documented abnormal clinical signs. These data are not included in the statistical analysis; however, they are included in results for completeness. The most observed clinical signs in this group included weight loss, lethargy, labored breathing, neurologic abnormalities, decreased appetite or decreased fecal output, and otitis externa (Table 4).

Histopathological examination was performed on a smaller subset ($n = 15$) of the 150 animals evaluated in the retrospective study. The animals with histopathology records included a mix of animals that were and were not documented to have abnormal clinical signs before early death. Abnormalities were most commonly found in the liver, lung, heart, kidney, pancreas, and spleen of these animals (Table 5). The most common pathology findings were hepatic steatosis ($n = 8$), bronchopneumonia ($n = 7$), pancreatitis ($n = 6$), and right ventricular cardiomyopathy ($n = 5$). Primary lesions observed included mild to marked diffuse hepatocellular vacuolation, marked congestion with inflammation and foreign vegetable material in the lungs, renal corticomedullary tubules with heavy influx of inflammatory cells, multifocal acute pancreatic necrosis with adjacent peritoneum/mesenteric fat necrosis and peritonitis, right ventricular dilation, splenic blood-filled medullary sinusoids, and epicardium/visceral pleura infiltrated by a neoplastic process. The chinchilla sacrificed for diagnostic testing was noted to have moderate to marked congestion in the liver with minimal evidence of steatosis, moderate to marked pulmonary congestion with minimal vascular leakage, and moderate to marked renal congestion. These histopathology changes are consistent with pathology appreciated as postmortem changes in animals euthanized with barbiturates.[12] Clinical and pathologic findings were too sparsely collected to infer any statistical associations.

**Table 4.** Frequency of clinical signs in chinchillas with detailed medical records ($n = 34$)

| Clinical signs[a] | *n* (%) |
|---|---|
| Weight loss | 12 (35.3) |
| Lethargy | 11 (32.4) |
| Labored breathing | 10 (29.4) |
| Neurologic abnormalities[b] | 7 (20.6) |
| Decreased appetite or decreased fecal output | 6 (17.6) |
| Otitis | 4 (11.8) |

[a]Of the animals with at least one clinical sign, most received treatment with liraglutide ($n = 33$).
[b]Neurological abnormalities included ataxia, seizure signs, stroke signs.

**Table 5.** Frequency of major organs with abnormal histopathology in chinchillas ($n = 15$)

| Histopathology | *n* (%) |
|---|---|
| Liver | 11 (73.3) |
| Lung | 8 (53.3) |
| Heart | 8 (53.3) |
| Kidney | 7 (46.7) |
| Pancreas | 6 (40) |
| Spleen | 5 (33.3) |

## Discussion

The intention of this retrospective analysis was to determine factors associated with this study's high early mortality rate in chinchillas. A number of potential contributing factors were examined, including anesthetic complications, husbandry, preexisting conditions, liraglutide exposure, and pneumatic pressure procedures. Liraglutide alone was associated with early death, and the association was not altered when controlling for sex, vendor, protocol, or refinement. The odds of exposure to liraglutide were almost 6-fold higher in animals that experienced early death compared with animals that did not experience early death. The results of the retrospective analysis support our hypothesis that treatment of chinchillas with liraglutide is associated with early mortality.

Although we have tried to explore the data by isolating and comparing the covariables, it is unrealistic to think that we have identified all of the possible covariables that could be influencing the outcome used in this retrospective study. One variable we were unable to fully eliminate as a potential covariable was the different formulations of liraglutide. The liraglutide used in the first protocol was compounded and the liraglutide used in the second protocol was an FDA-approved formulation. According to the *Guide for the Care and Use of Laboratory Animals*, pharmaceutical-grade substances should be used when available. Nonpharmaceutical grade substances can have impurities, as well as differences in pH, osmolality, and stability that can affect compatibility and efficacy. Because the formulation change coincided with the protocol change, we chose not to publish liraglutide formulation as a covariable. Some details were unavailable, or unknowable based on the nature of our retrospective approach. Prospective studies can be superior at identifying and controlling for variables expected to influence the study outcomes.

On histopathology ($n = 15$), the primary diagnosis observed included one or more of the following: hepatic steatosis, aspiration and nonaspiration bronchopneumonia, tubulointerstitial nephritis, acute necrotizing pancreatitis with peritonitis, congestive heart failure, congestive splenomegaly, and pericardial mesothelioma. The tubulointerstitial nephritis could generally be attributed to several causes, including bacterial or viral septicemia and toxicosis.[37] Histopathology suggested that the animals that developed tubulointerstitial nephritis experienced bacterial septicemia. Most of the splenic pathology consisted of congestive splenomegaly, which is a common finding in animals that have received ketamine or been given barbiturates.[2,4] Dilated cardiomyopathy, congenital septal defects, and valvular disease have all been previously reported in chinchillas[24]; however, there is no comprehensive literature characterizing the prevalence of cardiovascular conditions in chinchillas. The heart abnormalities found in this study are likely due to preexisting conditions in the animals and are not a direct effect of the experimental procedures. Pericardial mesothelioma was also considered a preexisting condition and is not thought to be associated with any experimental manipulation. Genetic and environmental differences might have had an impact on the overall health status of the animals being studied. Our animal program did not have genetic lineages of the animals received from the vendors nor did we perform genetic testing to further explore the genetic variations. Nevertheless, genetic differences between the animals received from each vendor may have influenced the outcome of this retrospective study, but we were unable to fully assess what effect it may have had.

We postulate that bronchopneumonia, hepatic steatosis, and pancreatitis are pathologic abnormalities directly associated

with the experimental manipulations administered during the study.

Liraglutide is documented to delay gastric emptying and decrease overall gastrointestinal motility in people,[26,27] which can lead to ileus and regurgitation. Delayed gastric emptying, along with general anesthesia that relaxes the gastric sphincters, can lead to aspiration of gastric materials into the lungs.[8,25] Liraglutide's mechanism of action and potential for aspiration pneumonia was the catalyst for a portion of the anesthesia refinements employed by the veterinary team. Chinchillas are hindgut fermenters that rely on the symbiotic relationship of gut bacteria (Gram-positive and anaerobic organisms) for digestion, nutrient absorption, and energy balance.[11] Liraglutide has been shown to decrease food intake in rodents due to the animals experiencing nausea.[21] In 2012, the Kanoski et al. study demonstrated that liraglutide produces nausea directly through CNS GLP-1 receptor activation, enabled by its ability to easily pass through the blood-brain barrier.[21]

The mechanism responsible for the high early mortality rates in chinchillas associated with liraglutide administration is postulated to have stemmed from a sequela of anorexia. We suspect that nausea and delayed gastric emptying played a role in the clinical presentation of anorexia. This knowledge presents an opportunity to reduce primary clinical signs by using preventative measures. There are 3 potential preventatives that could address the causes of anorexia: antiemetics, promotility medication, and supportive supplemental nutrition. This approach would address anorexia from 3 different aspects, that is, before, during, and after nausea and delayed gastric emptying occur. Regrettably, antiemetics and promotility therapies have not been consistently successful in their application in chinchillas. Further studies will need to be performed to demonstrate improved efficacy of these medications in chinchillas. However, theoretically there are several classes of drugs that could be used to provide nausea relief based on applications in other species. These medications include antihistamines, serotonin antagonists, neurokinin receptor antagonists, dopamine antagonists, benzodiazepines, and cannabinoids.[28,30] There are also several classes of drugs that could be used to help gastric motility, including cholinergic antagonists, dopamine antagonists, serotonergic agonists, and macrolides.[30] Supplemental nutrition can be offered in the form of diet gels, finely ground commercial formulas, and finely ground custom-made formulas. In theory, many of these applications may or may not be effective, but ultimately treatment options would need to be carefully chosen to reduce confounding effects to the research being performed.

For future studies evaluating the therapeutic potential of liraglutide in chinchillas we recommend the development of a clinical assessment rubric to help in guiding clinical care since signs can be both rare and subtle. The clinical assessment rubric could be supportive when evaluating the health of chinchillas being treated with liraglutide; however, chinchillas are adept at hiding illness, and therefore more invasive diagnostics may be needed to properly assess for interventions. Although we think that a clinical assessment rubric would help support animal welfare and improve experimental translation, it is not the only tool that can be relied on to evaluate the overall health and wellbeing of the chinchillas; diagnostic imaging (radiography, ultrasound, CT) and bloodwork should also be employed. Diagnostic imaging could be used to evaluate thoracic and abdominal structures. Radiographs could help assess the heart, lungs, liver, and gastrointestinal structures. Ultrasound could provide quick, real-time imaging to evaluate the liver, pancreas, and gastrointestinal motility and to perform an echocardiogram. Bloodwork would assess the hematologic and biochemical values. Some hematologic and biochemistry changes could include the following: dehydration (erythrocytosis, hyperalbuminemia, azotemia), hepatic steatosis (hyperglycemia, hyperproteinemia, elevated ALT, elevated sorbitol dehydrogenase), gastrointestinal inflammation (thrombocytosis or thrombocytopenia, leukocytosis, heterophilia, hyperproteinemia), renal insufficiency (azotemia, hyperproteinemia), and pancreatitis (leukocytosis, heterophilia, elevated ALP, elevated GGT).[9,31] This retrospective analysis did not include hematology or serum biochemistry analysis, in part because clinical presentation was rare and clinical progression was rapid. Bloodwork may have provided additional objective data to evaluate organ and systemic health. Future chinchilla studies may benefit by incorporating diagnostic bloodwork and imaging for monitoring purposes due to a lack of consistent clinical signs.

Research uses known information to explore different possibilities to relieve a pathologic condition or change a disease outcome. Part of the journey involves using new techniques, therapies, and animal models. It is difficult to predict how an animal study using these new approaches will progress. During this study, inferences were made about the cause of the illnesses, pathology, and early deaths. However, it was only possible to put the whole picture of what was happening together through retrospective analysis of all the different factors present. In short, observed clinical signs and histopathology demonstrate pathophysiological effects that are consistent with the mechanism of action of liraglutide, and the effects documented in other species, much of which is from human data. This retrospective case-control study illustrates the clinically significant impact that liraglutide had on the systemic health of chinchillas. It has also revealed a number of different approaches we can take to help reduce the mortality rate in future experimental exploration of GLP-1 agonists in chinchilla animal models.

## Acknowledgments

We thank Stanley D. Kosanke, DACVP, DACLAM for expert veterinary pathology interpretations as well as for time and effort spent consulting on historical pathology reports.

## Conflict of Interest

The author(s) have no conflict(s) of interest to declare.

## Funding

This work was internally funded.

## References

1. **Abd El-Rady NM, Ahmed A, Abdel-Rady MM, Ismail OI.** 2021. Glucagon-like peptide-1 analog improves neuronal and behavioral impairment and promotes neuroprotection in a rat model of aluminum-induced dementia. Physiol Rep **8**:e14651.
2. **Auger M, Fazio C, Swarte M, Bussières G, Schaefer D, Springer CM.** 2019. Administration of certain sedative drugs is associated with variation in sonographic and radiographic splenic size in healthy cats. Vet Radiol Ultrasound **60**:717–728.
3. **Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M.** 2008. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon like peptide 1 receptor-dependent and -independent pathways. Circulation **117**:2340–2350.
4. **Berry S.** 2015. Veterinary anesthesia and analgesia. Lumb and Jones 5th ed. Ames (IA): Wiley-Blackwell.
5. **Briyal S, Shah S, Gulati A.** 2014. Neuroprotective and anti-apoptotic effects of liraglutide in the rat brain following focal cerebral ischemia. Neuroscience **281**:269–281.

6. **Brodbelt DC, Blissitt KJ, Hammond RA, Neath PJ, Young LE, Pfeiffer DU, Wood JL.** 2008. The risk of death: The confidential enquiry into perioperative small animal fatalities. Vet Anaesth Analg **35**:365–373.
7. **CITES.** [Internet]. 2022. Appendices I, II and III. [Cited 17 October 2022]. Available at: https://cites.org/eng/app/appendices.php.
8. **Cotton BR, Smith G.** 1984. The lower oesophageal sphincter and anaesthesia. Br J Anaesth **56**:37–46.
9. **DeCubellis J.** 2016. Common emergencies in rabbits, guinea pigs, and chinchillas. Vet Clin North Am Exot Anim Pract **19**: 411–429.
10. **Fish R, Medicine ACoLA, Danneman PJ, Brown M, Karas A.** 2008. Anesthesia and analgesia in laboratory animals. New York (NY): Elsevier.
11. **Fox JG, Anderson LC, Otto G, Pritchett-Corning KR, Whary MT.** 2015. Laboratory animal medicine. San Diego (CA): Elsevier.
12. **Grieves JL, Dick EJ Jr, Schlabritz-Loutsevich NE, Butler SD, Leland MM, Price SE, Schmidt CR, Nathanielsz PW, Hubbard GB.** 2008. Barbiturate euthanasia solution-induced tissue artifact in nonhuman primates. J Med Primatol **37**:154–161.
13. **Hakon J, Ruscher K, Romner B, Tomasevic G.** 2015. Preservation of the blood brain barrier and cortical neuronal tissue by liraglutide, a long acting glucagon-like-1 analogue, after experimental traumatic brain injury. PLoS One **10**:e0120074.
14. **Han WN, Hölscher C, Yuan L, Yang W, Wang XH, Wu MN, Qi JS.** 2013. Liraglutide protects against amyloid-β protein-induced impairment of spatial learning and memory in rats. Neurobiol Aging **34**:576–588.
15. **Hansen HH, Barkholt P, Fabricius K, Jelsing J, Terwel D, Pyke C, Knudsen LB, Vrang N.** 2016. The GLP-1 receptor agonist liraglutide reduces pathology-specific tau phosphorylation and improves motor function in a transgenic hTauP301L mouse model of tauopathy. Brain Res **1634**:158–170.
16. **Hussein AM, Eid EA, Taha M, Elshazli RM, Bedir RF, Lashin LS.** 2020. Comparative study of the effects of GLP1 analog and SGLT2 inhibitor against diabetic cardiomyopathy in type 2 diabetic rats: Possible underlying mechanisms. Biomedicines **8**:43.
17. **Insuela DBR, Carvalho VF.** 2017. Glucagon and glucagon-like peptide-1 as novel anti-inflammatory and immunomodulatory compounds. Eur J Pharmacol **812**:64–72.
18. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
19. **International Union for Conservation of Nature.** [Internet]. 2015. Red list of endangered species. Available at: https://www.iucnredlist.org/species/4652/117975205.
20. **Jiang S, Welch P, Sanders S, Gan RZ.** 2022. Mitigation of hearing damage after repeated blast exposures in animal model of chinchilla. J Assoc Res Otolaryngol **23**:603–616.
21. **Kanoski SE, Rupprecht LE, Fortin SM, De Jonghe BC, Hayes MR.** 2012. The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide. Neuropharmacology **62**:1916–1927.
22. **Lee YS, Lee C, Choung JS, Jung HS, Jun HS.** 2018. Glucagon-Like peptide 1 increases β-cell regeneration by promoting α- to β-cell transdifferentiation. Diabetes **67**:2601–2614.
23. **Lee YS, Shin S, Shigihara T, Hahm E, Liu MJ, Han J, Yoon JW, Jun HS.** 2007. Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. Diabetes **56**:1671–1679.
24. **Linde A, Summerfield NJ, Johnston M, Melgarejo T, Keffer A, Ivey E.** 2004. Echocardiography in the chinchilla. J Vet Int Med **18**:772–774.
25. **McGrath JP, McCaul C, Byrne PJ, Walsh TN, Hennessy TPJ.** 1996. Upper oesophageal sphincter function during general anaesthesia. Br J Surg **83**:1276–1278.
26. **Novo Nordisk.** [package insert]. Victoza (liraglutide) injection. Available at: https://www.google.com/search?q=victoza+%28liraglutide%29+injection+package+insert&sca_esv=1b590d2ad2d1828a&sca_upv=1&rlz=1C1GCEB_enUS1043US1043&sxsrf=ACQVn0-91KN0O58rVMUZ892OA0juM6caTg%3A1712698689187&ei=QbUVZryEC7W90PEPiM-A2AQ&ved=0ahUKEwj8qOfni7aFAxW1HjQIHYgnAEsQ4dUDCBA&uact=5&oq=victoza+%28liraglutide%29+injection+package+insert&gs_lp=Egxnd3Mtd2l6LXNlcnAiLnZpY3RvemEgKGxpcmFnbHV0aWRlKSBpbmplY3Rpb24gcGFja2FnZSBpbnNlcnRIAFAAWABwAHgBkAEAmAEAoAEAqgEAuAEDyAEAmAIAoAIAmAMAkgcAoAcA&sclient=gws-wiz-serp.
27. **Novo Nordisk.** Saxenda 6 mg/mL solution for injection in pre-filled pen [package insert]. Available at: https://www.google.com/search?q=Saxenda+6+mg%2FmL+solution+for+injection+in+pre-filled+pen+package+insert&sca_esv=1b590d2ad2d1828a&sca_upv=1&rlz=1C1GCEB_enUS1043US1043&sxsrf=ACQVn09epis6rcByX5V2jdZx65zwCaVGCg%3A1712698807117&ei=t7UVZv_gBoCd0PEP4LKVqAg&ved=0ahUKEwi_mIWgjLaFAxWADjQIHWBZBYUQ4dUDCBA&uact=5&oq=Saxenda+6+mg%2FmL+solution+for+injection+in+pre-filled+pen+package+insert&gs_lp=Egxnd3Mtd2l6LXNlcnAiR1NheGVuZGEgNiBtZy9tTCBzb2x1dGlvbiBmb3IgaW5qZWN0aW9uIGluIHByZS1maWxsZWQgcGVuIHBhY2thZ2UgaW5zZXJ0SNYbUKoGWKMZcAF4AJABAJgB5AKgAfITqgEIMC4xMy4yLjG4AQPIAQD4AQGYAgagAqgIwgIKEAAYRxjWBBiwA8ICBhAAGBYYHsICCxAAGIAEGIoFGIYDwgIHECEYChigAcICBBAhGBWYAwCIBgGQBgiSBwcxLjMuMS4xoAexPQ&sclient=gws-wiz-serp.
28. **Parker LA, Rock EM, Limebeer CL.** 2011. Regulation of nausea and vomiting by cannabinoids. Br J Pharmacol **163**:1411–1422.
29. **Parthsarathy V, Hölscher C.** 2013. Chronic treatment with the GLP1 analogue liraglutide increases cell proliferation and differentiation into neurons in an AD mouse model. PLoS One **8**:e58784.
30. **Plumb DC, ed.** 2018. Plumb's Veterinary Drug Handbook: Desk 9th ed. Stockholm, WI: Wiley-Blackwell.
31. **Quesenberry K, Mans C, Orcutt C, Carpenter JW.** 2020. Ferrets, rabbits, and rodents: Clinical medicine and surgery. Philadelphia (PA): Elsevier.
32. **Saunders R, Harvey L.** 2012. Anaesthesia and analgesia in chinchillas. In Practice **34**:34–43.
33. **Shao N, Jiang S, Younger D, Chen T, Brown M, Rao KVR, Skotak M, Gan RZ, Chandra N.** 2021. Central and peripheral auditory abnormalities in chinchilla animal model of blast-injury. Hear Res **407**:108273.
34. **Suckow MA, Stevens KA, Wilson RP.** 2012. The laboratory rabbit, guinea pig, hamster, and other rodents. San Diego (CA): Elsevier.
35. **Tølbøl KS, Kristiansen MN, Hansen HH, Veidal SS, Rigbolt KT, Gillum MP, Jelsing J, Vrang N, Feigh M.** 2018. Metabolic and hepatic effects of liraglutide, obeticholic acid and elafibranor in diet-induced obese mouse models of biopsy-confirmed nonalcoholic steatohepatitis. World J Gastroenterol **24**:179–194.
36. **Trevino M, Lobarinas E, Maulden AC, Heinz MG.** 2019. The chinchilla animal model for hearing science and noise-induced hearing loss. J Acoust Soc Am **146**:3710.
37. **Zachary JF.** 2017. Pathologic basis of veterinary disease, 6th ed. St. Louis (MO): Elsevier.