PEER REVIEW HISTORY

eGastroenterology publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Endocrine Pathology in Young Cystic Fibrosis Rabbits
AUTHORS	Liang, Xiubin; Hou, Xia; Chen, Y. Eugene; Jin, Jian-Ping; Zhang, Kezhong; Xu, Jie

VERSION 1 - REVIEW

REVIEWER NAME	Norris, Andrew W.
REVIEWER AFFILIATION	N/A
REVIEWER CONFLICT OF INTEREST	I have no competing interests. A portion of my ongoing research is performed in competing models, but I have no related financial or employment stakes.
DATE REVIEW RETURNED	21-Jun-2024

GENERAL COMMENTS	This manuscript reports on pancreas related histology and function in a rabbit model of cystic fibrosis. This is an important subject to report, given that rodent models of cystic fibrosis do not recapitulate the pancreatic disease found in human cystic fibrosis. The results are generally straightforward, showing that this genetic rabbit model of cystic fibrosis demonstrates a degree of pancreatic histological pathology and some exhibit mild glucose intolerance. However, there are shortcomings in interpretation and reporting of the results. Major * The manuscript implies that glucose intolerance is related to a primary loss of beta-cells. However, the data do not establish this. An example of an alternative explanation could be that the smaller beta-islets are secondary to malabsorption (for example of this adaptive phenomena see PMID: 1451950). Since the CF rabbits are indeed underweight (reference #14), the small islets may indeed be an adaptive response to malabsorption. If the authors can show that the CF rabbits with glucose intolerance are the ones with the smallest islets, then perhaps the argument could be made that the small islets are related to glucose intolerance. This logic also applies for the lower insulin levels - this may be adaptive to the malnutrition in the rabbits. By definition, given the normal fasting blood sugar levels in the CF animals, the insulin level is appropriate and not "low". In fact, glucose intolerance can be a consequence of malnutrition in humans (e.g. PMID: 807787) and animals (PMID: 1451950). For this reason, it is suggested that (a) the manuscript report the weights of the CF versus WT rabbits used to assess pancreas histology and also for tests of glucose and insulin; (b) in the absence of data showing correlation between small islets and glucose intolerance, that causal statements linking the two be removed. * More details regarding morphometrics are needed. What portion of

	the pancreas was sampled? In figures 1 and 2, it is stated that these are representative images, but the numbers of independent animals sampled needs to be stated. * The pathology exhibited by the CF rabbits appears less severe and perhaps patchier than that seen in many / most CF humans. But details about age and maturation can impact this interpretation, as human pancreatic pathology in CF progresses over several years. For this reason, the age at time of examination should be detailed for all presented data. Also, the maturation timeline of rabbits should be mentioned at some point in the manuscript such as weaning age and age of sexual maturity. Some discussion about severity compared to human CF would be beneficial. Other * The title wording is imprecise, as it seems the manuscript is studying "pediatric endocrine disorders", but CFRD is a disease that is more common in adults. The authors probably meant something where "pediatric" modifies the age of the animals studied, perhaps along these lines: "Young rabbits exhibit endocrine pathology in a model of cystic fibrosis". * Lines 54 - 58 & 233: The text implies that rabbit research costs are substantially less than ferrets. This is misleading, as in many academic animal facilities, ferret and rabbit per diem costs are similar, and in fact are identical at many institutions. For example, at the authors' Wayne State Univ, per their current website, ferret per diem is $5.00 and rabbit is $4.15. Pigs and sheep are more expensive at $15.78 and rats much less so at $1.34. * Line 119: How do the authors know this material is "mucus"? Maybe a more precise word should be used, such as "eosinophilic material". * Methods: The diet of the CF and WT rabbits should be detailed. * Figure 3: Legend text uses INGET, whereas figure and text use INDET. This should be harmonized. * Lines 161-167: The WTs should be similarly stratified. Show the worst 4 versus best 6. * Elastase assay results should be mentioned in results, not just first mentioned in discussion. * It appears that fatty tissue replacement in the pancreas was not observed. This is a common finding in human CF pancreas. This should be noted in discussion. * Was insulin not measured during GTTs? * Figure 2C: are the discrete points from separate animals, or just represent different slides from the same animal? * Figure 2D: Details of N for the graph need to be stated. * Line 89: What methodology does the A1c Now+ meter use to measure A1c? Some methods of A1c measurement will not translate well between species. * More needs to be detailed about this specific CFTR mutant. Does it exhibit impaired chloride transport? How would it be classified in terms of CFTR mutation classes? If these details are not known, this should be explicitly stated. * Table 2: should differentiate between "unknown" and "N" (meaning known not to occur). Currently, both of these situations appear to be represented by blanks. * Some refs are missing page numbers (e.g. ref# 13 & 14)

REVIEWER NAME	Liu, Yu
REVIEWER AFFILIATION	Nanjing Medical University
REVIEWER CONFLICT OF INTEREST	This group and authors previously reported on the development of a cystic fibrosis rabbit model and characterization of disease

	manifestations including obstructive bowel disease, liver histopathological changes, and abnormal airway epithelium (JCI Insight, 2021;6:e139813; PNAS Nexus, 2022;2:pgac306; JCI Insight, 2024;9:e165826). The current study is a follow-up description of pancreatic morphology and endocrine phenotypic dysfunction in the CF rabbit model. The authors described pancreatic histological alterations and endocrine abnormalities in CF rabbits, including decreased insulin levels and impaired glucose metabolism. This work will be valuable for gastrointestinal and cystic fibrosis researchers.
DATE REVIEW RETURNED	22-Jun-2024

GENERAL COMMENTS	This manuscript is well-written and detailed, and the results are well-supported by relevant data. I have only a few minor comments. 1.The authors reported that juvenile CF rabbits exhibit pancreatic endocrine abnormalities and that CF rabbits spontaneously develop pancreatic lesions at an early age. It would be beneficial to include a definition and reference for the terms "young age" and "juvenile" in relation to rabbits in the introduction or discussion section. 2.Since blood glucose was tested at 0, 10, 20, 30, 60, 90, and 120 minutes, the IVGTT results in Figs. 3B, 3C, 3D, and supplementary Fig. 2 suggest using 30 for the major ticks' interval of the X-axis rather than 50. 3.For IVGTT data, the time point that has a statistically significant difference should be noted with a * or p value.

REVIEWER NAME	Yang, Ling
REVIEWER AFFILIATION	The University of Iowa Roy J and Lucille A Carver College of Medicine, Department of Anatomy and Cell Biology
REVIEWER CONFLICT OF INTEREST	No conflict of interest.
DATE REVIEW RETURNED	02-Jul-2024

GENERAL COMMENTS	Previously, the authors have developed a CF rabbit model by CRISPR/Cas9 mediated gene editing. Here, the author characterized the exocrine and endocrine pancreatic morphology, and assessed glucose and insulin homeostasis in the juvenile CF-9 rabbit. They found that in comparison to the wildtype animals, CF rabbits have pancreatic tissue damage characterized by inflammation and fibrosis, vascular degeneration, epithelium mucus secretory cell metaplasia, and pancreatic duct dilation. These exocrine abnormalities were associated with reduced area of the pancreatic islets. Systematically, the authors showed that young CF rabbits present early onset glucose intolerance and reduced insulin levels. This study is significant as it provides a novel animal model to investigate CF-related pancreatic pathologies. Given the fact that CF is associated with systemic metabolic disruption and the exocrine pancreas controls nutrient absorption, it would be important to investigate the body weight and food intake of these CF rabbits at different ages. Indeed, the authors previously reported that CF rabbit have growth retardation. The question is whether the reduced islet size in CF rabbit contribute to the small size of pancreas/body. Moreover, the authors should consider performing GSIS to directly evaluate pancreatic insulin secretion in vivo in CF and WT animals or using isolated islets from these rabbits. Lastly, the author claimed that there is substantial islet loss in CF rabbits. Apoptotic or cell death markers should be provided to support this conclusion.

	Specific comments: 1. Could the author discuss the higher inflammatory markers in younger CF rabbit compared to older ones in (Table 1)? Is there correlation between the inflammatory status with compromised glucose metabolism juvenile CF rabbits? 2. In Figure 1, what are the major cell type of these immune cells? 3. In Figure 2, the authors should provide cell death/apoptotic markers. 4. In Figure 3, could the authors provide ITT result? An in vivo or in vitro GSIS analysis could be included in this experimental setting.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1 (R1)

R1.1. The manuscript implies that glucose intolerance is related to a primary loss of beta-cells. However, the data do not establish this. An example of an alternative explanation could be that the smaller beta-islets are secondary to malabsorption (for example of this adaptive phenomena see PMID: 1451950). Since the CF rabbits are indeed underweight (reference #14), the small islets may indeed be an adaptive response to malabsorption. If the authors can show that the CF rabbits with glucose intolerance are the ones with the smallest islets, then perhaps the argument could be made that the small islets are related to glucose intolerance. This logic also applies for the lower insulin levels - this may be adaptive to the malnutrition in the rabbits. By definition, given the normal fasting blood sugar levels in the CF animals, the insulin level is appropriate and not "low". In fact, glucose intolerance can be a consequence of malnutrition in humans (e.g. PMID: 807787) and animals (PMID: 1451950). For this reason, it is suggested that (a) the manuscript report the weights of the CF versus WT rabbits used to assess pancreas histology and also for tests of glucose and insulin; (b) in the absence of data showing correlation between small islets and glucose intolerance, that causal statements linking the two be removed.

Response: Thank you for the insightful comments. We agree that our data do not support that the loss of beta-cells is the primary cause for the observed glucose intolerance phenotype. Our findings show that the CF rabbits have a decreased insulin level, reduced insulin secretion, and an indeterminate glucose tolerance (INDET) phenotype. We rewrote the part about the small islets and glucose intolerance in the revised manuscript. In particular, we changed the session title “Islet loss in the pancreas of CF rabbits” in the Results to “Islet morphology in the pancreas of CF rabbits” [line #161 in the clean copy]

R1.2. More details regarding morphometrics are needed. What portion of the pancreas was sampled? In figures 1 and 2, it is stated that these are representative images, but the numbers of independent animals sampled needs to be stated.

Response: The anatomy of the rabbit pancreas is different from that of humans. The marjory of Rabbit pancreas is the mesenteric type that is diffusely distributed in the mesentery of small intestine. In the present work, we collected the whole mesenteric part of pancreas in rabbits. We added this to the revised Methods. [line #82 in the clean copy]

For the n numbers for Figure 1 and 2, we have included the numbers of independent animals in the Figure legends. We also updated Figure 2D in which each dot represents one animal.

R1.3. The pathology exhibited by the CF rabbits appears less severe and perhaps patchier than that seen in many / most CF humans. But details about age and maturation can impact this interpretation, as human pancreatic pathology in CF progresses over several years. For this reason, the age at time of examination should be detailed for all presented data. Also, the maturation timeline of rabbits should be mentioned at some point in the manuscript such as

weaning age and age of sexual maturity. Some discussion about severity compared to human CF would be beneficial.

Response: Thank you for the suggestions. We now provided age information for all animals in the results. In Figure 1 and 2, all data were obtained from post-mortem animals. The ages of the animals are provided in Table 1. In Figure 3, the age information is provided in the text. [line #180 to 204 in the clean copy].

We also added a sentence of discussion about observed severity of the CF rabbit pancreatic pathology vs that of other CF animal models. [line #248 in the clean copy]

R1.4. The title wording is imprecise, as it seems the manuscript is studying "pediatric endocrine disorders", but CFRD is a disease that is more common in adults. The authors probably meant something where "pediatric" modifies the age of the animals studied, perhaps along these lines: "Young rabbits exhibit endocrine pathology in a model of cystic fibrosis".

Response: Thank you. We changed the title to: “Young rabbits exhibit endocrine pathology in a model of cystic fibrosis”.

R1.5. Lines 54 - 58 & 233: The text implies that rabbit research costs are substantially less than ferrets. This is misleading, as in many academic animal facilities, ferret and rabbit per diem costs are similar, and in fact are identical at many institutions. For example, at the authors' Wayne State Univ, per their current website, ferret per diem is $5.00 and rabbit is $4.15. Pigs and sheep are more expensive at $15.78 and rats much less so at $1.34.

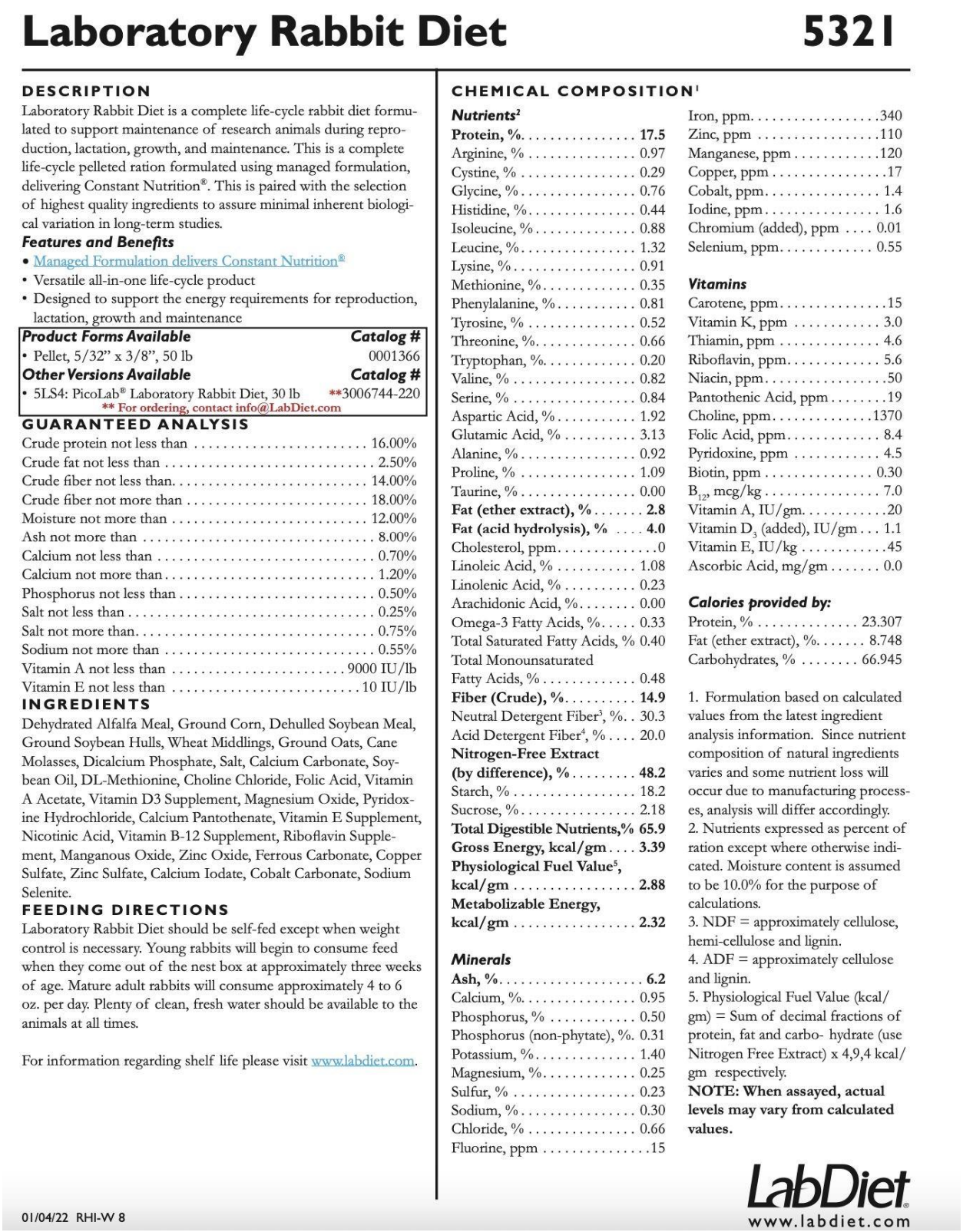
Response: Thank you. We wanted to say that ferret, in comparison to rabbit, is a relatively uncommon species and as a result it needs special skills of handling. We modified the text in the revision to avoid the confusion. [line #54 in the clean copy]

R1.6. Line 119: How do the authors know this material is "mucus"? Maybe a more precise word should be used, such as "eosinophilic material".

Response: Thanks. We agree to use “eosinophilic material” and have modified the text and figure lengend in the revised manuscript.

R1.7. Methods: The diet of the CF and WT rabbits should be detailed.
Response: We have included the diet information (Labdiet #5321) in the revised methods. Detailed composision of the diet is available at the manufactuer’s website.
https://www.labdiet.com/product/detail/5321-laboratory-rabbit-diet

A screenshot is provided here.



Laboratory Rabbit Diet 5321

DESCRIPTION

Laboratory Rabbit Diet is a complete life-cycle rabbit diet formulated to support maintenance of research animals during reproduction, lactation, growth, and maintenance. This is a complete life-cycle pelleted ration formulated using managed formulation, delivering Constant Nutrition®. This is paired with the selection of highest quality ingredients to assure minimal inherent biological variation in long-term studies.

Features and Benefits

- Managed Formulation delivers Constant Nutrition®
- Versatile all-in-one life-cycle product
- Designed to support the energy requirements for reproduction, lactation, growth and maintenance

Product Forms Available	***Catalog #***
• Pellet, 5/32" x 3/8", 50 lb	0001366
Other Versions Available	***Catalog #***
• 5LS4: PicoLab® Laboratory Rabbit Diet, 30 lb	**3006744-220

** For ordering, contact info@LabDiet.com

GUARANTEED ANALYSIS

Crude protein not less than	16.00%
Crude fat not less than	2.50%
Crude fiber not less than	14.00%
Crude fiber not more than	18.00%
Moisture not more than	12.00%
Ash not more than	8.00%
Calcium not less than	0.70%
Calcium not more than	1.20%
Phosphorus not less than	0.50%
Salt not less than	0.25%
Salt not more than	0.75%
Sodium not more than	0.55%
Vitamin A not less than	9000 IU/lb
Vitamin E not less than	10 IU/lb

INGREDIENTS

Dehydrated Alfalfa Meal, Ground Corn, Dehulled Soybean Meal, Ground Soybean Hulls, Wheat Middlings, Ground Oats, Cane Molasses, Dicalcium Phosphate, Salt, Calcium Carbonate, Soybean Oil, DL-Methionine, Choline Chloride, Folic Acid, Vitamin A Acetate, Vitamin D3 Supplement, Magnesium Oxide, Pyridoxine Hydrochloride, Calcium Pantothenate, Vitamin E Supplement, Nicotinic Acid, Vitamin B-12 Supplement, Riboflavin Supplement, Manganous Oxide, Zinc Oxide, Ferrous Carbonate, Copper Sulfate, Zinc Sulfate, Calcium Iodate, Cobalt Carbonate, Sodium Selenite.

FEEDING DIRECTIONS

Laboratory Rabbit Diet should be self-fed except when weight control is necessary. Young rabbits will begin to consume feed when they come out of the nest box at approximately three weeks of age. Mature adult rabbits will consume approximately 4 to 6 oz. per day. Plenty of clean, fresh water should be available to the animals at all times.

For information regarding shelf life please visit www.labdiet.com.

CHEMICAL COMPOSITION[1]

Nutrients[2]	
Protein, %	**17.5**
Arginine, %	0.97
Cystine, %	0.29
Glycine, %	0.76
Histidine, %	0.44
Isoleucine, %	0.88
Leucine, %	1.32
Lysine, %	0.91
Methionine, %	0.35
Phenylalanine, %	0.81
Tyrosine, %	0.52
Threonine, %	0.66
Tryptophan, %	0.20
Valine, %	0.82
Serine, %	0.84
Aspartic Acid, %	1.92
Glutamic Acid, %	3.13
Alanine, %	0.92
Proline, %	1.09
Taurine, %	0.00
Fat (ether extract), %	**2.8**
Fat (acid hydrolysis), %	**4.0**
Cholesterol, ppm	0
Linoleic Acid, %	1.08
Linolenic Acid, %	0.23
Arachidonic Acid, %	0.00
Omega-3 Fatty Acids, %	0.33
Total Saturated Fatty Acids, %	0.40
Total Monounsaturated Fatty Acids, %	0.48
Fiber (Crude), %	**14.9**
Neutral Detergent Fiber[3], %	30.3
Acid Detergent Fiber[4], %	20.0
Nitrogen-Free Extract (by difference), %	**48.2**
Starch, %	18.2
Sucrose, %	2.18
Total Digestible Nutrients,%	**65.9**
Gross Energy, kcal/gm	**3.39**
Physiological Fuel Value[5], kcal/gm	**2.88**
Metabolizable Energy, kcal/gm	**2.32**

Minerals	
Ash, %	**6.2**
Calcium, %	0.95
Phosphorus, %	0.50
Phosphorus (non-phytate), %	0.31
Potassium, %	1.40
Magnesium, %	0.25
Sulfur, %	0.23
Sodium, %	0.30
Chloride, %	0.66
Fluorine, ppm	15
Iron, ppm	340
Zinc, ppm	110
Manganese, ppm	120
Copper, ppm	17
Cobalt, ppm	1.4
Iodine, ppm	1.6
Chromium (added), ppm	0.01
Selenium, ppm	0.55

Vitamins	
Carotene, ppm	15
Vitamin K, ppm	3.0
Thiamin, ppm	4.6
Riboflavin, ppm	5.6
Niacin, ppm	50
Pantothenic Acid, ppm	19
Choline, ppm	1370
Folic Acid, ppm	8.4
Pyridoxine, ppm	4.5
Biotin, ppm	0.30
B_{12}, mcg/kg	7.0
Vitamin A, IU/gm	20
Vitamin D_3 (added), IU/gm	1.1
Vitamin E, IU/kg	45
Ascorbic Acid, mg/gm	0.0

Calories provided by:	
Protein, %	23.307
Fat (ether extract), %	8.748
Carbohydrates, %	66.945

1. Formulation based on calculated values from the latest ingredient analysis information. Since nutrient composition of natural ingredients varies and some nutrient loss will occur due to manufacturing processes, analysis will differ accordingly.
2. Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purpose of calculations.
3. NDF = approximately cellulose, hemi-cellulose and lignin.
4. ADF = approximately cellulose and lignin.
5. Physiological Fuel Value (kcal/gm) = Sum of decimal fractions of protein, fat and carbo- hydrate (use Nitrogen Free Extract) x 4,9,4 kcal/gm respectively.

NOTE: When assayed, actual levels may vary from calculated values.

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R1.8. Figure 3*:* Legend text uses INGET, whereas figure and text use INDET. This should be harmonized.

Response: Sorry for the typo. We changed the legend text in the revised manuscript.

R1.9. Lines 161-167: The WTs should be similarly stratified. Show the worst 4 versus best 6. Response: Thank you. Following your suggestion, we dividied the WT animals into two groups: the worst 4 (worst-4-WT) vs. the best 6 (best-6-WT). The AUC values of the CFRD-like CF animals are still higher than those of the worst-4-WT (and the best-6-WT). We added this data in the new Supplementary Figure 3C.

R1.10. Elastase assay results should be mentioned in results, not just first mentioned in discussion.

Response: Agreed and moved the elastase assay results to the Results section.

R1.11. It appears that fatty tissue replacement in the pancreas was not observed. This is a common finding in human CF pancreas. This should be noted in discussion.

Response: Thank you for your suggestions. We indeed observed the fatty infiltration in exocrine pancreatic tissue of one CF rabbit (1 out 5, 20%) with severe phenotypes, similar to what has been described in CF patients (Radiology 1999; 210:611-615) and CF pigs (J Pathol 2016; 238: 311-320). We also note that this phenotype is similarly reported in a CF ferret study as a late stage (Phase III) CF pancreatic lesion, where much of the exocrine pancreas is replaced by adipocytes in (PMID: 29366680). We have included this data in the updated new Supplementary Figure 2.

R1.12. Was insulin not measured during GTTs?
Response: No, we did not. This is because the GTT assay was performed using test strips with a glucose monitor , therefore no blood serum samples were collected. We added this as one technical pitfall of the current study in the Discussion. [line #278 in the clean copy]

R1.13. Figure 2C: are the discrete points from separate animals, or just represent different slides from the same animal?

Response: The discrete points represent separate animals in Figure 2C.

R1.14. Figure 2D: Details of N for the graph need to be stated.
Response: It is provided in the revision.

R1.15. Line 89: What methodology does the A1c Now+ meter use to measure A1c? Some methods of A1c measurement will not translate well between species.

Response: The methodology of A1c meter is provided in the revised Methods. [line #101 in the clean copy]

We also added a caution about the potential cross-reactivity issue related to the A1c assay kit.

R1.16. More needs to be detailed about this specific CFTR mutant. Does it exhibit impaired chloride transport? How would it be classified in terms of CFTR mutation classes? If these details are not known, this should be explicitly stated.

Response: The animals carry CFTR-9 mutation. This mutation is predicted to be a Class II mutation, similar to the CFTR-F508del mutation. We included this information in the revision. [line #275 in the clean copy]

R1.17. Table 2: should differentiate between "unknown" and "N" (meaning known not to occur). Currently, both of these situations appear to be represented by blanks.

Response: We updated Table 2 in the revision following your suggestion.

R1.18. Some refs are missing page numbers (e.g. ref# 13 & 14)
Response: We added the missing page numbers to the references including ref#13 and 14.

Reviewer #2 (R2)
This manuscript is well-written and detailed, and the results are well-supported by relevant data. I have only a few minor comments.
R2.1. The authors reported that juvenile CF rabbits exhibit pancreatic endocrine abnormalities and that CF rabbits spontaneously develop pancreatic lesions at an early age. It would be beneficial to include a definition and reference for the terms "young age" and "juvenile" in relation to rabbits in the introduction or discussion section.

Response: Thank you. We added the definition of “young age” in this revision. [line #72 in the clean copy]

R2.2. Since blood glucose was tested at 0, 10, 20, 30, 60, 90, and 120 minutes, the IVGTT results in Figs. 3B, 3C, 3D, and supplementary Fig. 2 suggest using 30 for the major ticks’ interval of the X-axis rather than 50.

Response: We greatly appreciate reviewer’s suggestion. We have revised the figures following your suggestions.

R2.3. For IVGTT data, the time point that has a statistically significant difference should be noted with a * or p value.

Response: We have included p values for each time point in the IVGTT figures.

Reviewer #3 (R3)
Previously, the authors have developed a CF rabbit model by CRISPR/Cas9 mediated gene editing. Here, the author characterized the exocrine and endocrine pancreatic morphology, and assessed glucose and insulin homeostasis in the juvenile CF-9 rabbit. They found that in

comparison to the wildtype animals, CF rabbits have pancreatic tissue damage characterized by inflammation and fibrosis, vascular degeneration, epithelium mucus secretory cell metaplasia, and pancreatic duct dilation. These exocrine abnormalities were associated with reduced area of the pancreatic islets. Systematically, the authors showed that young CF rabbits present early onset glucose intolerance and reduced insulin levels.

This study is significant as it provides a novel animal model to investigate CF-related pancreatic pathologies. Given the fact that CF is associated with systemic metabolic disruption and the exocrine pancreas controls nutrient absorption, it would be important to investigate the body weight and food intake of these CF rabbits at different ages. Indeed, the authors previously reported that CF rabbit have growth retardation. The question is whether the reduced islet size in CF rabbit contribute to the small size of pancreas/body. Moreover, the authors should consider performing GSIS to directly evaluate pancreatic insulin secretion in vivo in CF and WT animals or using isolated islets from these rabbits. Lastly, the author claimed that there is substantial islet loss in CF rabbits. Apoptotic or cell death markers should be provided to support this conclusion.

Specific comments:

R3.1. Could the author discuss the higher inflammatory markers in younger CF rabbit compared to older ones in (Table 1)? Is there correlation between the inflammatory status with compromised glucose metabolism juvenile CF rabbits?

Response: Thank you for this insightful question. The animals were euthanized when they became moribund. So the two animals of young ages were in fact the animals that suffered the most severe CF phenotypes (at an early age). This could have contributed to the observed higher levels of inflammatory markers in these two younger CF rabbits compared to older ones in Table

1. We included a discussion of this in the revision. [line #282 in the clean copy]

R3.2. In Figure 1, what are the major cell type of these immune cells?

Response: The primary type of inflammatory cells are Heterophils (the name of Neutrophils in rabbits). We added this in the revised Results section. [line #144 in the clean copy]

R3.3. In Figure 2, the authors should provide cell death/apoptotic markers.

Response: Overll there are very small number of cells that appear to be apoptotic as observed in the HE staining images, in both CF and WT sections. Therefore we did not conduct IF staining for apoptotic markers in the present work. This is likely reflecting the young age of the animals used in the current work. Follow up work on determining the extent of apoptotic deaths in the pancreatic tissues in CF vs WT animals of older animals are warranted. We included a discussion of this point in the revision. [line #287 in the clean copy]

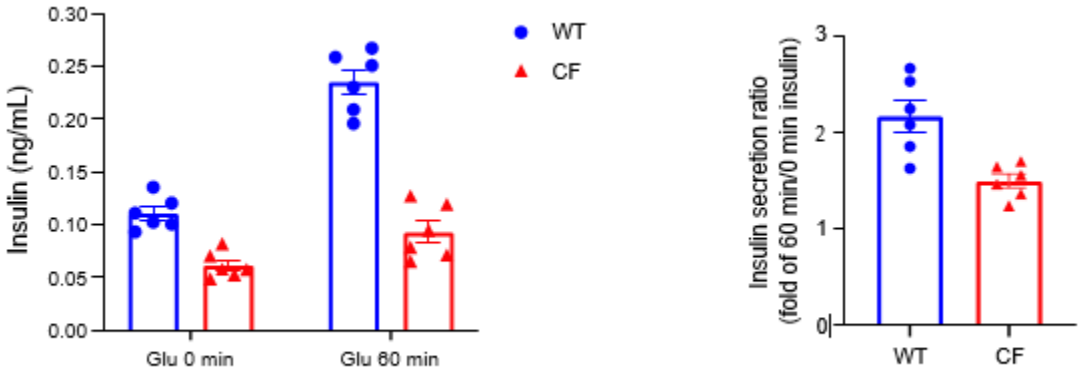
R3.4. In Figure 3, could the authors provide ITT result? An in vivo or in vitro GSIS analysis could be included in this experimental setting.

Response: In the present work, we did not do ITT. In an earlier work that we published in 2022 (PNAS Nexus, PMID: 36712930), the ITT results suggest that young CF rabbits do not suffer from insulin resistance despite the overall lower insulin levels.

With regard to GSIS, we unfortunately don't have blood samples at 10, 20, 30 min after glucose stimulation because we used only trace amount of blood samples in the IVGTT assay (also see response to R1.12). We only have sufficient serum samples at 0 min and 60 min after glucose stimultation. Using these samples, we compared the insulin levels and relative fold-change of insuline levels at 0 min vs 60 min in WT and CF rabbits.

The data suggest that (i) CF rabbits have lower insulin level compared with WT rabbits; (ii) CF rabbits secrete less insulin in response to glucose stimulation; and (iii) the fold-change data (60 min/0 min) also indicate that CF rabbits have impaired glucose-stimulated insulin secretion (GSIS), which is consistent

with the report in CF patient (DOI:10.1016/j.jcf.2016.05.005; PMID: 27289198). We have included this new data in Supplementary Figure 3A and B.



VERSION 2 - REVIEW

REVIEWER NAME	Norris, Andrew W.
REVIEWER AFFILIATION	N/A
REVIEWER CONFLICT OF INTEREST	I have no competing interests.
DATE REVIEW RETURNED	12-Aug-2024

GENERAL COMMENTS	The critiques have been reasonably addressed and just a few points remain. A major limitation of the work is that the assessment of insulin secretion is superficial. Insulin secretion is a complex process not always well captured by measurement of serum levels at two time-points during an IVGTT. This limitation needs to be better transmitted in the discussion. Recommend adding to discussion that the insulin secretion phenotyping is limited, and that deeper phenotyping such as examination of first phase secretion, hyperglycemic clamp, other insulin secretagogues, and ex vivo studies could yield additional insights. Minor suggestion: For Table 1: Table legend ideally should make clear the meaning of + and -. Most readers will be able to figure it out, but safer to specify. Typo: Page 15, top line: "severe" not "server"

REVIEWER NAME	Liu, Yu
REVIEWER AFFILIATION	Nanjing Medical University
REVIEWER CONFLICT OF INTEREST	There are no competing interest of this manuscript.
DATE REVIEW RETURNED	20-Aug-2024

GENERAL COMMENTS	There are no more comments to the author.

VERSION 2 – AUTHOR RESPONSE

Point by point response to egastro-2024-100102.R1- "Young rabbits exhibit endocrine pathology in a model of cystic fibrosis"

Reviewer #1 (R1)

The critiques have been reasonably addressed and just a few points remain.

R1.1. A major limitation of the work is that the assessment of insulin secretion is superficial. Insulin secretion is a complex process not always well captured by measurement of serum levels at two time-points during an IVGTT. This limitation needs to be better transmitted in the discussion. Recommend adding to discussion that the insulin secretion phenotyping is limited, and that deeper

phenotyping such as examination of first phase secretion, hyperglycemic clamp, other insulin secretagogues, and ex vivo studies could yield additional insights.

Response: Thank you for this insightful comment. We have included these points in the revised Discussion. [line 281-289 in the clean copy].

R1.2. Minor suggestion: For Table 1: Table legend ideally should make clear the meaning of + and -. Most readers will be able to figure it out, but safer to specify.

Response: Thank you. We added definition of +/- signs in the revision.

R1.3. Typo: Page 15, top line: "severe" not "server".
Response: Corrected. Thank you.

Reviewer #2 (R2)

Comments to the Author

There are no more comments to the author.