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Posgrado en Biología Experimental

“Cambios histológicos e inflamatorios en la vesícula biliar por el consumo crónico de una dieta alta en colesterol”

TESIS

Que para obtener el grado de

MAESTRA EN BIOLOGÍA EXPERIMENTAL

P R E S E N T A

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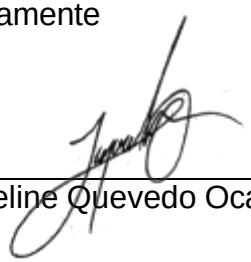

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Miembros del jurado

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Versión abreviada en español

Las enfermedades del hígado ocupan actualmente el cuarto lugar como causa de muerte en México, solo atrás de las enfermedades del corazón, la diabetes mellitus y las neoplasias malignas acorde al INEGI (https://www.inegi.org.mx/contenidos/saladeprensa/boletines/2024/EDR/EDR2023_En-Jn.pdf). Llama la atención que las 4 principales causas de muerte en el país estén estrechamente relacionadas con características distintivas, una de ellas es el componente metabólico, las cuatro causas de muerte tienen en común un metabolismo aberrante.

No es difícil comprender que los desórdenes metabólicos tienen como componente además de la genética, el consumo de dietas no óptimas que pueden incluir, pero no limitarse, a niveles elevados de carbohidratos, particularmente fructosa, niveles elevados de grasas saturadas y colesterol. Este último tiene particular relevancia en nuestro país debido a que la dieta promedio del mexicano es alta en este lípido.

El colesterol, si bien es una molécula vital para el ser humanos, el exceso en el cuerpo tiene serios impactos negativos en la salud.

Es bien sabido el papel de colesterol en la formación de membranas plasmáticas, en la formación de sales biliares y hormonas. Pero su naturaleza química lo hace particularmente difícil de manejar en un ambiente altamente hidrofílico. Adicionalmente, la biosíntesis, por medio de la ruta del mevalonato, es energéticamente muy cara y compleja, por lo que el sistema en mamíferos ha optado por obtenerla en la dieta dejando muy reprimida la síntesis *de novo* en las células humanas, con excepción de gónadas, donde la síntesis de novo se mantiene activa.

Existen desordenes genéticos relacionados con una síntesis elevada del colesterol, como la hipercolesterolemia familiar hereditaria, sin embargo, el

consumo elevado en la dieta representa un problema aún más serio dado al consumo creciente de este lípido en alimentos, como se ha mencionado.

El exceso en el consumo de colesterol puede tener la ventaja de ser controlado solo con medidas dietéticas, es decir, disminuyendo significativamente el consumo de alimentos de origen animal o incrementando el consumo de otros alimentos que coadyuven a su disminución como alimentos altos en fibras o en ácidos grasos de la familia omega 3 y 6.

Por otro lado, la desventaja es que los efectos dañinos del consumo elevado de alimentos altos en colesterol son silenciosos, no genera de manera directa obesidad, más que como un evento compensatorio para su manejo, elevando la síntesis de ácidos grasos, que se requieren para el empaquetamiento a nivel hepático, en proteínas de muy baja densidad (VLDL).

Adicionalmente, es bien sabido el efecto aterogénico del colesterol, con implicaciones a nivel del sistema circulatorio y cardiaco, pero que también impacta en otros órganos y sistemas.

En nuestro grupo de investigación hemos caracterizado intensamente los efectos tóxicos y dañinos del consumo de una dieta alta en colesterol a nivel hepático y pancreático.

Demostramos, que el consumo de una dieta alta en colesterol (HC) induce daño hepatocelular, particularmente a nivel de mitocondria, generando un decremento en ATP, estrés oxidante, pérdida en el potencial de membrana mitocondrial y cambios en la dinámica mitocondrial en general (Dominguez-Perez et al., 2019), por otro lado, animales de experimentación con esta dieta, indujeron igualmente estrés oxidante y en ratones deficiente de c-Met, el receptor del factor de crecimiento de hepatocitos (HGF) el daño fue mayor, induciendo un profundo cambio transcriptómicos indicando como principal consecuencia una colestasis intrahepática(Gomez-Quiroz et al., 2016), esto fue un primer indicio de un impacto severo a nivel de vías biliares y de la vesícula biliar.

Es bien sabido que la sobrecarga de grasa (Hernandez et al., 2015), particularmente el colesterol, potencia los efectos tóxicos de otros agentes como el alcohol (Lopez-Islas et al., 2016), o los metales pesados como el cadmio (Rosales-Cruz et al., 2018).

Como se ha mencionado el exceso de colesterol en los sistemas celulares induce estrés oxidante por un mecanismo dependiente de la mitocondria, esto puede en gran medida agravar el problema en el consumo crónico de colesterol, tal y como es en la dieta del mexicano. Adicionalmente se ha observado con dietas HC inducen procesos inflamatorios que pueden agravar aún más el daño oxidante (Simoni-Nieves et al., 2021)

Un escenario con gran infiltrado inflamatorio, estrés oxidante, y daño crónico puede condicionar a estados que derivan en transformación celular. Datos recientes de nuestro grupo comprueban que el consumo de una dieta HC induce procesos carcinogénicos a nivel (Enriquez-Cortina et al., 2017) que derivan en cambios transcriptómicos profundos indicando carcinomas con alta agresividad y mal pronóstico (Simoni-Nieves et al., 2021). El cuadró que se encontró se caracterizó por un gran infiltrado de macrófagos, particularmente tipo M2 o pro-tumorales, angiogénesis, activación de rutas inflamatorias que median a la activación de Stat3, que puede generar, además, resistencia a la muerte, como lo demostramos previamente (Dominguez-Perez et al., 2019)entre otros procesos.

Así pues, el colesterol de presenta como una seria complicación en el trato gastrointestinal y el hígado en particular.

Es bien sabido que el colesterol tiene rutas de excusión por ruta biliar, aunque la circulación enterohepática genera que más del 90% de las sales biliares regresen al hígado.

Nuestro grupo reportó que una dieta alta en colesterol en ratones conduce a cambios en la arquitectura histológica de la vesícula biliar, litiasis mínima e

inflamación, esto con el consumo de solo 2 días con una dieta HC con 2% de colesterol y 0.5% de colato de sodio.

La vesícula biliar es un órgano accesorio del hígado que tiene una presentación histológica similar al intestino. La vesícula biliar vista a bajo aumento tiene tres capas principales: la mucosa interna compuesto por un foro de células epiteliales columnares, la túnica muscular o lámina propria, y su capa externa de tejido conjuntivo llamada adventicia externa o serosa. La mucosa tiene muchos pliegues o rugosidades de la mucosa, pero no son lo suficientemente largos como para ser considerados vellosidades.

En aumentos mayores, se puede ver que el epitelio de la mucosa está revestido de células cilíndricas simples que se superponen a la lámina propia, que contiene tejido conjuntivo denso e irregular, muchas células inmunitarias y pequeños capilares.

Los pliegues de la mucosa de la vesícula biliar pueden tener un aspecto similar al de partes del intestino, como se ha dicho, pero pueden distinguirse por la ausencia de criptas intestinales en la base de los pliegues de la mucosa o células globet.

Algunos de los pliegues de la mucosa son lo suficientemente profundos como para dar el aspecto de puentes cruzados cuando se ven al microscopio. Es un buen marcador de daño la pérdida de esta arquitectura, como se demuestra en el presente trabajo.

Dado que la vesícula es el receptáculo de las salas biliares y el contenido de agentes xenobióticos que produce el hígado, así como del colesterol, es importante conocer los impactos en este órgano accesorio causado por el consumo de una dieta HC, de tal forma que permita comprender la razón por la cual de incrementan los casos de cáncer de vesícula (GBC) en México, para poder distinguir entre subtipos de tumores, y poder identificar marcadores de diagnóstico temprano. De esta forma pues, se pretende dar un primer paso hacia un problema aún mayor, que es el GBC.

Estos hallazgos, junto con lo que hemos reportado a nivel hepático, particularmente en cáncer nos han llevado a generar la siguiente hipótesis.

Hipótesis.

El consumo crónico de dietas altas en colesterol induce un proceso de inflamación en la vesícula biliar, que inducen daño y cambios morfológicos en el tejido epitelial.

Pregunta de investigación

¿El consumo crónico de colesterol dietético genera inflamación y cambios histológicos en la vesícula biliar?

Objetivos particulares

- Identificar los cambios morfológicos e histológicos a nivel de la vesícula biliar de ratones alimentados con una dieta rica en colesterol.
- Identificar cambios inflamatorios en la vesícula biliar de ratones alimentados con una dieta rica en colesterol.

Resultados

El presente proyecto tuvo como objetivo estudiar el efecto del consumo crónico de una dieta rica en colesterol sobre la vesícula biliar, estudiar los cambios celulares, tisulares y moleculares en este órgano, particularmente de una posterior expansión de este estudio al contexto del GBC. Se separaron 48 ratones C57BL/6 en machos y hembras, y cada cohorte se dividió en tres grupos para analizar la vesícula biliar en tres momentos diferentes (1, 3 y 8 meses) para abordar los cambios dependientes del tiempo en este tejido causados por el consumo. de una dieta alta en colesterol (2% colesterol). Presentaré y discutiré los principales hallazgos en cada momento.

Mes 1 de tratamiento

Los ratones, machos y hembras, mostraron un comportamiento normal sin cambios relevantes en los ratones.

Curiosamente, sólo los ratones HC macho mostraron un aumento notable de grasa visceral en el momento del sacrificio (Figura 3A). Se registró el peso semanal de los ratones y no se observaron diferencias estadísticas significativas (Figura 2B).

En ambos grupos, el hígado no presentó cambios en color, peso o tamaño (Figura 4 A-C).

Curiosamente, noté un grado de atrofia en la vesícula biliar en algunos animales, pero no se logró encontrar significancia estadística en el peso o la relación entre el peso de la vesícula biliar y el cuerpo (Figura 4 B-E).

Como no encontré diferencias en la observación macroscópica del hígado, quise saber si a nivel sérico se podía ver algún signo de daño hepático que explicara por qué la vesícula biliar aumentó de tamaño. El análisis del suero en los ratones macho no mostró diferencias estadísticas en GOT o AST, GPT o ALT, GGT, colesterol total (T-cho), HDL (HDL-Cho) y triglicéridos (Figura 5).

Aunque el aumento de la vesícula biliar podría estar relacionado con un aumento esperado en el flujo biliar, mi objetivo fue analizar el posible impacto histológico. Decidí realizar una inspección histológica de este tejido bajo tinción con hematoxilina-eosina.La mucosa en los ratones macho NT consta de un epitelio columnar simple y una lámina propia, con una presencia mínima de pliegues (Figura 6A). La histología es similar a la del epitelio normal de la vesícula biliar del ratón.

En particular, el grupo HC exhibió cambios significativos, incluido un marcado aumento en los pliegues en el epitelio columnar y alteraciones en el revestimiento epitelial (Figura 6; flechas amarillas; observadas a 630x). Estas alteraciones, observadas después de un mes de dieta HC, indican un daño celular potencial.

Los hallazgos sugirieron fuertemente una inflamación en curso. Me indicaron que analizara marcadores celulares de macrófagos y neutrófilos mediante inmunofluorescencia en la vesícula biliar. El análisis de microscopía (Figura 7A-

B) demostró una presencia significativa de células positivas para MPO (neutrófilos) en tejido obtenido de ratones tratados con una dieta HC, pero no con macrófagos (Figura 8).

Este resultado demuestra que, en las primeras etapas, la dieta HC induce respuestas inflamatorias en la vesícula biliar, representada por neutrófilos, pero no por macrófagos, en ratones macho.

El daño observado en la mucosa de la vesícula biliar sugiere una alteración en la adhesión entre células mediada por uniones estrechas. Para ganar más confianza, analicé la β-catenina por IF. Con respecto a los animales NT, encontré una deslocalización de β-catenina desde la membrana al citoplasma en ratones HC (Figura 9A y B). Luego, analizamos el área bajo la curva del análisis de barrido lineal y encontramos una mayor acumulación de β-catenina en ratones tratados con una dieta HC. Este resultado muestra que la dieta HC induce un desplazamiento de β-catenina después de un mes de tratamiento en ratones macho.

Evalué los parámetros bioquímicos séricos de las hembras. El análisis del suero no mostró diferencias estadísticas en ninguno de los aspectos evaluados (Figura 5).

El análisis microscópico de tinción H&E de la vesícula biliar femenina (Figura 10) reveló una estructura de tejido anormal. En comparación con las muestras NT, observé un incremento notable en el pliegue de la mucosa con crestas más prominentes. La lámina propia se engrosó y algunas áreas presentaron un crecimiento irregular de epitelio columnar, sugiriendo anaplasia (círculo amarillo).

También evaluamos si esta pérdida de arquitectura tisular estaba relacionada con un proceso inflamatorio, específicamente por reclutamiento de neutrófilos y macrófagos, asociado al infiltrado inmune en las enfermedades biliares. En ratones hembra, solo observamos reclutamiento de neutrófilos en el tejido de la vesícula biliar, como se ve en la Figura 11, sin respuesta de macrófagos en este momento del tratamiento (Figura 12).

Esto demuestra que la dieta HC induce respuestas inflamatorias a nivel de la vesícula biliar en ratones hembra y macho, pero esto está mediado por neutrófilos, como se reporta en otras enfermedades similares donde cristalizan compuestos químicos, en este caso el colesterol y otros, como la gota, el urato.

En conjunto, estos resultados muestran que la dieta HC induce una rápida respuesta en el tejido de la vesícula biliar que podría condicionar el desarrollo de tumores posteriormente. Por eso es necesario monitorear a estos ratones durante largos períodos para evaluar su progreso y encontrar algunos marcadores que puedan ser útiles en el ámbito clínico.

Mes 3 de tratamiento

Para continuar la investigación, analizamos otra cohorte bajo la dieta HC durante tres meses. Hallazgos similares en este momento se obtuvieron en la inspección macroscópica y el peso corporal en ratones macho (Figura 13), al igual que el hígado y la vesícula biliar; sin embargo, observé una disminución en el tamaño tanto en hembras como en machos, pero sin cambios significativos.

Una vez más, los parámetros bioquímicos no mostraron diferencias, con todas las pruebas bajo valores basales. Se estudiarán más animales para corroborar estos valores, ya que tres meses con esta dieta deberían presentar daño hepatocelular.

Sin embargo, esta investigación se centra en abordar los efectos sobre la vesícula biliar. La vesícula biliar masculina bajo la dieta HC durante tres meses indujo una disminución significativa en los pliegues del revestimiento epitelial columnar; casi no estaba presente, pero curiosamente había zonas de superposición en células con una arquitectura diferente, asemejándose a un epitelio escamoso (Figura 15), en comparación con los animales NT que exhiben un epitelio único y continuo. Revestimiento del epitelio columnar.

El IF de la MPO no reveló infiltración de neutrófilos en animales HC, pero la presencia de macrófagos fue notable en este momento (Figura 17)

En el caso de la hembra, el análisis H&E reveló un panorama completamente diferente. Las hembras a los tres meses de tratamiento exhibieron crestas de la mucosa prominentes y más gruesas en las hembras. También observé los mismos cambios en la arquitectura, lo que sugiere metaplasia probablemente inducida por un incremento en la proliferación y diferenciación celular.

Se realizarán más experimentos para confirmar la metaplasia y la proliferación. El análisis del infiltrado inflamatorio principal reveló un incremento considerable de neutrófilos, determinado mediante tinción con MPO (Figura 19), y macrófagos (Figura 20). Estos resultados indican claramente un empeoramiento de la inflamación y que, según los tipos celulares determinados como posibles, el estrés oxidativo podría estar en curso.

Mes 8 de tratamiento

En este momento del tratamiento, observé una notable deposición de grasa visceral tanto en hembras como en machos, pero fue más prominente en los machos. También observé cambios significativos en el peso corporal en machos HC a los seis meses de tratamiento (Figura 21), lo que indica un impacto crónico de la dieta HC en la salud de los ratones.

El hígado HC exhibió un color pálido en este momento, lo que indica esteatosis. Aun así, los pesos del hígado y la vesícula biliar y la relación vesícula biliar/hígado no fueron significativos debido a la pequeña cohorte del estudio (Figura 22).

El análisis de la tinción H&E reveló hallazgos notables en ratones macho. La vesícula biliar se contrajo, particularmente por un aumento significativo en la lámina. Observé regiones con superposición celular, probablemente metaplasia (círculo rojo), y áreas con pérdida de continuidad del revestimiento epitelial, sugestivas de daño celular (Figura 23).

A los ocho meses de tratamiento, el IF de β−catenina reveló una deslocalización significativa de esta proteína de la membrana plasmática, sugiriendo un estímulo

de reparación crónica que podría conducir a la probable metaplasia observada en la tinción H&E, este efecto fue más prominente en los machos.

Un ligero pero significativo incremento en los neutrófilos (Figura 25) marcó inflamación en este momento, pero en los machos, los macrófagos disminuyeron (Figura 26).

La tinción H&E mostró que las hembras presentaron menos daño que los machos (Figura 27); la lámina propia era considerablemente más gruesa con crestas aumentadas. El solapamiento de células, sugestivo de una metaplasia, se presenta a los ocho meses. Un incremento de neutrófilos y macrófagos marcó la inflamación en las hembras, esta última sin valores significativos (Figura 30).

Conclusión

Aunque el presente trabajo puede presentarse como una investigación simple en términos técnicos, es muy relevante y tiene implicaciones clínicas específicas. El consumo crónico de una dieta HC condiciona un proceso inflamatorio dinámico en el tiempo, marcado por neutrófilos y macrófagos, sin que ello signifique que otros tipos celulares, como los linfocitos, no estén implicados; esto es parte de las limitaciones del presente proyecto que serán saldadas en la siguiente etapa de la investigación.

La inflamación observada en el tejido de la vesícula biliar se relacionó con cambios significativos en la arquitectura histológica, no solo a nivel del epitelio columnar sino también en la lámina propia.

El consumo de una dieta HC conduce a procesos inflamatorios críticos y cambios morfológicos en la vesícula biliar que podrían formar metaplasia y la probable o eventual evolución de un GBC (Roa et al., 2022).

INTRODUCTION

According to the INEGI (https://www.inegi.org.mx/contenidos/saladeprensa/boletines/2024/EDR/EDR2023_En-Jn.pdf), liver diseases currently occupy fourth place as a cause of death in Mexico, only behind heart diseases, diabetes mellitus, and malignant neoplasms. It is striking that the country's four leading causes of death are closely related to distinctive characteristics; one of them is the metabolic component; the four causes of death have an aberrant metabolism in common.

It is not difficult to understand that metabolic disorders have as a component, in addition to genetics, the consumption of non-optimal diets that can include, but are not limited to, high levels of carbohydrates, particularly fructose, high levels of saturated fats, and cholesterol. The latter is particularly relevant in our country because the average Mexican diet is high in this lipid (Gomez-Quiroz & Roman, 2022).

Although cholesterol is a vital molecule for humans, excess in the body severely impacts health. The role of cholesterol in forming plasma membranes and creating bile salts and hormones is well known. However, its chemical nature makes it particularly difficult to handle in a highly hydrophilic environment. Additionally, through the mevalonate route, biosynthesis is energetically very expensive and complex, so the mammalian system has chosen to obtain it in the diet, leaving de novo synthesis in human cells repressed, except gonads, where de novo synthesis remains active.

There are genetic disorders related to high cholesterol synthesis, such as hereditary familial hypercholesterolemia(Arnold & Koenig, 2023); however, high

consumption in the diet represents an even more severe problem given the increasing consumption of this lipid in foods, as mentioned(Backes & Hilleman, 2024).

Excess cholesterol consumption can be controlled only with dietary measures, that is, significantly reducing the consumption of foods of animal origin or increasing the consumption of other foods that contribute to its reduction, such as foods high in fiber or in fatty acids of the omega 3 and 6 families(Castellanos-Tapia et al., 2020).

On the other hand, the disadvantage is that the harmful effects of high consumption of foods high in cholesterol are silent; it does not directly generate obesity other than as a compensatory event for its management, increasing the synthesis of fatty acids, which are required for packaging at the liver level, into very low-density proteins (VLDL)(van Zwol et al., 2024).

The atherogenic effect of cholesterol is well known, with implications at the circulatory and cardiac systems level, but it also impacts other organs and systems.

In our research group, we have intensely characterized the toxic and harmful effects of consuming a diet high in cholesterol in the liver and pancreas (Dominguez-Perez et al., 2016; Dominguez-Perez et al., 2019; Enriquez-Cortina et al., 2017; Lopez-Islas et al., 2016).

We demonstrate that the consumption of a diet high in cholesterol (HC) induces hepatocellular damage, particularly at the mitochondrial level, generating a decrease in ATP, oxidant stress, loss in mitochondrial membrane potential, and changes in mitochondrial dynamics in general (Dominguez-Perez et al., 2016), on the other hand, experimental animals with this diet also induced oxidative stress. The damage was more significant in mice deficient in c-Met, the hepatocyte growth factor (HGF) receptor, causing a profound change. Transcriptomics indicates intrahepatic cholestasis is the main

consequence(Gomez-Quiroz et al., 2016); this was the first indication of a severe impact on the bile ducts and gallbladder.

Fat overload (Hernandez et al., 2015), particularly cholesterol, enhances the toxic effects of other agents, such as alcohol (Lopez-Islas et al., 2016) or heavy metals, such as cadmium (Rosales-Cruz et al., 2018).

As mentioned, excess cholesterol in cellular systems induces oxidative stress through a mechanism dependent on the mitochondria; this can significantly aggravate the problem of chronic cholesterol consumption, such as in the Mexican diet. Additionally, it has been observed that HC diets induce inflammatory processes that can further aggravate oxidative damage (Simoni-Nieves et al., 2021).

A scenario with an extensive inflammatory infiltrate, oxidative stress, and chronic damage can condition states that lead to cellular transformation.

Recent data from our group prove that the consumption of an HC diet induces carcinogenic processes at the liver level (Enriquez-Cortina et al., 2017; Simoni-Nieves et al., 2021) that lead to profound transcriptomic changes, indicating highly aggressive carcinomas with a poor prognosis (Simoni-Nieves et al., 2021). The condition was characterized by a prominent infiltrate of macrophages, particularly type M2 or pro-tumoral, angiogenesis, and activation of inflammatory pathways that mediate the activation of Stat3, which can also generate resistance to death, as we previously demonstrated (Dominguez-Perez et al., 2016), among other processes.

Thus, cholesterol is a severe complication of the gastrointestinal tract and the liver. It is well known that cholesterol has an excretion route through the biliary route, although enterohepatic circulation generates more than 90% of the bile salts return to the liver.

Our group reported that a high cholesterol diet in mice leads to changes in the histological architecture of the gallbladder, minimal lithiasis, and inflammation,

with the consumption of only two days with an HC diet with 2% cholesterol and 0.5% sodium cholate(Lopez-Reyes et al., 2018).

The gallbladder is an accessory organ of the liver that receives the bile from the hepatic bile ducts to form the common hepatic and cystic ducts. After one stimulus, the gallbladder drains the content into the common bile duct to the ultimate destination in the duodenum (Figure 1).

The gallbladder has a histological presentation similar to the intestine. The gallbladder seen at low magnification has three main layers: the internal mucosa composed of a forum of columnar epithelial cells; the muscular tunica or lamina propria; and its outer layer of connective tissue called external adventitia or serosa. The mucosa has many mucosal folds or ridges, but they are not long enough to be considered villi (Konstantinoff et al., 2024).

At higher magnifications, the mucosal epithelium is lined with simple cylindrical cells that overlie in the lamina propria, which contains dense, irregular connective tissue, many immune cells, and small capillaries (Figure 1)(Konstantinoff et al., 2024; Roa et al., 2022).

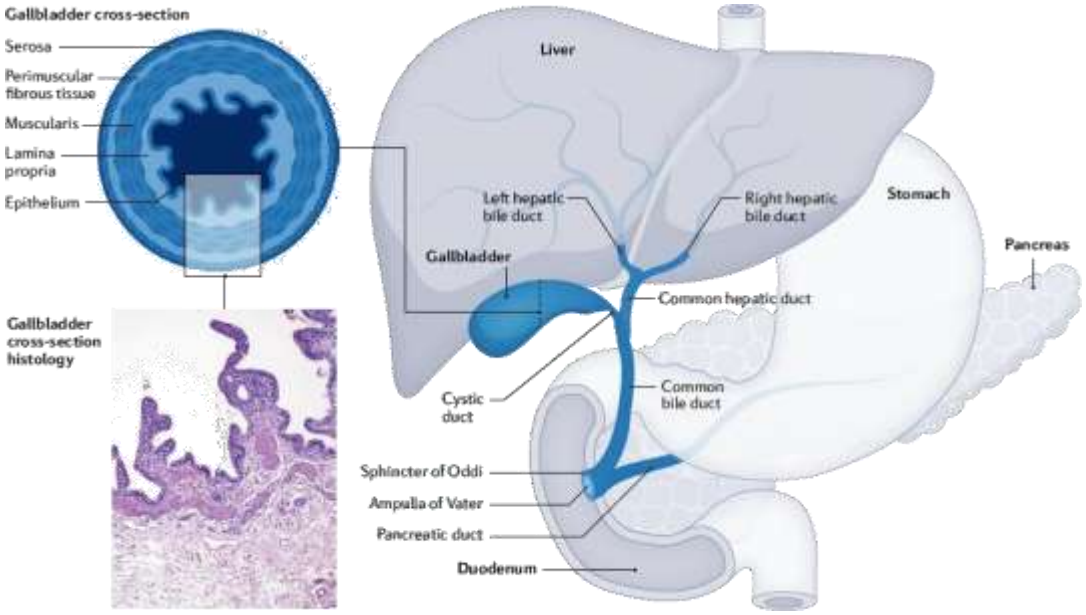


Figure 1. Anatomy and disposition of the gallbladder (Roa et al., 2022)

As mentioned, the mucosal folds of the gallbladder may look similar to parts of the intestine. Still, they can be distinguished by the absence of intestinal crypts or goblet cells at the base of the mucosal folds(Konstantinoff et al., 2024).

Some mucosal folds are deep enough to resemble crossed bridges when viewed under a microscope. The loss of this architecture is a good marker of damage, as demonstrated in the present work.

Given that the gallbladder is the receptacle of the bile chambers and the content of xenobiotic agents produced by the liver, as well as cholesterol, it is essential to know the impacts on this accessory organ caused by the consumption of an HC diet in such a way that it allows understanding the reason why cases of gallbladder cancer (GBC) are increasing in Mexico, to be able to distinguish between tumor subtypes, and to identify early diagnostic markers. In this way, it is intended to take a first step towards an even bigger problem: the GBC(Roa et al., 2022).

Based on this context, I hypothesize:

HYPOTHESIS.

Chronic consumption of diets high in cholesterol induces inflammation in the gallbladder, which damages and morphologically changes the epithelial tissue.

RESEARCH QUESTION

Does chronic consumption of dietary cholesterol generate inflammation and histological changes in the gallbladder?

GENERAL OBJECTIVE

Characterize the histological and inflammatory changes in the gallbladder of mice fed with a high-cholesterol diet at 1, 3, and 8 months of consumption.

SPECIFIC OBJECTIVES

Identify morphological and histological changes in the gallbladder in mice fed a cholesterol-rich diet at different times.

Identify and characterize the immune infiltration in the gallbladder of mice fed a cholesterol-rich diet.

MATERIAL AND METHODS

Animal Treatment Conditions

Male and female C57BL76 mice aged 8-12 weeks were maintained in a pathogen-free environment with controlled temperature and a 12-hour light-dark cycle. They were cared for according to the ethical guidelines of the Universidad Autónoma Metropolitana (UAM), the National Institutes of Health of the United States (NIH), and under the Mexican Official Standard (NOM-062-ZOO-1999) for the care and use of laboratory animals. Before being fed a high-cholesterol diet, mice were fed a balanced diet (Labdiet Rodent 5001).

Animals subjected to a cholesterol-enriched diet were fed the same standard diet supplemented with cholesterol (2% w/w). Both diets and water were provided *ad libitum*. Animal work was conducted in the National Institute of Cardiology Ignacio Chávez's animal facility. At the end of treatments, animals were euthanized under avertin narcosis. The gallbladders were obtained and fixed in 10% neutral formalin, the liver tissue in 4% neutral formalin, and the blood, serum, and liver tissue were obtained for further studies.

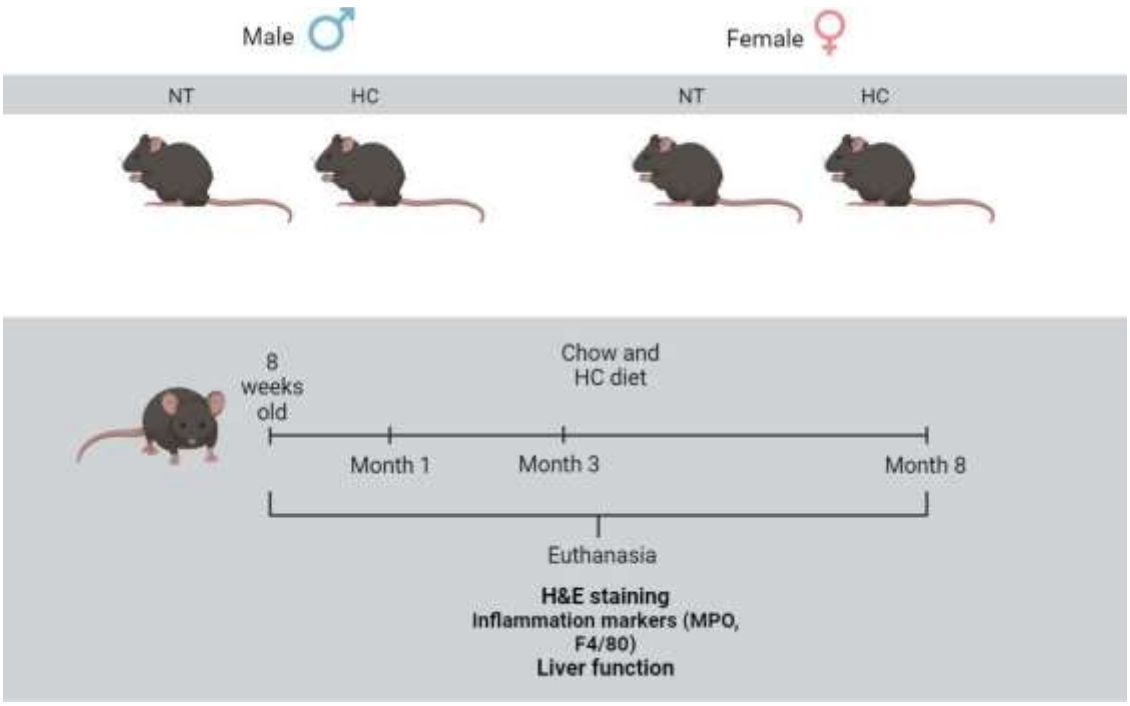


Figure 2. Experimental design followed in the present study. Male and female C57BL76 mice aged 8-12 weeks subjected to a cholesterol-enriched diet were fed the same standard diet supplemented with cholesterol (2% w/w).

Gallbladder assessment by ultrasonography

An expert radiologist from the Rehabilitation Hospital conducted all examinations. Mice were shaved with a shaving machine. Ultrasound gel was applied after cleaning the area with a dry paper towel. The best images were selected and recorded. All USG examinations were performed using a General Electric healthcare model LOGIQe with a GE L10-22-RS linear array probe.

Serum biochemical profile

After the animals were anesthetized, blood was obtained from the orbital venous plexus. Samples were centrifuged at 3000 rpm for 10 min at 4^{o}C. Serum biochemical parameters, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total serum cholesterol (T-Cho), and Triglycerides (TG) were measured using the SpotChem EZ automated analyzer (ARKRAY, USA Clinical Diagnostics).

Histology by Hematoxylin & Eosin staining

The histology analysis was performed as previously reported(Salas-Silva et al., 2020); representative serial sections of 3 to 5 µm thickness cut from tissue blocks embedded in paraffin were overlaid on slides. For analysis, slides were kept at 60°C for 10 min and then deparaffinized with xylene for 10 min. For rehydration, sections were immersed in an ethanol train from 100% to 70% and water for 5 min. After this, Harris Hematoxylin was used for 5 min, followed by washing with water and then washing with acidic ethanol (1%) for 2 sec. It was rewashed with water, and the slides were immersed in alcoholic eosin solution for 2 min. Finally, an ethanol train (70-100%) was used reversely to dehydrate the tissue, ending with xylene and mounting resin.

Immunofluorescence

AS previously reported (Gerardo-Ramirez et al., 2019), the paraffin-embedded tissue was cut into 3 to 5 µm sections. Subsequently, these were washed with PBS-tween for 5 min. Specific antibodies (Table 1) were diluted in PBS with 0.1% horse serum and incubated overnight at 4°C in a humid chamber. Tissues were rewashed with PBS and incubated with DAPI for 5 min. Finally, slides were examined, and the number of positive cells was quantified. Slides were observed in a Carl Zeiss Axiovert. A10 epifluorescent microscope

Antibody	**Brand**	**# cat**	**Dilution**
F4/80	Abcam	Ab60343	1:1000
MPO	GeneTex	GTX54393	1:1000

Table 1. Antibodies used for Immunofluorescence

Statistical analysis

All experiments were carried out with at least four animals. Data are expressed as the mean ± SEM. The t-test was performed to compare means, two-way ANOVA between treatment groups, and the Tukey-Kramer test. The GraphPad Prism 8.2.1 Software for Mac OsX (GraphPad Software, San Diego, California, USA) was used for data processing. Differences were considered significant at $p \leq 0.05$.

RESULTS

The present project aimed to study the effect of the chronic consumption of a cholesterol-enriched diet on the gallbladder, to study the cellular, tissue, and molecular changes in this organ, particularly of a later expansion of this study to the context of the GBC. 48 C57BL/6 mice were separated into males and females, and each cohort was in three groups to analyze the gallbladder at three different times (1, 3, and 8 months) to address the time-dependent changes in this tissue caused by the consumption of a high cholesterol diet (2% cholesterol). I will present and discuss the main findings at each time.

Month 1 of treatment

Mice, male and female, exhibited normal behavior with no relevant changes in the mice. Interestingly, only male HC mice showed a noticeable increase in visceral fat at the time of sacrifice (Figure 3A). The mice's weekly weight was recorded, and no significant statistical differences were observed (Figure 3B).

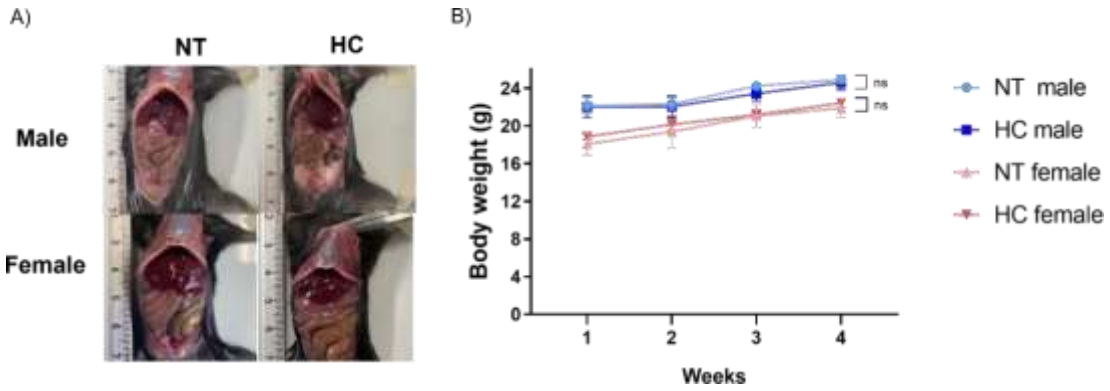


Figure 3. Gross inspection of mice at one month under the HC diet. A) *Visceral inspection of experimental animals at one month of the study. Representative images of four animals in each group. B) Body weight throughout one month of treatment. Each point is the average of four animals per group.*

In both groups, the liver exhibited no changes in color, weight, or size, which could indicate a relevant degree of steatosis (Figure 4 A-C).

Interestingly, I noticed a degree of atrophy in the gallbladder in some animals, but I could not find statistical significance in the weight or the gallbladder-to-body weight ratio (Figure 4A, D-E).

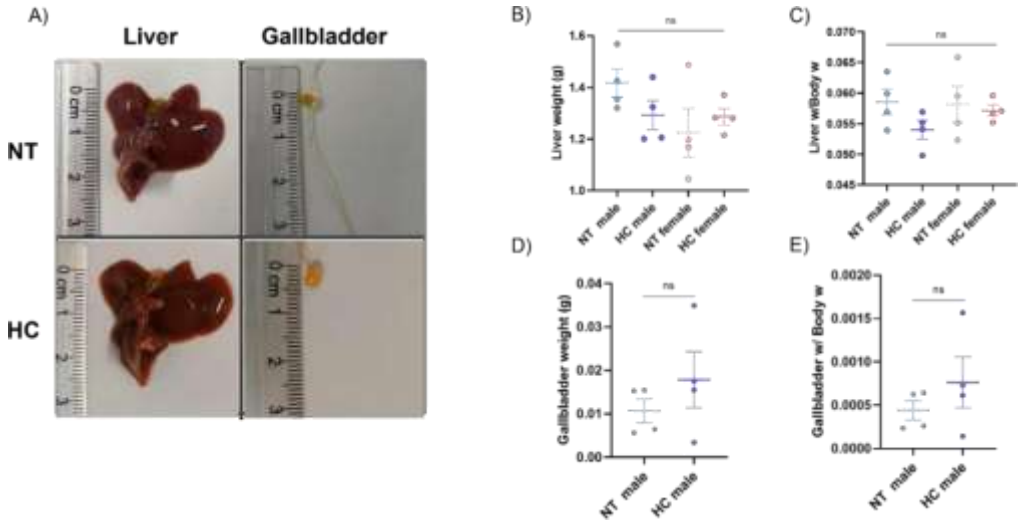


Figure 4. Liver and gallbladder inspection*. A) the size and appearance of the liver and gallbladder in male mice. B) liver weight, and C) liver-to-body weight ratio. D) gallbladder weight,*

and E) the gallbladder-to-body weight ratio (n=4), ns: no significant. Each point is the average of four animals per group.

Because I did not find differences in the macroscopic observation of the liver, I wanted to know whether, at the serum level, we could see some sign of liver damage that would explain why the gallbladder increased in size. The serum analysis in the male mice did not show statistical differences in GOT or AST, GPT or ALT, GGT, total cholesterol (T-cho), HDL (HDL-Cho), and triglycerides (Figure 5).

Although the gallbladder increment could be related to an expected increment in the biliary flux, I aimed to analyze the possible histological impact. I decided to perform a histological inspection of this tissue under hematoxylin-eosin staining (Figure 6).

The mucosa in the NT male mice consists of a simple columnar epithelium and a lamina propria, with a minimal presence of folds (Figure 6A). The histology is similar to that of the normal gallbladder epithelia in the mouse. Notably, the HC group exhibited significant changes, including a marked increase in folds in the columnar epithelium and disruptions in the epithelial lining (Figure 6; orange arrows; observed at 630x). These alterations, observed after one month under the HC diet, indicate potential cellular damage.

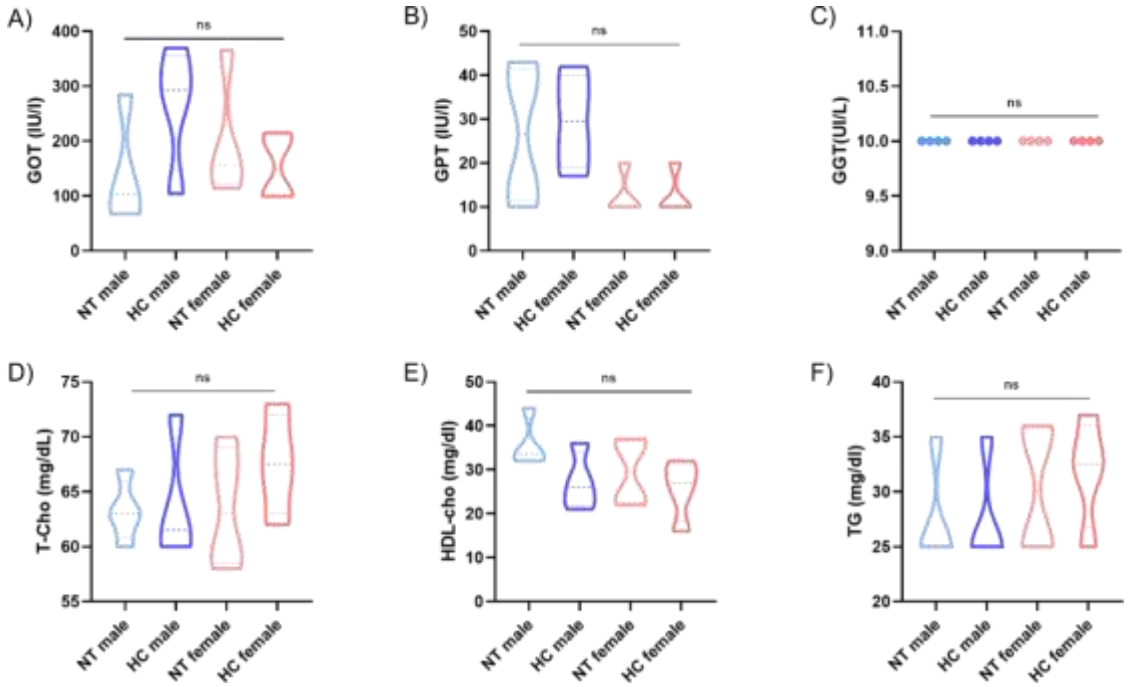


Figure 5. Liver function test*. Quantification of serum values in male HC and NT mice. A serum analysis was performed on a SpotChem device including parameters such as A) GOT, B) GPT, C) GGT, D) Total cholesterol (T-cho), E) HDL cholesterol (HDL-Cho), and F) Triglycerides. (n=4), ns: not significant.*

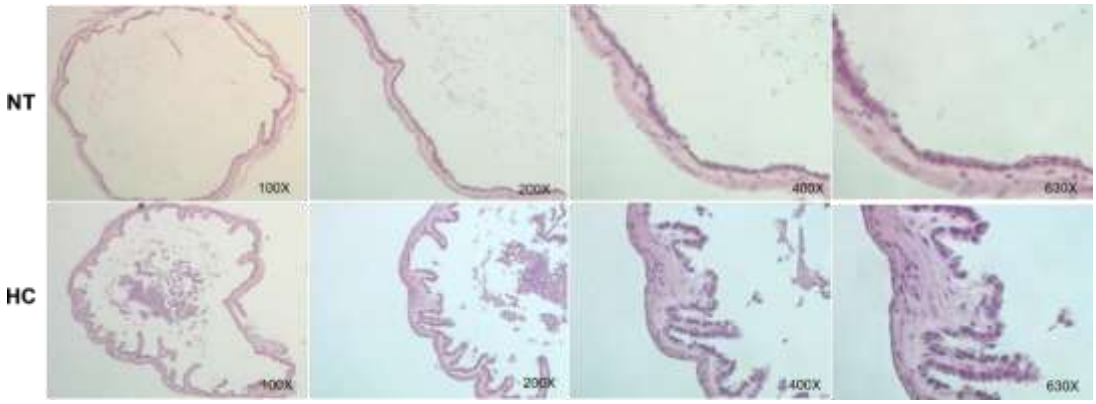


Figure 6. Microscopic inspection by Hematoxylin-Eosin staining of the male mouse gallbladder*. The gallbladder tissue is observed with hematoxylin and eosin staining, thickening of the epithelium, and changes in the accommodation of the cells. Epithelium disruption (orange arrow). Images are representative of four experimental animals.*

The findings strongly suggested an ongoing inflammation. I was directed to analyze macrophage and neutrophil cellular markers by immunofluorescence in

the gallbladder. The microscopy analysis (Figure 7A-B) demonstrated a significant presence of MPO-positive cells (neutrophils) in tissue obtained from mice treated with an HC diet but not macrophages (Figure 8).

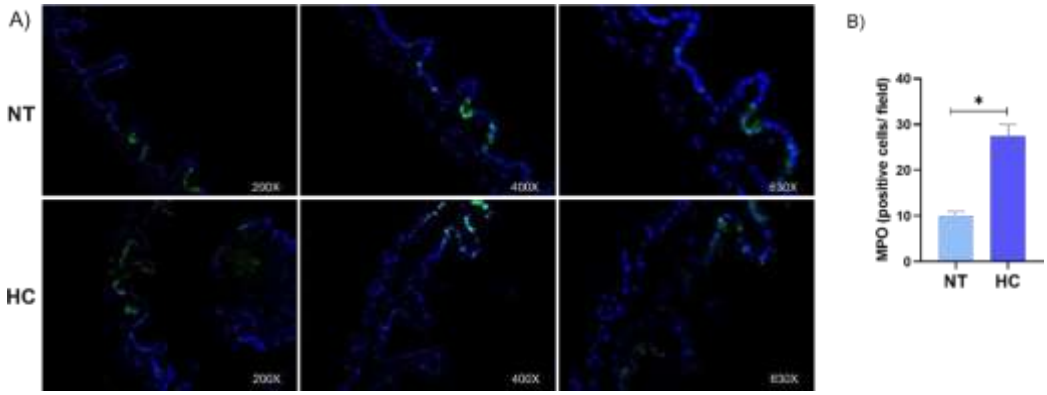


***Figure 7. Immunofluorescence of neutrophils in the gallbladder of male mice**. A) Representative images of myeloperoxidase (MPO, green) positive cells in the fixed gallbladder tissue, and B) the quantification of MPO shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Representative images of four mice. Each column represents an average ± SEM. * p<0.05.*

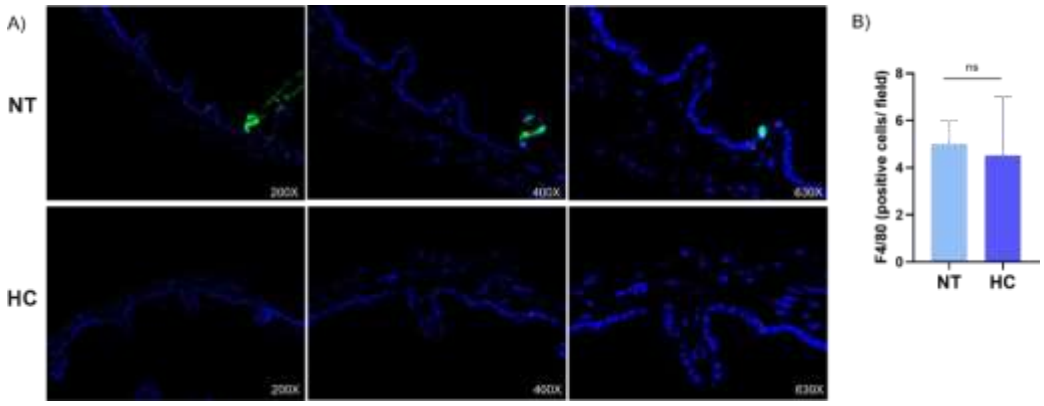
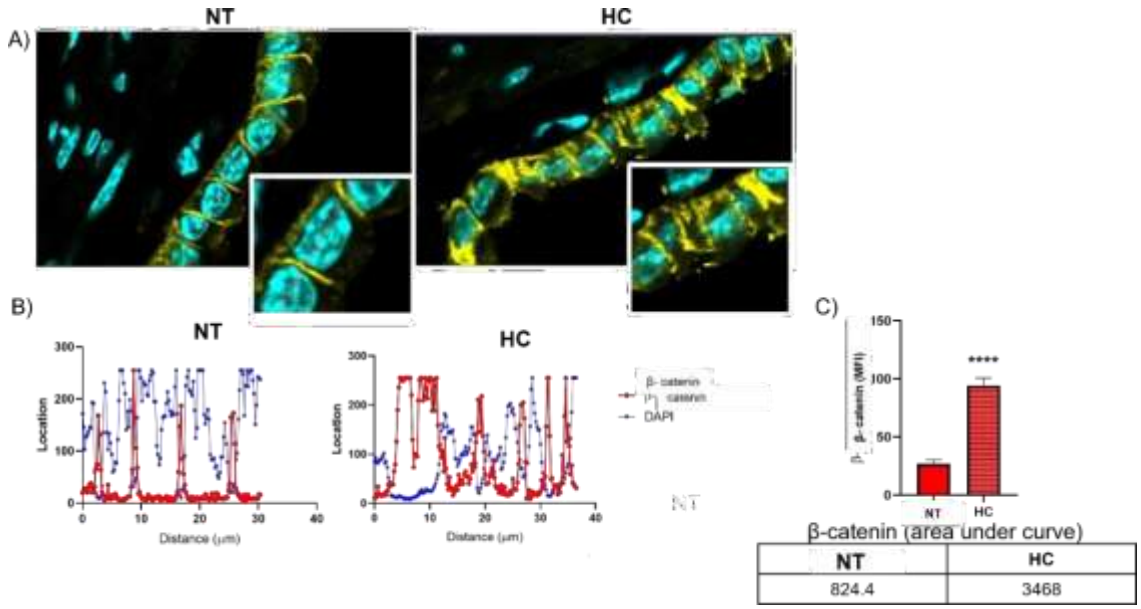


***Figure 8. Immunofluorescence of macrophages in the gallbladder of male mice**. A) Representative images of F4/80 (green) positive cells in the fixed gallbladder tissue, and B) F4/80 quantifying shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Each column represents an average ± SEM. ns: not significant.*

This result demonstrates that, at the early stages, the HC diet induces inflammatory responses in the gallbladder, represented by neutrophils but not macrophages, in male mice.

The damage observed in the gallbladder mucosa suggests disruption in the cell-to-cell adhesion mediated by tight junctions. To gain more confidence, I analyzed β-catenin by IF.

Regarding the NT animals, I found a delocalization of β-catenin from the membrane to the cytoplasm in HC mice (Figure 9A and B). Then, we analyzed the area under the curve of the linear sweep analysis and found a higher accumulation of β-catenin in mice treated with an HC diet. This result shows that the HC diet induces a displacement of β-catenin after one month of treatment in male mice.



β-catenin (area under curve)

NT	HC
824.4	3468

***Figure 9. β-catenin localization in the gallbladder of male mice**. A) Representative immunofluorescence images of β-catenin staining. B) Confocal line-scanning analysis for DAPI and β-catenin; C) Quantification of the area under the curve obtained from the line-scan analysis. **** $p < 0.00005$.*

I evaluated the female mice's serum biochemical parameters. The serum analysis did not show statistical differences in any aspects assessed (Figure 4).

The microscopic H&E staining analysis of the female gallbladder (Figure 10) revealed abnormal tissue structure. Compared with NT samples, I observed a remarkable increment in mucosa folding with more prominent crests. The lamina

propria thickened, and some areas presented an irregular growth of columnar epithelium, suggesting anaplasia (yellow circle).

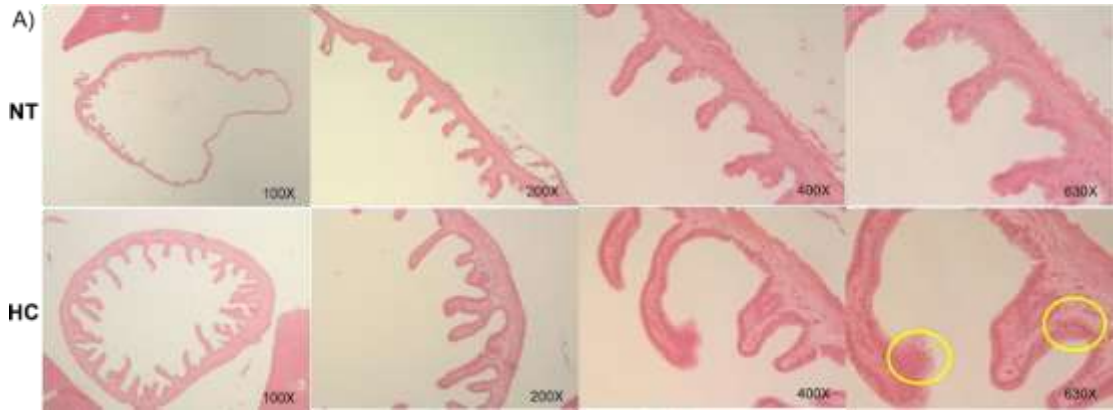


Figure 10. Microscopic inspection by Hematoxylin-Eosin staining of the female mouse gallbladder*. The gallbladder tissue is observed with hematoxylin and eosin staining, thickening of the epithelium, and changes in the accommodation of the cells. Epithelium disruption (yellow arrow). Images are representative of four experimental animals.*

We also evaluated whether this loss of tissue architecture was related to an inflammatory process, specifically by neutrophil and macrophage recruitment, associated with the immune infiltrate in biliary diseases. In female mice, we only observed neutrophil recruitment in gallbladder tissue, as seen in Figure 11, with no macrophage response at this treatment time (Figure 12).

This demonstrates that the HC diet induces inflammatory responses at the gallbladder level in female and male mice, but this is mediated by neutrophils, as reported in other similar diseases where chemical compounds crystallize, in this case, cholesterol and others, such as gout, the urate.

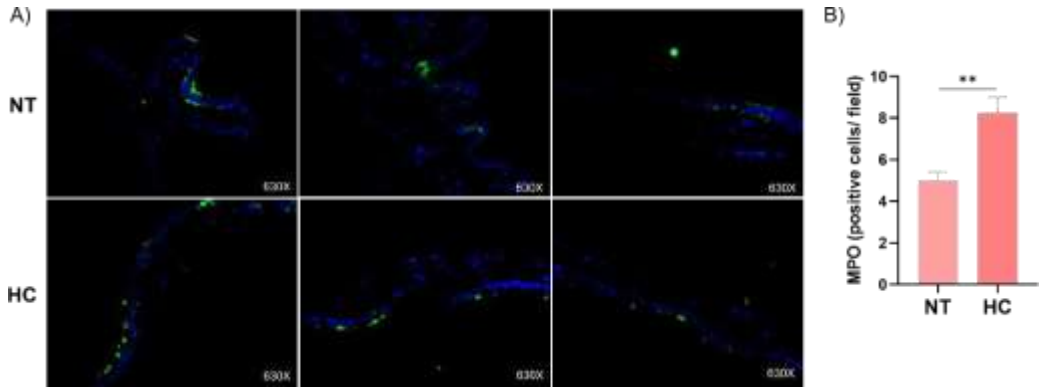


Figure 11. Immunofluorescence of neutrophils in the gallbladder of female mice*. A) Representative images of myeloperoxidase (MPO, green) positive cells in the fixed gallbladder tissue, and B) the quantification of MPO shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Representative images of four mice. Each column represents an average ± SEM. ** p<0.01.*

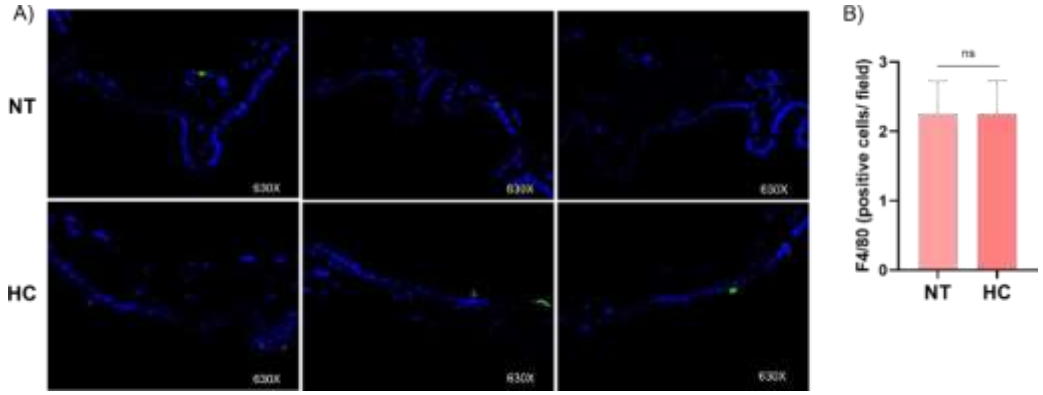


Figure 12. Immunofluorescence of macrophages in the gallbladder of female mice*. A) Representative images of F4/80 (green) positive cells in the fixed gallbladder tissue, and B) F4/80 quantifying shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Each column represents an average ± SEM. ns: not significant.*

Taken together, these results show that the HC diet induces a rapid response in the gallbladder tissue that could condition the development of tumors later. This is why it is necessary to monitor these mice for long periods to evaluate their progress and find some markers that could be helpful in the clinical setting.

Month 3 of treatment

To continue the research, we analyzed another cohort under the HC diet for three months. Similar findings at this time were obtained in the gross inspection and body weight in male mice (Figure 13), the same as the liver and gallbladder; however, I observed a decrease in size in both females and males but with no significant changes (Figure 14).

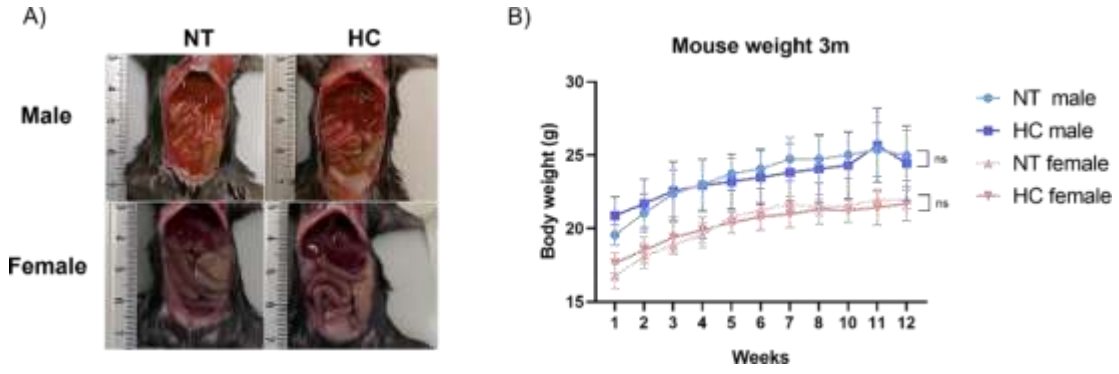


Figure 13. Gross inspection of mice at three months under the HC diet. A) *Visceral inspection of experimental animals at one month of the study. Representative images of four animals in each group. B) Body weight throughout one month of treatment. Each point is the average of four animals per group. ns: no significative.*

Once again, biochemical parameters exhibited no difference, with all tests under basal values. More animals will be studied to corroborate these values, as three months on this diet should present hepatocellular damage.

However, this research is focused on addressing the effects on the gallbladder.

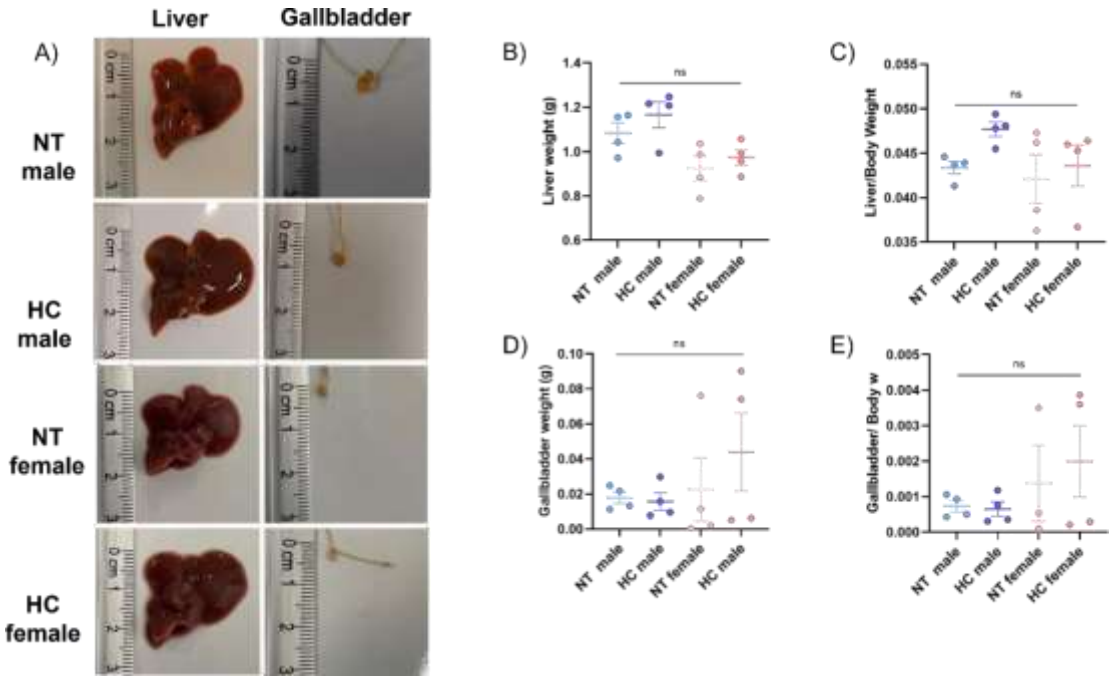


Figure 14. Liver and gallbladder inspection at three months under the HC diet*. A) the size and appearance of the liver and gallbladder in male mice. B) liver weight, and C) liver-to-body weight ratio. D) gallbladder weight, and E) the gallbladder-to-body weight ratio (n=4), ns: no significant. Each point is the average of four animals per group.*

Male gallbladder under the HC diet for three months induced a significant decrement in folds of columnar epithelial lining; almost it was not present, but interestingly, there were zones with overlapping in cells with a different architecture, resembling a squamous epithelium (Figure 15), compared with NT animals which exhibit a single and continue columnar epithelium lining.

Figure 15. Microscopic inspection by Hematoxylin-Eosin staining of the male mouse gallbladder at three months under the HC diet*. The gallbladder tissue is observed with hematoxylin and eosin staining, thickening of the epithelium, and changes in the accommodation of the cells. Images are representative of four experimental animals.*

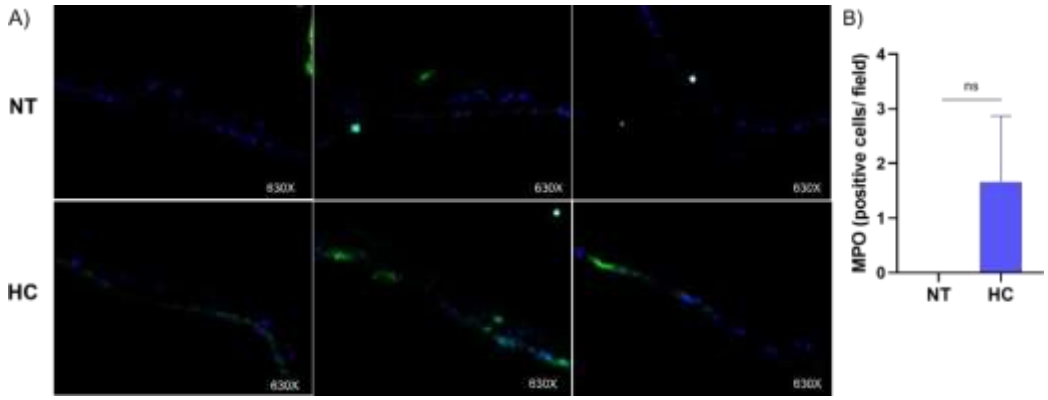


Figure 16. Immunofluorescence of neutrophils in the gallbladder of male mice at three months under the HC diet*. A) Representative images of myeloperoxidase (MPO, green) positive cells in the fixed gallbladder tissue, and B) the quantification of MPO shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Representative images of four mice. Each column represents an average ± SEM. ns: not significant.*

The IF of the MPO not revealed neutrophil infiltration in HC animals, but the presence of macrophages was remarkable at this time (Figure 17).

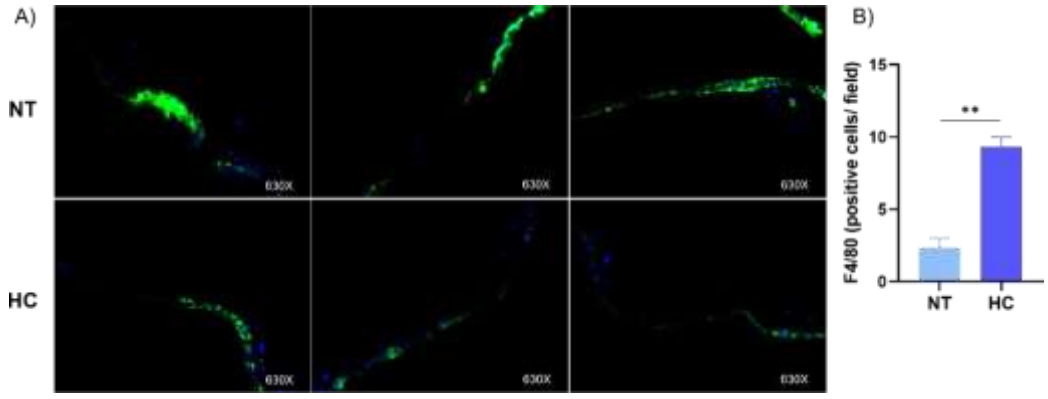


Figure 17. Immunofluorescence of macrophages in the gallbladder of male mice at three months under the HC diet*. A) Representative images of F4/80 (green) positive cells in the fixed gallbladder tissue, and B) F4/80 quantifying shows a significant presence of infiltrated macrophages in the gallbladder treated with the HC diet. Each column represents an average ± SEM. ns: ** p<0.01.*

In the case of the female, the H&E analysis revealed a completely different panorama. Females at three months of treatment exhibited prominent and thicker crests of the mucosa in females I also observed the seme architecture changes, suggesting metaplasia probably induced by an increment in cell proliferation and differentiations.

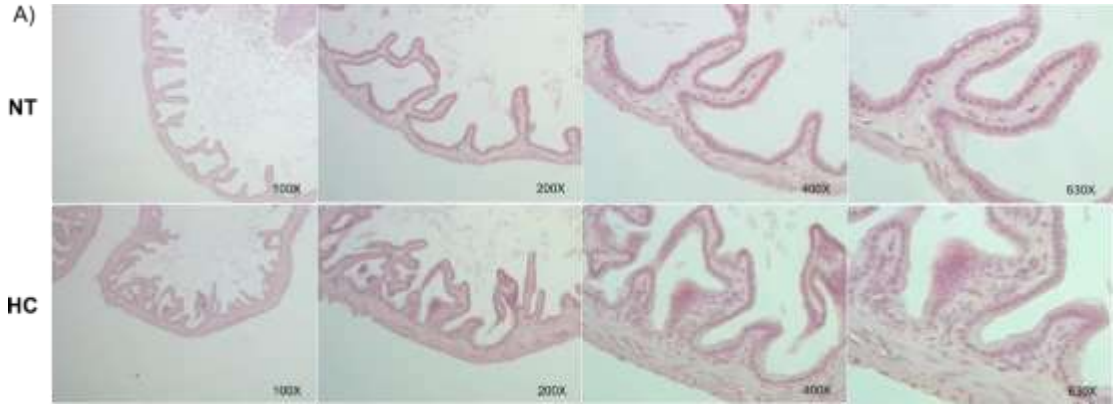


Figure 18. Microscopic inspection by Hematoxylin-Eosin staining of the female mouse gallbladder*. The gallbladder tissue is observed with hematoxylin and eosin staining, thickening of the epithelium, and changes in the accommodation of the cells. Images are representative of four experimental animals.*

More experiments will be conducted to confirm the metaplasia and proliferation. The analysis of the main inflammatory infiltrate revealed a considerable increment in neutrophils, determined by MPO staining (Figure 19), and macrophages (Figure 20). These results clearly indicate a worsening inflammation that, according to the cellular types determined as possible, oxidative stress could be on course.

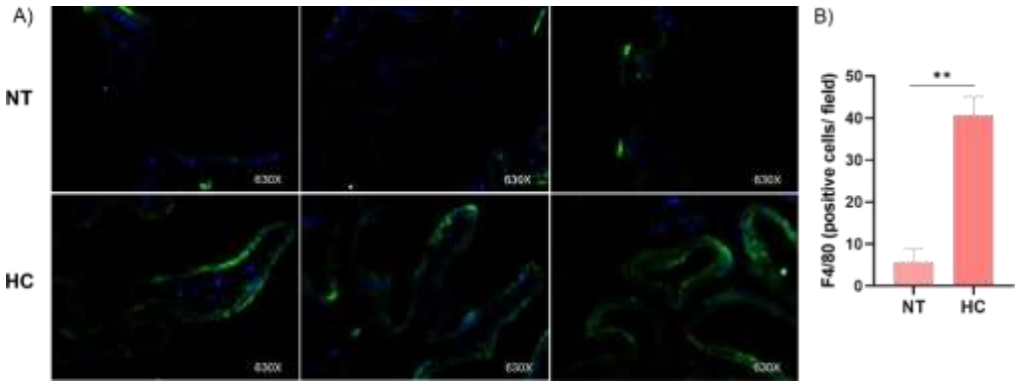


Figure 19. Immunofluorescence of neutrophils in the gallbladder of female mice at three months under the HC diet*. A) Representative images of myeloperoxidase (MPO, green) positive cells in the fixed gallbladder tissue, and B) the quantification of MPO shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Representative images of four mice. Each column represents an average ± SEM. * p<0.05.*

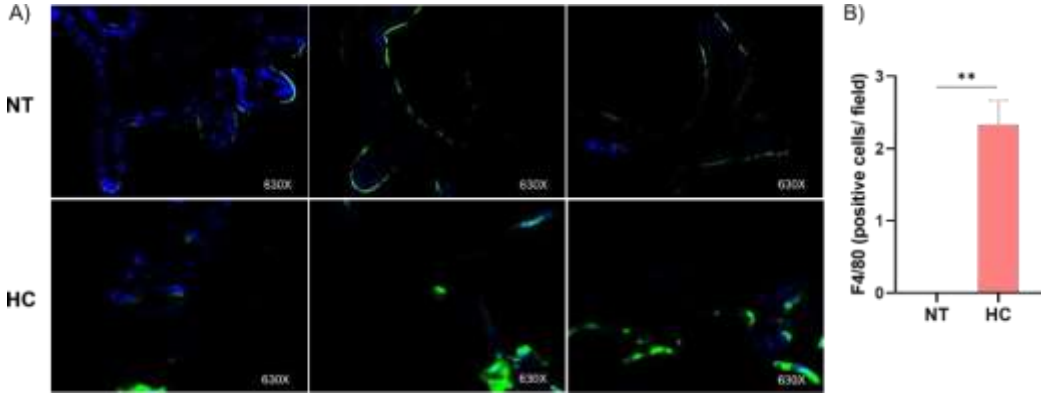


Figure 20. Immunofluorescence of macrophages in the gallbladder of female mice at three months under the HC diet *A) Representative images of F4/80 (green) positive cells in the fixed gallbladder tissue, and B) F4/80 quantifying shows a significant presence of infiltrated macrophages in the gallbladder treated with the HC diet. Each column represents an average ± SEM. ** p<0.01.*

Month 8 of treatment

At this time of treatment, we observed remarkable visceral fat deposition in both females and males, but it was more prominent in males than females. I also observed significant changes in body weight in HC males at six months of treatment (Figure 21), indicating a chronic impact of the HC diet on mice's health.

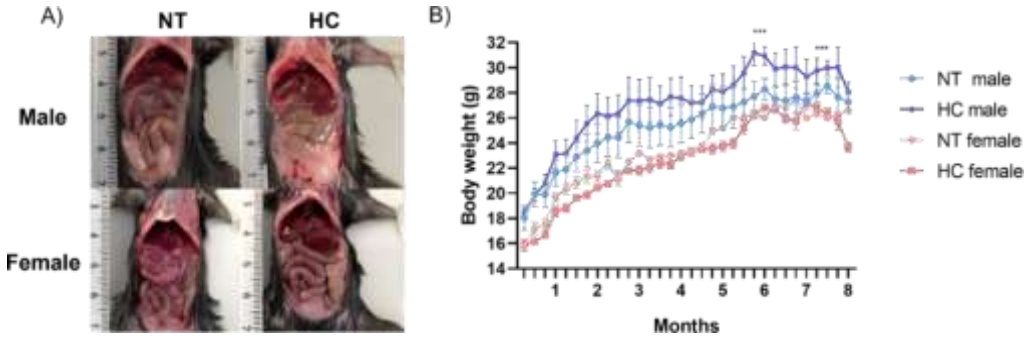


Figure 21. Gross inspection of mice at eight months under the HC diet. A) *Visceral inspection of experimental animals at eight months of the study. Representative images of four animals in*

each group. B) Body weight throughout one month of treatment. Each point is the average of four animals per group. *** p<0.0005.

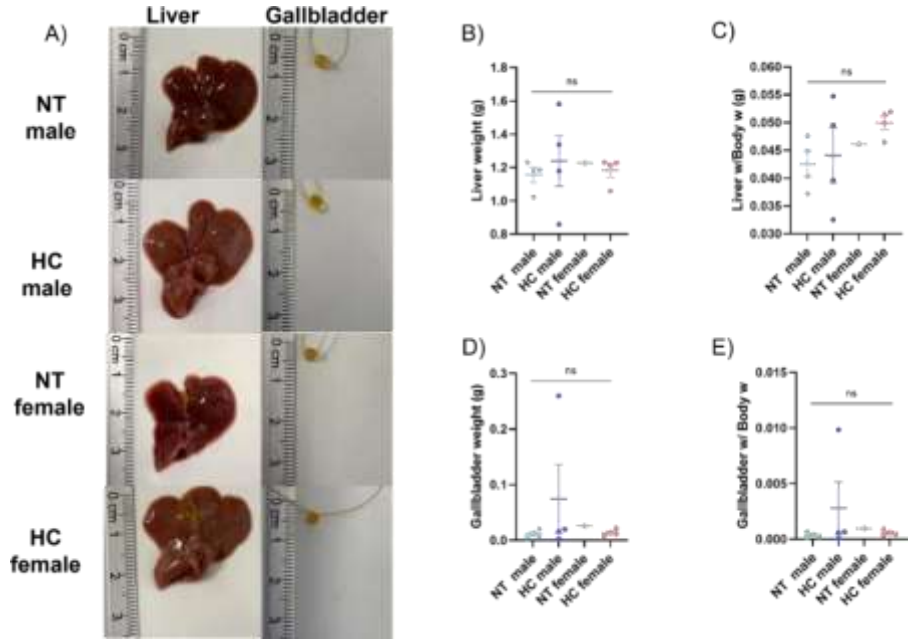


Figure 22. Liver and gallbladder inspection at eight months under the HC diet*. A) the size and appearance of the liver and gallbladder in mice. B) liver weight, and C) liver-to-body weight ratio. D) gallbladder weight, and E) the gallbladder-to-body weight ratio (n=4), ns: no significant. Each point is the average of four animals per group.*

The HC liver exhibited a pale color at this time, indicating steatosis. Still, the liver and gallbladder weights and the gallbladder-to-liver ratio were not significant due to the small cohort of the study (Figure 22).

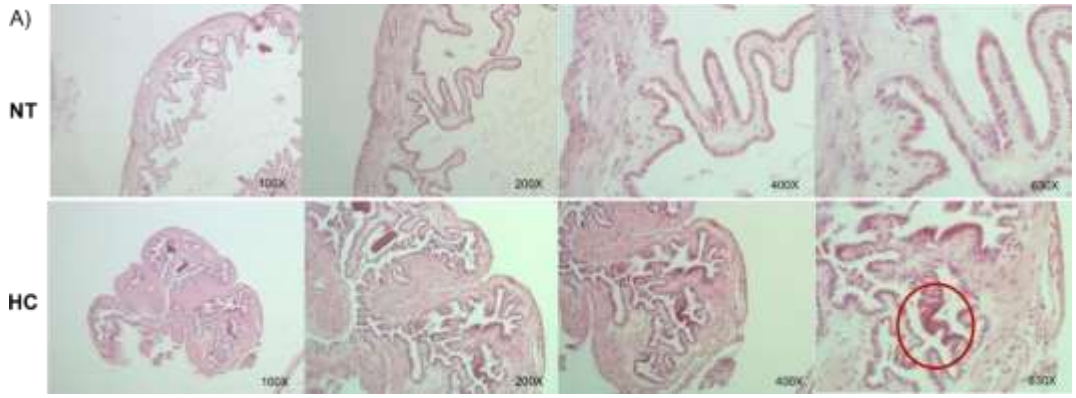


Figure 23. Microscopic inspection by Hematoxylin-Eosin staining of the male mouse gallbladder at eight months under the HC diet*. The gallbladder tissue is observed with hematoxylin and eosin staining, thickening of the epithelium, and changes in the place of the cells. Epithelium disruption (green arrow). The red circle is suggestive of metaplasia. Images are representative of four experimental animals.*

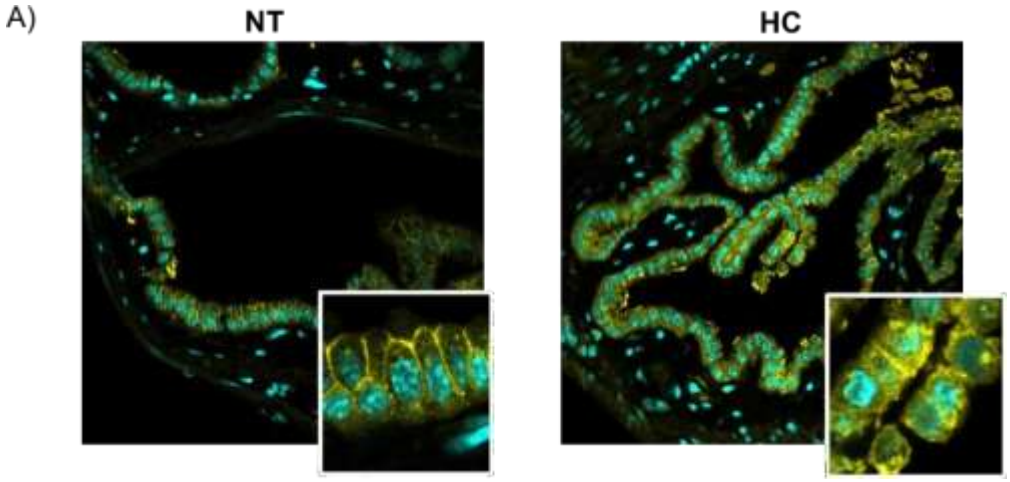


Figure 24. β-catenin localization in the gallbladder of male mice at eight months of treatment*. A) Representative immunofluorescence images of β-catenin staining. Inset, electronic magnification.*

The analysis of the H&E staining revealed remarkable findings in male mice (Figure 23). The gallbladder was contracted, particularly by a significant increment in the lamina propria.

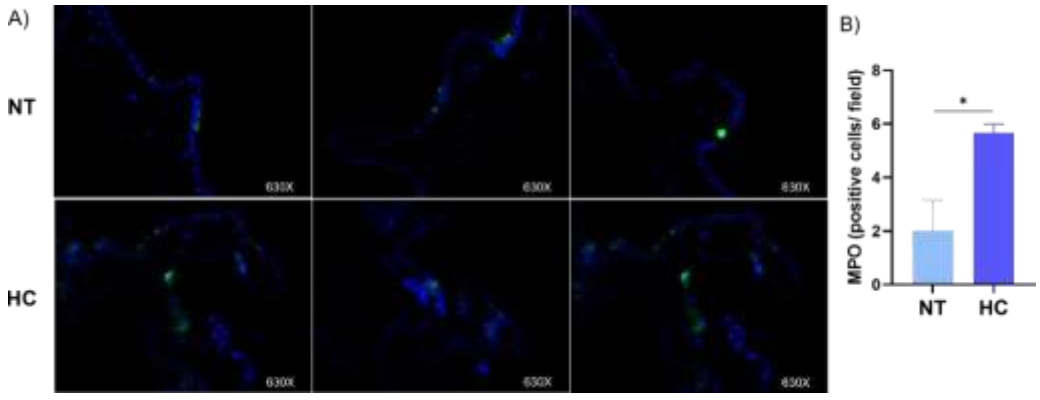


Figure 25. Immunofluorescence of neutrophils in the gallbladder of male mice at eight months under the HC diet*. A) Representative images of myeloperoxidase (MPO, green) positive*

*cells in the fixed gallbladder tissue, and B) the quantification of MPO shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Representative images of four mice. Each column represents an average ± SEM. * p<0.05.*

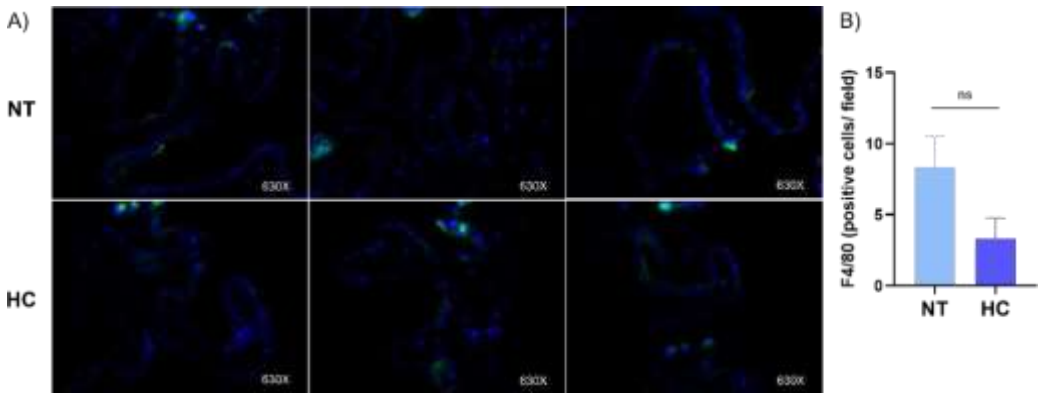


Figure 26. Immunofluorescence of macrophages in the gallbladder of male mice at eight months under the HC diet*. A) Representative images of F4/80 (green) positive cells in the fixed gallbladder tissue, and B) F4/80 quantifying shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Each column represents an average ± SEM. ns: not significant.*

I observed regions with cellular overlapping, probably metaplasia (red circle), and areas with loss of continuity of the epithelial lining, suggestive of cell damage.

At eight months of treatment, the IF of β–catenin revealed a significant delocalization of this protein from the plasma membrane, suggesting a chronic repair stimulus that could lead to the probable metaplasia observed in the H&E staining, this effect was more prominent in males (Figure 24) than females (Figure 28).

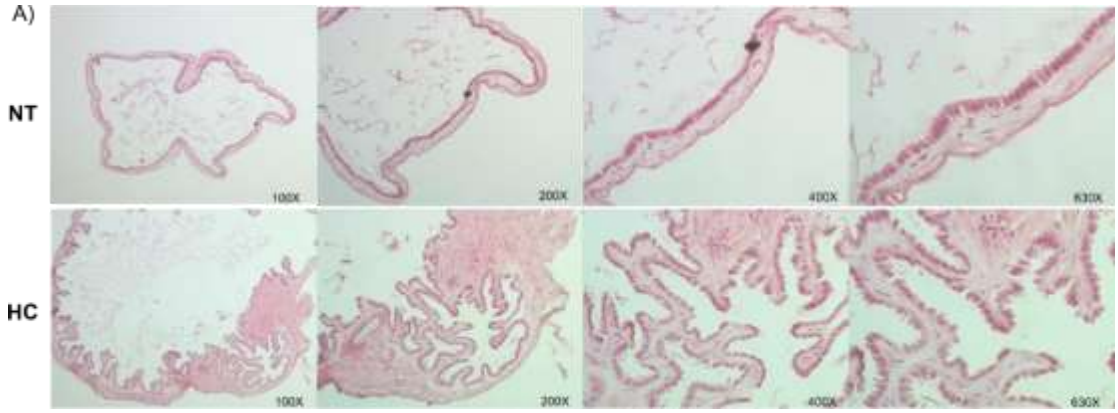


Figure 27. Microscopic inspection by Hematoxylin-Eosin staining of the female mouse gallbladder at eight months under the HC diet*. The gallbladder tissue is observed with hematoxylin and eosin staining, thickening of the epithelium, and changes in the accommodation of the cells. Images are representative of four experimental animals.*

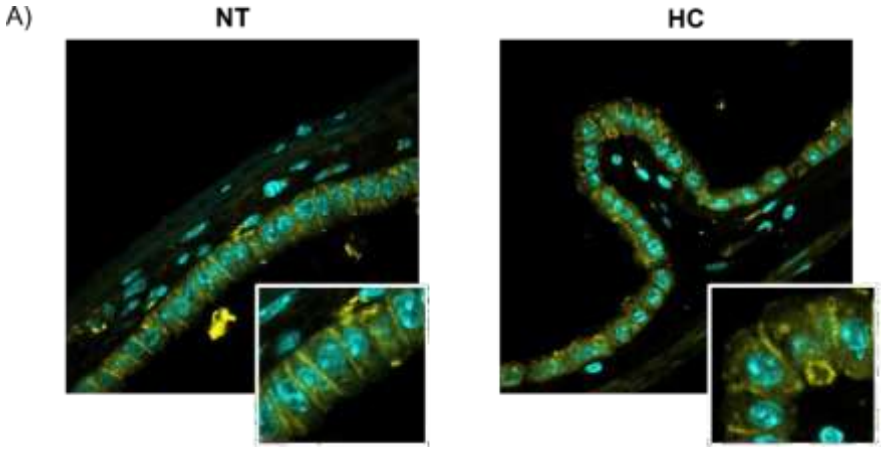


Figure 28. β-catenin localization in the gallbladder of female mice at eight months of treatment*. A) Representative immunofluorescence images of β-catenin staining. Inset, electronic magnification.*

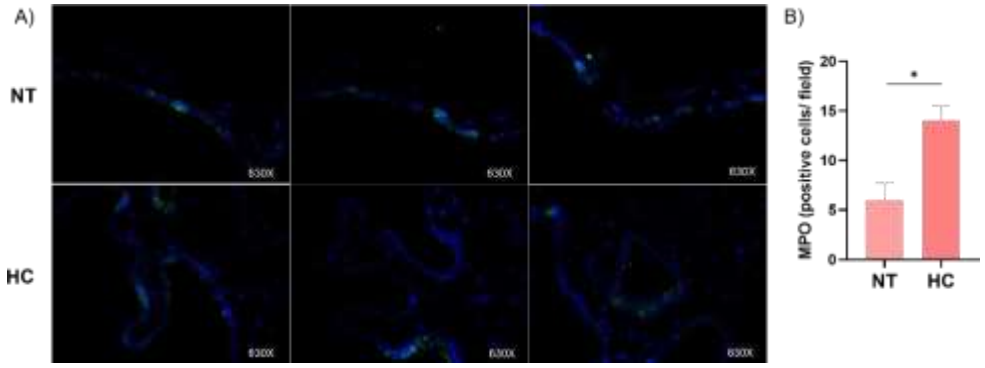


Figure 29. Immunofluorescence of neutrophils in the gallbladder of male mice at eight months under the HC diet*. A) Representative images of myeloperoxidase (MPO, green) positive cells in the fixed gallbladder tissue, and B) the quantification of MPO shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Representative images of four mice. Each column represents an average ± SEM. * p<0.05.*

A slight but significant increment in neutrophils (Figure 25) marked inflammation at this time, but in males, macrophages were decreased (Figure 26).

The H&E staining showed that females presented less damage than males (Figure 27); the lamina propria was considerably thicker with increased crests. The overlapping of cells, suggestive of a metaplasia, is presented at eight months. An increment in neutrophils and macrophages marked the inflammation in females, the last one with no significant values.

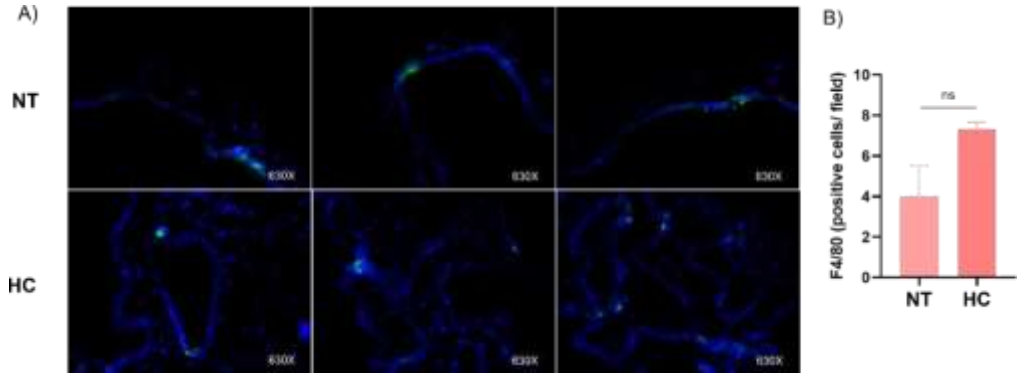


Figure 30. Immunofluorescence of macrophages in the gallbladder of female mice*. A) Representative images of F4/80 (green) positive cells in the fixed gallbladder tissue, and B) F4/80 quantifying shows a significant presence of infiltrated neutrophils in the gallbladder treated with the HC diet. Each column represents an average ± SEM. ns: not significant.*

Discussion

Diets high in cholesterol represent a serious health problem in Mexico and in Western countries such as the United States. Although the impacts of hypercholesterolemia at the cardiovascular and hepatic levels are known, little is known about the gallbladder level and its implications in cellular transformation processes in the gallbladder epithelium. This work is presented as the first with this approach, trying to answer the question of what effects the consumption of a diet high in cholesterol generates on the vesicular epithelium. Above all, we are interested in the process of chronic inflammation with a probable implication of carcinogenic onset.

Due to the previous finding of epithelial thickening, it was questioned whether these changes could be due to an inflammatory response. Bile is known to be concentrated and acidified in the luminal epithelium of the gallbladder, increasing the solubility of cholesterol and calcium salts, which is a prerequisite for forming gallstones.

The data obtained at 1, 3, and 8 months show a clear progression of gallbladder damage. However, we did not find conclusive data in the serum markers; the damage at a microscopic level is evident. Regarding this point, these serum markers will be verified in the coming months. However, a possible explanation for this apparent biochemical stability is due to the extraordinary capacity of the liver to compensate for these physiological changes with relative ease.

The changes or deterioration of the gallbladder tissue were dependent on time. The formation of mucosal ridges, not only in number but also in size, was evident in all animals subjected to the HC diet.

It was interesting to see the presence of possible metaplasia in the histology. There are two possible scenarios for the GBC onset: the sequence metaplasia-dysplasia-carcinoma and the sequence adenoma-carcinoma (Roa et al., 2022).

The first sequence has been related to 95% of the GBC cases, which is relevant to the present findings for the future progression of the present study.

The molecular impact was early; 1 month after treatment, a delocalization of β–catenin was observed from the plasma membrane to the cytoplasm and probably to the nucleus, where it is required to initiate repair processes. This aspect, if maintained over time, could have serious implications in the initiation of cancer because β–catenin at the nuclear level can display oncogenic functions. This aspect continued to be observed at eight months, where this effect is most prominent. It has been reported that mutated β–catenin is frequently found in bile duct cancers (Rashid et al., 2001) so that this finding may have severe implications for advanced disease. The consumption of an HC diet affected the dynamics of β–catenin, an aspect that has not been reported in this tissue.

Another extremely relevant aspect was the characterization of the inflammatory infiltrate. At this stage, we decided to focus only on the characterization of the infiltrate at the level of neutrophils (cells positive for myeloperoxidase, MPO) and macrophages (cells positive for the F4/80 protein).

The results were dynamic, but interestingly, I found that neutrophils have a more significant presence in the early stages of a month of HC diet, which is consistent with other reports. The neutrophils are responsible for giving the first counterattack, especially when there is the formation of crystals, presumably cholesterol, an aspect that also occurs in gout disease, where the neutrophils try to phagocytose the urate crystals, a sign known as the “Martini olive.”

In homeostasis, neutrophils travel through the body and can enter into the bile ducts(Nicolas-Avila et al., 2017). However, inflammatory conditions, such as gout and neutrophils, are responsible for packaging uric acid crystals. A similar mechanism occurs in the gallbladder during the formation of gallstones, agglomerating calcium and cholesterol crystals (Munoz et al., 2019).

Over time, this moves towards an infiltrate mainly made of macrophages, which already speaks of a more critical cellular commitment. The difference between

males and females was notable, presenting a differential infiltrate that remains to be validated as experiments conducted in the following research stage that I will develop as part of my Ph.D. studies on the subject.

The project is the initial research step aimed at characterizing the molecular signature induced by cholesterol, which can display an oncogenic or tumor-promoting potential in the vesicle, as we have reported at the hepatocellular level.

The mouse data will allow us to use a comparative functional genomics approach to identify, in humans, the transcriptomic signature of cholesterol to identify a subtype of GBC conditioned to the presence of cholesterol caused by the consumption of HC diets.

Conclusion

Although the present work can be presented as simple research in technical terms, it is very relevant and has specific clinical implications. The chronic consumption of an HC diet conditions an inflammatory process that is dynamic over time, marked by neutrophils and macrophages, without this meaning that other cell types, such as lymphocytes, are not involved; this is part of the limitations of the present project that will be settled in the next stage of the investigation.

The inflammation observed in the gallbladder tissue was related to significant changes in the histological architecture, not only at the level of the columnar epithelium but also in the lamina propria.

Consumption of an HC diet leads to critical inflammatory processes and morphological changes in the gallbladder that could form metaplasia and the probable or eventual evolution of a GBC(Roa et al., 2022).

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Casa abierta al tiempo
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ACTA DE EXAMEN DE GRADO

No. 00257
Matrícula: 2223803096

Cambios histológicos e inflamatorios en la vesicula biliar por el consumo crónico de una dieta alta en colesterol.

En la Ciudad de México, se presentaron a las 10:00 horas del día 2 del mes de octubre del año 2024 en la Unidad Iztapalapa de la Universidad Autónoma Metropolitana, los suscritos miembros del jurado:

DRA. MONSERRAT GERARDO RAMIREZ
DR. BENJAMIN PEREZ AGUILAR
DRA. VERONICA SOUZA ARROYO
DR. CARLOS ALEJANDRO MARTINEZ ARMENTA

Bajo la Presidencia de la primera y con carácter de Secretario el último, se reunieron para proceder al Examen de Grado cuya denominación aparece al margen, para la obtención del grado de:

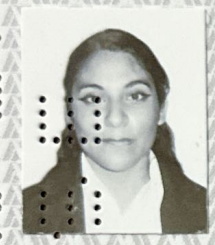
MAESTRA EN BIOLOGÍA EXPERIMENTAL

DE: JAQUELINE QUEVEDO OCAMPO

y de acuerdo con el artículo 78 fracción III del Reglamento de Estudios Superiores de la Universidad Autónoma Metropolitana, los miembros del jurado resolvieron:

aprobar

Acto continuo, la presidenta del jurado comunicó a la interesada el resultado de la evaluación y, en caso aprobatorio, le fue tomada la protesta.



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ALUMNA

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