Biochemical and histological characterisation of an experimental rodent model of non-alcoholic steatohepatitis – Effects of a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist and a glucagon-like peptide-1 analogue

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ABSTRACT

Background: Non-alcoholic steatohepatitis (NASH) is a prevalent disease that is highly associated with the metabolic syndrome and type II diabetes. The development of in vivo models that reflect all nuances of the human NASH pathology is essential for drug discovery and development. We aimed to further characterise a dietary induced model of NASH both biochemically and histologically. In addition, we also investigated whether pioglitazone and liraglutide, drugs that have both been investigated as potential NASH treatments, could modulate the pathological changes induced by the NASH diet. Furthermore, to aid the translation of data from pre-clinical in vivo models, we aimed to adapt the NASH Clinical Research Network (CRN) histological score system for use in rodent studies.

Methods: Sprague Dawley rats were fed a high-fat diet (HFD) for 9 weeks, after which they were switched to a high fat, high cholesterol and cholate diet (HFCC) for 12 weeks. The rats were divided into treatment groups, receiving either 30 mg/kg pioglitazone p.o. SID or liraglutide s.c. 200 μg/kg BID or the respective vehicles. Serum levels of triglycerides (TG), cholesterol (Chol), LDL, HDL, AST and ALT, as well as body weight were assessed in all subjects. Upon termination, the liver was weighed and evaluated histologically using modified NASH-CRN criteria.

Results: HFCC feeding induced severe hepatic injury and hepatomegaly as indicated by significant increases in AST, ALT and an increased liver weight. Additionally, HFCC feeding induced dyslipidaemia, significant increases in circulating cholesterol and LDL were observed. No obesogenic effect of the HFCC diet was observed, though the diet did induce insulin resistance. Histological analysis showed that the HFCC diet induced several NASH like features, though it did not induce the development of severe fibrosis. However, microgranulomas were often prevalent in addition to lobular inflammatory foci. Pioglitazone showed little efficacy upon both biochemical and histological features. However, liraglutide induced weight loss, improved glycaemic control, reduced ALT and AST and showed some beneficial effects upon steatosis and lobular inflammation.

Conclusion: Similar to previous reports we have shown that the atherogenic diet, HFCC, induces a phenotype akin to that seen in human NASH patients. Despite inducing all histological features of NASH, HFCC feeding does not promote the development of significant fibrosis within rodents. Pioglitazone and liraglutide have been investigated as potential NASH treatments. Within this model of NASH we have shown that pioglitazone has little efficacy, whereas liraglutide reduced the levels of circulating aminotransferases and had some beneficial effects upon NASH histological parameters.
1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome, hence the prevalence of NAFLD has been increasing in concordance with that of obesity and type II diabetes [1]. Estimates place the global prevalence of NAFLD at 25% and NAFLD is expected to become the primary indication for liver transplantation in the USA by 2020 [1,2]. NAFLD is defined by the accumulation of fat in the liver, so called hepatic steatosis, in the absence of excessive alcohol consumption. Several histologic manifestations of the disease exist under the umbrella term of NAFLD, the least severe being simple steatosis, meaning the occurrence of lipid droplets in at least 5% of hepatocytes. A more severe histologic manifestation of NAFLD is the progressive form non-alcoholic steatohepatitis (NASH). NASH is diagnosed via histological analysis of a liver biopsy and is defined by the presence of steatosis, hepatocyte ballooning and lobular inflammation [3]. Patients with NASH have higher fibrogenic activity and are at increased risk of developing cirrhosis and liver failure [4,5]. The pathogenesis driving the progression of hepatic steatosis to NASH is still unclear; the multi-parallel hypothesis proposes that the process is multifactorial and that various conditions such as lipotoxicity, oxidative stress and mitochondrial dysfunction act in unison [6,7]. It is usually believed that NASH triggers the development of liver fibrosis, which ultimately can lead to the most severe histologic manifestation of NAFLD, liver cirrhosis [8,9].

At present, no pharmacological NASH therapy has been approved by the FDA. Hence, there is an urgent need for the development of therapies. Reliable animal models that reflect all nuances and subtleties of the human condition are required for the development of NASH treatments. An ideal dietary-induced NASH model must exhibit all defining features of human NASH patients, such as obesity, insulin resistance, steatohepatitis and various other metabolic and histological abnormalities [10]. The severity of fibrosis has been shown to be the most prognostic histological feature within NAFLD patients; hence, the development of fibrosis is an important feature which models of NASH should aim to replicate [11-13]. Various diets have been investigated as potential NASH models, though many are unable to replicate all features of the human condition [10]. One promising candidate is the atherogenic high fat, high cholesterol and cholate diet (HFCC), which has been shown by Heebell et al. to be capable of replicating the histological features of NASH [14]. The high concentration of cholesterol in the diet induces the progression of hepatic steatosis to NASH [15]. The inclusion of a high concentration of cholate further drives the progression to NASH, cholate has been shown to increase the hepatic accumulation of cholesterol [16].

Several drugs have been investigated as potential NASH treatments [17-21], the most characterised of which is the thiazolidinedione pioglitazone [17,22-24]. Pioglitazone is a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist and has been approved for use as an anti-diabetic drug [25]. As patients with NASH are highly likely to be diabetic or pre-diabetic, investigators explored whether pioglitazone as an anti-diabetic drug [25]. As patients with NASH are highly likely to be diabetic or pre-diabetic, investigators explored whether pioglitazone could be a potential treatment for NASH within the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Non-alcoholic Steatohepatitis (PIVENS) study [3,17]. Pioglitazone showed minimal efficacy upon the primary endpoints of the study; however, there is evidence that pioglitazone could be beneficial to some patients [17,24]. Aithal et al reported that non-diabetic NASH patients treated with pioglitazone had a significant decrease in hepatocellular injury and fibrosis stage when compared to placebo after 12 months of treatment [24]. Similarly, liraglutide is a drug that has been approved for use in diabetic patients; however, its mode of action differs from pioglitazone [26]. Liraglutide is a glucagon-like peptide-1 analogue and acts to improve blood glucose control and ameliorate insulin resistance [26]. Liraglutide has shown some promise as a potential treatment for NASH, and showed in a phase II study to increase the likelihood of resolution of NASH compared to placebo [18]. While both drugs have been approved by the FDA, their use has been associated with side effects such as pancreatitis in the case of liraglutide and an increased risk of bone fractures in the case of pioglitazone use.

In this study, we aimed to biochemically and histologically characterise the dietary induced model of NASH utilised by Heebell et al. and to investigate the efficacy of pioglitazone and liraglutide within this model [14].

2. Methods

2.1. Animals

All animal procedures were performed in accordance with guidelines from the Animal Welfare Division of the Danish Ministry of Justice under the institutional license issued to Nordic Bioscience (2016-15-0201-00910). Male Sprague Dawley rats (Envigo, Venray, the Netherlands) were obtained at 6 weeks of age and housed in the standard type IV cage (2 rats per cage, standard wood chips enriched with red-tinted huts, nest material and sticks) at the Nordic Bioscience animal facility (21–23 °C, 55–65% relative humidity, 12-h light/dark cycle) with ad libitum access to food and water. Male rats were used as they are more susceptible to develop hepatic steatosis [27]. From arrival to study start, the rats were fed a 60 kcal% fat diet (HFD) (5.1 kcal/g) (#S81Y, TestDiet, London, UK). After 9 weeks of HFD feeding the rats were randomised into groups and fed the test diets for 12 weeks. Control rats were fed a 10 kcal% fat (mostly cocoa butter) diet (3.80 kcal g⁻¹) (#D09052201, Research Diet, New Brunswick, NJ, USA) (n = 10) and the high fat, high cholesterol and cholate fed rats (HFCC) were fed a 65 kcal% fat (mostly cocoa butter) (5.30 kcal g⁻¹) with 2% cholesterol and 0.5% cholate added (#D09052204, Research Diet, New Brunswick, NJ, USA).

HFCC fed animals were randomly divided into treatment groups receiving either; vehicle (0.5% Sodium carboxymethyl cellulose in saline via gavage (n = 8) or saline injections subcutaneously) (n = 10), pioglitazone (Beijing Ablepharmatech, Beijing, China) 30 mg/kg via oral gavage (4 ml/kg) once a day (n = 10) or liraglutide (Bachem, Bubendorf, Switzerland) 200 μg/kg via subcutaneous injections (1 ml/kg) twice a day (n = 10).

Food intake and body weight were monitored once weekly throughout the entire study period. Aspartate transaminase (AST) and alanine transaminase (ALT) as well as circulating lipoproteins, cholesterol and triglycerides were measured in serum sampled every 2 weeks after the initiation of the study. Further, an oral glucose tolerance test (OGTT) was performed after 11 weeks of dietary induction and treatment. The rats received glucose per oral gavage (p.o.) (2 g kg⁻¹). Blood samples were collected from the tail vein before glucose challenge (0 min) and 15, 30, 60, and 120 min post glucose challenge in the OGTT.

2.2. Histology

Upon termination of the animals, the liver tissue along with three different fat deposits (epididymal, perirenal and inguinal adipose tissue) were excised, weighed, fixed in formalin and embedded in paraffin. 5 μm sections from liver tissue were stained with Picrosirius Red and Haematoxylin & Eosin according to standard protocols. Sections of liver tissue were taken towards the front of the lobe. Histopathological changes to the liver were assessed by a medical doctor (MFB), supervised by a liver pathologist (SD) using a modified NASH-CRN score system similar to that described by Liang et al. [28,29]. After preliminary assessment, however, it was deemed necessary to adjust elements of the NASH-CRN score system for use in rodents. The number of inflammatory foci, particularly the number of microgranulomas that were present within the tissue far exceeded the scale of the NASH-CRN hence lobular inflammation was scored on a continuous scale rather than ordinal scale.
The degree of macrovesicular steatosis was determined as the percentage of hepatocytes containing fat vacuoles was determined and scored as follows; grade 0: < 5%, grade 1: 5%–33%, grade 2: > 33–66%, grade 3: > 66%. Microvesicular steatosis was characterised by contiguous patches with at least 10 hepatocytes with steatotic microvesicles (the diameter is typically equal to or smaller than the diameter of the nuclei) and graded as follows; grade 0 (No patches of hepatocytes with microvesicular steatosis) and grade 1 (at least 2 patches of hepatocytes with microvesicular steatosis identified). The severity of lobular inflammation was determined by assessing five randomly selected fields at 100 x magnification and counting the number of inflammatory foci inside the liver lobuli within these five fields of view, which was then divided by the number of fields of view. Inflammatory foci were defined as groups of inflammatory cells, mostly lymphocytes, though occasionally a few neutrophilic granulocytes or plasma cells were observed. Importantly, also microgranulomas were counted as inflammatory foci, and in some instances, microgranulomas represented the main type of inflammatory foci. The presence of microgranulomas was determined by assessing two randomly selected areas, and in each area, 10 fields of view at 100 x magnification were examined. Microgranulomas were defined as rounded foci of various sizes consisting of macrophage-like cells with bright cytoplasm, possibly containing necrotic material. For the assessment of portal inflammation, two representative areas were selected randomly, each of which was examined to assess the degree of portal inflammation, which was graded on the following scale; grade 0 = none to minimal portal inflammation and grade 1 = more than minimal portal inflammation. We used the criteria defined by Kleiner et al. to assess the severity of fibrosis [28]; stage 0 = no fibrosis, stage 1 A = mild pericellular fibrosis predominately in zone 3, no portal/periportal fibrosis, stage 1B = moderate pericellular fibrosis in zone 3, +/– pericellular fibrosis in zone 1, no portal/periportal fibrosis, stage 1C = Portal/periportal fibrosis, no pericellular / perisinusoidal fibrosis, stage 2 = Pericellular and portal/periportal fibrosis, often with one or few fibrotic bridges, stage 3 = Fibrosis with frequent bridging and stage 4 = Presence of frequent bridging and regenerative nodules. Prominent hepatocyte ballooning was a feature which was rarely observed, the severity of ballooning was assessed using the following scale; grade 0 = no ballooning, grade 1 = few ballooned cells and grade 2 many ballooned cells/prominent ballooning.

Hepatic collagen content was assessed using the total collagen assay (hydroxyproline-based) according to manufacturer’s instructions (QuickZyme, Leiden, the Netherlands).

2.3. Statistics

All data are presented as means ± standard deviation. Differences between control and HFCC vehicle groups were assessed using analysis of variance followed by post hoc Tukey’s multiple comparison test. Differences between treated HFCC-fed animals and HFCC-fed animals receiving vehicle was determined by student’s t-test. All analyses were performed using GraphPad Prism software (GraphPad Prism, San Diego, CA). A value of p < 0.05 was considered statistically significant.

3. Results

3.1. HFCC diet induces hepatomegaly and maintains HFD-induced obesity

The animals were placed on HFD upon arrival to induce obesity and after 9 weeks of feeding they were switched to the experimental (high fat, high cholesterol and cholate) HFCC diet or to a control diet. HFCC feeding did not induce significant weight change when compared to the lean control (Fig. 1A). Despite the lack of obesogenic effect, the HFCC fed animals consumed significantly more calories over the course of the study when compared to the lean control (Fig. 1B). HFCC feeding did not significantly increase the size of various fat depots when compared to the lean control and in some cases, the size decreased significantly due to HFCC feeding (Fig. 1C, D). HFCC feeding significantly reduced the weight of the inguinal and epididymal adipose tissue compared to the lean control (p = 0.0009, p = 0.008). Animals fed the HFCC diet developed significant hepatomegaly when compared to the lean control (p = < 0.0001) (Fig. 1E).

3.2. HFCC feeding induces significant dyslipidaemia, insulin resistance and hepatic injury

When compared to the lean control the HFCC fed animals had significantly higher levels of circulating cholesterol and low-density lipoprotein (LDL) (p < 0.0001) (Fig. 2A–B, E–F), however circulating levels of high-density lipoprotein (HDL) and triglycerides were unchanged (Fig. 2C–D, G–H). The 9 week period of HFD diet induced insulin resistance in all animals which was maintained by the HFCC diet phase of the study. Fasting blood glucose (FBG) was assessed at all time points (except baseline) and was elevated within the HFCC-fed rats compared to the control (Supplementary material). After 11 weeks of HFCC feeding, an oral glucose tolerance test (OGTT) was performed. Compared to the control group, HFCC-fed rats were significantly more insulin resistant (p = 0.002) (Fig. 3A, C). However, after correction for baseline FBG the difference between control and HFCC was no longer observed (Fig. 3D). Markers of liver function, ALT and AST, were significantly elevated within the HFCC-fed animals, when compared to control (p = 0.0003) and continued to increase through the study period (Fig. 4A, B).

3.3. Adaptation of the NASH-CRN scoring system for use in rodents

The adapted scoring system was then used by a blinded medical doctor, supervised by a liver pathologist, to assess sections obtained from all animals that completed the study. HFCC feeding induced substantial macrovesicular steatosis compared to control fed rats (Figs. 5B and 6 A). In addition, HFCC fed animals were more likely to exhibit microvesicular steatosis (Fig. 6B).

Portal inflammation, a feature often associated with severe hepatic injury in humans, was not observed within any of the experimental groups (Fig. 6C). Despite the lack of portal inflammation, the number of inflammatory foci within the liver lobules of the HFCC-fed animals was substantial compared to the control group (Figs. 5B and 6 D). Within this study, and in accordance with Heebøll et al., we found that HFCC feeding induced ballooning in few of the hepatocytes (Fig. 6E). Microgranulomas probably represent early forms of lipogranulomas [30] and have been shown to be associated with severe hepatic steatosis [31]. The presence of microgranulomas was more common within animals fed the HFCC diet, when compared to the lean control fed group (Fig. 6F).

We used the criteria described by Kleiner et al. to grade the severity of fibrosis within this rodent study. Most cases were graded with stage 1 A mild perisinusoidal fibrosis (58% of all animals); some were graded with stage 1B moderate perisinusoidal fibrosis, though this was less common (38% of all animals) (Fig. 6G). Only three animals within the control group were observed to have no fibrosis (stage 0). We subsequently confirmed the histological fibrosis assessment by performing a hydroxyproline assay upon collected liver tissue, no difference in collagen content was observed between any of the experimental groups (Fig. 7).

3.4. Modest effects of pioglitazone and liraglutide on dietary induced NASH

Pioglitazone treated animals gained significantly more weight compared to vehicle as expected (p < 0.0001), conversely liraglutide induced significant relative weight loss in HFCC fed rats (p < 0.0001) (Fig. 1A). Treatment with pioglitazone reduced the size of the liver when compared to vehicle (p = 0.006) (Fig. 1E). Despite the induced
weight loss, liraglutide appeared to have no effect upon the weight of the various adipose tissues or the HFCC-induced hepatomegaly (Fig. 1C–F).

Treatment with liraglutide appeared to have no effect upon the blood lipid profile. In contrast, pioglitazone significantly reduced and stabilised circulating triglyceride levels (p < 0.0001) and induced slight reductions in cholesterol levels (p = 0.06) (Fig. 2A, E, D, H). Both pioglitazone and liraglutide appeared to ameliorate the slight HFCC-induced insulin resistance, however only liraglutide significantly lowered the tAUC compared to vehicle (p 0.03) (Fig. 3B, C). Pioglitazone had no effect upon the HFCC-induced damage, as measured by ALT and AST. Liraglutide significantly reduced and stabilised the serum levels of

Fig. 1. Changes in body weight, calorie consumption and tissue weights. A) HFCC-feeding does not have any obesogenic effect compared to control. Pioglitazone treatment significantly increases body weight whereas liraglutide significantly decreases body weight compared to respective vehicles. B) Animals fed HFCC diet consumed significantly more calories. C–D) HFCC-feeding does not increase the size of fat deposits, epididymal adipose tissue (EAT) and inguinal adipose tissue (IAT). E) Significant hepatomegaly was induced by HFCC-feeding, treating with pioglitazone significantly reduced the size of the liver. * p < 0.0332, ** p < 0.0021, *** p < 0.0002, **** p < 0.0001.

Fig. 2. HFCC-feeding induces dyslipidaemia. A–D) Changes in serum levels of cholesterol, HDL, LDL and triglycerides over the course of the experiment. E) Circulating levels of cholesterol are significantly elevated in HFCC-fed rats. F) No changes in HDL levels were observed. G) HFCC-feeding significantly increase circulating LDL. H) No differences in serum triglyceride level was observed between control and HFCC fed animals, pioglitazone treated rats had significantly lower levels of triglycerides. * p < 0.0332, ** p < 0.0021, *** p < 0.0002, **** p < 0.0001.
ALT and AST (p = 0.02, p = 0.03) (Fig. 4C, D).

Pioglitazone had little effect upon the severity of macrovesicular steatosis. In contrast and in keeping with the effect on weight loss, liraglutide reduced the degree of steatosis in 33% of animals (Fig. 6A). Furthermore when compared to vehicle, Liraglutide treated animals were more likely to be scored grade 2 macrovesicular steatosis instead of grade 3 (44% vs 11%) (Fig. 6A). The prevalence of microvesicular steatosis was similar in all HFCC fed groups, except for the pioglitazone treated group where it was absent (Fig. 6B). There was no discernible effect of pioglitazone upon the level of lobular inflammation. Liraglutide decreased the severity of lobular inflammation, with 33% of subjects having 0–5 inflammatory foci present in the lobules (Fig. 6D). Treating with pioglitazone or liraglutide had no effect upon the prevalence of ballooning within HFCC-fed rats (Fig. 6E). Liraglutide did not have any effect on the number of subjects presenting microgranulomas, whereas pioglitazone increased the number of subjects with microgranulomas (Fig. 6F). Both treatments had no effect upon fibrosis as determined by histology and by assessment of collagen content of the liver (Figs. 6G and 7).

4. Discussion

The present study details the biochemical and histopathological characteristics of an atherogenic NASH diet in rats and the modification of the NASH-CRN histological scoring system for use in rodent studies. Furthermore, we investigated the effects of drugs previously investigated as potential NASH treatments, pioglitazone and liraglutide, within this model.

The HFCC diet induced significant hepatic injury and replicated features of human NASH, most notably elevated circulating transaminases, hepatic steatosis, lobular inflammation and hepatocyte ballooning. The NASH-CRN scoring system was modified to account for the
severe lobular inflammation induced by the HFCC-diet. Within the HFCC model of NASH we were unable to replicate the beneficial effects of pioglitazone seen within NASH patients [17,23,24]. In contrast, liraglutide reduced body weight, improved insulin resistance, lowered circulating transaminase levels and improved histological features of NASH, all of which has been seen within treated NASH patients [18].

In concordance with Heebøll et al. and Thomsen et al., animals fed the HFCC diet developed features indicative of the human NASH.
pathology such as; hepatomegaly, elevated circulating amino-transferase levels, steatosis and lobular inflammation [14,32]. Both studies noted that the atherogenic diet alone has no obesogenic effect; to circumvent this we fed the rats HFD for 9 weeks prior to study initiation and HFCC feeding. Such an approach has several benefits; the rats are obese and aged at the beginning of the study, reflecting the characteristics of NAFLD and NASH patients. In contrast to previous studies, we found that HFCC-fed rats were significantly more insulin resistant when compared to controls [14,32]. We postulate that the insulin resistance seen within this study may be mediated by the 9 weeks of HFD feeding that preceded the experimental HFCC feeding phase similar to what was seen by Hjuler et al. [33]. Interestingly HFCC feeding maintains the insulin resistant state, though it should be noted that the difference between HFCC and control was small. Within the human pathology, there are indications for the role of cholesterol in NASH [34,35]. This model uses a cholesterol concentration that is substantially higher than that seen within a typical human diet. Whilst being artificial, the high concentration of cholesterol is necessary for the rapid onset on NASH within this model.

NASH is characterised by the histological presence of steatosis, lobular inflammation and hepatocyte ballooning [3]. All these features were observed within HFCC-fed animals, though ballooning was marginal and only observed in a fraction of subjects, which is consistent with previous findings regarding rodent models of NASH [29,36]. Hepatic steatosis and NASH is a slowly progressing disease that takes years for affected patients to develop advanced liver fibrosis and exhibit serious clinical outcomes. Hence, accurately modelling such a slow progressing disease within rodents within a restricted timeframe is challenging. Liver fibrosis is the most valuable prognostic histological feature of liver biopsy within NASH patients [11–13]. The severity of liver fibrosis dictates the likelihood of an adverse clinical outcome [11,12]. Here we observed that the HFCC-feeding did not induce a pro-fibrogenic response, in contrast to Hheeboll et al. who observed that HFCC feeding upregulated markers of fibrosis such as Col1α1. Subsequent analysis of age-match chow-fed rats indicated that the 9 week period of HFD feeding was a key driver in the development of stage 1 fibrosis within the control group (Data not shown).

Both pioglitazone and liraglutide have been investigated as potential treatments for patients with NASH, the treatments showed modest efficacy within this experimental model of NASH. Both compounds were biologically active as exemplified by their actions upon body weight and, in the case of pioglitazone, circulating levels of triglycerides. Patients treated with pioglitazone typically gain 4–5 kg in weight, within this study we found that pioglitazone treated rats gained more weight than the respective control [17]. In addition, circulating levels of triglycerides dropped markedly in animals treated with pioglitazone, which has previously been observed within patients treated with pioglitazone [37]. In contrast to the findings of clinical studies and other experimental models of NASH [17,24,38,39], pioglitazone did not seem to have an effect on histological features of NASH and on the elevated circulating levels of ALT and AST induced by HFCC-feeding. In agreement with the findings of the LEAN clinical study by Armstrong et al. and a rodent study by Tølbo et al., body weight and the levels of circulating aminotransferases dropped significantly in rats treated with liraglutide, indicating that the compound was active. Liraglutide has
been shown to significantly reduce the severity of steatosis in humans [18], and this was observed in this study. A beneficial effect of liraglutide on the severity of lobular inflammation and microgranulomas, an effect not seen within NASH patients [18], was also observed. Pioglitazone and liraglutide are both clinically available drugs that have been approved for use in diabetic patients; hence, it is unsurprising that both compounds, to varying degrees,ameliorated the insulin resistance induced by HFCC-feeding.

5. Conclusion

In conclusion, we have shown that the atherogenic diet, HFCC, induces a phenotype in rats that is akin to that seen in human NASH patients. However, HFCC feeding did not promote the development of significant fibrosis within rodents during the study period. It would be interesting to evaluate whether a longer study time (at least 26–52 weeks) would have resulted in the development of fibrosis. Within this model of NASH we have shown that pioglitazone and liraglutide have little efficacy upon the induced histological changes. Despite any histological changes, liraglutide did reduce the levels of circulating aminotransferases, indicating a beneficial effect upon hepatic pathophysiology. In addition to the investigation into the HFCC NASH model and the efficacy of two compounds within this model.

Authors’ contributions

SJD, DJL, KH, PH, MAK, SB and SC conceived of and designed the study. SJDD, DJL, SD, STH, KH, PH, MAK, SB and SC contributed to data analysis and interpretation. SJD wrote the manuscript. All authors critically reviewed the manuscript for intellectual content. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

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