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Effect of quercetin administration during the first two weeks post-weaning on the development of non-alcoholic fatty liver disease and dyslipidaemia in Sprague Dawley rats fed a high fructose diet

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Abstract. Hepatic steatosis and dyslipidaemia are associated with excessive fructose consumption. We investigated the effect of quercetin intake during the early pre-weaning period on metabolic dysfunction caused by a high fructose diet. Sprague Dawley rats, 21-day-old, were weaned onto standard rat chow and randomly allocated to four groups which either water or 20% fructose solution to drink with or without quercetin (100 mg/kg body mass). Quercetin was administered for two weeks. Thereafter, rats continued on their respective diets for six weeks without quercetin. Terminally, serum triglyceride concentrations were not significantly different ($p > 0.05$) between males across groups. However, females receiving quercetin alone had lower serum triglyceride levels than those receiving fructose ($p < 0.01$). Quercetin increased the incidence of hepatic steatosis in female rats. Quercetin intake in the immediate post-weaning period may prevent hypertriglyceridemia. However, female rats receiving quercetin alone are predisposed to hepatic steatosis associated with a high fructose diet.

Key words: Post-weaning — Dyslipidaemia — High-fructose diet — Phytochemicals

Introduction

Fructose consumption has been associated with the rise in obesity and its allied complications, such as non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome (Coronati et al. 2022). There has been a significant increase in fructose consumption over the past 40 years (Pereira et al. 2017). This increase has mainly been due to the consumption of high-fructose corn syrup, used in sweetened beverages that are even consumed by children (Giussani et al. 2022). The harmful metabolic effects of fructose consumption have been observed as early as a few months after birth in children whose mothers consumed a sugar-rich diet during pregnancy (Hu and Malik 2010; Zheng et al. 2016). These metabolic alterations are shown to have lifelong effects such as transcriptome alterations to the normal functioning of the kidney, heart and brain, later in adulthood (Chao et al. 2016). The consumption of a fructose-rich diet has been shown to induce modifications in the expression of genes and receptors that code for enzymes involved in lipid and glucose metabolism. These include acetyl-Co-A-carboxylase, malic enzyme and glucose-6-phosphate dehydrogenase (G6PDH) (Taylor et al. 1967; Anderson and Kauffman 1973).

Early postnatal life is a crucial developmental window where health outcomes in later life can be programmed (Ekelund et al. 2007). Indeed, the Developmental Origins of Health and Disease (DOHaD) hypothesis emphasises the relationship between the peri-conceptual, foetal, and early phases of life, with either the manifestation of obesity and related metabolic disorders in later life (Vickers 2014) or, improved health through the programming of genes and/or altered gene expression (Reddy and Mbewu 2016). The early post-weaning period is characterised by

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profound hormonal and enzymatic changes. It is during this period that lipogenic enzymes, for example, acetyl-Co-A-carboxylase, malic enzyme, decarboxylating and G6PDH appear for the first time in the liver and adipose tissue (Taylor et al. 1967; Anderson and Kauffman 1973). Therefore, surges in the expression and activity of lipogenic enzymes during this developmental period may increase the risk of obesity and metabolic disease development later in life (Conceição et al. 2015).

Obesity is strongly associated with the development of NAFLD (Divella et al. 2019). Non-alcoholic fatty liver disease is caused by the infiltration of fatty droplets into hepatocytes without substantial alcohol consumption (Powell et al. 2021). It can range from simple steatosis to steatohepatitis and when inflammation is present, this can result in liver failure (Luci et al. 2020). Increased physical activity and dietary changes have been prescribed for the management of obesity and its associated complications such as dyslipidaemia and NAFLD (Munteanu et al. 2016). However, currently, no single pharmacological agent is specifically dedicated to the non-invasive treatment of NAFLD. Furthermore, current pharmacological agents prescribed to manage NAFLD aim to treat underlying conditions and may be associated with unpleasant side effects (Pennisi et al. 2019). Patients may require multiple drugs to target the key risk factors. Therefore, there is a need to investigate more natural, potential alternatives that are broadly biologically active such as phytochemicals.

The phytochemical quercetin is a natural polyphenolic flavonoid compound reported to possess anti-obesity, antithrombotic, antioxidant, antimicrobial, anti-inflammatory and hepatoprotective properties (Kawabata et al. 2015; Zhao et al. 2017). In the current study, we investigated whether quercetin, administered during the developmentally plastic early post-weaning period, has prophylactic potential on the development of NAFLD and dyslipidaemia in Sprague Dawley rats fed a high fructose diet. A 20% fructose solution provided *ad libitum* was used as the model for high fructose diet intake (Sandeva et al. 2015; Fakhoury-Sayegh et al. 2019).

Materials and Methods

This study was conducted in adherence with ARRIVE guidelines and was approved by the Animal Research Ethics Committee of the University of the Witwatersrand (Ethics clearance number: 2017/02/07B).

Animals, housing and general care

Sixty-four (32 males and 32 females), 21-day-old Sprague Dawley rat pups were used in the study. The rats were randomly allocated to one of four treatment groups, with 16 rats in each group (8 males and 8 females). The sample size was based on previous studies. The rats were housed individually in transparent Perspex cages containing wood shavings for bedding, in the Research Animal Facility of the University of the Witwatersrand. The bedding was replaced once a week. A standard 12-h light/12-h dark cycle (lights switched on between 7.00 and 19.00) and room temperature of 24 ± 2°C was maintained during the study period.

Study design

This prospective interventional study was divided into two major phases. In the first phase, which extended from when the rats were weaned at 21-day-old until they were 35-day-old, the rat pups were randomly allocated to one of four treatment groups replicated by sex. 1) Control group: standard rat chow (SRC), tap water to drink *ad libitum* and plain gelatine cubes, twice *per* day; 2) Quercetin-only group: received SRC, tap water to drink *ad libitum* and gelatine cubes containing quercetin (200 mg/kg body mass) (Liu et al. 2020), twice *per* day; 3) Fructose group: received SRC and 20% fructose drinking solution as drinking fluid (Meirelles et al. 2011) *ad libitum* to induce metabolic dysfunction and plain gelatine cubes, twice *per* day; 4) Fructose+quercetin group: received SRC, 20% fructose drinking solution *ad libitum* as drinking fluid and gelatine cubes containing quercetin (100 mg/kg body mass), twice daily to investigate whether quercetin intake in the early post-weaning period protects against fructose-induced metabolic dysfunction. The gelatine cubes, with and without quercetin, were placed into the rat cages on a white piece of paper to condition the rats. The rats were observed until they ate the cubes.

From post-natal day 36, the rats continued to the second phase of the study where they continued to receive SRC and either normal tap water or 20% fructose solution as drinking fluid, as *per* group allocation, but without gelatine cubes and their associated treatments. The second phase of the study lasted for 42 days. Fluid intake was measured for five days before termination. However an incomplete data set was collected. Figure 1 shows a summary of the group allocations and treatment regimens followed during the two experimental phases.

Gelatine cube and fructose drinking solution preparation

Gelatine cubes were prepared according to Kamerman et al. (2004) with slight modification of sugar content wherein 8 g gelatine (Sheridans gelatine, Libstar Operations (Pty) Ltd, Dunkeld, South Africa), 8 g brown sugar (Selati, RCL Foods Ltd, Westville, South Africa) and 3 ml Bovril (Beefy Bovril, Pioneer Foods (Pty) Ltd, South Africa), into 100 ml of hot water. Quercetin powder (Sigma-Aldrich Co., St. Louis,

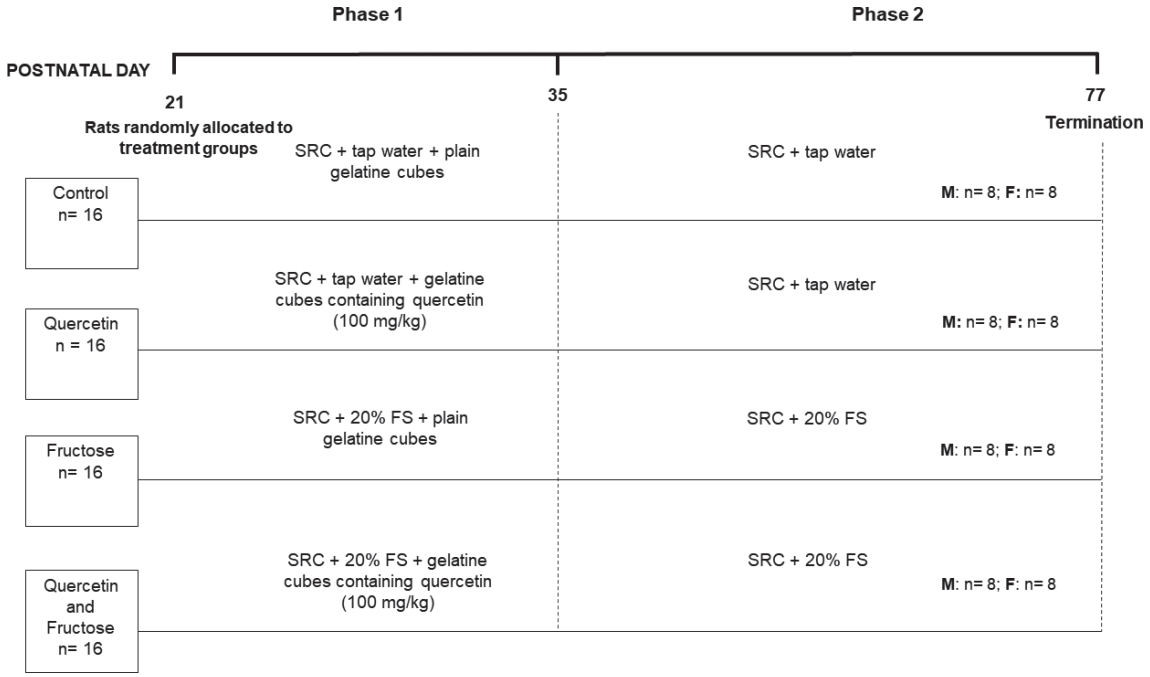


Figure 1. Summary of the group allocations and treatment regimens followed during the two experimental phases. M, male; F, female; SCR, standard rat chow; FS, fructose solution.

USA) (dose adjusted to rat body mass) was mixed with 0.5% dimethyl sulfoxide (DMSO) (Sigma-Aldrich Co., St. Louis, USA) as a solvent before adding it to the gelatine mixture. The mixture was then poured into 2 ml moulds and allowed to set in a refrigerator at 4°C.

The twenty percent (20%) fructose drinking solution was prepared by adding 400 g fructose powder (Fructose, Nature's Choice, Randvaal, South Africa) to 2000 ml of hot water and two drops of red food colouring (Robertson Food Colouring, Libstar Operations (Pty) Ltd, Dunkeld, South Africa). This was then mixed until fully dissolved. Two drops of green food colouring (Robertson Food Colouring, Libstar Operations (Pty) Ltd, Dunkeld, South Africa) were also added to the tap water provided to the other groups to distinguish it from the red-coloured fructose drinking solution. Bottles with drinking fluid (water or 20% fructose) were changed twice a week.

Body mass measurements

An electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan) was used to weigh the rats daily during the first two weeks to monitor growth performance and adjust the amount of administered quercetin to ensure a consistent dosage relative to body mass. Thereafter the frequency of weighing was reduced to twice a week.

Food and fluid intake

Food and fluid intake was measured from post-natal day 70 (last week of the study). Food intake was measured by computing the weight difference between feed initially provided to the rats and remaining feed weighed on the day using an electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan). Fluid intake was measured by measuring the difference between fluid offered 532 ml and that remaining after 24 hours. The average absolute daily feed and fluid intake were then computed for each treatment group. Additionally, the feed and fluid intake relative to body weight was also computed.

Terminal procedures

At the end of the second phase of the study, the rats were fasted overnight, where only tap water was provided. Blood was collected following a pinprick to the tail, and fasting glucose was measured using a calibrated glucometer (Contour plus Blood glucose monitoring system, Bayer (Pty) Ltd., Praha, Czech Republic). The rats were then euthanized using a sodium pentobarbital (Euthapent, Kyron Laboratories, Johannesburg, South Africa) overdose (200 mg/kg body mass injected intraperitoneally). Liver and blood samples were then collected for further analysis.

Blood parameters

Blood samples were collected by cardiac puncture into vacutainer coagulant-coated tubes (Vacuette tube, Greiner Bio-One, Kremsmünster, Austria). The blood was then centrifuged (1500 × *g* for 20 min) to obtain serum for the clinical biochemistry analyses (triglycerides (TGs), alanine transaminase (ALT), and high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol). The serum was then stored at −20°C until assays were performed.

Clinical biochemistry assays

Serum TGs and the surrogate marker of liver function, ALT were measured using an IDEXX Chemistry Analyser (IDEXX VetTest® Clinical Chemistry Analyzer, IDEXX Laboratories Inc., USA).

Serum HDL and LDL levels were determined using an ELISA kit (HDL and LDL/VLDL Cholesterol Assay Kit, Cell Biolabs, Inc., San Diego, USA) according to manufacturers' instructions. A fluorescent microplate reader (Cytation 5 Image Multi-Mode reader, Bio Tek Instruments, Vermont, USA) was used to read the microplates at the end of the assay processes.

Total cholesterol was calculated using the formula by Friedewald calculation equation: LDL = Total cholesterol – (Triglyceride/2.2 mmol/l) – HDL.

Tissue parameters

Body adiposity

Visceral and epididymal fat (male rats) deposition was quantified by dissecting and then weighing the absolute mass of the mesenteric, retroperitoneal and epididymal (males only) fat pads using a balance (Precisa 310 M, Precisa Gravimetrics AG, Dietikon, Switzerland). The relative mass (to body mass) of these fat depots was then computed.

Liver histology

The liver was excised from the euthanized rats and weighed. The left lobe of the liver was removed and preserved in 10% phosphate-buffered formalin (PBF). After fixation in the PBF, the liver samples were processed for histology using an automated tissue processor (Citadel 2000, Thermo Fisher Scientific, Runcorn, UK). The liver samples were then embedded in paraffin wax and sectioned using a microtome (Leica Biosystems Instruments (Pty), Ltd, model RM 2125, Wetzlar, Germany). Sections were subsequently mounted onto glass slides and stained with haematoxylin and eosin. Coverslips were then mounted and sections were histologically examined using a light compound microscope (Axioskop 2 plus microscope, Carl Zeiss (Pty) Ltd, Göttingen, Germany), provided with a couple-charged-device camera (AxioCam HRc, Carl Zeiss (Pty) Ltd, Göttingen, Germany) and image capture software (ZEN 2011, Carl Zeiss (Pty) Ltd, Göttingen, Germany). The liver slides were scored for the presence of micro-and macrosteatosis, as described by Brunt et al. (2011), whose scoring criteria are summarised in Table 1. The scoring was done by the researcher while blinded to the source groups of the rats.

Table 1. Microsteatosis and macrosteatosis score cut-off values

	Grade			
	0	1	2	3
Microsteatosis	<5%	5–33%	34–66%	>66%
Macrosteatosis	<5%	5–33%	34–66%	>66%

Hepatic lipid accumulation was semi-quantitatively analysed by determining the percentage area affected in each camera field (Brunt et al. 2011).

Statistical analysis

Parametric data are expressed as mean ± standard deviation. Non-parametric liver steatosis scores are expressed as median (interquartile range). GraphPad Prism version 5 software (Graph-pad Software Inc., San Diego, USA) was used for data analysis and graph plotting. Daily dietary intake was compared using a two-way ANOVA, for both male and female rats, separately. This was followed by Tukey's *post hoc* test for comparison of the means. Average total feed intake was analysed using a one-way ANOVA for both male and female rats, separately. This was followed by Tukey's *post hoc* test for comparison of the means. Differences were considered statistically significant when $p \leq 0.05$. The Kruskal-Wallis test was used to compare NAFLD steatosis scores between groups, for male and female rats, separately, followed by Dunn's *post hoc* test to compare the medians.

Results

Body mass

There were no significant differences ($p > 0.05$) across treatment groups in the initial body mass of both male (Fig. 2A) and female (Fig. 2B) rats, nor in their body mass at the end of the first phase of the study (day 35). All rats (both male and female) grew significantly during the first and second phases of the study ($p < 0.0001$). However, at the end of the second phase of the study period, after 8 weeks, the male (Fig. 2A) rats receiving fructose and those receiving fructose and quercetin had significantly lower final body masses than the rats receiving only quercetin ($p < 0.05$). There were no significant differences in final body mass between female treatment groups ($p > 0.05$) (Fig. 2B).

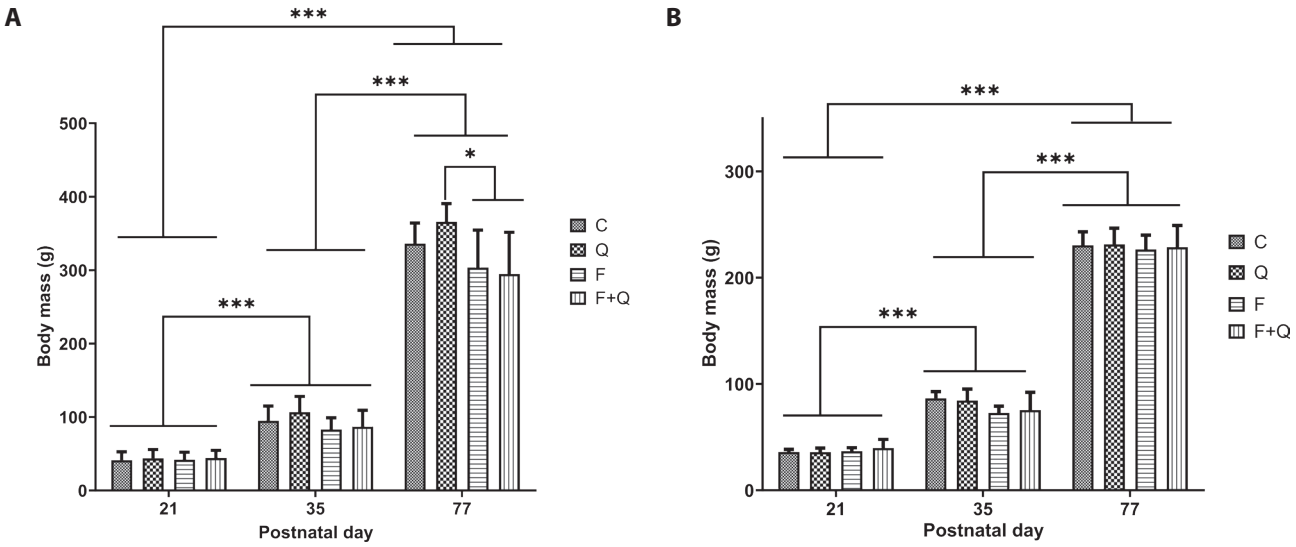


Figure 2. The effects of orally administered quercetin on the body mass of male (**A**) and female (**B**) Sprague Dawley rats fed a high fructose diet for eight weeks. Initial mass (postnatal day 21), body mass following the first phase (at postnatal day 35) and terminal body mass (postnatal day 77) was measured. Data represented as mean ± standard deviation (n = 8 in each group); * $p < 0.05$, *** $p < 0.0001$, **** $p < 0.0001$. C, control group; Q, quercetin-only group; F, fructose group; F+Q, fructose+quercetin group. For more information, see Materials and Methods section.

Circulating metabolites

Table 2 shows the effects of orally administered quercetin on circulating metabolites of male and female Sprague Dawley rats fed a high fructose diet for eight weeks. No significant differences in fasting blood glucose levels ($p > 0.05$) were observed amongst male and female rats. There were no significant differences observed in serum HDL cholesterol for both male and female rats, and LDL cholesterol (in female rats) ($p > 0.05$). Amongst male rats, significant differences were observed in total cholesterol and HDL cholesterol, with Fructose group having significantly higher serum concentrations than Control, Quercetin-only and Fructose+quercetin groups ($p < 0.05$). Significant differences were also observed between Fructose+quercetin groups having significantly higher LDL cholesterol levels than the Control and Quercetin-only groups ($p < 0.05$). Similarly, female rats in Quercetin-only group had significantly lower

Table 2. Circulating metabolites of male and female Sprague Dawley rats after eight weeks intervention

	C	Q	F	F + Q
Male				
Glucose (mmol/l)	3.58 ± 0.36	3.63 ± 0.35	3.78 ± 0.47	3.59 ± 0.29
Triglycerides (mmol/l)	0.42 ± 0.16^{abd}	0.42 ± 0.16^{abd}	0.70 ± 0.11^{c}	0.44 ± 0.51^{abd}
HDL (μM)	6.50 ± 0.58	6.05 ± 0.59	6.17 ± 0.48	7.32 ± 1.82
LDL (μM)	1.67 ± 0.23^{abc}	1.70 ± 0.13^{abc}	1.86 ± 0.20^{abc}	2.16 ± 0.31^{ad}
Total cholesterol (mmol/l)	0.20 ± 0.07^{abd}	0.20 ± 0.07^{abd}	0.32 ± 0.05^{c}	0.20 ± 0.02^{abd}
Female				
Glucose (mmol/l)	3.60 ± 0.23	3.78 ± 0.18	3.68 ± 0.24	3.52 ± 0.52
Triglycerides (mmol/l)	0.45 ± 0.19^{ab}	0.34 ± 0.15^{a}	0.74 ± 0.30^{b}	0.65 ± 0.24^{ab}
HDL (μM)	9.18 ± 1.58	9.76 ± 1.55	9.27 ± 2.89	12.00 ± 1.052
LDL (μM)	1.83 ± 0.47	1.84 ± 0.46	2.73 ± 0.85	3.07 ± 1.07
Total cholesterol (mmol/l)	0.25 ± 0.09^{ab}	0.17 ± 0.67^{a}	0.36 ± 0.16^{b}	0.29 ± 0.08^{ab}

Data represented as mean ± standard deviation (n = 5 in each group). Values in a row with different superscripts are significantly different ($p < 0.05$). C, Control group; Q, Quercetin-only group; F, Fructose group; F + Q, Fructose+quercetin group; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol. For more details, see Materials and Methods section.

Table 3. Absolute and relative adiposity of male and female Sprague Dawley rats after eight weeks of intervention

	C	Q	F	F + Q
Male				
Absolute visceral fat (g)	7.07 ± 1.58	8.24 ± 2.07	6.66 ± 2.64	6.34 ± 2.77
Relative visceral fat (%)	2.09 ± 0.35	2.23 ± 0.43	2.22 ± 0.74	2.06 ± 0.59
Absolute epididymal fat (g)	2.15 ± 0.43	2.39 ± 0.35	1.69 ± 0.44	4.51 ± 8.03
Relative epididymal fat (%)	0.64 ± 0.09	0.65 ± 0.06	0.56 ± 0.14	1.37 ± 2.28
Female				
Absolute visceral fat (g)	8.39 ± 1.49	7.88 ± 1.43	8.26 ± 1.64	8.99 ± 2.75
Relative visceral fat (%)	3.63 ± 0.56	3.39 ± 0.45	3.63 ± 0.57	3.88 ± 0.87

Data represented as mean ± standard deviation (n = 8 in each group). For abbreviations, see Table 2.

serum TG and total cholesterol levels than those in Fructose group ($p < 0.05$).

Adiposity

Table 3 shows the effects of orally administered quercetin on the adiposity of male and female Sprague Dawley rats fed a high fructose diet for eight weeks. In both males and females, no significant differences ($p > 0.05$) in the absolute and relative (to body mass) visceral fat masses were observed. In male rats, no significant difference was observed in epididymal fat mass between treatment groups ($p > 0.05$).

Effect of orally administered quercetin on serum biomarker of liver function

No significant differences in serum ALT were observed between treatment groups of male (Fig. 3A) and female (Fig. 3B) Sprague Dawley rats fed a high fructose diet for eight weeks.

Liver steatosis scores

Figures 4A and 4B are photographs of liver histological sections from representative male and female rats from each dietary group. In male rats (Fig. 4A), steatosis was observed in groups receiving fructose alone and the steatosis observed in those receiving fructose and quercetin appeared more pronounced than that observed in the groups receiving quercetin alone and those in the Control group. However, in female rats (Fig. 4B), subjectively, the steatosis appeared more pronounced in the groups receiving quercetin alone and fructose alone, compared to the Control and Fructose+quercetin groups.

Nonetheless, following objective assessment based on NAFLD Activity Scoring (NAS) criteria, no significant differences in the overall and micro-and macro steatosis scores

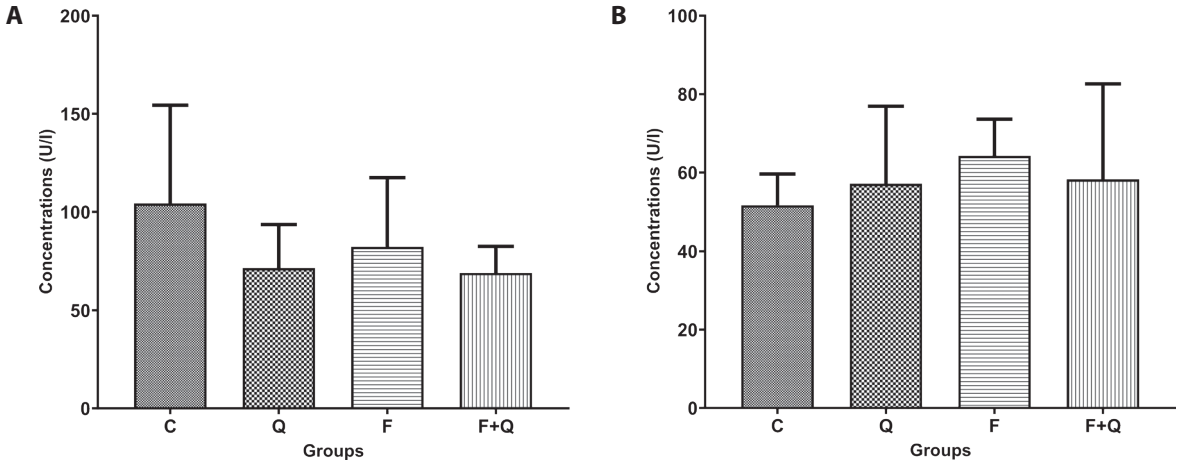


Figure 3. Serum biomarker of the liver function – alanine aminotransferase (ALT) – of male (**A**) and female (**B**) Sprague Dawley rats after eight weeks of receiving either a normal or high fructose diet, with or without quercetin. Data represented as mean ± standard deviation (n = 8 in each group). For other abbreviations, see Figure 2.

were observed across all treatment groups ($p > 0.05$) in both males and females (Table 4), respectively.

Percentage distribution of steatosis

The percentage distribution was determined to further explore the distribution of steatosis within and across the groups. Table 5 shows the percentage of male and female Sprague Dawley rats with micro-steatosis after being fed a high fructose diet for eight weeks. A greater percentage of male rats administered fructose with or without quercetin had micro-steatosis compared to the other groups. Female rats in Control group had varying degrees of steatosis. Quercetin-only group had the greatest percentage of rats with severe steatosis (steatosis score 3).

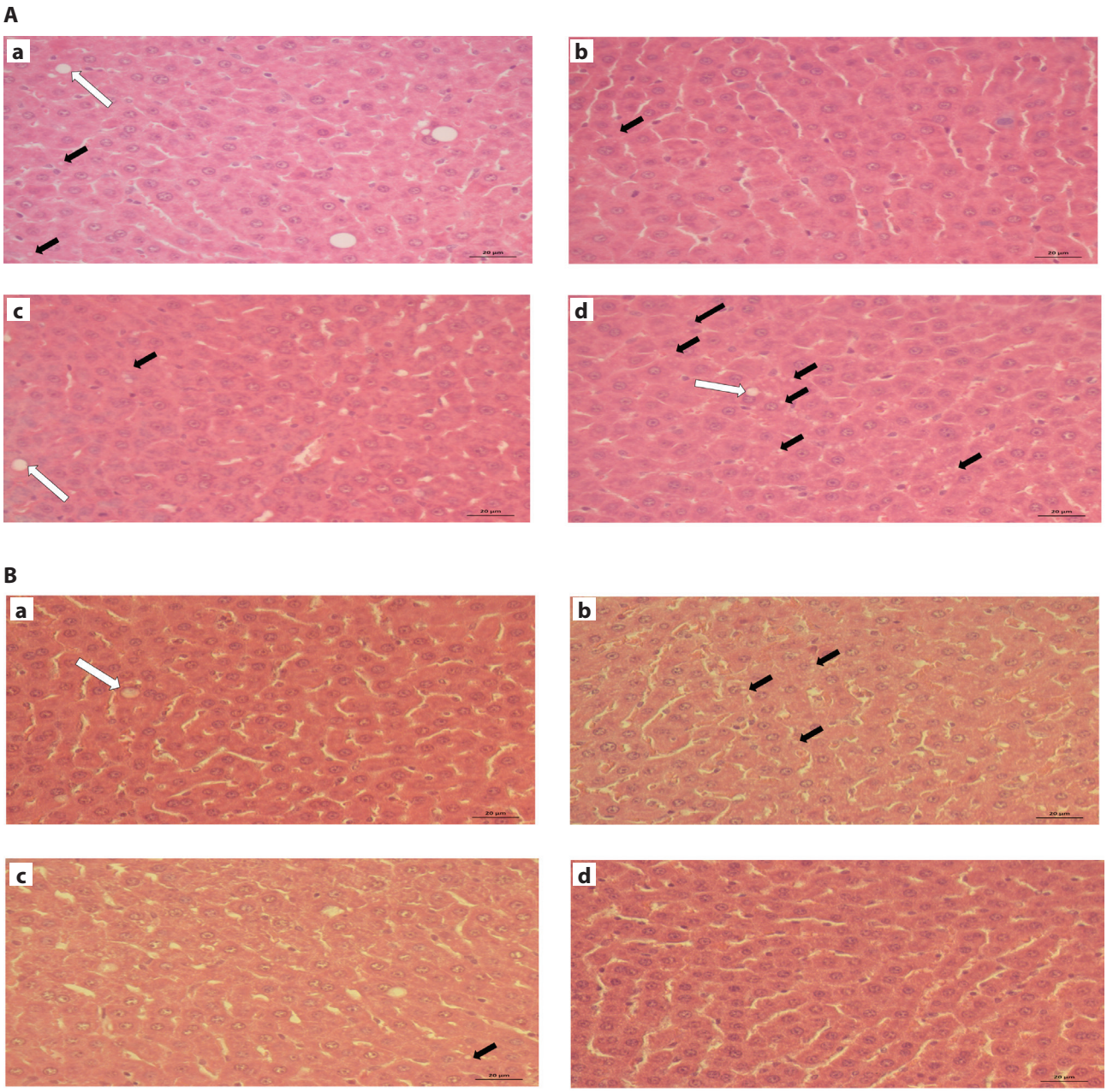


Figure 4. Liver histology representative sections (haematoxylin and eosin stain, 40× magnification) obtained from male (**A**) and female (**B**) Sprague Dawley rats after eight weeks of receiving either a normal or high fructose diet, with or without quercetin. Black arrows indicate micro-steatosis and empty arrow indicate macro-steatosis. **a**, Control group; **b**, Quercetin-only group; **c**, Fructose group; **d**, Fructose+quercetin group.

Table 4. Liver microsteatosis, and macrosteatosis scores by grade of male and female Sprague Dawley rats fed after eight weeks of intervention

	C	Q	F	F + Q
Male				
Microsteatosis	0.0 (0.00–0.75)	0.50 (0.00–1.75)	1.00 (0.00–1.75)	2.00 (0.25–2.75)
Macrosteatosis	0.0 (0.00–0.00)	0.0 (0.00–0.00)	0.0 (0.00–0.00)	0.0 (0.00–0.00)
Female				
Microsteatosis	1.00 (0.25–2.75)	1.50 (1.00–3.00)	1.00 (0.25–2.75)	0.50 (0.00–2.00)
Macrosteatosis	0.0 (0.00–0.00)	0.0 (0.00–0.00)	0.0 (0.00–0.00)	0.0 (0.00–0.00)

Data represented as median (interquartile range); $n = 8$ in each group. For abbreviations, see Table 2.

Food and fluid intake

Supplementary Figure S1 shows the average daily food and fluid intake of female and male rats over the last five days before termination. There was no significant difference in the fluid intake within female and male treatment groups during the five-day pre-terminal period ($p > 0.05$). There was no significant difference in the feed intake in female rat groups on all pre-terminal days ($p > 0.05$) except on pre-terminal day two, where Quercetin-only group consumed significantly less feed than rats in Fructose group ($p < 0.01$).

There was no significant difference in the feed intake on day one and day four amongst all male treatment groups. However, Quercetin-only group consumed significantly more feed than Fructose group on days two ($p < 0.01$) and day five ($p < 0.001$). Quercetin-only group consumed significantly more feed than rats in Fructose+quercetin group on days two, three and five ($p < 0.05$). No significant difference was observed in fluid intake amongst treatment groups for all male rats during the five-day period ($p > 0.05$).

Table 5. The effects of orally administered quercetin on the percentage distribution of microsteatosis in male and female Sprague Dawley rats after eight weeks of intervention

	Microsteatosis score (grade)			
	0	1	2	3
Male				
C	75	12.5	0	12.5
Q	50	37.5	12.5	0
F	37	37.5	25	0
F + Q	25	12.5	37.5	25
Female				
C	25	25	25	25
Q	12.5	37.5	12.5	37.5
F	25	37.5	12.5	25
F + Q	37.5	25	25	12.5

Data is represented as a percentage ($n = 8$ in each group). For grades, see Table 1; for abbreviations, see Table 2.

Supplementary Figure S2 shows the average of the total daily food and fluid intake of female and male rats over the last five days before termination. Female rats in all other treatment groups did not have any significant differences in average food intake for the five-day period ($p > 0.05$). However, male rats in Control ($p < 0.05$) and Quercetin-only ($p < 0.05$) groups consumed significantly more feed than rats consuming fructose with or without quercetin. There were no significant differences in average fluid intake among male and female rats across all treatment groups ($p > 0.05$).

Discussion

The current study investigated the effects of dietary supplementation with quercetin (during the early post-weaning period) on high fructose (20% fructose drinking solution) induced metabolic derangements in growing male and female Sprague Dawley rat pups. This study is distinctive in that it evaluated the potential prophylactic benefits of intervention with quercetin during the early post-weaning period against diet-induced metabolic derangements. The early post-weaning period is of particular interest because it is during this period that enzymes involved in metabolism, such as fatty acid synthase, appear for the first time in rat livers days after weaning (Morishita et al. 2014). The enzymatic activity of enzymes 6-phosphogluconate dehydrogenase and G6PDH has previously been shown to increase by 30% at weaning from their steady pre-weaning levels (Taylor et al. 1967; Hahn and Novak 1975; Bazin and Lavau 1982).

Although no statistically significant differences in micro- and macro-steatosis scores were observed between treatment groups, fructose consumption seems to predispose male rats to the development of hepatic steatosis, since there was a greater percentage of male rats administered fructose, with or without quercetin, that had micro-steatosis compared to the other groups of males. This is similar to findings in previous literature that have reported that fructose consumption in drinking water predisposes male Wistar albino rats weighing 280–300 g treated with high fructose

for 10 days to developing macrovesicular and microvesicular steatosis at the end of the treatment period (Armutcu et al. 2005). Although the aforementioned study was conducted in adult rats, even the short-term consumption of fructose had an adverse effect on the liver. Ackerman et al. (2005) also reported that high fructose intervention (60% fructose in rat pellets) predisposed male Sprague Dawley rats, weighing 200–250 g to moderate micro- and macro-vascular fat deposition following 5-week dietary intervention.

Unlike male rats, female rats in Fructose and Fructose+quercetin group had a lower percentage of micro-steatosis rats than other treatment groups not receiving fructose. Quercetin-only group of female rats had the greatest percentage of micro-steatosis. This could suggest that female rats treated with quercetin during weaning are more predisposed to the development of NAFLD later in life. Administration of quercetin alone did not affect the clinical biomarkers assessed or result in any other adverse health outcomes.

In the current study, no differences in body mass gain were observed between treatment groups over the eight-week study period, which commenced from weaning to adolescence, phases which are characterised by rapid growth. This suggests that it was unlikely that the high fructose diet or treatment regimen had any negative effects on the health of the rats, as measured by growth performance. This result is in accordance with a previous study from our lab (Donaldson et al. 2019) where it was observed that after eight weeks of high-fructose/high-cholesterol feeding, the female Sprague Dawley rats did not show any significant differences in final body mass between groups. No significant difference in body weight was also reported by Zarfeshani et al. (2011) between Control group and Fructose group where 6- to 8-week-old male Sprague Dawley consumed 21% w/v fructose in drinking water for 10 weeks. Larsen et al. (2013) also reported no significant change in body weight compared to control, following a 26-week intervention with 10% fructose in water solution, in 5-week-old male Wistar rats, fed a controlled diet contained 68% energy from glucose (corn starch or maltodextrin), 21% casein, and 12% corn oil and a fructose-rich diet that contained 50% energy from fructose, 18% energy from glucose (corn starch or maltodextrin), 21% casein, and 12% energy from corn oil.

In this present study, we did however observe that male rats receiving fructose and those receiving fructose and quercetin had significantly lower final body masses than the rats receiving only quercetin. This finding differs from a previous study by Oron-Herman et al. (2008) that showed that male Sprague Dawley rats fed a 60% fructose-rich diet, 21% protein, 5% fat, 8% cellulose, and standard vitamins and mineral mix diet exhibited an increased body mass gain after seven weeks of intervention; However, no significant differences were observed between Fructose group and Control group.

Fructose consumption has also been reported to induce adverse health effects associated with visceral fat accretion (O'Neill and O'Driscoll 2015; Popkin and Hawkes 2016) and thus increased susceptibility to dyslipidaemia and its associated complications. Mamikutty et al. (2014) reported that 20% fructose drinking solution consumption in male Wistar rats over eight weeks increased caloric intake and significantly increased visceral adiposity. Ramos et al. (2017) also found that dietary intervention with 20% fructose drinking solution in 30-day-old male Wistar rats for 90 days resulted in a significantly higher percentage of visceral fat compared to Control group which only received plain drinking water. However, in the current study, we found that there was no significant difference in the visceral fat deposits between rats in the various treatment groups. The lack of fructose-induced changes in visceral fat mass observed in the current study may be because rats were treated at a younger age compared to previous studies. Compared to older rodents, younger rodents have higher basal metabolic rates due to their higher muscle tissue, thus higher energy expenditure. Due to increased energy demand for growth and maintenance of body temperature in younger rats, they also have a higher basal metabolic rate than older rats (Kibler and Brody 1942). As a result, adult rats store more extra energy as fat, while younger rodents use it to sustain normal bodily functions and growth. In the early postnatal period and young age, organ development is crucial and the body maintains physiological homeostasis to protect the rats from diet-induced derangements. Since new cells are rapidly produced and homeostatic balance is maintained, the rats are protected from the adverse effects of diet-induced derangements.

Increased visceral obesity and metabolic syndrome were previously thought to result in the development of NAFLD. However, it has been shown that NAFLD can occur without the development of obesity or changes in lipid profiles (Abdelmalek et al. 2010). Indeed, in the current study, the interventions did not result in differences in the HDL and LDL (in female rats), beyond the normal ranges reported for rats. Male rats did show a significant difference in TGs and total cholesterol serum levels with Fructose group having significantly higher serum concentration levels than the other treatment groups. These findings are similar to the finding by Elkayam (2003) where fructose consumption elevates serum concentrations of TGs and total cholesterol in male Sprague Dawley rats, weighing 200–250 g receiving either a control diet: 21% protein, and 5% fat (of total energy, % kcal), sodium 0.49%, and potassium 0.49%, or a diet enriched with 60% fructose for 8 weeks. The study reported a significant difference in plasma levels of TGs and total cholesterol plasma with Fructose group having significantly higher plasma levels than Control group. In the current study, it was also observed that male rats in Fructose+quercetin group had significantly higher serum LDL cholesterol levels than both Control group and Quercetin-only group. These elevations in serum lipid content are likely due to fructose consumption inducing de

novo lipogenesis, leading to elevated blood lipid content even after short-term exposure to a high fructose diet.

Although female rats receiving fructose did show a significant increase in serum concentrations of TGs and total cholesterol compared to Quercetin-only group, these increases were not above levels of normal ranges for rodents. Nevertheless, it may be of clinical significance in terms of long-term metabolic health.

Upon assessing the liver histology, it was found that although there was no significant difference in the overall hepatic micro- or macro-steatosis scores amongst treatment groups, fructose consumption was associated with an increased percentage of male rats with micro-steatosis. Of potential concern was the observation that quercetin administration alone in female rats, during the early post-weaning period, increased the percentage of rats presenting with micro-steatosis. It can therefore be speculated that the administration of quercetin for two weeks, during the early post-weaning period predisposes female rats to the development of micro-steatosis. These findings were different from those observed by Kim et al. (2015) where six-week-old male C57BL/6 mice were fed a high-fat diet (lard and soybean oil 60%; carbohydrate 20%; protein 20%) and treated with quercetin (100 mg/kg) for nine-weeks had reduced obesity-induced hepatic lipid accumulation. The authors attributed the reduced hepatic lipid accumulation to quercetin-inducing heme oxygenase-1 (HO-1) *via* the nuclear factor erythroid 2-related factor 2 (Nrf-2) pathway, thus enhancing mitochondrial oxidative capacity.

The consumption of a fructose-rich diet has been associated with elevated activity of ALT, a liver enzyme that serves as a marker of liver damage, therefore its serum concentration can be used to assess hepatotoxicity (Liu et al. 2014; Aulbach and Amuzie 2017). Studies have found that although serum ALT concentrations can indicate liver disease, they do not always indicate the presence of significant liver disease or other MetS components in rodents (Perés Wingeyer et al. 2008; Kang et al. 2011). In the current study, serum ALT levels remained normal, this may be due to the lack of severe liver damage. This may be due to the similar hepatic steatosis scores observed between groups. There must be more than 50% damage to the liver before its functionality is compromised (Tucker and Heaton 2005) because the liver has a large residual functional capacity. Therefore, it can be assumed that although there was micro-steatosis, it was not substantial enough to induce an elevation in serum ALT.

Although incomplete data is reported, the supplementary data shows that over the five-day period there were no significant differences in the consumption of fructose and water across all dietary groups in males and females; however, we observed that in the last five days before termination, male rats consuming fructose consumed significantly less feed in three of the five-day period than rats in Quercetin-only group. Although not significantly different on day five we observed that male rats in Fructose+quercetin group consumed more fructose solution and less feed. This could be a result of rats maintaining an energy balance, thus less feed was consumed. Hence the daily data show variances in feed and fluid intake. To further elucidate these daily variances, the average of the total feed and fluid intake were computed and showed that male rats on the high fructose diet consumed less feed than those on water. This finding is concurrent with studies from Ramos et al. (2017) where it was observed that 30-day-old male Wistar rats consuming 20% fructose drinking solution consume less feed than rats consuming a control diet with tap water for 60 days and 90 days of intervention. Similar findings were reported by Miranda et al. (2019) where 35-day-old male Wistar rats consuming a 7% fructose in drinking solution for 12 weeks, consumed significantly less feed than rats in Control group after 12 weeks of dietary intervention. This reported reduced food intake by rats receiving fructose may be because rats are more prone to maintaining an energy balance and so the energy deficit that may otherwise be experienced because of consuming less feed, is compensated for by the high energy contained in the fructose. These studies also reported that rats consuming fructose has a significantly greater energy intake than rats in Control group, despite the lower feed intake. Similar to our study there was no significant differences in the final weigh at the end of the study period.

Conclusion

High dietary fructose intake is a recognised and established model for metabolic dysfunction. However, in the current study, administration of 20%, fructose *ad libitum* for 8 weeks from weaning did not cause the overt development of aspects of metabolic syndrome such as altered growth performance, increased adiposity, and deleterious changes in surrogate biomarkers of health.

The administration of quercetin during the early post-weaning period did not impact body mass or markers of general health. However, the administration of quercetin alone seems to have predisposed female rats to the development of hepatic steatosis. When administered during the early post-weaning period, these observations suggest that quercetin could result in NAFLD in adulthood. Of note in this study is the sexually dimorphic response of the rats to fructose administration and their susceptibility to hepatic steatosis development.

Thus, in conclusion, this study has highlighted the importance of considering both sexes when investigating the potential use of alternative interventions for treating NAFLD and its associated disorders, as there seem to be differences in reactions to interventions based on gender.

The study also highlighted the fact that liver steatosis can begin to develop independently of other generalised metabolic disorders.

Conflicts of interest. The authors have no conflicts of interest to declare.

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