Coronary Artery and Myocardium Injuries Induced by a High Cholesterol and Fructose Diet in Growing Male and Female Wistar Rats

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RESEARCH ARTICLE

Abstract
Cardiovascular disease (CVD) are mainly consequent of atherosclerosis. Men develop CVD at a young age, this risk increases in women at an older age. Several studies have been carried out on male rats, but experiments on growing rats especially female are rare. The objective of this study was to investigate the effect of a high cholesterol and high fructose diet on the coronary artery and myocardium in growing male and female rats Young Wistar rats were divided into control groups fed a standard diet, cholesterol groups supplemented with 3% cholesterol (ChD), and cholesterol-fructose groups supplemented with 3% cholesterol and 15% fructose (ChFrD) for 14 weeks. Each group consists of male (n=6) and female (n=6) rats. We found, in comparison with corresponding controls rats, that both ChD and ChFrD diets caused a significant hyperglycemia and dyslipidaemia. In hearts supernatants, we highlighted increases of total lipids, malondialdehyde and Catalase assays. The histopathological examination showed a disorganization of the myocardial structure, arterial walls damage and endothelium injuries. Our study showed that ChD and ChFrD diets, caused weight, biochemical, oxidative and tissue disturbances that could lead to CVD in both young male and female Wistar rats even during the growing period.

Keywords: Coronary artery; myocardium; Cholesterol-fructose; male and female Wistar rats.

INTRODUCTION
Cardiovascular diseases (CVDs) are the first cause of premature death worldwide. CVDs cause 17.3 million deaths annually, this number will increase to approximately 23.6 million in 2030 (Mozaffarian et al., 2016). CVDs comprise multiple maladies, such as coronary heart disease, hypertension, stroke, and peripheral vascular diseases. Furthermore, atherosclerosis (AS) is the main cause of morbidity and mortality in CVDs (Deng et al., 2019). AS plays a predetermining role in the pathogenesis of the two most common CVDs – cardiac ischemia and cerebrovascular disease (Fatkhullina et al., 2016). The primary initiating event in AS is Low Density Lipoprotein cholesterol (LDLc) accumulation in the subendothelial matrix. In native coronary vessels AS is characterized by sub-intimal accumulation of smooth muscle cells, foamy lipid laden macrophages, extracellular matrix formation and angiogenesis. Dietary factors play a key role in the development of various human diseases including atherosclerotic disease (El-Sabban et al., 2008). The change of eating habits has led to a high consumption of sugary drinks, animal foods and less tedious...
products to prepare (Daboné et al., 2013), as well as a decrease in the consumption of fruits and vegetables (Pérez-Escamilla et al., 2013). In young people, the risk of developing CVDs is partly linked to their eating habits (Mikkilä et al., 2008; Ndlovu et al., 2019). Children tend to develop poor eating habits associated with a decrease in regular physical activity (Bechiri and Agli, 2012), which unfortunately are the main factors responsible for the increased risk of early onset of metabolic diseases: obesity, diabetes (DM), arterial hypertension, dyslipidemia, metabolic syndrome (MetS). Dyslipidemia refers to a lipoprotein metabolism disorder involving elevated concentrations of serum lipids, such as Total Cholesterol (TC), LDLc and Triglycerids (TG), and a marked lowering of High Density Lipoprotein cholesterol (HDLc) (Hill, 2006). In addition, hypercholesterolemia is associated with chronic low-grade inflammation, which acts as a key contributor to the initiation and development of dyslipidemia-related vascular diseases (Lumeng and Saltiel, 2011).

Many studies on adult male rats, that received a high energy diet, describe elevated plasma TC, TG, LDLc, HDLc, glucose (Glu), and insulin which lead to the development of DM, MetS and cardiovascular injuries. However, there are only few reports on the effect of high energy diets in young rats and female rats. In this study, we tested the effect of a diet enriched with cholesterol (Ch) alone or with fructose (Fr) in male Wistar (MW) and female Wistar (FW) rats. We attempted to establish the development of dyslipidaemia and hyperglycaemia, which may be precursors of DM. In parallel, we measured oxidative statue by malondialdehyde (MDA) and Catalase (CAT) assay. At the end of experiments, we observed the biochemical and histopathological injuries of the coronary arteries and myocardium. In addition, attempts were made to understand whether the changes appeared both MW and FW rats in growth.

MATERIALS AND METHODS

Animals and housing

6 to 7 week old Wistar rats, MW and FW, were obtained from the animal breeding division of Algeria Pasteur Institute (IPA, Algiers). Rats were housed at the Kouba-Higher Normal School (ENS-Kouba, Algiers) animal facility under controlled conditions of humidity, lighting, and temperature (50±10 % RH, 12-h light/dark, 23±2°C). All experiments were carried out in compliance with the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA) following approval by the local Ethical Committee of the Houari Boumediene University of Sciences and Technology (USTHB), Algeria (Agreement Number 45/DGPG/DVA/SA.14).

Reagents

The used chemicals and reagents in this study were of analytical high quality and were from Sigma (St. Louis, MO), Merck (Mannheim, Germany), Biochemical (Germany) and (Troika Pharmaceuticals, India). Corn oil and Fructose were purchased from local trend.

Dyslipidemia induction

After an adaptation period, MW rats weighing 136.33±6.76 g and FW rats weighing 126.33±5.71 g were randomly divided into six groups (n = 6 per group). The MW control (MC) and FW control (FC) groups were fed with a standard diet (SD). The MW Ch (MChD) and FW Ch (FChD) groups were daily supplemented by gavage with an hyperlipidic mixture. The MW Ch-Fr (MChFrD) and FW Ch-Fr (FChFrD) groups were daily force-fed with the hyperlipidic mixture and have free access to Fr solution. The hyperlipidic mixture consists of corn oil with 3% Ch and 1.5% sodium cholate. The Fr solution consists of 15% Fr in distilled water (Vogel, 2008). During the whole experiment which lasted 14 weeks, all rats have access to pellets and city water ad libitum. The pellets were provided by the national office of cattle feed, ONAB-Bejaia (East Algeria). They are composed of 49.80% carbohydrates, 23.50% proteins, 5% lipids and 5.7% mineral-vitamin complex. Control rats, MW and FW, were placed in the same experimental conditions and were daily gavaged with city water.

Animals were weighed at the beginning, initial body weight (IBW) and at the end of experiments, final body weight (FBW). The weight gains percentage (WG) was calculated with the formula: WG (%) = [(FBW-IBW)/IBW] x100.

At the end of experiments, blood samples were performed, after a 12 hour fasting period, on animals through puncture at the retro-orbital sinus under anesthesia with intraperitoneal injection of 150 mg/kg body weight ketamine hydrochloride. The blood was collected either in heparin-coated tubes for biochemical measurements and in EDTA-coated tubes for the measurement of oxidative parameters. The immediately blood sampled is centrifuged at 3500 t for 15 minutes and stored at -20°C until analysis. After euthanizing animals with ketamine hydrochloride, hearts were quickly removed and hearts weights (HW) were determined. The relative heart weight (RHW) was calculated with the formula:

\[ \text{RHW} = \left( \frac{\text{HW}}{\text{FBW}} \right) \times 100. \]
Tissue sampling/preparation for subsequent analyses
Each animal heart were divided in two pieces: one piece was fixed in 10% formaldehyde for histological analysis and the other one were homogenized [1/10 (w/v)] in physiological saline [0.9% (w/v NaCl)] using a Polytron homogeniser (T25 UltraTurrax; IKAWerke, Staufen, Germany). The homogenates were then centrifuged at 15000 tr/min for 20 min at 4°C and the supernatant were collected and stored at −20 °C until used for total lipids (T Lip), MDA and CAT assays.

Biochemical analysis
Biochemical parameters and oxidative stress analysis were measured by spectrophotometry (Jenway spectrophotometer, UK) and with (AE-600 Biochemical analyser, Erma inc. - Japan). Common plasma biochemical parameters, i.e. Glu, T Lip, TC, TG, HDLc, LDH and CK-MB levels were measured using standard and colorimetric commercial kits (SPINREACT, SantEsteve De Bas (GI). SPAIN), following the manufacturer's protocols. The LDLC fractions were estimated using the Friedewald formula: LDLc (mg/dl) = TC–(HDLc+TG/5) and the cholesterol in VLDLc is estimated by difference: VLDLc (mg/dl) = TC–(HDLc+LDLc).

MDA and CAT Assay
Plasma MDA concentration was measured according to the method of (Ohkawa et al., 1979). An aliquot of 100 µL was added to a reaction mixture containing 50 µL of 8.1% sodium dodecyl sulfate, 375 µL of 20.0% acetic acid (pH 3.5), 375 µL of 0.8% thiobarbituric acid. Samples were then boiled for 1h at 95°C and centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured at 532 nm, and MDA content was expressed as µmol/ml in plasma and as µg/100 mg per heart tissue (ε = 1.56 × 105 mmol/L/cm).

The CAT assay was performed according to the method described by Aebei (1984). In a spectrophotometer cell, put 500 µl of 0.2% H2O2 solution; add 950 µl of 0.1 M PBS buffer, then 50 µl of heart tissue supernatant. After mixing, the absorbance was measured at 240 nm, and CAT content was expressed as µg/100mg per heart tissue (ε = 39.4 µmol/L/cm).

Histological analysis
Hearts were fixed in 10% formol and embedded in paraffin. Sections at five µm were stained with hematoxylin-eosin (HE) and examined under light microscopy (Leica, 2010). Microscopic observation was performed under in order to verify alterations in the tissues. Digital images were obtained using optical microscope Leica connected to the AmScop 3.7 camera.

Statistical analysis
Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, US). All values are expressed as means±the standard error of the means (SEM). To identify significant differences in the effects of the diets, the normality of the distribution for the variables was tested by the Shapiro-Wilk test. Statistical analysis was carried out using one-way analysis of variance (ANOVA). Differences were considered significant at p<0.05.

RESULTS
ChD and ChFrD induce weight changes in growing MW and FW rats
After 14 weeks of experiments, we showed that FBW of both MW and FW rats in ChD and ChFrD-feed groups were less than FBW of controls rats (Table 1). This decrease of FBW is significant between MChFrD and MC groups. However, the WG (%) showed significant decrease in MChD, FChD, MChFrD and FChFrD groups compared to those of MC and FC rats (Table 1). The HW of MChFrD and FChFrD rats, showed a significant decrease when compared to their corresponding controls rats, while the RHW shows a highly significant difference of its levels in MChFrD group when compared to MChD group (Table 2).

ChD and ChFrD induce blood biochemicals changes in growing MW and FW rats

In blood: When we compare to the SD-feed groups, the ChD and ChFrD induced a significant increase of blood Glu in MChD, FChD, MChFrD and FChFrD groups. Furthermore, the ChD and ChFrD disturb the lipid status which exhibited significant increase of TC, TG, LDLc and VLDLc with a significant decrease of HDLc levels. We also noted a significant increase of LDH and CK-MB levels. In addition, the oxidative parameters, MDA results, were significantly increased (Table 3).

In heart supernatant: In comparison with MC and FC groups, T Lip assay showed a very significant increase in the MChD, FChD, MChFrD and FChFrD groups. Additionally, MDA and CAT assays carried out on the heart supernatant exhibited significantly increased levels in MW and FW rats supplemented with ChD and ChFrD diets (Table 2).
ChD and ChFrD induce histo-pathological changes in growing MW and FW rats

At the CAs level: The CAs are of musculoelastic arteries type. Their tunics are: intima, media and adventicia. In MW and FW ChD groups, we observed a rearrangement of the intima, the media and the adventitia accompanied by the presence of optically empty amorphous space [Figure 1 (c and d)]. In MW and FW ChFrD groups, we noted a rearrangement of the intima, a thickening of the Lei, the rupture of the endothelium and Lei, optically empty amorphous spaces at the level of the media and a reworking of the adventitia with cellular infiltration.

![Figure 1](image_url)

**Figure 1.** Histopathological changes in MW and FW rats CAs after 14 weeks of experiments. Control rats (A, Scale Bar = 50 µm and B, Scale Bar = 20 µm). ChD rats (C, Scale Bar = 50 µm and D: Scale Bar = 20 µm) intima rearrangement (red and blue arrow) the media (light blue arrow) and the adventitia (black arrow). ChFrD groups (E, Scale Bar = 50 µm and F, Scale Bar = 20 µm) intima rearrangement (dark blue arrow), Lei thickening (red arrow), endothelium and Lei rupture (pink arrow), optically empty amorphous spaces (light blue arrow) adventitia reworking (black arrow) with cellular infiltration (white arrow). HE staining

At the level of myocardium: In MW and FW ChD groups, the histological sections, showed complete disorganization of the cell architecture. Cardio myocytes became thinner and take a wavy shape; this change in shape is due to the destruction of the connective framework that maintains cell cohesion, it is probably a dilated cardiomyopathy. This form, favoured by the lack of cohesion between the cells, is believed to be due to the alteration of the extracellular matrix. In addition, we noted the presence of interstitial oedemas [Figure 2 (c and d)]. In MW and FW ChFrD groups we observed alterations marked by a thinner aspect of the cardiomyocytes, presence of interstitial oedemas, the presence of hemorrhagic focus and probable apoptotic nuclei [Figure 2 (e and f)].

No obvious pathological changes were detected in the main CAs [Figure 1 (a and b)] and in the myocardium of the control rats [Figure 2 (a and b)].
Table 1. ChD and ChFrD diets effects on body weight and weight gain (%) of Wistar male and female rats in growth, after 12 weeks of experiment

<table>
<thead>
<tr>
<th>Parametrs</th>
<th>Male Wistar rats</th>
<th>Female Wistar rats</th>
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<tbody>
<tr>
<td></td>
<td>MC</td>
<td>MChD</td>
</tr>
<tr>
<td>Initial body weight (BW; g)</td>
<td>136.33 ± 6.76</td>
<td>130.75 ± 4.31</td>
</tr>
<tr>
<td>Final body weight (FBW; g)</td>
<td>285.67 ± 6.5</td>
<td>255.50 ± 18.15</td>
</tr>
<tr>
<td>Weight gain (WG; %)</td>
<td>112.05 ± 10.72</td>
<td>106.43 ± 18.65*</td>
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</table>

Data are expressed as mean ± standard deviation (SEM); (n = 6). MC: Male Control, MChD: Male Cholesterol Diet, MChFrD: Male Cholesterol Fructose Diet, FC: Female Control, FChD: Female Cholesterol Diet, FChFrD: Female Cholesterol Fructose Diet.

* * p ≤ 0.05 significant; ** ** p ≤ 0.01 very significant; *** *** p ≤ 0.001 highly significant.

Table 2. ChD and ChFrD diets effects on heart weight, relative heart weight and supernatant heart tissue total lipids (T Lip), malondialdehyde (MDA) and catalase (CAT) of Wistar male and female rats in growth, after 12 weeks of experiment

<table>
<thead>
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<th>Parametrs</th>
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<th>Female Wistar rats</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MC</td>
<td>MChD</td>
</tr>
<tr>
<td>Heartweight ((HW; g))</td>
<td>0.84 ± 0.07</td>
<td>0.8 ± 0.01</td>
</tr>
<tr>
<td>Relative HW (RHW; %)</td>
<td>0.3 ± 0.02</td>
<td>0.28 ± 0.00</td>
</tr>
<tr>
<td>T Lip (mg/100g per heart tissue)</td>
<td>131.97 ± 16.17</td>
<td>199.85 ± 6.66 **</td>
</tr>
<tr>
<td>MDA (µg/100mg per heart tissue)</td>
<td>450.25 ± 138.82</td>
<td>873.93 ± 89.53 *</td>
</tr>
<tr>
<td>CAT (µg/100mg per heart tissue)</td>
<td>88.10 ± 8.74</td>
<td>352.97 ± 81.53*</td>
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</table>

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Data are expressed as mean ± standard deviation (SEM); (n = 6). MC: Male Control, MChD : Male Cholesterol Diet, MChFrD : Male Cholesterol Fructose Diet, FC : Female Control, FChD : Female Cholesterol Diet, FChFrD : Female Cholesterol Fructose Diet.

* * p ≤ 0.05 significant; ** * p ≤ 0.01 very significant; *** * * p ≤ 0.001 highly significant.

MChD vs MC, MChFrD vs MC, FChD vs FC, FChFrD vs FC.

MChD vs MChFrD; FChD vs FChFrD.

Table 3. ChD and ChFrD diets effects on blood biochemical parameters of Wistar male and female rats in growth, after 12 weeks of experiment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male Wistar rats</th>
<th>Female Wistar rats</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MC</td>
<td>MChD</td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>75.50±3.15</td>
<td>211.33±2.33***</td>
</tr>
<tr>
<td>TL (mg/dl)</td>
<td>200.70±16.50</td>
<td>267.85±7.2*</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>70.33±4.87</td>
<td>230.70±50.97*</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>15.68±6.47</td>
<td>64.62±8.91*</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>56.65±5.47</td>
<td>29.71±5.07*</td>
</tr>
<tr>
<td>MDA (μmol/ml)</td>
<td>106.81±20.6</td>
<td>215.81±8.69**</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>401.83±106.81</td>
<td>811.70±112.38*</td>
</tr>
<tr>
<td>CK-MB (U/l)</td>
<td>108.14±7.84</td>
<td>236.51±18.95**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (SEM); (n = 6). MC: Male Control, MChD : Male Cholesterol Diet, MChFrD : Male Cholesterol Fructose Diet, FC : Female Control, FChD : Female Cholesterol Diet, FChFrD : Female Cholesterol Fructose Diet.

* * p ≤ 0.05 significant; ** * p ≤ 0.01 very significant; *** * * p ≤ 0.001 highly significant.

MChD vs MC, MChFrD vs MC, FChD vs FC, FChFrD vs FC.

MChD vs MChFrD; FChD vs FChFrD.
**DISCUSSIONS**

Koceir et al. (2009) observed in Algeria, increasingly deviations in life-style alimentary, from the Mediterranean-Cretan model to American fast-food model and even worse. Inspired by the model of our society, where the number of people receiving inappropriate food increase, the sedentary lifestyle and the food abounds while energy expenditure decreases, especially among the youngest, compromising their nutritional status, we aimed to study the cardio-vasculo-metabolic repercussions of Ch alone or with Fr, in growing MW and FW rats. Both ChD and ChFrD diets lowered significantly the FBW and WG of treated rats. Our results corroborate with those of Zidani et al. (2017) who found growth retardation in growing animals supplemented with hyperlipidique diets. However, Bray et al. (2019) obtained in adult Wistar rats, feed with high ChD, a weight rises. Despite the low FBW of treated rats with ChD and ChFrD, the HW, as well as their RHW, were found to be significantly high, thus indicating a modification of their structures and/or activities. Our results corroborate those of Bitam et al. (2004) and Zidani et al. (2017).

Biochemical parameters showed that the MW and FW supplemented rats, with both ChD or ChFrD, develop a hyperglycemia and dyslipidemia. Our results agree with those of Hamlat et al. (2008) who found a slight hyperglycaemia in Wistar rats supplemented with hyperlipidic diet. Berdja et al. (2016) found increased blood Glu and decreased insulin in Psamommys obesus on a high carbohydrate-only diet. The results of the lipid balance show an increase in TC, TG, LDLc and VLDLc in male and female rats regardless of the high calorie diet to which they are subjected, while HDLc is found to be reduced. However, LDLc are found to be much higher in females in comparison with males from batches supplemented with the ChD or ChFrD, conversely, HDLc are found to be significantly reduced in males compared to females treated with the two diets. Furthermore, Bitam et al. (2004) recorded an increase in HDLc, LDLc and VLDLc in rats supplemented with 5% fresh vegetable oil. The increases of both CT, TG, LDLc and VLDLc are consistent with previous studies (Hamlat et al., 2008; Aïnouz et al., 2015; Echeverría et al., ...
2019) and correspond well with the pattern observed in human dyslipidemia, including elevated TG (Zimmet et al., 2005). In rabbits fed with standard diet supplemented with 2% Ch for 8 weeks Bozoky et al. (2006) showed elevated serum TC and LDLc levels. However, Son et al. (2007) obtained an elevated TC level, a decrease in HDLc and no change in TG in rats supplemented with 1% Ch. Similar results were obtained by Ainouz et al. (2015) and Zidani et al. (2017), who observed an increase in TC and LDLc in plasma in animals supplemented with vegetable oil and Ch essentially, which may be due to an increase of Ch absorption at the level of the intestine due to its emulsification by sodium cholates (Morozova et al., 2004). The Ch and Fr present in our two diets, might increase the production of TG and TC by the liver and might decrease the catabolism of LDLc by the repression of their receptors (Nicolosi, 1997). In Japanese patients, Akihiro Endo et al. (2021) concluded LDL-c was a residual risk for recurrent-ACS even after recommended standard LDL-C lowering management target values had been achieved. Indeed, Fr is distinguished from other sugars by its ability to induce intracellular ATP depletion, nucleotide turnover as well as uric acid production. Supplementation of rodents with high-dose fructose and sucrose causes rapid installation of lipid deposits in the liver as well as insulin resistance, while in muscles lipid deposits and insulin resistance appear after several weeks (Lecouttre et al., 2013). In addition, sodium cholate exerts an effect on the activity of cholesteryl ester hydrolase which regulates lipid metabolism in liver cells (Winkler et al., 1992).

LDH and CK-MB highlighted a significantly increase in rats supplemented with ChD and ChFrD when compared with control groups. Moreover, the MDA signed a significant increase in MW and FW rats supplemented with ChD and ChFrD; LDH and MDA increase in animals fed by high calorie diet (Bitam et al., 2004; Berdja et al., 2016).

In hearts supernatents, T Lip, MDA and CAT showed a significant increase in rats supplemented with ChD and ChFrD in comparison with their SD-feed rats. Khalaf and El Mansy (2019) found CAT levels close to that of control rats while MDA was found to be very high in rats with heart disease.

The histopathological study of heart both MW and FW rats, supplemented for 14 weeks with the ChD and ChFrD, revealed the establishment of a pathophysiology illustrated by cardiovascular remodelling, thus meeting the objective of our work. Thus, at the myocardium level, histological examination revealed the presence of alterations marked interstitial oedemas, haemorrhage, optically empty spaces in the form of vesicles, cardiomyocytes with probably apoptotic nuclei and a complete disorganisation of the cell architecture which may be the cause of the probable installation of dilated cardiomyopathy. This accumulation of oedemas can explain the rise of RHW. Concerning the vascularisation of the myocardium, we observed a rupture of the vascular endothelium, and the internal elastic boundary and a rearrangement of the media and the adventitia. The risk of silent myocardial ischemia and CAs stenosis was assessed by Kartikey et al. (2010).

On the other hand, we observed an inflammation characterised by cells infiltration in CAs of treated rats. Scientific evidence is mounting to confirm that inflammation at the vascular level plays a critical role in the pathophysiology of AS and CVD (Balogh et al., 2019). It is widely accepted that innate and adaptive immunity are important for the initiation and progression of the atherosclerotic process. The elements involved are monocytes, macrophages, T and B lymphocytes (Moriya, 2019; Prediman, 2019). According to Favier (2003) inflammation is an important source of oxygen radicals produced directly by activated phagocytic cells, which are the site of a phenomenon called oxidative explosion consisting of the activation of the complex of NADPH oxidase, an enzyme capable of using molecular oxygen to produce large amounts of superoxide anions at the cell membrane level, which we have seen in our results of MDA and CAT assays which are disturbed in blood and hearts supernatants. In addition, Khalaf and El Mansy (2019) found CAT levels close to control rats CAT and highlighted a rise MDA in rats with histological lesions similar to histological myocardium results of MW and FW rats.

In fact, in the etiology of atherosclerosis, high fat in general and high Ch in particular are recognized as classic risk factors for CVD (Han et al., 2002; Kan-Merchant et al., 2002). The presence of DM with lower values of HDL-c (Lin et al., 2021) caused left ventricular ejection fraction. The thickening of intima media of the CAs, which observed in ChD and ChFrD rats, could be due to the accumulation of substances of a glycoprotein or lipid nature in the interlamellar spaces. This thickening is associated with abnormal collagen synthesis by fibroblasts or vascular smooth muscle cells (Bassett et al., 2006).

**CONCLUSIONS**

In this study, we conclude that the diet enriched with Ch alone or with Fr in MW and FW rats, even in growth, induced dyslipidaemia and histopathological injuries in CAs and myocardium. Those tissue damages may initiate atherosclerosis pathologies, which is the cause of CVD. All these changes are common to both male and female rats in growth.

**Author Contributions:** LA and DB have conceived and designed the study. MZ, HR, and LA were responsible for sample collection, macroscopic and histopathological interpretation, and the manuscript writing under the guidance of DB. LA, SB, MZ and KC worked out a major part of the analysis protocols and carried out the laboratory examinations. SB and LA carried out the statistical analysis. All authors read and approved the final manuscript.
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Conflicts of Interest
The authors declare that they do not have any conflict of interest.

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