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The role of oxidative stress in alterations of hematological parameters and inflammatory markers induced by early hypercholesterolemia

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ABSTRACT

Aims: The investigation of the effects of a high cholesterol diet (HD) for a short-time period on hematological parameters and the potential role of oxidative stress and inflammation markers.
Main methods: Rabbits were fed either a control diet or a diet containing 1% cholesterol (HD) for 5–6 weeks. The plasma lipid levels, C reactive protein (CRP), total red blood cells (RBC), total white blood cells (WBC), platelet count, packed cell volume (PCV) and leukocyte formula were determined. Oxidative stress was evaluated by the thiobarbituric acid reactive substances (TBARS), total glutathione and GSH serum level measurements. The osmotic fragility and the membrane fluidity of erythrocytes were determined. The levels of total cholesterol and TBARS were also measured in the erythrocyte membrane suspension.
Key findings: A decrease in the RBC and PCV was observed in rabbits fed on HD. The membrane rigidity and osmotic fragility were increased, and the morphological changes caused by the HD and TBARS levels in the erythrocyte membrane may account for this phenomenon. The inflammatory markers as the CRP levels, the platelet count, the WBC and the neutrophils were increased. The TBARS and GSH levels in the serum were increased and decreased, respectively.
Significance: This study shows that feeding rabbits an HD for a short time induces hematological alterations, disturbances in the oxidant–antioxidant balance and an increase of inflammatory markers. These findings support the importance of the early correction or prevention of high cholesterol levels to disrupt the process leading to the development of cardiovascular diseases.

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Introduction

Hyperlipidemia, or a high level of serum cholesterol, is an important risk factor in the development of premature atherosclerosis (Riccioni and Sblendorio, 2012). Atherosclerosis is a primary cause of coronary and cardiovascular diseases (Hirobe et al., 1982; Jensen et al., 1967). Considerable data support that atherosclerosis is a chronic inflammatory process that is induced and perturbed by lipid accumulation (Glass and Witztum, 2001; Ross, 1999).

Oxygen free radicals are involved in the pathogenesis of hypercholesterolemic atherosclerosis, mainly by oxidizing low-density lipoproteins (LDL) (Sorescu et al., 2002; Chisolm and Steinberg, 2000). A marked and focal increase in the LDL concentration in the arterial wall was found in rabbits after 16 days of high cholesterol feeding (Schwenke and Carew, 1989). The cells of the arterial wall secrete oxidative products from multiple pathways that can modify the LDL trapped in the subendothelial space and initiate lipid oxidation. An oxidized LDL (oxLDL) activates an NFκB-like transcription factor and induces the expression of genes containing NFκB binding sites. The protein products of these genes initiate an inflammatory response that initially leads to the development of the fatty streak (Berliner et al., 1995). oxLDL also induces the expression of adhesion molecules by the endothelial cells, leading to the attachment of monocytes that migrate to the subendothelial space, where they differentiate into macrophages. The macrophages express scavenger receptors that bind oxLDL. The cytoplasm of the macrophages becomes full of lipid droplets, and the subsequent development of "foam cells" takes place. These cells produce cytokines and reactive oxygen species (ROS), which enhance the inflammatory process (Ross, 1993). This is the mechanism that links hypercholesterolemia, inflammation and oxidative stress with atherosclerosis.

It is known that severe hypercholesterolemia leads to alterations in hematological parameters, including an increase in the cholesterol content of erythrocytes, platelets, leukocytes, endothelial and smooth muscle

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cells (Balkan et al., 2002; Gleason et al., 1991). Severe hypercholesterolemia also leads to anemia (Akahane et al., 1896; Abdelhalim, 2010). Red blood cells have potent antioxidant protection consisting of enzymatic and nonenzymatic pathways that modify highly ROS into less reactive intermediates (Çimen, 2008). Because ROS are related to the development of cardiovascular diseases such as atherosclerosis (De Rosa et al., 2010), anemia may be a risk factor for these pathologies (Pereira and Sarnak, 2003).

Even though murine models are currently the most extensively used species for atherosclerosis research due to the relative ease of genetic manipulation, the rabbit is the species perhaps more sensitive to dietary cholesterol overload (Getz and Reardon, 2012). The rabbit is the only animal that can exhibit hypercholesterolemia within a few days of the administration of a high cholesterol diet (Yanni, 2004). Although various studies have reported differences in the biochemical and hematological parameters related to hypercholesterolemia in rabbits (Abdelhalim and Moussa, 2010; Pessina et al., 1981), there are no reports regarding the influence of oxidative stress and inflammation markers in rabbits fed a high cholesterol diet for a short-term.

The aim of this study was to investigate the effects of a high cholesterol diet for a short-time period on hematological parameters and to investigate the potential role of oxidative stress and inflammation markers.

Material and methods

Animals

The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Tucuman. All animal care and experimental protocols were performed according to the Guide for the Care and Use of Laboratory Animals (NIH Publication 86 to 23, revised 1985). Male hybrid Flanders rabbits from a slaughterhouse initially weighing 850–1000 g were used in this study. They were housed individually in a humidity- and temperature-controlled room with a 12-hour light cycle. The rabbits were fed 100 g/d of standard rabbit chow. After a one-week acclimation period, the rabbits were randomly divided into two groups. The control rabbits ($n = 12$) were fed a standard chow diet (CD), which is an appropriate maintenance diet for a normal adult rabbit. The hypercholesterolemic rabbits ($n = 12$) were fed a diet containing 1% cholesterol (SIGMA, St Louis, USA) for 5–6 weeks with free access to tap water (HD). Only male rabbits were used to avoid the secondary variability of sex differences in this experimental model. The animals were weighed daily throughout the experimental period.

Collection of blood

At the end of the 5–6 week dietary intervention, the animals fasted overnight and were sacrificed under ketamine (75 mg/kg) anesthesia. A direct cardiac puncture was used to collect blood samples, and the samples were placed into 2 polypropylene tubes: one tube was for the serum, and one tube was for the plasma. The blood for the plasma was collected in EDTA or citrate. The serum was separated by allowing the blood to clot at 37 °C and centrifuging at 3000 rpm for 10 min.

Determination of biochemical parameters

The total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were measured using colorimetric reactions with commercial kits (Wiener, Rosario, Argentina). The C-reactive protein (CRP) was determined by a quantitative turbidimetric test (Wiener, Rosario, Argentina), according to the manufacturer's instructions.

Determination of hematological parameters

The total red blood cells (RBC), total white blood cells (WBC), numbers of platelets, hemoglobin concentration (Hb), and erythrocytes indices such as mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean platelet volumes (MPV) were examined using an automated cell counter through standard methods (Wintrobe, 2009). The packed cell volume (PCV) was determined by the standard microhematocrit method and was expressed in percentages. The blood smear was performed with a drop of fresh blood. The monolayer obtained was fixed with methanol and stained with Giemsa. The blood cells were analyzed under a microscope at 10×, 40× and 100× magnification. The individual cells were examined, and their morphology was characterized and recorded to obtain the differential WBC (leukocyte formula). Photographs were taken with a digital camera incorporated into the microscope.

Determination of lipid peroxidation in serum

Lipid peroxidation was evaluated by measuring the thiobarbituric acid reactive substances (TBARS). Briefly, 700 μL of trichloroacetic acid (TCA 10%) was added to 500 μL of serum. The mixture was centrifuged (15 min; 10,000 rpm; 4 °C), and the supernatant (500 μL) was reacted with 500 μL of 0.67% (w/v) thiobarbituric acid (TBA). The samples were heated at 100 °C for 10 min. After cooling, the absorption of malondialdehyde (MDA)–TBA chromogen was measured spectrophotometrically at 540 nm. The TBARS concentration was calculated using MDA as a standard. The results are expressed as μmol of TBARS/mg of protein, as determined by Lowry's method.

Measurement of GSH and total glutathione serum level

The serum proteins were precipitated by adding 10% TCA. The mixture was centrifuged (15 min; 10,000 rpm; 4 °C). The supernatant was incubated with 0.3 mM NADPH and 6 mM 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) for 2 min at room temperature to determinate the total glutathione levels (TGL). Glutathione reductase (0.077 U) was added, and the mixture was incubated for 35 min. The supernatant (200 μL) was incubated for 15 min with 6 mM DTNB and buffer phosphates (pH 7.4) to calculate the reduced glutathione (GSH). The absorption was measured at 412 nm.

The GSH and TGL were calculated using a GSH standard curve.

The oxidized glutathione (GSSG) was calculated as GSSG = TGL − GSH.

The results are expressed as μg/mg protein, as determined by Lowry's method.

Serum protein electrophoresis

The total serum protein concentration was determined by Lowry's method. Aliquots from each rabbit sample containing equal amounts of protein (23–50 μg) were resuspended in SDS-containing sample buffer and heated at 100 °C for 5 min. Twenty micrograms of the proteins were loaded onto a 10% SDS-polyacrylamide gel. The electrophoresis conditions were 2 h, 30 mA and 140 V (Mini Protean II, Bio-Rad, USA). The electrophoresis gel was stained with Coomassie blue. The protein bands were quantified using the software Quanti-Scan (5.1).

Suspension of erythrocyte membranes

A suspension of erythrocyte membranes was obtained from whole blood with 2% EDTA at 0–4 °C. After 15 min of centrifugation at 2500 rpm, the plasma and the buffy coat were discarded. A disruption was induced by adding a cold hypotonic buffer (phosphate buffer 20 mOsm; pH 7.4 with 1 mM EDTA Na_2) at a 1:20 volume ratio. After

mixing the erythrocytes and the hemolysis buffer, the suspension was left for 20 min, and the hemolysate was centrifuged for 15 min at 35,000 ×*g*. The hemoglobin was removed by 3–4 washing steps in the same buffer. The suspension was kept in the same buffer at 4 °C. The membrane protein concentrations were determined by Lowry's method.

The TC and lipid peroxidation in the erythrocyte membranes were determined with the methods previously described. The results are expressed as mg of cholesterol/mL of suspension of erythrocyte membranes, mg of cholesterol/mg of total protein in a suspension of erythrocyte membranes and μmoles of TBARS/mg of total proteins in a suspension of erythrocyte membranes.

Osmotic fragility of red blood cells

The osmotic resistance (fragility) of the red blood cells was determined according to Parpart et al. (1947). Briefly, 0.02 mL of citrated whole blood was added to an osmotic resistance series. The series was prepared from a buffered 10% NaCl stock solution. Each tube contained 1 mL of the NaCl buffered solution at different osmotic values. After mixing, the blood and the salt solution were equilibrated for 1 h at room temperature. The readings were performed according to Parpart et al. (1947).

Measurement of erythrocyte membrane fluidity

The membrane fluidity was assessed through steady-state fluorescence polarization analyses using the lipid soluble fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). The probe was used at 500 μM in MeCN (methyl cyanide) prepared daily, stored and protected from light. The erythrocyte suspensions were homogenized and incubated in the dark for 30 min at 25 ± 5 °C in PBS labeled solutions under agitation. The fluorescence anisotropy values were determined in an ISS PC1 Photon Counting Spectrofluorometer using a 350 nm excitation filter and a 450 nm emission filter. All the filters were equipped with polarizers. The controls containing only the fluorophores were concurrently examined to correct for the interference of the measurement induced by light scattering and intrinsic fluorescence. The fluorescence anisotropy for DPH was determined as follows:

$$R = Ivv\text{-}GIvh/Ivv + 2GIvh,$$

where Ivv and Ivh indicate the emitted fluorescence intensities in the vertical and horizontal planes, respectively, when the excitation radiation is polarized vertically. G denotes the grating factor, a parameter determined for the instruments.

Statistical analysis

The Kolmogorov–Smirnov goodness-of-fit test was used to test for normal distribution. The results are expressed as the mean ± standard error (SEM). The differences in the mean values between the two diet groups were tested by the unpaired Student's *t*-test. Correlation analyses were evaluated by Pearson's correlation coefficient. The data were analyzed using GraphPad Prism 3.0 software. The results were considered significant when $p < 0.05$.

Results

Effects of HD on biochemical and hematological parameters

The rabbits fed the HD showed higher plasma levels of TC, LDL-C, TG and CRP than animals fed the CD. At the end of the experiment, no differences were found in the body weights between the diet groups (Table 1). HD induced the following hematological changes: a- a significant increase in the WBC and platelet count; b- the neutrophils were increased because of a lymphocyte decrease (Table 2); c- a reduction in the RBC counts, a decrease of the PCV and an increase of the MCHC (Table 3); d- the presence of acanthocytes and fragmentocytes. The total serum proteins were significantly increased in rabbits fed the HD. The electrophoresis revealed that the increase was caused by the serum albumin (Table 4).

We found that the serum levels of CRP were positively correlated with the WBC ($r = 0.88$, $p < 0.05$) and negatively correlated with the PCV ($r = -0.71$, $p < 0.01$).

Table 1
Weight and serum biochemical parameters from rabbits fed a control diet (CD) or a high cholesterol diet (HD).

	CD	HD
TC (mg/dL)	59.0 ± 5.6	871.8 ± 45.2**
LDLc (mg/dL)	23.8 ± 3.2	666.3 ± 99.1**
HDLc (mg/dL)	28 ± 5	35 ± 9
TG (mg/dL)	91.8 ± 14.4	222.3 ± 32.6**
CRP (mg/L)	5.1 ± 2.9	11.1 ± 2.9*
Weight (g)	2190 ± 100	2040 ± 170

Total cholesterol (TC), low density lipoprotein (LDLc), high density lipoprotein (HDLc), triglycerides (TG), and C reactive protein (CRP). Values are means ± SEM of 12 rabbits.
* $p < 0.05$.
** $p < 0.001$ indicates statistically significant differences between rabbits fed a HD and rabbits fed a CD (unpaired *t* test).

Effect of HD on oxidative stress

As shown in Figs. 1, 2a, b and c, the TBARS levels, TGL and GSSG level of serum were higher and the GSH serum level was significantly lower in rabbits fed the HD compared with rabbits fed the CD.

The TBARS levels were significantly correlated with the WBC ($r = 0.60$, $p < 0.05$) and the PCV ($r = -0.75$, $p < 0.05$).

Effect of HD on erythrocyte parameters

Osmotic fragility of red blood cells

The rabbits fed the HD showed increases in the osmotic fragility. The NaCl concentration to induce the initial hemolysis was: HD 8.7 ± 0.1 g/L vs. CD 7.2 ± 0.4 g/L ($n = 8$, $p < 0.05$). Total hemolysis was observed with the HD at 2.15 ± 0.50 g/L NaCl and the CD at 3.09 ± 0.37 g/L NaCl.

Measurement of erythrocyte membrane fluidity

The fluidity in the erythrocyte membranes from rabbits fed the HD (0.385 ± 0.033) was significantly lower than from rabbits fed the CD (0.342 ± 0.013, $n = 8$, $p < 0.05$).

Other parameters measured in the suspensions of erythrocyte membrane

The mg of cholesterol/mL of suspension of erythrocyte membranes (0.21 ± 0.03) was significantly increased in rabbits fed the HD compared with the control CD (0.10 ± 0.02; $n = 8$, $p < 0.05$).

The mg of cholesterol/mg of proteins and TBARS in the suspension of erythrocyte membranes were significantly greater in the HD rabbits (Figs. 3 and 4).

Table 2
Leukocyte formula from rabbits fed a control diet (CD) or a high cholesterol diet (HD).

	CD (%)	HD (%)
Staff	1 ± 1	1 ± 1
Segmented	15 ± 2	32 ± 6*
Eosinophils	3 ± 1	2 ± 1
Basophils	0 ± 0	0 ± 0
Lymphocytes	65 ± 4	49 ± 7*
Monocytes	6 ± 1	5 ± 1

Data are expressed as means ± SEM of 12 rabbits.
* $p < 0.05$ indicates statistically significant differences between rabbits fed a CD and rabbits fed a HD (unpaired *t* test).

Table 3
Hematological parameters from rabbits fed a control diet (CD) or a high cholesterol diet (HD).

	CD	HD
WBC ($\times 10^3$ μL)	3.87 ± 0.91	9.18 ± 2.87*
Platelets ($\times 10^3$ μL)	187.83 ± 46.87	313.67 ± 20.62*
RBC ($\times 10^6$ μL)	5.59 ± 0.20	4.96 ± 0.25*
PCV %	37.46 ± 1.02	33.68 ± 1.14*
Hb (g/dL)	10.86 ± 0.34	10.73 ± 0.34
(MCV) fL	68.22 ± 0.87	67.65 ± 1.29
(MCH) pg	20.10 ± 0.31	21.20 ± 0.68
(MCHC) g/dL	29.46 ± 0.29	31.33 ± 0.68*

WBC: total white blood cells. RBC: total red blood cells. PCV: packed cell volume. Hb: hemoglobin concentration. (MCV): mean cell volume. (MCH): mean cell hemoglobin. (MCHC): mean cell hemoglobin concentration. Data are expressed as means ± SEM of 12 rabbits.
* $p < 0.05$ indicates statistically significant differences between rabbits fed a CD and rabbits fed a HD (unpaired *t* test).

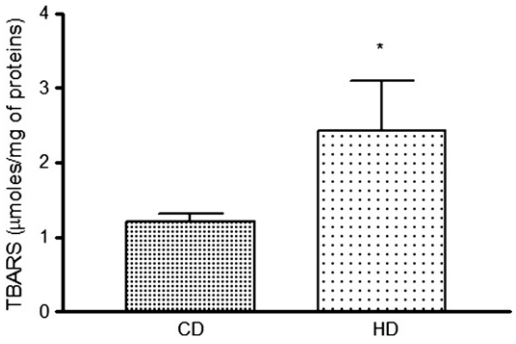

Fig. 1. The effects of a high fat diet on the serum levels of the thiobarbituric acid substances (TBARS) in rabbits fed a control diet (CD) or a high cholesterol diet (HD). The values shown represent the mean ± SEM (n = 12). The bars marked with * are significantly different ($p < 0.05$; unpaired *t* test).

Discussion

This study demonstrated that the consumption of an HD over a short time period could lead to RBC alterations linked to an increase in the oxidative state and inflammatory markers.

It has been reported that hypercholesterolemia modifies the RBC lipid composition, decreases membrane fluidity, alters permeability and the antioxidant system (Chisolm and Steinberg, 2000; Çimen, 2008) and increases hemolysis (Akahane et al., 1896) and RBC lipid peroxidation (Özdemirler et al., 2001). Abdelhalim (2010) demonstrated a significant decrease in the hemoglobin concentration, the percentage of hematocrit and the RBC count in rabbits. In agreement with these authors, the present rabbit model of early hypercholesterolemia showed hematological alterations that included a lower RBC count and hematocrit percentage. Walter et al. (2002) reported an underestimation of the hemoglobin concentration in blood samples caused by a high WBC count or lipid levels when blood cell automated analyzers are used. The hematimetric index that relates the hemoglobin and hematocrit (MCHC) is not affected by these factors. The MCHC was increased in rabbits fed the HD compared with rabbits fed the CD, even though there were no differences in the Hb concentration observed. An increase in oxidative stress has been suggested as a cause of the anemia induced by an HD (Balkan et al., 2004; Abdelhalim and Moussa, 2010). Balkan et al. (2002) found no changes in the erythrocyte lipid peroxide levels. In this study, feeding rabbits an HD for a short time increased the levels of lipid peroxidation in both the plasma and in erythrocytes membranes. Another possible mechanism for the anemia is the alteration in the physical state (fluidity) of the erythrocyte membrane induced by the high cholesterol level. Cholesterol is thought to condense and rigidify the plasma membrane by restricting the random motion of the lipid and protein molecules (Gleason et al., 1991). According to these data, the present work shows: A) increased cholesterol levels in the erythrocyte membrane (this was in disagreement with Akahane et al., 1986), B) increased fluorescence anisotropy and decreased membrane fluidity, C) alterations in the erythrocyte morphology (presence

Table 4
Plasma protein concentrations from rabbits fed on a control diet (CD) or a high cholesterol diet (HD).

Band number	CD (g/L)	HD (g/L)
1	4.84 ± 1.09	7.56 ± 1.57
2	11.58 ± 1.89	18.83 ± 3.70
3	5.50 ± 0.54	5.72 ± 1.06
4	2.40 ± 0.75	4.26 ± 0.91
5 (Serum albumin)	50.40 ± 3.56	62.39 ± 5.34*
Total	99.41 ± 4.82	127.27 ± 4.08*

1, 2, 3, 4, 5: protein bands obtained from serum electrophoresis and stained with Coomassie blue. Concentrations were calculated with the software Quanti-Scan (5.1). Total protein concentration was determined with Lowry's method. Data are expressed as means ± SEM of 12 rabbits.
* $p < 0.05$ indicates statistically significant differences between rabbits fed a CD and rabbits fed a HD (unpaired *t* test).

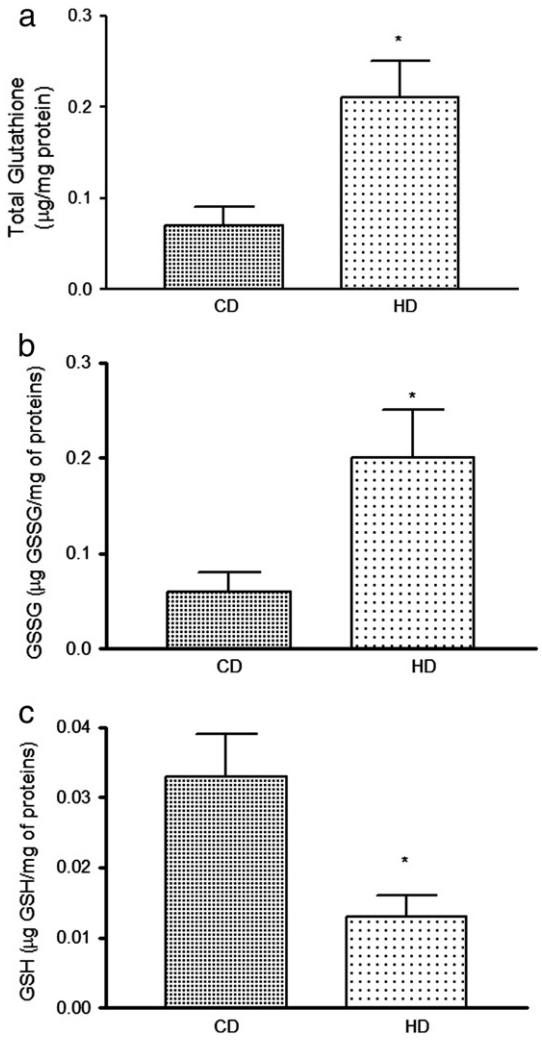

Fig. 2. The effects of a high fat diet on the serum levels of (a) total glutathione, (b) oxidized glutathione (GSSG) and (c) reduced glutathione (GSH) in rabbits fed a control diet (CD) or a high cholesterol diet (HD). The values shown represent the mean ± SEM (n = 12). The bars marked with * are significantly different ($p < 0.05$; unpaired *t* test).

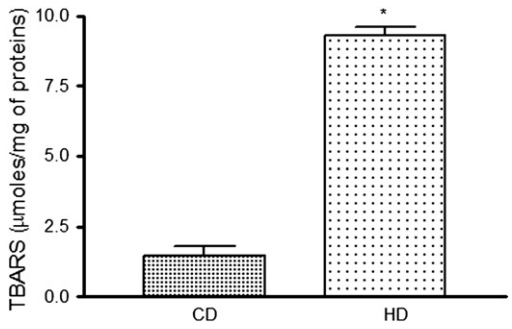

Fig. 3. The effects of a high fat diet on the levels of the thiobarbituric acid substances (TBARS) in the erythrocyte membranes from rabbits fed a control diet (CD) or a high cholesterol diet (HD). The values shown represent the mean ± SEM (n = 12). The bars marked with * are significantly different ($p < 0.05$; unpaired *t* test).

of acanthocytes and fragmentocytes), D) increased erythrocyte osmotic fragility that may be attributed to changes in the ionic motion through the membrane induced by the decreased fluidity. All together, these results suggest that feeding rabbits an HD for a short time may increase the destruction of the RBCs by lipid peroxidation (Vaya et al., 1995) and decrease the erythrocyte membrane fluidity. Considering that anemia has been recently recognized as a risk factor for cardiovascular diseases (Felker et al., 2004), the results from the present study support the early treatment of hypercholesterolemia even in healthy asymptomatic individuals.

Inflammatory factors play an important role in the development and progression of atherosclerotic lesions. CRP, a classic acute-phase reactant, is a sensitive marker of inflammation. Increased levels of CRP have been shown in several studies to be associated with atherosclerosis and coronary artery disease (Danesh et al., 2004). A considerable data support a causative role for reactive oxygen species (ROS) in these inflammatory processes. The ROS-induced production of inflammatory mediators such as CRP activates the pro-thrombotic factors and platelets (Zhang et al., 2012). Consequently, the present study showed that early hypercholesterolemia induces changes in the oxidative state, which are manifested as an increase in the lipid peroxidation capacity and a decrease in the antioxidant defense capacity of the glutathione system. The plasma CRP levels and platelet counts were higher in rabbits fed the HD. The higher WBC count may indicate a clinical or subclinical in vivo inflammatory status. An increased pro-inflammatory state enhances the activation of WBC and endothelial cells, thereby promoting platelet aggregation and thrombus formation (Ross, 1993; Waqar and Mahmood, 2010; Kabiri and Setorki, 2012). A significantly increased WBC count, neutrophil percentage and platelet count were observed in the present model of early hypercholesterolemia. The present results suggest that feeding rabbits an HD for a short time may increase the inflammatory activity by causing an imbalance between oxidant and antioxidant mechanisms. According to the concept of continuum cardiovascular (Dzau et al., 2006), the increase in oxidative stress and inflammatory factors produces endothelial dysfunction by initiating a series of events that results in the pathology of the target-organ. The present results strongly support the treatment of asymptomatic hypercholesterolemia for the prevention of cardiovascular diseases.

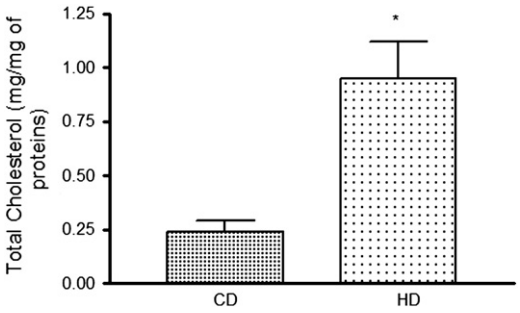

Fig. 4. The effects of a high fat diet on the cholesterol levels of the erythrocyte membranes from rabbits fed a control diet (CD) or a high cholesterol diet (HD). The values shown represent the mean ± SEM (n = 12). The bars marked with * are significantly different ($p < 0.05$; unpaired *t* test).

The present study found that feeding rabbits an HD increased the total serum proteins. The electrophoretic study revealed that hyperproteinemia was mainly due to an increase in the albumin concentrations. Dubach and Hill (1946) reported the effect of sustained hypercholesterolemia on the lipids and proteins in the plasma of the rabbit. They found no changes in the total protein levels in rabbits fed an HD. The disagreement with our results may be due to the different percentages of cholesterol added to the diet, the methodology of preparing the food and the age of the rabbits.

It is known that cholesterol may play an important role in liver disease. An HD induces a non-alcoholic fatty liver disease that can progress to hepatic failure. The mechanism proposed includes the imbalance of the oxidant/antioxidant state (Mehta et al., 2002). Under such conditions, the cells are the most sensitive to inflammatory factors, and hepatocyte apoptosis is increased (Mari et al., 2006). The results from the present study suggest that early hypercholesterolemia did not decrease the hepatic protein synthesis ability. Feeding rabbits an HD for a short time might not induce hepatotoxicity. This hypothesis must be demonstrated with further studies.

Conclusion

This study demonstrates that feeding rabbits an HD for a short time induces hematological alterations, disturbances in the oxidant–antioxidant balance and increases the inflammatory factors. The physiology of the blood cells is altered both as a consequence of the short-term exposure to high levels of serum cholesterol and lipid exchange between the plasma lipoprotein and the RBC membrane and because of the increased oxidative stress. An excess of erythrocyte membrane cholesterol decreased membrane fluidity and modified the RBC morphology. These cholesterol-induced alterations may account for the increased erythrocyte destruction. All these processes occur early and could be responsible for the progression of cardiovascular diseases. The present findings illustrate the adverse consequences of short durations of hypercholesterolemia and support the importance of early correction or prevention of high cholesterol levels to disrupt the pathophysiological process leading to heart disease.

Conflict of interest statement

No conflict of interest.

Acknowledgments

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