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LDL exposure disrupts mitochondrial function and dynamics in a hippocampal neuronal cell line

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ABSTRACT

Hypercholesterolemia has been associated with cognitive dysfunction and neurodegenerative disease. Moreover, this metabolic condition disrupts the blood-brain barrier, allowing Low-Density Lipoprotein (LDL) to enter the Central Nervous System. Thus, we investigated the effects of LDL exposure on mitochondrial function in a mouse hippocampal neuronal cell line (HT-22). HT-22 cells were exposed to human LDL (50 and 300 µg/mL) for 24 hours. After this, intracellular lipid droplet (LD) content, cell viability, cell death, and mitochondrial parameters were performed. We found that the higher LDL concentration LDL increases LD content compared to control. Both concentrations increased the number of Annexin V-positive cells, indicating apoptosis. Moreover, in mitochondrial parameters, the exposure of LDL on hippocampal neuronal cell line leads to a decrease in mitochondrial complexes I and II in both concentrations tested and a reduction in Mitotracker™ Red fluorescence and Mitotracker™ Red and Mitotracker™ Green ratio in the higher concentration, indicating dysfunction in the mitochondria. The LDL incubation induces mitochondrial superoxide production and a decrease in superoxide dismutase activity in the lower concentration in HT-22 cells. Finally, hippocampal neuronal cell line exposed to LDL exhibit an increase in the expression of genes associated with mitochondrial fusion (OPA1 and Mitofusin 2) in the lower concentration. In conclusion, our findings suggest that LDL exposure induces mitochondrial dysfunction and modulation in mitochondrial dynamics in the hippocampal neuronal cells.

Keywords: Hypercholesterolemia; brain dysfunction; LDL-cholesterol; mitochondria; HT-22 cells.

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1 INTRODUCTION

Hypercholesterolemia is a metabolic disorder characterized by high levels of plasmatic cholesterol [1]. It is already established that elevated plasmatic cholesterol levels are risk factors for developing atherosclerotic cardiovascular disease and stroke [2]. Moreover, in the last decades, hypercholesterolemia has been associated with the development of cognitive impairments characteristic of neurodegenerative diseases, such as Alzheimer's disease [3–6]. The lipoprotein more associated with health prejudice caused by hypercholesterolemia is the low-density lipoprotein (LDL)[7]. This lipoprotein is responsible for transporting cholesterol from the blood to the tissues [8, 9] ; however, it is easily oxidized in peripheral tissues[10].

The exact mechanism by which hypercholesterolemia leads to neuronal damage and, consequently, cognitive impairment is unclear, but some mechanisms have been proposed. Experimental studies in hypercholesterolemic rodents demonstrated alterations in the cholinergic system [11–13], reduced mitochondrial metabolism [13–16], increases in reactive species production [13–17] and alterations in antioxidant enzymes activity [14, 15, 17, 18] in different brain regions.

Cholesterol metabolism in Central Nervous System (CNS) occurs independently of peripheral metabolism since the plasma lipoproteins cannot cross the blood-brain barrier (BBB) [19]. Therefore, under normal conditions, brain cholesterol is derived from astrocytes [20, 21]. However, hypercholesterolemia is associated with BBB disruption [13, 22, 23], as well as neuroinflammation [11, 13, 24, 25], therefore allowing the entry of compounds of the peripheral system to the CNS, such as LDL and inflammatory factors [26, 27].

In vitro studies have already demonstrated that LDL causes an increase in reactive species production in human neuroblastoma cells [28], disturbs the structure and function of endolysosomes, as well as increases the Aβ production in primary neurons [29]. However, the direct effect of LDL on mitochondria from neurons is unclear. The neurons have high energetic demands to exercise their functions and require that their mitochondria are viable [30–33]. The mitochondria are responsible for several metabolic functions, such as ATP production. Moreover, the mitochondria are the primary source of reactive species production, mainly in oxidative phosphorylation [34]. Thus, when something causes mitochondrial dysfunction, especially mitochondrial complexes alteration, it leads to energetic deficits and oxidative stress [35].

Mitochondria are remarkably dynamic organelles that control their size, morphology, and number through fusion and fission, these processes are known as mitochondrial dynamics [36]. The maintenance of the mitochondrial organization, function, and morphology is complex, and it is orchestrated by a group of proteins

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that maintain the equilibrium between form and function by coordinating their activities [37]. These proteins are dynamin-related protein1 (Drp1), mitofusin (Mnf) 1 e 2, and Optic Atrophy 1 (OPA1). For mitochondrial fusion, the main proteins associated are Mnf 1 e 2, which mediate outer mitochondrial membrane fusion, while OPA1 mediates the inner mitochondrial membrane fusion [36]. The main protein associated with mitochondrial fission is Drp1 [38]. Therefore, herein, we investigated the effects of LDL exposure in HT-22 cells, mainly on mitochondrial function.

2 MATERIALS AND METHODS

2.1 Cell Culture and Experimental Design

HT-22 cells (Mouse Hippocampal Neuronal cell line) were grown in Dulbecco’s modified Eagle’s medium (DMEM, Sigma, D7777) containing 10% fetal bovine serum (CRIPION, SP, Brazil) and 100 IU penicillin/streptomycin (Sigma, P0781), at 37 °C in 5% CO_2 and 95% air in a humidified atmosphere. HT-22 cells were seeded at the density of $2x10^4/cm^2$, and after 24 hours of growing, HT-22 cells were exposed to LDL (50 or 300 μg/ml) for 24 hours. The concentrations were chosen from previous experiments that observed alterations caused by LDL exposure in neurons [28, 29]. All experiments were repeated at least three times. LDL was isolated from normolipidemic human serum by discontinuous density-gradient ultracentrifugation in KBr solutions containing 30 mmol/l EDTA as described by de Bem et al [39]. The concentration of LDL was determined from the total protein concentration, and protein content was quantified by the method described by Lowry et al. [40], using bovine serum albumin as standard. Ethical Committee project approved under number 4557728.

2.2 AdipoRed™ assay

Intracellular lipid droplets were quantified using the AdipoRed™ Assay Reagent (Lonza, PT-7009) according to the manufacturer's protocol. Briefly, after LDL exposure, the cells were prewashed with PBS once and incubated with the AdipoRed™ Reagent for 15 min (1:40). Data acquisition and analysis were performed in FACSCalibur™ flow cytometer (BD Bioscience, San Jose, CA, USA). Data were analyzed using FlowJo XV version 10 (FlowJo LLC)

2.3 Cell viability assay

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The cell viability was assessed by the reduction of 3-(4,5-Dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. After LDL exposure, we added a MTT (Sigma, M2128) solution (5 mg/mL dissolved in PBS sterile) to the medium, reaching a final concentration of 0.5 mg/mL. The cells were incubated for three hours at 37ºC, and the formazan formed by the reduction of MTT was dissolved in dimethyl sulfoxide (DMSO). Results are expressed as the percentage of control cells. All experiments were performed in technical triplicate. This protocol was adapted from Farias et al [41].

2.4 Cell Density

To assess cell density and determine the effects of LDL exposure on cell survival, we utilized the sulforhodamine B (SRB) assay. After the LDL exposure, HT-22 cells were stained with 0.4% sulforhodamine B (Sigma, S1402) in acetic acid 1% for one hour at room temperature. Excess unbound SRB was removed by washing the cells five times with distilled water. The stained cells were then dissolved in 1% SDS, and the absorbance was measured at 560 nm using the SpectraMax® M5 (Molecular Devices). Results were expressed as a percentage of control.

2.5 Lactate dehydrogenase (LDH) activity

LDH release was performed to test the loss of plasma membrane integrity. After LDL exposure, the culture medium was collected, centrifuged, and analyzed. The LDH activity was performed using the LDH diagnostic kit according to the manufacturer's instructions (BioTecnica).

2.6 AnnexinV positive-cells

The FITC Annexin V (QuatroG, 100034) was performed for cell death analysis following the manufacturer's instructions. Samples were incubated in a binding buffer containing Annexin-V FITC and PI for 15 minutes in the dark at room temperature. As a positive control, cells were frozen and then stained. Data acquisition and analysis were performed using a FACSCalibur™ flow cytometer (BD Bioscience, San Jose, CA, USA). Data were analyzed using Data were analyzed using FlowJo XV version 10 (FlowJo LLC).

2.7 Mitochondrial Complexes activities

Mitochondrial complex I (NADH dehydrogenase) activity was measured by the NADH-dependent ferric reduction rate at 420 nm, as described by Cassina and Radi [42]. The activity was calculated in nanomoles per

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minute per milligram of protein. The complex II activity was measured according to Fischer et al [43] by the decrease in absorbance due to the reduction of 2,6-dichloroindophenol (DCIP). The results were expressed as nanomoles per minute per milligram of protein.

2.8 Mitotracker™ Red and Green

Mitochondrial mass and membrane potential were evaluated by Mitotracker™ Green (MTG - Invitrogen™, M7514) and Red (MTR-Invitrogen™, M7512) dye, respectively. Therefore, it was possible to establish a relationship between MTR and MTG fluorescence to estimate the rate of mitochondrial function [44]. After LDL exposure, HT-22 cells were harvested using trypsin. Then, cells were resuspended and incubated for 20 minutes in the dark with 100 nM of MTG and 100 nM of MTR diluted into pre-warmed (37 °C) Hank's Balanced Salt Solution (HBSS). The samples were analyzed using a FACSCalibur™ flow cytometer (BD Bioscience, San Jose, CA, USA) and data were analyzed using FlowJo XV version 10 (FlowJo LLC).

2.9 MitoSOX™

For quantitation of mitochondrial superoxide generation, cells were loaded with MitoSOX™ Red (Invitrogen™, #M36008). After LDL exposure, the cells were prewashed with PBS once loaded with Mitosox Red (500nM) in HBSS for 30 min. Cells were then washed. Fluorescence intensity was then measured at 510/580 nm in SpectraMax® M5 (Molecular Devices)[45].

2.10 Superoxide dismutase (SOD) activity

SOD activity was determined using the RANSOD kit (Randox, SD125). This method employs xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a formazan dye that is assayed by spectrophotometric analysis at 505 nm at 37 °C in lysed cells. SOD activity is expressed as U/mg of protein. One unit of SOD causes a 50 % inhibition of the rate of reduction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride under the conditions of the assay.

2.11 Gene expression analysis (RT-qPCR)

RNA extraction was performed using TRIzol® Reagent (ThermoFisher Scientific, USA) and 2-Mercaptoethanol (Sigma-Aldrich, M3148) following the manufacturer's protocol and Santos et al. [46]. RNA

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concentration and purity were quantified using the I-Quant equipment (Loccus, BR), with purity verified through the ratio of absorbances at 260nm and 280nm (A260/A280). The cDNA synthesis reaction was then performed using 2 μg of RNA for each sample with the High-Capacity cDNA Reverse Transcription® kit (Thermo Fisher Scientific, USA).

Gene-specific primers were designed using IDT Design software (Integrated DNA Technologies Inc., USA), ensuring no secondary structures were generated. Primer efficiency was evaluated to confirm the absence of nonspecific amplifications. Subsequently, gene expression analysis for proteins involved in mitochondrial dynamics: Mitofusin 1 (MFN1), mitofusin 2 (MFN2), optic atrophy protein 1 (OPA1), and dynamin-related protein 1 (DRP-1) and β-actin housekeeping gene was conducted using the sequences shown in Table 1. RT-qPCR reactions were performed in triplicate using the PowerUp™ SYBR® Green Master Mix kit (Thermo Fisher Scientific, USA), following the manufacturer's instructions. The results were analyzed using the 2−ΔΔCT method.

Table 1. Sequence of primers used for RT-qPCR

Gene	Primer sequences
MFN1	Forward: 5’ GGT GGA AAT ACA GGG CTA CAG 3’
	Reverse: 5’ ACA CTC AGG AAG CAG TTG G 3’
MNF2	Forward: 5’ ATG TCC CTG CTC TTT TCT CG 3’
	Reverse: 5’ TCC AGT TCT GTG TTC CTG TG 3’
OPA1	Forward: 5’ ACG ACA AAG GCA TCC ACC 3’
	Reverse: 5’ GAG CAA TCA TTT CCA GCA CAC 3’
DRP1	Forward: 5’ TCA ATA AGC TGC AGG ACG TC 3’
	Reverse: 5’ TTC TGG TGA AAC CTG GAC TAG 3’
β-actin	Forward: 5’ TATGCCAACACAGTGCTGTCTGG 3’
	Reverse: 5’ TACTCCTGCTTGCTGATCCACAT 3’

2.12 Statistical analyses

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All experiments were performed on at least three occasions. Results were expressed as mean ± standard error of the mean (SEM). One-way ANOVA with Dunnet post hoc tests was used for data analysis, and a significant difference was defined as $p < 0.05$.

3 RESULTS

3.1 LDL exposure leads to an increase in intracellular lipid droplet content in the hippocampal neuron cell line.

First, we evaluated the intracellular lipid droplet content after 24 hours of LDL exposure. We observed an increase in the intracellular lipid droplet content at the higher concentration of LDL (Fig. 1a and 1b), confirming the LDL uptake by HT-22 cells.

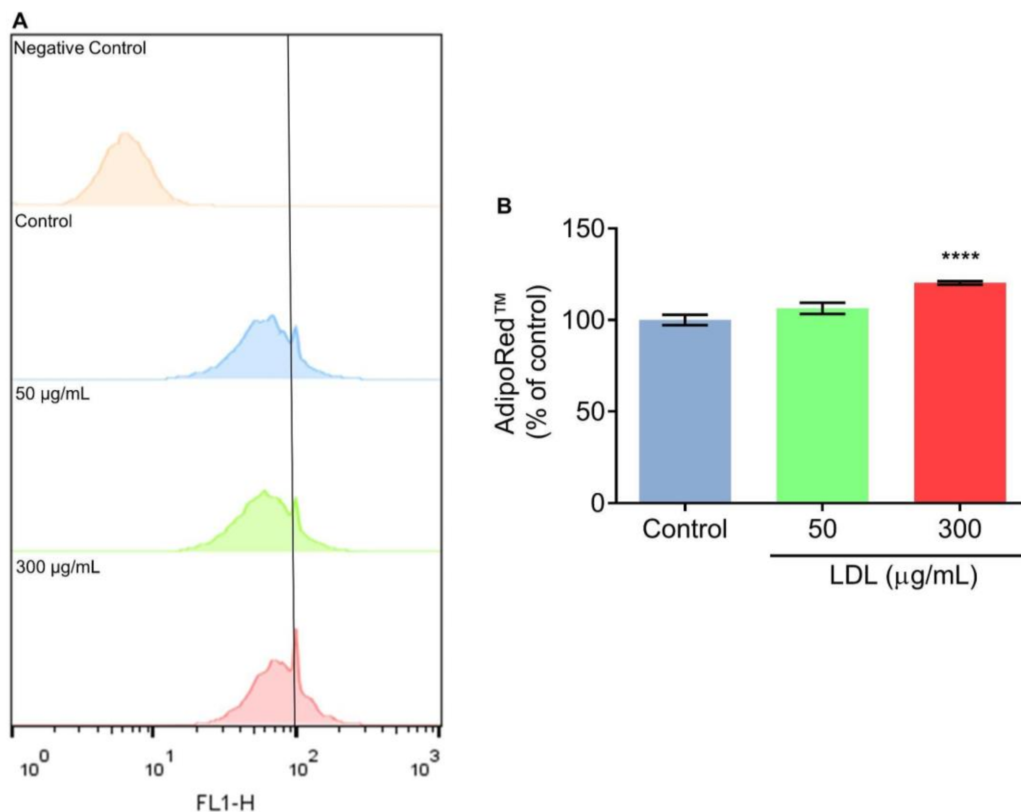

Fig. 1 LDL exposure increases lipid droplets in HT-22 cells. (a) Representative histogram of lipid droplets assessed by AdipoRed™. (b)Lipid Droplets. Data were expressed as mean ± SEM. Statistical analyses were performed using a One-way ANOVA/Dunnett post hoc test. ****p<0.0001 compared to control group.

3.2 LDL exposure induces an increase in AnnexinV-positive cells in the hippocampal neuron cell line

Exposure to LDL did not affect the viability of hippocampal neuronal cell line, as assessed by the MTT (Fig. 2a) and SRB assays (Fig. 2b). However, when we measured the cellular death by Annexin, we observed that

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LDL exposure (50 and 300 ug/ mL) leads to a significant increase of AnnexinV-positive cells in HT-22 cells (Fig. 2 d and e), suggesting that LDL induces apoptosis. Moreover, HT-22 cells exposed to LDL have no alteration in extracellular LDH activity, a parameter for necrosis (Fig. 2c).

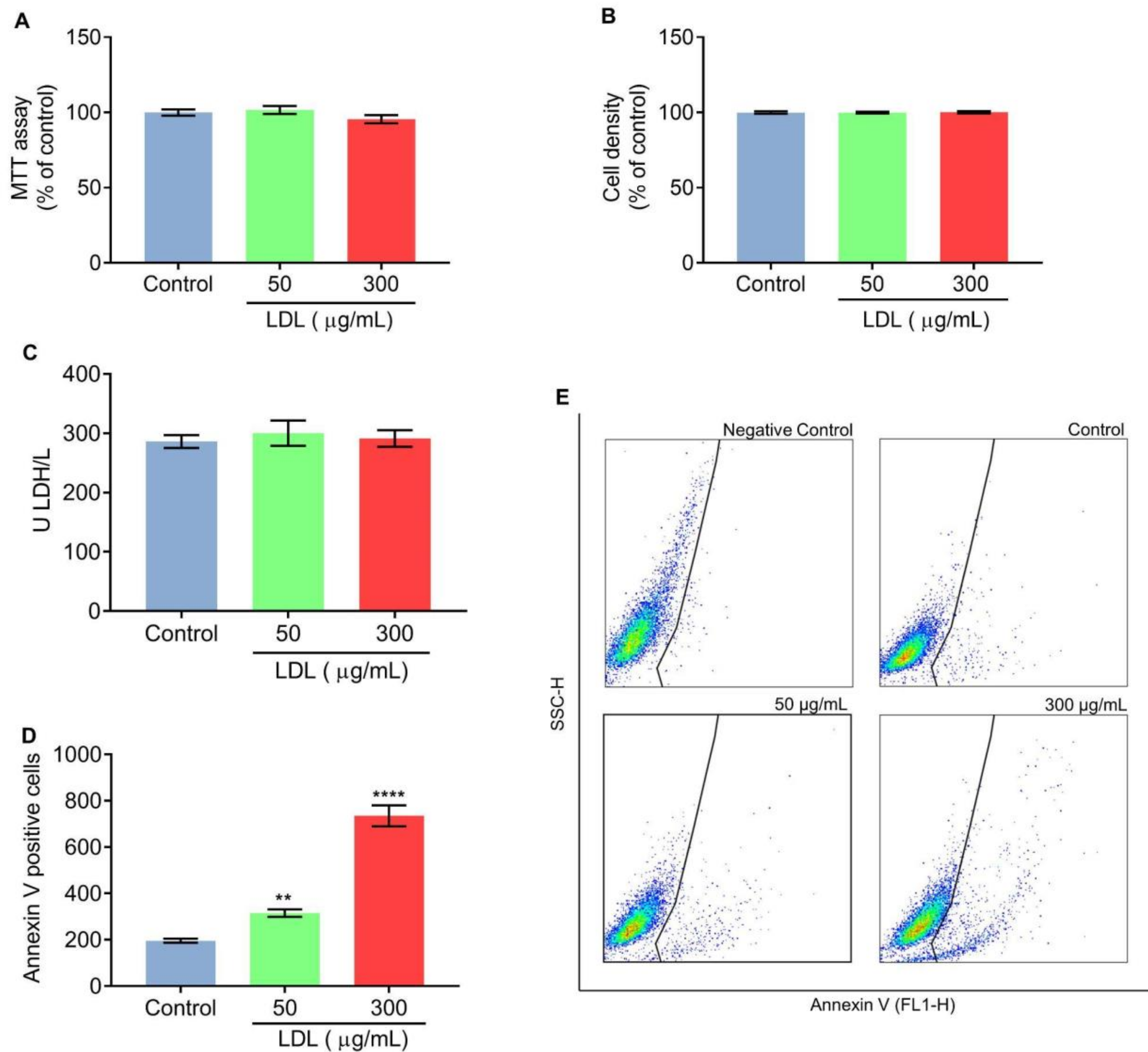

Fig. 2 LDL induces an increase in AnnexinV-positive cells in HT-22 cells. (a)The Cell viability was measured using an MTT assay. (b) SRB assay was used to measure cell density. (c) Extracellular LDH activity. (d) and (e) AnnexinV-positive cells, (d)graphic analysis, (e) Dot plot. Data were expressed as mean ± SEM. Statistical analyses were performed using a One-way ANOVA/Dunnett post hoc test. **$p<0.01$, ****$p<0.0001$ compared to control group.

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3.3 LDL exposure leads to a reduction in mitochondrial complexes activities and alteration in mitochondrial membrane potential in HT-22 cells

The effect of LDL on the mitochondria of the hippocampal neuron cell line was evaluated. We observed that the exposure to LDL leads to a decrease in mitochondrial complexes I (Fig. 3a) and II (Fig. 3b) in both concentrations (50 and 300 µg/mL) in HT-22 cells. Afterward, the measurement of mitochondrial mass (MTG) and potential (MTR) was made by flow cytometry. We observed that LDL induces a significant reduction in MTR fluorescence in the concentration of 300 µg/mL (Fig. 3d) without altering the mitochondrial mass (Fig. 4e). We also observed that the exposure to LDL at 300 µg/mL decreases significantly the MTR/MTG ratio (Fig. 3f). Figure 3C shows a fluorescence shift to the left and down upon exposure to 300 µg/mL of LDL in HT-22 cells, indicating mitochondrial damage induced by LDL.

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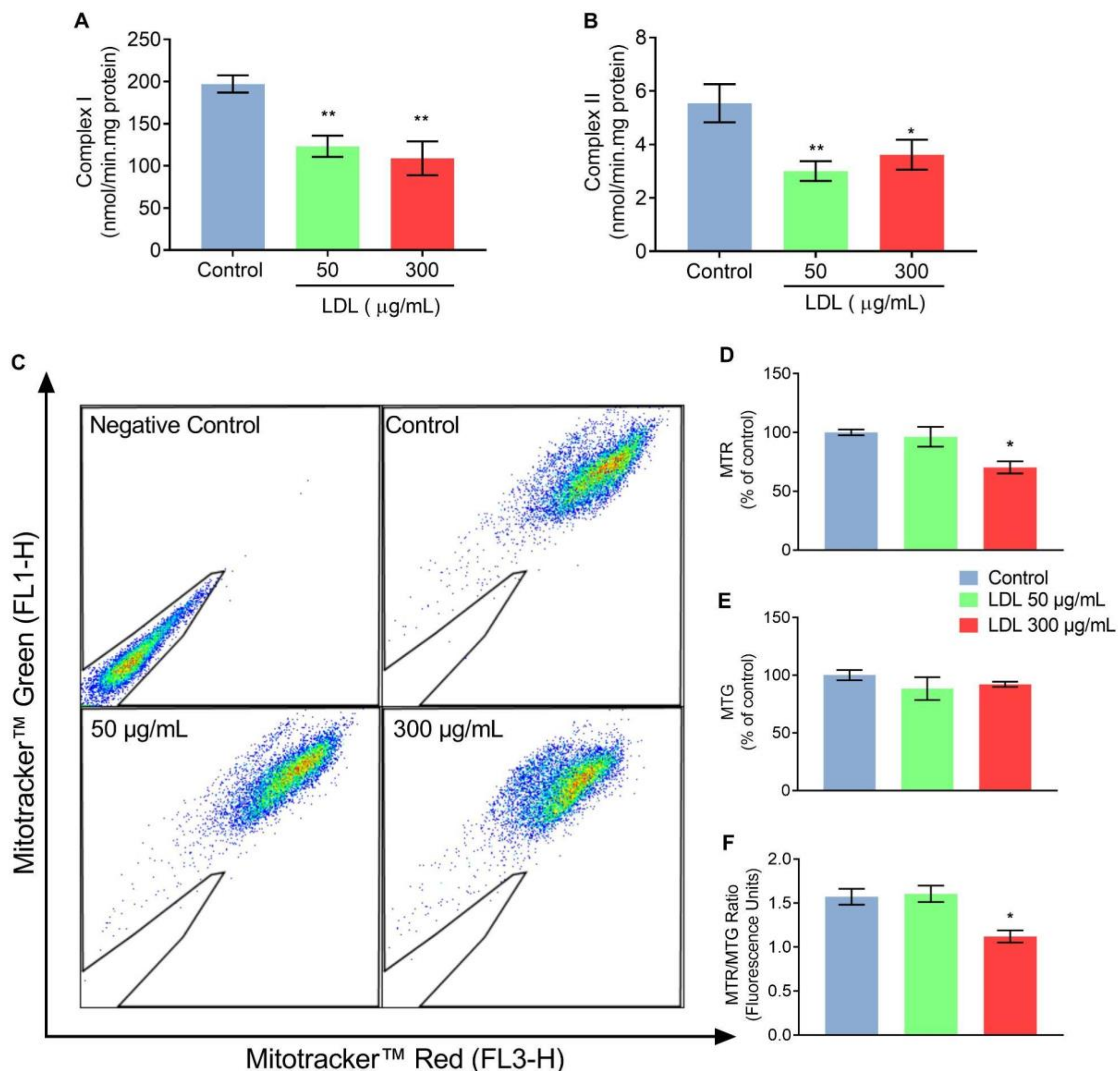

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Fig. 3 LDL induces mitochondrial dysfunction in the hippocampal neuron cell line. (a) Mitochondrial complex I activity. (b) Mitochondrial complex II activity. (c) Dot plot of Mitotracker™ Red and Green. (d) Graphic representation of Mitotracker™ red fluorescence (% of control). (e) Graphic representation of Mitotracker™ green fluorescence (% of control). (f) Mitotracker™ Red and Mitotracker™ green ratio. Data were expressed as mean ± SEM. Statistical analyses were performed using a one-way ANOVA/Dunnett post hoc test. **$p<0.01$, ****$p<0.0001$ compared to control group. Legend: MTR - Mitotracker™ Red; MTG - Mitotracker™ Green

3.4 LDL exposure induces an increase in the production of mitochondrial superoxide production and reduces superoxide dismutase activity in HT-22 cells

It has been established that mitochondrial dysfunction increases reactive species production [47]. Then, we evaluated the effect of LDL on mitochondrial superoxide production and Superoxide Dismutase activity. Our results demonstrated that the lower concentration (50 ug/mL) of LDL induces a significant increase in mitochondrial superoxide production (Fig. 4a), as well as a significant decrease in Superoxide dismutase activity (Fig. 4b). These results indicated that LDL induces an oxidative environment in HT 22 cells.

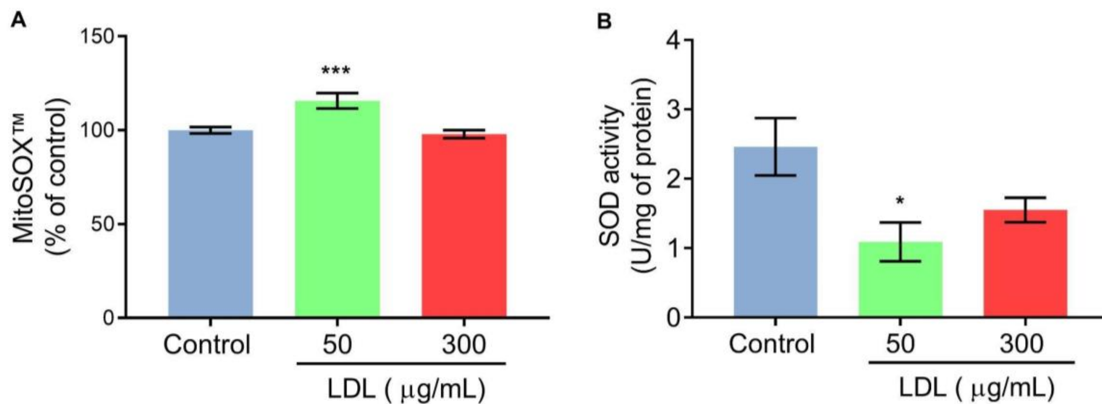

Fig. 4 LDL exposure induces an oxidative environment in the hippocampal neuron cell line. (a)Mitochondrial superoxide production assessed by MitoSOX™. (b) Superoxide Dismutase (SOD) activity. Data were expressed as mean ± SEM. Statistical analyses were performed using a one-way ANOVA/Dunnett post hoc test. *$p<0.05$, ***$p<0.001$ compared to control group.

3.5 LDL exposure induces an increase in the expression of genes associated with mitochondrial fusion in HT-22 cells

We evaluated the effect of exposure to LDL on the expression of genes that code for proteins associated with mitochondrial dynamics. We observed that at the lower concentration of LDL (50 ug/mL), there was a significant increase in the gene expression of OPA1 (Fig. 5B), a protein associated with inner mitochondrial membrane fusion, and MFN2 (Fig. 5d), a protein associated with outer mitochondrial membrane fusion [48]. The LDL exposure did not significantly change the DRP1 and MNF1 expression.

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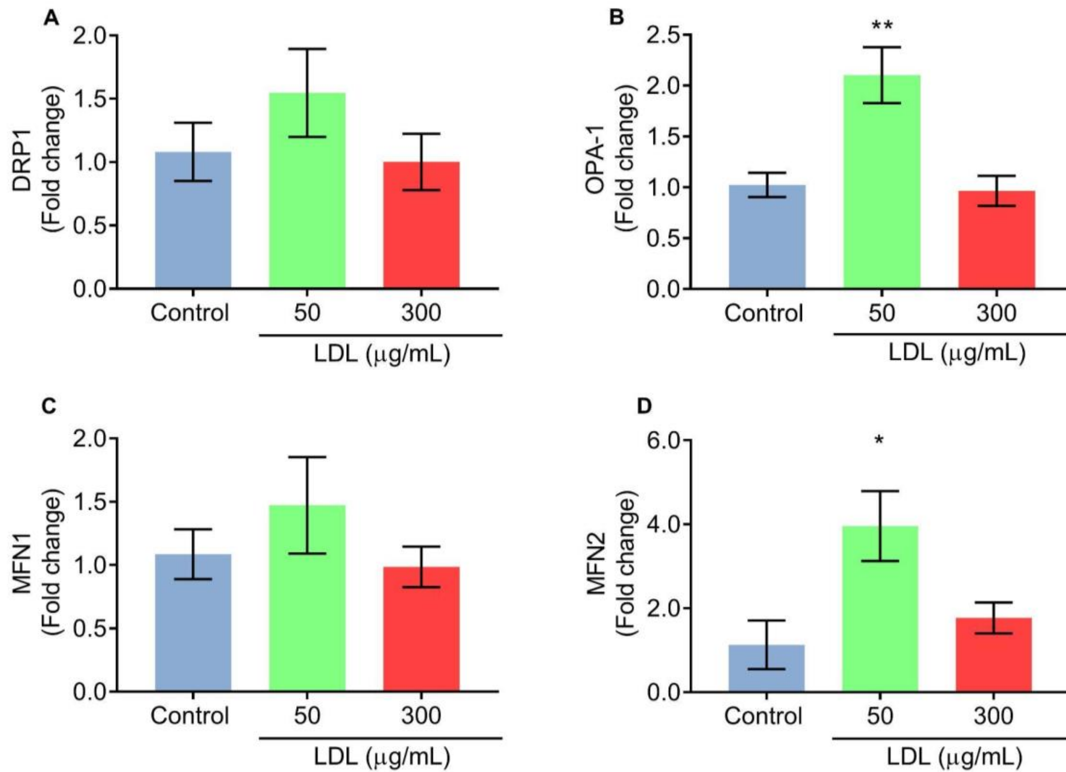

Fig. 5 LDL exposure increases the expression of genes associated with mitochondrial fusion in the hippocampal neuron cell line. (a) DRP1 expression, (b) OPA-1 expression. (c) MFN1 expression. (d) MFN2 expression. Data were expressed as mean ± SEM. Statistical analyses were performed using a one-way ANOVA/Dunnett post hoc test. *p<0.05, **p<0.01 compared to control group.

4 DISCUSSION

Hypercholesterolemia has been experimentally linked with BBB breakdown and cognitive impairment. [13, 22, 23]. Importantly, the BBB dysfunction dysfunction allows the leakage to brain parenchyma of peripheral system compounds, including LDL-cholesterol [26]Moreover, the hippocampus, a brain area critical for memory, is particularly vulnerable to damage induced by hypercholesterolemia [13, 17, 24, 49]. However, the exact mechanism by which high LDL cholesterol levels lead to neuronal damage and subsequent dementia remains unclear. We hypothesize that neurons exposed to LDL will have impaired mitochondrial homeostasis, which may lead to the activation of cell death mechanisms.

Using a Hydrophilic Stain Nile Red (AdipoRed™), we verified that in the higher concentration of LDL, the intracellular lipid droplet was increased in HT-22 cells. The Nile red stains neutral and polar lipids, including triacylglycerol and cholesteryl esters[50, 51]. In health conditions, neurons do not present high lipid droplet contents since they have a lower capacity to use lipids for energy production[52]. However, lipid droplets are

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increased in neurons in several neurodegenerative diseases [53–56], and it is associated with cognitive impairment [57].

Next, the cellular viability assay showed that LDL does not alter the cell viability of HT-22 cells assessed by the MTT assay. Similarly, it was previously demonstrated that LDL does not alter this parameter in HT-22 cells (100 μg/ml) using the CCK-8 assay [58], nor in hippocampal neural precursor cells (25 to 200μg/ml), performed by the resazurin assay [59]. Similar experiments using the MTT assay on SHSY-5Y cells (neuroblastoma cells) and embryonic cortical neurons were performed by Dias et al [60] and Sugawa et al. [61], respectively. Dias et al [60] demonstrated that LDL does not alter the MTT assay in different concentrations (0.8, 1.6, 4.0, 8.0 μg/ml) and times of exposure (2 and 16h). The concentrations tested by Dias et al. [60] were significantly lower than those used in this work. However, Sugawa et al. [61] demonstrated that, after 24 hours, 100μg/ml of LDL did not alter MTT assay results in embryonic cortical neurons.

We also observed that LDL causes no alteration in the necrosis parameter in HT-22 cells, assessed by extracellular LDH activity. While LDL does not alter cellular viability or necrosis levels, we observed an increase in Annexin V staining, suggesting apoptosis of HT-22 cells in both concentrations. Annexin V is a dye-labeled phosphatidylserine (PS)-binding protein [62]. PS is localized exclusively on the inner leaflet of the cell membrane [63, 64]. After induction of apoptosis, PS is flipped to the outer membrane and acts as an “eat me” signal to recruited phagocytes [65–67]. This increase in Annexin V fluorescence can indicate that LDL is leading to apoptosis. However, it is important to mention that Annexin V staining alone is not sufficient to definitively confirm cell death since the plasmatic membrane can be intact. A previous study using primary cortical neurons of rats observed TUNEL-positive staining after exposure to 100 μg/ml LDL, suggesting apoptosis [61]. In contrast, Engel et al [59] did not find evidence of pyknotic nucleus presence, another indicator of apoptosis, in hippocampal neural precursor cells treated with different concentrations of LDL (25 to 200 μg/ml). These findings suggest that LDL can induce neurotoxicity and possible apoptosis in some neuron cells, but further studies are needed to confirm this hypothesis.

Neurons require properly functioning mitochondria to maintain their activity, as they have high ATP demands [68]. Mitochondria have several metabolic functions, mainly combining electron transport along the electron transport chain with oxygen consumption and generation of ATP [69]. These organelles are also strictly involved with apoptosis [70, 71]. Our previous work using hypercholesterolemic animals pointed out a negative correlation between cholesterol levels and complex I and II activities in the cerebral cortex [14]. Moreover, Paul and Borah [13] demonstrated a reduction in complex I and II activities in different brain areas, including the

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hippocampus, in an experimental model of hypercholesterolemia. Consistent with these findings, our current data demonstrate that LDL exposure in HT-22 cells caused a decrease in both complex I and II activities, suggesting that LDL impairs the activity of these respiratory chain complexes.

To better understand the effect of LDL on mitochondria, we assessed the mass and mitochondrial potential with Mitotracker™ Green and Red, respectively, using flow cytometry. MTR gets across the cellular membrane and accumulates in active mitochondria, depending on their oxidative activity. In addition, MTG labels mitochondria independently of the membrane potential, providing a readout related solely to mitochondrial mass. Therefore, by performing the ratio between MTR and MTG, it is possible to establish the rate of mitochondrial function [72, 73]. Our data suggests that exposure to a higher concentration of LDL (300ug/mL) in HT-22 cells was associated with a reduction in MTR fluorescence. Nonetheless, LDL at 300 µg/ml reduced the MTR/MTG ratio in the neurons, suggesting a possible mitochondrial alteration. The reduction in the MTR/MTG ratio can suggest a mitochondrial swelling, which, together with a decrease in mitochondrial membrane potential, may be correlated with neuronal death [74–76].

It has been known that impairment of mitochondria caused by decreases in the mitochondrial complex is responsible for producing reactive species, such as superoxide anions [77–80]. Here, we found that LDL (50µg/ml) exposure increases mitochondrial superoxide anion production in HT-22 cells. Moreover, a previous study demonstrated in other brain cells, such as astrocytes and microglia cultures, that LDL causes a significant increase in reactive species generation [81], as well in human neuroblastoma cells (SHSY-5Y) [28]. It is important to mention that we observed that LDL induces a decrease in complex I and II activity in both concentrations tested, however, there was an increase in mitochondrial superoxide production only in the cells exposed to 50 µg/mL of LDL. It can be explained by some antioxidant modulation, mainly for Superoxide Dismutase (SOD) activity. We observed that at 50ug/mL, LDL leads to a decrease in SOD activity, suggesting that superoxide production is increased at this exposure concentration. It was in agreement with previous studies that have demonstrated that hypercholesterolemia leads to a decrease in SOD activity in the hippocampus of rodents [82–84].

Hypercholesterolemia has been associated with oxidative stress in both peripheral and central nervous systems [14, 15, 17, 85]. An oxidative environment, characterized by high levels of ROS, is associated with changes in mitochondrial dynamics [86], including an increase in mitochondrial fusion [87]. Our results demonstrated that the lower concentration of LDL leads to an increase in the expression of OPA-1 and Mnf2 genes, both associated with mitochondrial fusion [48]. Mitochondrial fusion occurs when mitochondria merge their outer and inner mitochondrial membranes, resulting in mitochondrial elongation. Fusion facilitates the

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distribution and mixing of mtDNA, metabolites, proteins, and lipids, acting as a protective mechanism against partially dysfunctional mitochondria by diluting damaged components [88].

In summary, the lower concentration of LDL triggered an increase in mitochondrial superoxide production and a decrease in antioxidant defense. This increase in reactive species appears to induce a modulation in mitochondrial fusion as evidenced by the increased expression of genes associated with fusion at this LDL concentration. Interestingly, the lower LDL concentration did not alter the MTR/MTG ratio. This might be explained by the modulation of mitochondria fusion, potentially preserving the mitochondrial membrane potential [89]. Our findings suggest a link between hypercholesterolemia and cognitive impairment, possibly mediated by LDL-induced mitochondrial dysfunction. Notably, we demonstrate that not only oxidized LDL but also the non-oxidized form of lipoprotein, can cause neuronal damage.

Nevertheless, further investigation is essential to definitively determine the impact of LDL on neuronal death pathways, as well as mitochondrial images, to confirm mitochondrial fusion. Furthermore, the potential influence of LDL on microglia and astrocytes, and the subsequent effects of mediators released by these glial cells on neurons, cannot be disregarded and warrant further exploration.

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