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Therapeutic Potential of Bovine Lactoferrin to Reduce Brain Malondialdehyde Levels in Hyperlipidemia-Induced Sprague-Dawley Rats

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Abstract
Hyperlipidemia is a medical condition that can trigger various diseases, one of which is a neurodegenerative or neurological disease. Lactoferrin is known to have multiple protective activities, one of which is antioxidant. This study aimed to determine the potential of lactoferrin bovine to decrease brain MDA levels in Sprague-Dawley (SD) rats induced by hyperlipidemia. This study was an in vivo experimental study using 24 male SD rats divided into six groups: normal, negative control, positive control, low-dose bovine lactoferrin (LLF), intermediate-dose bovine lactoferrin (ILF), and high-dose bovine lactoferrin (HLF). The diet in the normal group was the standard diet, and the other groups were induced by a high-fat diet (HFD). The intervention for the positive control group was simvastatin. In contrast, LLF, ILF, and HLF groups were given bovine lactoferrin doses of 100, 200, and 400 mg/kg BW, respectively. After seven weeks, all rats were necropsied, and their brains were taken to be tested for malondialdehyde (MDA) levels with an MDA assay kit using a spectrophotometer. Data was then analyzed using the Shapiro-Wilk and Levene test, which was continued with one-way ANOVA and post-hoc Tukey test. There were significant differences ($p<0.05$) between the negative control group (269.99±13.50 nmol/g weight) and every bovine lactoferrin group (219.92±22.99 nmol/g weight, 151.60±23.43 nmol/g weight, 158.16±12.33 nmol/g weight, respectively) in reducing brain MDA levels. In summary, all bovine lactoferrin groups (100, 200, and 400 mg/kgBW) significantly reduced brain MDA levels of SD rats in hyperlipidemia conditions.

Keywords: Bovine lactoferrin; Brain MDA; Hyperlipidemia; Lipid peroxidation

1. INTRODUCTION

Hyperlipidemia is one of the conditions that need attention and caution. Based on Indonesia Basic Health Research in 2018, data on the population in Indonesia aged ≥15 years with a high proportion of total cholesterol levels (≥240 mg/dl) was 7.6%, low HDL (high-density lipoprotein) levels (<40 mg/dl) was 24.3%, high LDL (low-density lipoprotein) levels

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(160-189 mg/dl) was 9.0%, and high triglyceride levels (200-499 mg/dl) was 13.8% (National Institute of Health Research and Development Indonesian Ministry of Health, 2018). Hyperlipidemia is characterized by increased serum total cholesterol, LDL, triglyceride, or even a decrease in HDL levels (Nelson, 2013; Yang et al., 2017). Currently, statin (HMG-CoA reductase inhibitor) is recommended as the first-line treatment in most hyperlipidemia patients (Grundy et al., 2019). However, taking a statin cannot be separated from the side effects, such as rhabdomyolysis, diarrhea, myopathy, hepatotoxicity, and increased risk of diabetes (Pinal-Fernandez et al., 2018).

High cholesterol and increased free cholesterol levels induce oxidative stress, forming reactive oxygen species (ROS) (Amiya, 2016; Dias et al., 2014). In addition, the structure of the lipid raft, which is part of the cell membrane, will also be disturbed (Amiya, 2016). The continuous increase in intracellular ROS levels will cause the formation of oxidative stress, disrupting the body's cellular working mechanism (Forrester et al., 2018; Rodwell et al., 2015). Oxidative stress in hyperlipidemia can cause disturbances and damage various body organs, such as the heart, liver, kidneys, and brain (Chan et al., 2015; Shichiri, 2014). Oxidative stress plays a role in developing various diseases, including neurodegenerative or neurological diseases, such as Alzheimer's, Parkinson's, Huntington's, and multiple sclerosis (Phaniendra et al., 2015; Pizzino et al., 2017). The brain is the central nervous system (CNS), which is very sensitive to oxidants due to the brain's high lipid content and oxygen demand, as well as low antioxidant levels (Phaniendra et al., 2015). Some brain parts, like the hippocampus, prefrontal cortex, substantia nigra, and corpus striatum, are sensitive to free radicals (Phaniendra et al., 2015; Zlatković et al., 2014).

ROS resulted in the formation of lipid peroxidation in cell membranes (Ayala et al., 2014). Lipid peroxidation is a chain reaction due to the continuous formation of ROS/free radicals that can potentially cause damage (Rodwell et al., 2015). Lipid peroxidation is formed by an interaction between free radicals and polyunsaturated fatty acids, especially in phospholipid membranes susceptible to free radicals (Catalá & Díaz, 2016). Malondialdehyde (MDA) is a secondary product of lipid peroxidation and has relatively stable properties, so it is often used as a biomarker for lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) to produce a pink chromogen (Ayala et al., 2014).

Antioxidants can reduce or control lipid peroxidation levels (Rodwell et al., 2015). Antioxidant activity can be found in lactoferrin (or lactotransferrin). Lactoferrin (Lf) is an iron-binding glycoprotein with a molecular weight of about 80 kDa capable of binding two Fe^{3+} to prevent the effects of oxidative stress. Lf can be found in cow's milk, neutrophil granules, or secreted from the human body, such as colostrum, tears, saliva, genital secretion, and urine (Kell et al., 2020; Superti, 2020).

Many studies have been carried out to assess the activity of various health markers, such as in reducing blood total cholesterol and triglyceride levels (Jusni et al., 2022), serum 8-isoprostane level (Faridvand et al., 2017), serum and liver MDA levels (Chen et al., 2016), and serum homocysteine and leptin levels (Nozari et al., 2018), as well as its ability to increase

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serum Paraoxonase1 level (Faridvand et al., 2017). These studies have highlighted the antioxidant and anti-inflammatory properties of Lf. Unfortunately, there was a lack of research on the effects of Lf on brain MDA levels. Therefore, this study aimed to determine the potential of bovine lactoferrin (bLf) in reducing brain MDA levels of male Sprague-Dawley (SD) rats in hyperlipidemia conditions. The findings of this study could suggest that Lf may serve as an alternative treatment for hyperlipidemia with minimal side effects and easy accessibility.

2. MATERIALS AND METHODS

2.1. Ethical clearance

The experiment was conducted after approval by the Chairman of the Ethical Clearance Commission of the School of Medicine and Health Sciences Atma Jaya Catholic University of Indonesia (protocol number: 07/11/KEP-FKIKUAJ/2021), and all procedures have complied with international standards for animal experimentation.

2.2. Research design

This experimental animal study was conducted at the Biochemistry and Pharmacology Laboratory and Animal House School of Medicine Atma Jaya Catholic University of Indonesia, Jakarta.

2.3. Sample size determination

The total number of rats used in this study was determined using the formula by Charan and Khantaria (2013), i.e., E = total number of animals - total number of groups. E is considered adequate between 10-20. A total of 24 rats were used in this study, comprising six groups. Thus, the E value is 18, which is considered adequate for this study.

2.4. Study subjects

The subjects used for this experimental study were Sprague-Dawley (SD) rats aged five weeks, weighing 150-200 g, and obtained from the Indonesian Food and Drug Administration Laboratory Service. A total of 24 (n=24) male SD rats were randomly divided into six experimental groups, each consisting of four (n=4) SD rats. One rat (n=1) is added as a reserve in each group to prevent dropout. Thus, there were 30 (n=30) SD rats in this study. The first group (normal group) was fed a standard rat diet. The second group (negative control/NC) was fed with a high-fat diet (HFD) containing 15% sucrose, 5% cow's fat, and 80% quail egg yolk at a dose equivalent to 1.5 g/150 g body weight (BW) per day and addition of 0.01% propylthiouracil (PTU). The third group (positive control/PC) was given HFD, PTU, and 1.5 mg/150 gBW simvastatin. The fourth (low-dose bovine lactoferrin/LLF), fifth (intermediate-dose bovine lactoferrin/ILF), and sixth (high-dose bovine lactoferrin/HLF) group were given HFD, PTU, and bLf doses of 100, 200, and 400 mg/kg BW, respectively. Bovine lactoferrin (bLf) was purchased from Xi'an Ruisaen Biotechnology Co., Ltd., as pinkish-white crystals slightly soluble in water with 99% purity (RSN201119). All rats were also given drinking water ad libitum and were acclimatized for five days before being treated. NC, PC, LLF, ILF, and

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HLF group rats were induced with HFD and PTU for 23 days to achieve hyperlipidemia. Then, they proceeded with intervention (simvastatin or bLf) for 26 days based on the provisions of each group. Meanwhile, the normal group is given a standard rat diet for seven weeks (49 days).

2.5. Measurements and laboratory analysis

After seven weeks of research, four rats (n=4) from each group were necropsied to obtain the rat's prefrontal cortex part of the brain to be tested for malondialdehyde (MDA) levels using the MDA Assay Kit. MDA Assay Kit was obtained from Beijing Solarbio Science and Technology. The components of the MDA Assay Kit were extraction reagent (liquid 50 mL×1), reagent I (liquid 30 mL×1), reagent II (Powder×2), MDA working reagent (add 15 mL of reagent I to reagent II and dissolve by heating at 70℃ and mix thoroughly), and reagent III (liquid 10 mL×1).

Before being necropsied, the rats were anesthetized by intramuscular injection with ketamine/xylazine. The brains taken were washed in ice-cold 0.9% NaCl, wiped with a paper filter, and obtained the prefrontal cortex part weighing 0.1 g. The weighted brain was mixed with 1 mL of extraction reagent to be homogenized on an ice bath and then centrifuged at 8000 ×g for 10 minutes at 4℃ using a Nüve NF 800R centrifuge to obtain the supernatant. There were two kinds of tubes used for testing in the spectrophotometer, namely test tube (mixture of 200 µL sample supernatant, 600 µL MDA working reagent, and 200 µL reagent III) and blank tube (mixture of 200 µL distilled water, 600 µL MDA working reagent, and 200 µL reagent III). The mixture was then incubated at 100℃ for 60 minutes. Next, a centrifuge was done at 10.000 ×g for 10 minutes at room temperature to remove insoluble materials. Finally, using a Shimadzu UV-1900i spectrophotometer, we collected 200 µL of supernatant in a 1 mL glass cuvette to measure the absorbance at 450, 532, and 600 nm. The MDA calculation formula (nmol/g weight) follows what is stated in the MDA assay kit.

2.6. Statistical analysis

SPSS software version 23 was used for statistical analysis, and R-4.2.2 was used for diagram visualization. Data results are presented as mean ± SD. In this study, parametric assumptions, including normality (Shapiro-Wilk) and homogeneity (Levene) test, were satisfied ($p>0.05$). Thus, a parametric test was established using a one-way analysis of variance (ANOVA) test for statistical significance analysis among the group means. The Tukey posthoc test assessed the significance of differences between groups. In all instances, a p-value less than 0.05 ($p<0.05$) was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Results

This study showed that the ILF group had the lowest average brain MDA level, while the NC group had the highest brain MDA level. The result of the ANOVA test in this study showed significant differences between groups ($p<0.05$), so it could be continued with Tukey's post-hoc test (Figure 1).

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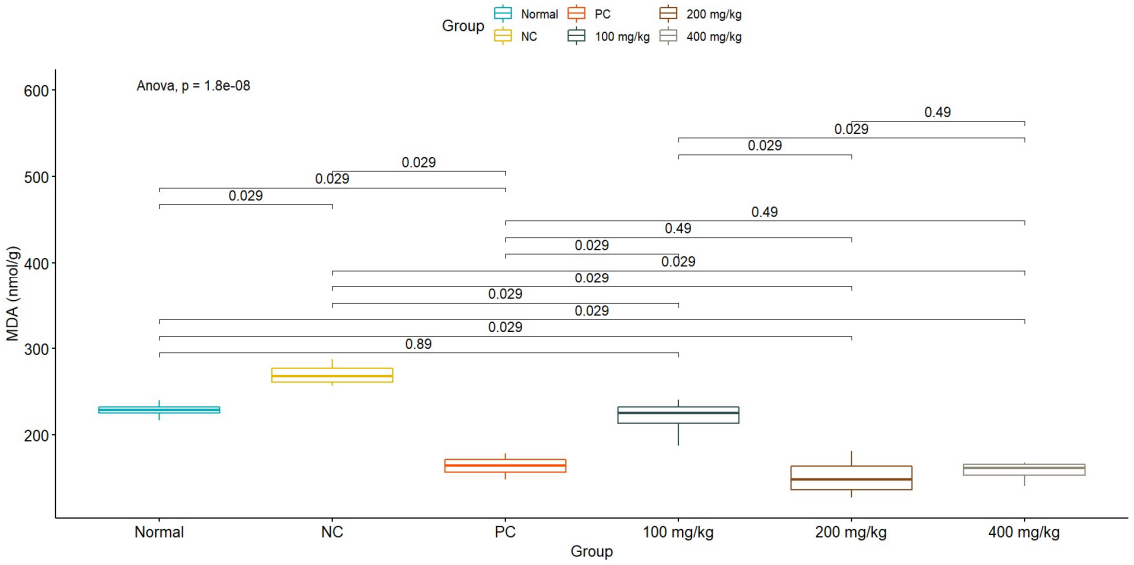

Figure 1. ANOVA and Tukey's post-hoc test result on brain MDA levels in male Sprague-Dawley rats brain (prefrontal cortex) after 49 days. Data results are presented as mean ± SD, and a p-value less than 0.05 ($p<0.05$) was considered statistically significant. *Description*: Negative control (NC); Positive control (PC); 100 mg/kg (LLF); 200 mg/kg (ILF); and 400 mg/kg (HLF).

As shown in Figure 1, there was a significant difference ($p<0.05$) between the normal and NC groups. Brain MDA levels showed a significant difference ($p<0.05$) between the NC group and PC group and also the NC group and all bLf groups (LLF, ILF, and HLF groups). Figure 1 also showed that brain MDA level had a significant difference ($p<0.05$) between the PC group and LLF group, where brain MDA level was lower in the PC group, while between the PC group and ILF and HLF groups showed no significant difference ($p>0.05$). In addition, differences were found significantly between the LLF ILF and HLF groups ($p<0.05$).

3.2. Discussion

In this study, the significant difference ($p<0.05$) between the normal group and NC group indicates that inducing hyperlipidemia with a high-fat diet (15% sucrose, 5% cow's fat, and 80% quail egg yolk) to increase brain MDA levels was successful. All bovine lactoferrin (bLf) groups [LLF (100 mg/kgBW), ILF (200 mg/kgBW), and HLF (400 mg/kgBW)] were able to reduce brain MDA levels of Sprague-Dawley (SD) rats in hyperlipidemia condition significantly ($p<0.05$). The comparison between the PC group and all bLf groups shows that the ILF and HLF groups cannot significantly reduce brain MDA levels in hyperlipidemia ($p>0.05$) compared to the PC group. However, the PC group can significantly ($p<0.05$) reduce brain MDA levels in hyperlipidemia conditions compared to the LLF group. Besides that, ILF and HLF groups significantly reduced brain MDA levels in hyperlipidemia ($p<0.05$) than the LLF group.

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Another published study supporting this study's results that the authors have conducted with other students by testing various indicators in SD rats induced by HFD was from Jusni et al. (2022), which showed that bLf doses of 100, 200, and 400 mg/kg BW could reduce rats' blood total cholesterol and triglyceride levels in hyperlipidemia condition. Besides that, all doses of bLf in the study also lowered the histopathological steatosis score and activated the Kupffer cell score of rats' fatty liver induced by hyperlipidemia. Several previous studies have also examined the activity of bLf as an antioxidant. However, they did not test brain MDA levels, one of which was a study that has been conducted by Faridvand et al. (2017), which showed that bLf at a dose of 200 mg/kg was able to reduce the value of 8-isoprostane and increase the value of Paraoxonase1 (PON1) in serum in a group of rats induced by high cholesterol diet. 8-isoprostane can be used as a biomarker against lipid peroxidation or oxidative stress (Chandra et al., 2016), and PON1 can act as an antioxidant to inhibit lipid peroxidation (Wu et al., 2022). In addition, research by Chen et al. (2016) showed a significant decrease in the mean value of serum and liver MDA levels after being given a low dose (0.5% bovine lactoferrin) and also a high dose of bLf (1% bovine lactoferrin) in mice induced by high cholesterol diet. Nozari et al. (2018) also conducted the effect of bLf at a dose of 200 mg/kgBW, which could act as an antioxidant to significantly reduce or improve serum homocysteine and leptin levels in rats induced by a high-cholesterol diet. Increased serum homocysteine and leptin levels correlate with the formation of ROS or oxidative stress.

Lactoferrin can reduce MDA levels in hyperlipidemia because the brain augments the expression of lactoferrin receptors in the brain. One of the conditions that can increase the expression of lactoferrin receptors in the brain is a neurodegenerative disease (Khan et al., 2020; Li & Guo, 2021). Lactoferrin (Lf) receptors are found on the intestinal mucosa, intestinal lymphatic tissue cells, and BBB (blood-brain barrier) endothelial cells (Superti, 2020). Lf will be digested in the stomach into smaller molecules after passing the oral route. The digestion results will be absorbed by the intestines and transported through the bloodstream to the central nervous system and ultimately to the brain. Lf is known to penetrate the BBB through a process of transcytosis mediated by the Lf receptor (Khan et al., 2020; Superti, 2020).

Neurodegenerative diseases are associated with increased exposure to oxidative stress (Phaniendra et al., 2015). Dysregulation of brain cholesterol levels in hyperlipidemia also increases the risk of neurodegenerative diseases (Vance, 2012). This foundational theory is supported by research conducted by Brooks et al. (2017), Chun et al. (2020), and Zhao et al. (2017), which shows that the condition of hyperlipidemia/hypercholesterolemia can contribute to the development of neurodegenerative diseases. On the other hand, this study showed a significant difference ($p<0.05$) in brain MDA levels between the NC group and PC group, which indicates that simvastatin has antioxidant activity to reduce brain MDA levels of SD rats in hyperlipidemia conditions. A systematic review and meta-analysis by Zinellu et al. (2019) also showed that statins could reduce systemic MDA levels. The study by Zhang et al. (2015) also showed that simvastatin significantly reduces rabbits' hippocampus MDA levels in hypercholesterolemia.

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This study did not encounter any side effects in rats after being given bLf at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW, which is supported by the United States Food and Drug Administration and has determined bLf as a food ingredient that is safe for consumption or can be called GRAS (Generally Recognized as Safe) (Gaynor, 2015; Superti, 2020). One product is an isolate from whey protein derived from cows' milk with lactoferrin as one of the primary ingredients. It has been used as a food ingredient for infants and toddlers and has been designated GRAS (Gaynor, 2015). The highest dose studied in rats using the product was up to 2,000 mg/kg BW by Forster et al. (2014), and no side effects were found in rats in the study. Therefore, 2,000 mg/kgBW can still be defined as a NOAEL (no-observed-adverse-effect level).

This study only measured MDA as the lipid peroxidation biomarker, even though other lipid peroxidation biomarkers such as isoprostane and 4-hydroxynonenal (HNE) can be tested. Therefore, we suggest testing other lipid peroxidation biomarkers in the brain in hyperlipidemia conditions to obtain comparable results.

4. CONCLUSION

Bovine lactoferrin (bLf) doses of 100, 200, and 400 mg/kgBW significantly reduced Sprague-Dawley rats' brain malondialdehyde (MDA) levels in hyperlipidemia conditions. However, in this study, bLf doses of 200 and 400 mg/kgBW showed the potential to have the same effect as simvastatin in reducing brain MDA levels.

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

REFERENCES

Amiya, E. (2016). Interaction of hyperlipidemia and reactive oxygen species: Insights from the lipid-raft platform. *World Journal of Cardiology*, *8*(12), 689–694. https://doi.org/10.4330/wjc.v8.i12.689

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Medicine and Cellular Longevity*, *2014*, e360438. https://doi.org/10.1155/2014/360438

Brooks, S. W., Dykes, A. C., & Schreurs, B. G. (2017). A High-Cholesterol Diet Increases 27-Hydroxycholesterol and Modifies Estrogen Receptor Expression and Neurodegeneration in Rabbit Hippocampus. *Journal of Alzheimer's Disease: JAD*, *56*(1), 185–196. https://doi.org/10.3233/JAD-160725

Catalá, A., & Díaz, M. (2016). Editorial: Impact of Lipid Peroxidation on the Physiology and Pathophysiology of Cell Membranes. *Frontiers in Physiology*, *7*, 423. https://doi.org/10.3389/fphys.2016.00423

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Chan, P. T., Matanjun, P., Yasir, S., & Tek Song, T. (2015). Oxidative stress biomarkers in organs of hyperlipidaemic and normal rats fed tropical red seaweed, Gracilaria changii. *Journal of Applied Phycology*, *28*. https://doi.org/10.1007/s10811-015-0670-x

Chandra, M., Panchatcharam, M., & Miriyala, S. (2016). Biomarkers in ROS and Role of Isoprostanes in Oxidative Stress. In *Free Radicals and Diseases*. IntechOpen. https://doi.org/10.5772/64706

Charan, J., & Kantharia, N. D. (2013). How to calculate sample size in animal studies? *Journal of Pharmacology & Pharmacotherapeutics*, *4*(4), 303–306. https://doi.org/10.4103/0976-500X.119726

Chen, H.-A., Chiu, C.-C., Huang, C.-Y., Chen, L.-J., Tsai, C.-C., Hsu, T.-C., & Tzang, B.-S. (2016). Lactoferrin Increases Antioxidant Activities and Ameliorates Hepatic Fibrosis in Lupus-Prone Mice Fed with a High-Cholesterol Diet. *Journal of Medicinal Food*, *19*(7), 670–677. https://doi.org/10.1089/jmf.2015.3634

Chun, Y. S., & Chung, S. (2020). High-Cholesterol Diet Decreases the Level of Phosphatidylinositol 4,5-Bisphosphate by Enhancing the Expression of Phospholipase C (PLCβ1) in Rat Brain. *International Journal of Molecular Sciences*, *21*(3), E1161. https://doi.org/10.3390/ijms21031161

Dias, I., Polidori, M. C., & Griffiths, H. (2014). Hypercholesterolaemia-induced oxidative stress at the blood-brain barrier. *Biochemical Society Transactions*, *42*, 1001–1005. https://doi.org/10.1042/BST20140164

Faridvand, Y., Nozari, S., Asoudeh-Fard, A., Karimi, M.-A., Pezeshkian, M., Safaie, N., & Nouri, M. (2017). Bovine lactoferrin ameliorates antioxidant esterase activity and 8-isoprostane levels in high-cholesterol-diet fed rats. *International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin- Und Ernahrungsforschung. Journal International De Vitaminologie Et De Nutrition*, *87*(3–4), 201–206. https://doi.org/10.1024/0300-9831/a000516

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q., & Griendling, K. K. (2018). Reactive Oxygen Species in Metabolic and Inflammatory Signaling. *Circulation Research*, *122*(6), 877–902. https://doi.org/10.1161/CIRCRESAHA.117.311401

Forster, R., Bourtourault, M., Chung, Y. J., Silvano, J., Sire, G., Spezia, F., Puel, C., Descotes, J., & Mikogami, T. (2014). Safety evaluation of a whey protein fraction containing a concentrated amount of naturally occurring TGF-β2. *Regulatory Toxicology and Pharmacology*, *69*(3), 398–407. https://doi.org/10.1016/j.yrtph.2014.05.003

Gaynor, P. (2015). GRAS Exemption Claim for Vitalarmor® GF-100, a Basic Whey Protein Isolate, for Use as an Ingredient in Term Infant Formulas and Toddler Formulas. Available from: https://www.fda.gov/media/97006/download [Accessed 1st April 2022]

Grundy, S. M., Stone, N. J., Bailey, A. L., Beam, C., Birtcher, K. K., Blumenthal, R. S., Braun, L. T., de Ferranti, S., Faiella-Tommasino, J., Forman, D. E., Goldberg, R., Heidenreich, P. A., Hlatky, M. A., Jones, D. W., Lloyd-Jones, D., Lopez-Pajares, N., Ndumele, C. E., Orringer, C. E., Peralta, C. A., & Yeboah, J. (2019). 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*, *139*(25), e1082–e1143. https://doi.org/10.1161/CIR.0000000000000625

Jusni, L.F.J., Chandra, V., Djuartina, T., Notario, D., Arieselia, Z., & Hananta, L. (2022). Potential Antihyperlipidemia Effect of Lactoferrin in Hyperlipidemia-Induced Male Sprague–Dawley Rats. *Makara J Health Res*, 26(3), 204-209. https://doi.org/10.7454/msk.v26i3.1387

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Kell, D. B., Heyden, E. L., & Pretorius, E. (2020). The Biology of Lactoferrin, an Iron-Binding Protein That Can Help Defend Against Viruses and Bacteria. *Frontiers in Immunology*, *11*, 1221. https://doi.org/10.3389/fimmu.2020.01221

Khan, A. I., Liu, J., & Dutta, P. (2020). Bayesian inference for parameter estimation in lactoferrin-mediated iron transport across blood-brain barrier. *Biochimica Et Biophysica Acta. General Subjects*, *1864*(3), 129459. https://doi.org/10.1016/j.bbagen.2019.129459

Li, Y.-Q., & Guo, C. (2021). A Review on Lactoferrin and Central Nervous System Diseases. *Cells*, *10*(7), 1810. https://doi.org/10.3390/cells10071810

National Institute of Health Research and Development Indonesian Ministry of Health (2018). *Laporan Nasional Riset Kesehatan Dasar (RISKESDAS)* 2018. Available from: http://repository.bkpk.kemkes.go.id/3514/1/Laporan%20Riskesdas%202018%20Nasional.pdf [Accessed 10th December 2021]

Nelson, R. H. (2013). Hyperlipidemia as a Risk Factor for Cardiovascular Disease. *Primary Care*, *40*(1), 195–211. https://doi.org/10.1016/j.pop.2012.11.003

Nozari, S., Fathi Maroufi, N., Nouri, M., Paytakhti Oskouei, M., Shiralizade, J., Yekani, F., Mamipour, M., & Faridvand, Y. (2018). Decreasing serum homocysteine and hypocholesterolemic effects of Bovine lactoferrin in male rat fed with high-cholesterol diet. *Journal of Cardiovascular and Thoracic Research*, *10*(4), 203–208. https://doi.org/10.15171/jcvtr.2018.35

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian Journal of Clinical Biochemistry*, *30*(1), 11–26. https://doi.org/10.1007/s12291-014-0446-0

Pinal-Fernandez, I., Casal-Dominguez, M., & Mammen, A. L. (2018). Statins: Pros and cons. *Medicina Clinica*, *150*(10), 398–402. https://doi.org/10.1016/j.medcli.2017.11.030

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, *2017*. https://doi.org/10.1155/2017/8416763

Rodwell, V. W., Bender, D. A., Botham, K. M., Kennelly, P. J., & Weil, P. A. (2015). *Harper's illustrated biochemistry* (Thirtieth edition). McGraw-Hill Education.

Shichiri, M. (2014). The role of lipid peroxidation in neurological disorders. *Journal of Clinical Biochemistry and Nutrition*, *54*(3), 151–160. https://doi.org/10.3164/jcbn.14-10

Superti, F. (2020). Lactoferrin from Bovine Milk: A Protective Companion for Life. *Nutrients*, *12*(9), Article 9. https://doi.org/10.3390/nu12092562

Vance, J. E. (2012). Dysregulation of cholesterol balance in the brain: Contribution to neurodegenerative diseases. *Disease Models & Mechanisms*, *5*(6), 746–755. https://doi.org/10.1242/dmm.010124

Wu, C.-C., Cheng, Y.-H., Chen, K.-H., & Chien, C.-T. (2022). Deep Sea Water-Dissolved Organic Matter Intake Improves Hyperlipidemia and Inhibits Thrombus Formation and Vascular Inflammation in High-Fat Diet Hamsters. *Life*, *12*(1), 82. https://doi.org/10.3390/life12010082

Yang, W., Shi, H., Zhang, J., Shen, Z., Zhou, G., & Hu, M. (2017). Effects of the duration of hyperlipidemia on cerebral lipids, vessels and neurons in rats. *Lipids in Health and Disease*, *16*. https://doi.org/10.1186/s12944-016-0401-6

Zhang, G., Li, M., Xu, Y., Peng, L., Yang, C., Zhou, Y., & Zhang, J. (2015). Antioxidation Effect of Simvastatin in Aorta and Hippocampus: A Rabbit Model Fed High-Cholesterol Diet. *Oxidative Medicine and Cellular Longevity*, *2016*, e6929306. https://doi.org/10.1155/2016/6929306

Zhao, X.-S., Wu, Q., Peng, J., Pan, L.-H., Ren, Z., Liu, H.-T., Jiang, Z.-S., Wang, G.-X., Tang, Z.-H., & Liu, L.-S. (2017). Hyperlipidemia-induced apoptosis of hippocampal neurons in

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apoE(−/−) mice may be associated with increased PCSK9 expression. *Molecular Medicine Reports*, *15*(2), 712–718. https://doi.org/10.3892/mmr.2016.6055

Zinellu, A., Paliogiannis, P., Usai, M. F., Carru, C., & Mangoni, A. A. (2019). Effect of statin treatment on circulating malondialdehyde concentrations: A systematic review and meta-analysis. *Therapeutic Advances in Chronic Disease*, *10*. https://doi.org/10.1177/2040622319862714

Zlatković, J., Todorović, N., Bošković, M., Pajović, S. B., Demajo, M., & Filipović, D. (2014). Different susceptibility of prefrontal cortex and hippocampus to oxidative stress following chronic social isolation stress. *Molecular and Cellular Biochemistry*, *393*(1–2), 43–57. https://doi.org/10.1007/s11010-014-2045-z

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