

Effect of Essential Unsaturated Fatty Acids on Structure and Function of Catalase at High Glucose Concentration



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Published Online August 21, 2017

Keywords: Antioxidant, Catalase,
Essential unsaturated fatty acid,
In vitro, Glycation



Abstract

Background: Assessment of changes in structure and activity of catalase, as an antioxidant enzymatic protein, in combination with glucose and essential unsaturated fatty acids is the aim of present study.

Materials and Methods: In order to investigate the activity and structure of catalase, a solution of this enzyme with 10 mg/mL concentration in phosphate buffer (0.1 M) and pH = 7.4, in the presence and absence of 50 mmol glucose was prepared, filtered, and then incubated for 4 months at 37°C with and without fatty acids including linolenic, linoleic, and arachidonic acids, separately. Samples were taken from each tube of solution, every 14 days for 4 months, to assess the fluorescence emission, circular dichroism (CD) and activity.

Results: According to the results, catalase showed increase in fluorescence emission and decrease in activity after incubation with glucose in comparison with pure protein. Moreover, catalase showed alteration in CD after incubation with glucose during 4 months. After incubation with glucose and each of the mentioned unsaturated fatty acids, alterations of catalase were neared toward normal level in fluorescence emission, CD, and activity in comparison with pure protein.

Conclusion: Functional and structural protection of catalase against damages from hyperglycemic environment with addition of essential unsaturated fatty acids was proved in our investigation.

Received May 20, 2017; Revised July 16, 2017; Accepted August 11, 2017

Introduction

An important risk factor in progression of microvascular and macrovascular complications in diabetes is oxidative stress. Hyperglycemia induces the production of mitochondrial reactive oxygen species (ROS) in endothelial cells of both large and small vessels. ROS production leads to the increase in the formation of advanced glycation end products (AGEs), which can cause activation of a number of proinflammatory pathways, associated with the pathogenesis of cardiovascular complications of diabetes.¹ Glycemic control can decrease the mortality rate by impairing the glycation of lipoproteins, enzymes, and other components of lipid metabolism, and inhibiting AGEs formation.^{2,3} Chemical chaperones (such as amino acids, polyols like glycerol and amino compounds like polyamines) are small molecules that can prevent the change in protein structures and functions due to glycation.^{4,5} Some previous studies illustrated that the essential unsaturated fatty acids play

a key role as antioxidants and increase the inherent anti-oxidative activity.^{6,7} Linolenic acid is one of the essential unsaturated fatty acids, which has significant anti-oxidative effects.⁸⁻¹⁰ Beneficial effects of unsaturated fatty acids in lowering the risk of cardiovascular diseases have been demonstrated.¹¹⁻¹³ Additionally, some studies showed that arachidonic acid, another essential unsaturated fatty acid, has a protective role against the reduction of other fatty acids and also it preserves physiological mechanisms in animal models.¹⁴⁻¹⁶ Besides, essential unsaturated fatty acids can increase the anti-oxidative activity of catalase which is an essential antioxidant enzyme for protecting cells against damages.^{8,9} Catalase can convert hydrogen peroxide to oxygen and water. This antioxidant enzyme can normalize renal dysfunction in diabetes such as albuminuria and glomerular hypertension and glomerular pathologies.¹⁷ Protein glycation (non-enzymatic glycosylation) due to hyperglycemia is one of the most important mechanisms that changes proteins

and leads to structural changes and dysfunction of the protein. Glycation of catalase causes structural damage and impairs physiological function of this enzyme.^{18,19} However, the non-glycated setting of this anti-oxidant can protect other components against oxidative stress and also can decrease the risk of cardiovascular diseases.^{20,21} In this study, we set out to assess the efficacy of some essential unsaturated fatty acids including arachidonic acid, linoleic acid and linolenic acid on structural and physiological function of catalase as an antioxidant enzyme in an in vitro setting. Due to high prevalence of cardiovascular diseases and diabetic nephropathy in type 2 diabetes, the presence of essential unsaturated fatty acids in the diet of these patients has been suggested for lowering the risk of renal and cardiovascular diseases.

Material and Methods

Assessment of the structural changes of catalase (an enzymatic protein) was performed with fluorimetry and circular dichroism (CD) spectra. Using 2 laboratorial methods to distinguish the structural changes of protein in this study could be analyzed from 2 different features. Comparison of the final results with each other increased the accuracy of the measurements and integrity of the data. Fluorescence spectroscopy gives us information about the rate of changes of protein fluorescence with a definite wavelength in specific laboratory conditions. The structural changes were evaluated by maintaining the conditions and through the measurements of emission from the excited proteins. We used Shimadzu Spectrofluorometer RF-5000 (Japan, Kyoto) at excitation and emission wavelengths of 350 and 440 nm, respectively. CD spectroscopy measured the changes in secondary structure of protein. The changes of optical rotation of protein at a definite wavelength, which was subjected to polarized light, demonstrate the structural changes.

In order to study the nature of the activity of enzymatic protein (catalase), its solution with 10 mg/mL in

phosphate buffered saline (PBS) with concentration of 0.1 M and pH = 7.4, in the presence and absence of filtrated glucose (with 50 M concentration) and afterwards it was incubated for the duration of 4 months at 37°C and in the presence and absence of linoleic, linolenic and arachidonic acids (as essential unsaturated fatty acids) with 0.5% volume-weight concentration. In a time span of 14 days, until the end of 4 months, samples were taken from each test tube related to catalase solution and kept at -80°C.

The activity of enzymatic protein (catalase) in this study was assessed by using related activity assay kit. Measurement of catalase activity in the presence and absence of high concentrated glucose and in the presence and absence of unsaturated fatty acids was performed. Analyzing of data and drawing the curves were performed by using of SPSS version 22.0 (IBM Corporation, New York, United States) and 2013 EXCEL software.

Results

In this study, the effects of linoleic and linolenic and arachidonic acids on structure and activity of catalase under the condition of presence and absence of highly concentrated glucose were studied. To this end, solution of catalase (incubated for 4 months) with highly concentrated glucose in the presence and absence of essential unsaturated fatty acids, was studied (For 3 sample types; i.e. enzyme alone, enzyme with glucose, and finally enzyme with both glucose and fatty acids).

Assessment of Enzymatic Structure Through Fluorescence Spectroscopy Method

Figure 1 shows the changes in fluorescence emission of glycated forms of catalase in the presence and absence of essential unsaturated fatty acids, at an excitement wavelength of 35 nm and emission wavelength of 440 nm. Results of fluorimetry indicated that glycation of catalase and formation of glycoforms in the presence of glucose

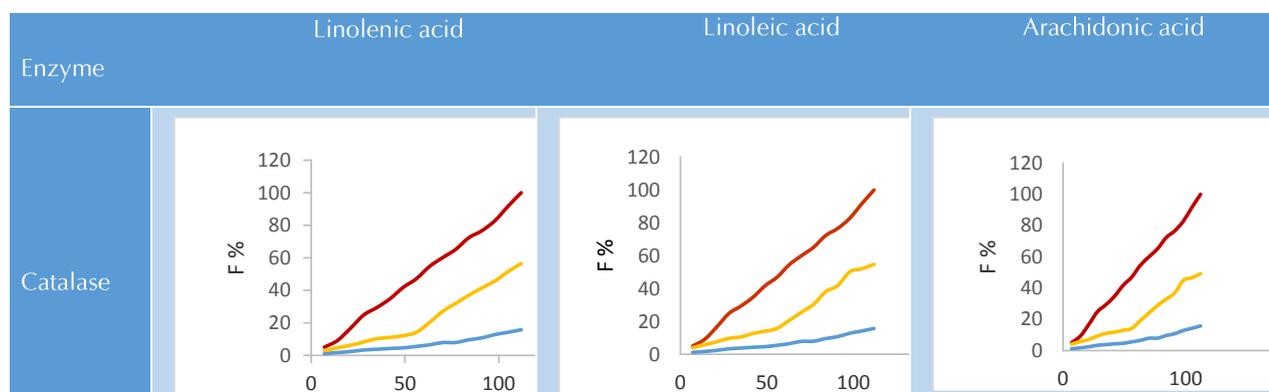


Figure 1. Percentage of Fluorescence Intensity (F %) of Catalase (C), Alone, After Incubation With Glucose and After Incubation With Glucose and Each Fatty Acid in Order (Linolenic acid [L1], Linoleic acid [L2], Arachidonic acid [L3]) Over Time (100 days). The excitation and emission wavelengths were 350 nm and 440 nm. Blue line: enzyme alone, Red line: enzyme + glucose, Orange line: enzyme + glucose + fatty acid .

gradually increased simultaneously with the increment of incubation time. However, addition of essential unsaturated fatty acids into catalase solution resulted in significant reduction in fluorescence emission by the end of 4 months.

Assessment of Enzymatic Structure Through Circular Dichroism Method

Based on Figure 2, data gathered from the study of CD spectrum relating to the samples of catalase at the end of 4 months of incubation showed a comparative impairment of secondary structure of catalase in the presence of glucose and slowing down in the trend of glycation process and preservation of secondary structure of protein in the presence of essential unsaturated fatty acids. The results of this observation put emphasis on the subject that secondary structure of catalase, in presence of glucose distance from natural conformation, but in the presence of essential unsaturated fatty acids, it tended to approach normal value.

Assessment of Enzymatic Activity Through the Colorimetric Method

The role of glucose and essential unsaturated fatty acids in enzymatic activity of catalase is shown in Figure 3. This figure demonstrates that the activity of catalase enzyme in the presence of glucose decreased, while in the presence of essential unsaturated fatty acids, this activity tended towards normal rate.

Discussion

Hyperglycemia induces mitochondrial ROS production that causes vascular complications such as nephropathy and cardiovascular diseases in diabetes.^{21,22} Hence, prevention of diabetes complications has always drawn the attention of researchers. Meanwhile, one method which is vastly studied is using therapeutic supplements. Studies have shown that the supplements which have natural biochemical structure are prescribed to the patients along with the main diabetes drugs. Structure of these supplements is similar to the corresponding

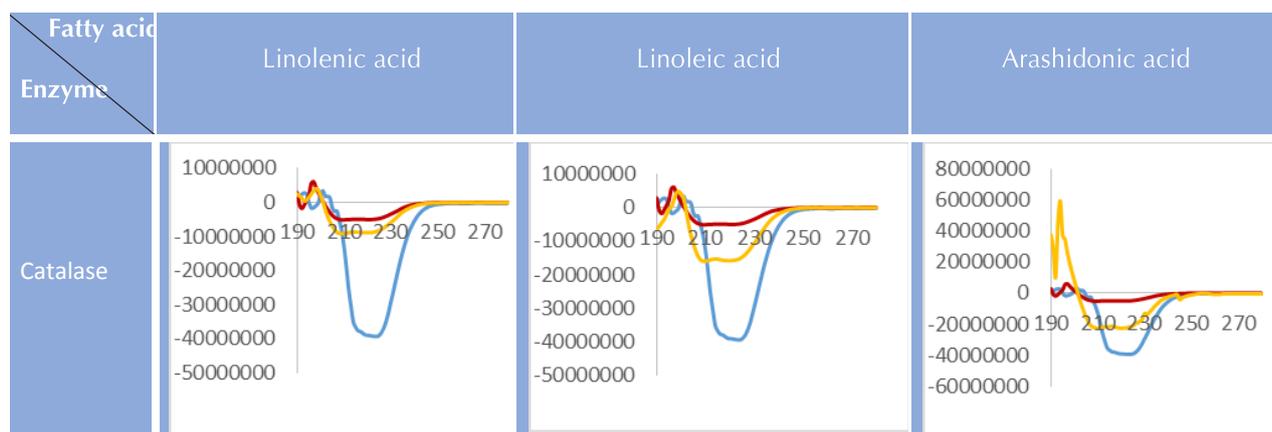


Figure 2. Changes in Circular Dichroism of Catalase Alone, After Incubation With Glucose and After Incubation With Glucose and Each Fatty Acid in Order (Linolenic Acid [L1], Linoleic Acid [L2], Arashidonic Acid [L3]). Blue line: enzyme alone, Red line: enzyme + glucose, Orange line: enzyme + glucose + fatty .

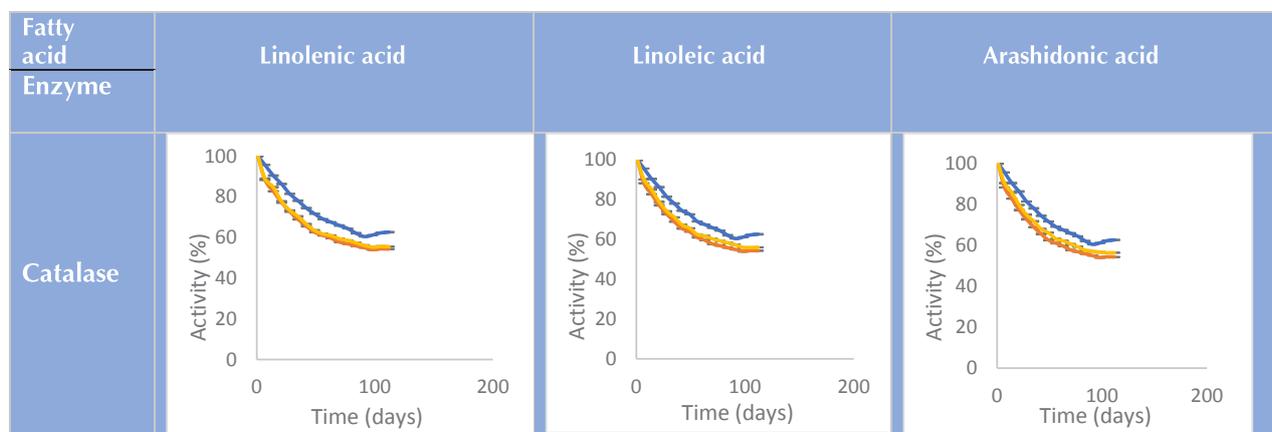


Figure 3. Changes in Activity Of Catalase Alone, After Incubation With Glucose And After Incubation With Glucose and Each Fatty Acid in Order (Linolenic Acid [L1], Linoleic Acid [L2], Arashidonic Acid [L3]). Blue line: enzyme alone, Red line: enzyme + glucose, Orange line: enzyme + glucose + fatty .

structure of biochemical compounds in human body.²² Essential unsaturated fatty acids are among these supplements. It has become clear that these fatty acids act as antioxidants.^{6,23} However, the related biochemical mechanisms have not been determined precisely. Our study is set up to make some progress in this respect. Using of linolenic and arachidonic acid led to a decrease in the rate of cardiovascular complications in animal models.^{10,15} In this study, we demonstrated that linoleic acid is capable of reinforcing the antioxidant system too. Also a comprehensive assessment in relation to the essential unsaturated fatty acids on the structure and activity of catalase was carried out in an in vitro setting. Similar studies were conducted to observe the activity of this antioxidant enzyme. The important role of catalase to reduce or inhibit infectious diseases has also been demonstrated. These diseases include kinds of free radicals that damage the body and catalase, as an antioxidant enzyme, can be effective to treat the mentioned diseases.^{8,11,16} In this study, we assessed not only the activity of catalase, but also their structure through fluorimetry and CD methods. In this study, it became clear that the normal structure of enzymatic protein (catalase) changed apparently when it was exposed to high concentration of glucose. However, when the essential unsaturated fatty acids were incubated with the glycosylated enzyme (glycosylated catalase), the structure of the glycosylated catalase showed significant change towards normal conformation, that was indicated through fluorimetry and CD spectroscopy.

Based on the results showed in Figure 1, essential unsaturated fatty acids were capable of causing fluorescence emission in glycosylated enzymes (glycosylated catalase) to tend towards normal rate. The rate of fluorescence emission in enzymatic protein was considered as the normal emission. The fluorescence rate is one of the indices which distinguish glycosylated form of catalase from the normal state.

According to the results shown in Figure 2, the essential unsaturated fatty acids were capable of causing tendency in CD spectra of glycosylated enzymatic protein (catalase) to approach to the normal rate. Destruction of protein's secondary structure in the presence of glucose and controlling glycation and maintaining the normal structure of protein in the presence of essential unsaturated fatty acids is shown in this figure. The net rate of CD spectrum of catalase was considered as the normal rate. The rate of CD spectrum is one of the indices which distinguish glycosylated form of catalase from the normal state.

According to the result shown in Figure 3 about catalase, essential unsaturated fatty acids were capable of causing tendency in the rate of glycosylated enzyme activity of catalase toward the normal activity rate. The net rate of enzymatic protein activity was considered as the normal activity rate. Assessment of enzymatic activity on the side

of enzymatic structure study has enriched the final results of this observation.

The research on structure-activity of antioxidant enzymatic protein such as catalase, demonstrated the condition of glycation, in combination with essential unsaturated fatty acids of this enzyme and also the condition of simultaneous combination with both of them, shall be able to affect the structure and activity of protein. This effect can prevent the glycation process of catalase and take it back towards the normal state. A similar interpretation can be made in this regard about protein activity.

Conclusion

In this study, we found that the structure and function of catalase can be affected by essential unsaturated fatty acids against hyperglycemic condition. Decrease of activity and structural changes of antioxidant enzyme (catalase) in hyperglycemic state is one of the well-known mechanisms of diabetes. Hence, this study can help to recognize one of the mechanisms involved in the development of diabetes complications and these results can be regarded as paving the way for treatment of this disease.

Authors' Contributions

HM performed the laboratory experiments. PH handled the laboratory data. FDF conducted the review literature. NA edited the manuscript. MA acted as a statistic analyzer.

Ethical Approval

The present study has been performed as an in vitro investigation, without any contribution of human or animal, so there is not a need for ethical approval.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Financial Support

Alborz University of Medical Sciences supported the study (grant No. 2505836).

References

1. Stefano GB, Challenger S, Kream RM. Hyperglycemia-associated alterations in cellular signaling and dysregulated mitochondrial bioenergetics in human metabolic disorders. *Eur J Nutr.* 2016;55(8):2339-2345. doi:10.1007/s00394-016-1212-2.
2. Ryden L, Mellbin L. Glucose perturbations and cardiovascular risk: challenges and opportunities. *Diab Vasc Dis Res.* 2012;9(3):170-176. doi:10.1177/1479164112451581.
3. Jafarnejad A, Bathaie SZ, Nakhjavani M, Hassan MZ. Effect of spermine on lipid profile and HDL functionality in the streptozotocin-induced diabetic rat model. *Life Sci.* 2008;82(5-6):301-307. doi:10.1016/j.lfs.2007.11.015.
4. Jafarnejad A, Bathaie SZ, Nakhjavani M, Hassan MZ, Banasadeh S. The improvement effect of L-Lys as a chemical chaperone on STZ-induced diabetic rats, protein structure and function. *Diabetes Metab Res Rev.* 2008;24(1):64-73. doi:10.1002/dmrr.769.
5. Rajan RS, Tsumoto K, Tokunaga M, Tokunaga H, Kita Y,

- Arakawa T. Chemical and pharmacological chaperones: application for recombinant protein production and protein folding diseases. *Curr Med Chem*. 2011;18(1):1-15.
6. Kravchenko LV, Aksenov IV, Avren'eva LI, Beketova NA, Trusov NV, Guseva GV. [Effects of omega-3 polyunsaturated fatty acids on antioxidant capacity in rats]. *Vopr Pitan*. 2013;82(2):4-9.
 7. Karalis DG. The role of lipid-lowering therapy in preventing coronary heart disease in patients with type 2 diabetes. *Clin Cardiol*. 2008;31(6):241-248. doi:10.1002/clc.20226.
 8. Wardhani DH, Fucinos P, Vazquez JA, Pandiella SS. Inhibition kinetics of lipid oxidation of model foods by using antioxidant extract of fermented soybeans. *Food Chem*. 2013;139(1-4):837-844. doi:10.1016/j.foodchem.2013.01.049.
 9. Nawirska-Olszanska A, Kita A, Biesiada A, Sokol-Letowska A, Kucharska AZ. Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. *Food Chem*. 2013;139(1-4):155-161. doi:10.1016/j.foodchem.2013.02.009.
 10. Chen X, Zhang Y, Wang Z, Zu Y. In vivo antioxidant activity of *Pinus koraiensis* nut oil obtained by optimised supercritical carbon dioxide extraction. *Nat Prod Res*. 2011;25(19):1807-1816. doi:10.1080/14786419.2010.483229.
 11. Muldoon MF, Erickson KI, Goodpaster BH, et al. Concurrent physical activity modifies the association between n3 long-chain fatty acids and cardiometabolic risk in midlife adults. *J Nutr*. 2013;143(9):1414-1420. doi:10.3945/jn.113.174078.
 12. Kruse LG, Ogletree RL, Jr. Omega-3 fatty acids and cardiovascular risk. *J Miss State Med Assoc*. 2013;54(6):156-157.
 13. Proust F, Drescher O, Laouan-Sidi EA, Robinson E, Lucas M, Dewailly E. Omega-3 polyunsaturated fatty acid profiles and relationship with cardiometabolic risk factors in Cree (Eeyouch) of Northern Quebec. *Int J Circumpolar Health*. 2016;75:30361. doi:10.3402/ijch.v75.30361.
 14. Seychelles LH, Doiron K, Audet C, Tremblay R, Pernet F, Lemarchand K. Impact of arachidonic acid enrichment of live rotifer prey on bacterial communities in rotifer and larval fish cultures. *Can J Microbiol*. 2013;59(3):189-196. doi:10.1139/cjm-2012-0564.
 15. Le HD, Fallon EM, Kalish BT, et al. The effect of varying ratios of docosahexaenoic acid and arachidonic acid in the prevention and reversal of biochemical essential fatty acid deficiency in a murine model. *Metabolism*. 2013;62(4):499-508. doi:10.1016/j.metabol.2012.10.003.
 16. DL L. *Essential Fatty Acids*. Woodland Publishing; P.O. Box 160; 1997.
 17. Koya D, Hayashi K, Kitada M, Kashiwagi A, Kikkawa R, Haneda M. Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. *J Am Soc Nephrol*. 2003;14(8 Suppl 3):S250-253.
 18. Bakala H, Hamelin M, Mary J, Borot-Laloi C, Friguet B. Catalase, a target of glycation damage in rat liver mitochondria with aging. *Biochim Biophys Acta*. 2012;1822(10):1527-1534. doi:10.1016/j.bbadis.2012.05.016.
 19. Suravajjala S, Cohenford M, Frost LR, Pampati PK, Dain JA. Glycation of human erythrocyte glutathione peroxidase: effect on the physical and kinetic properties. *Clin Chim Acta*. 2013;421:170-176. doi:10.1016/j.cca.2013.02.032.
 20. Bagnyukova TV, Vasyukiv OY, Storey KB, Lushchak VI. Catalase inhibition by amino triazole induces oxidative stress in goldfish brain. *Brain Res*. 2005;1052(2):180-186. doi:10.1016/j.brainres.2005.06.002.
 21. Scheffer PG, Schindhelm RK, van Verschuer VM, et al. No effect of atorvastatin and simvastatin on oxidative stress in patients at high risk for cardiovascular disease. *Neth J Med*. 2013;71(7):359-365.
 22. Mirmiranpour H, Bathaie SZ, Khaghani S, Nakhjavani M, Kebriaeezadeh A. Investigation of the mechanism(s) involved in decreasing increased fibrinogen activity in hyperglycemic conditions using L-lysine supplementation. *Thromb Res*. 2012;130(3):e13-19. doi:10.1016/j.thromres.2012.04.010.
 23. Hunina LM, Chekman IS, Nebesna T, Horchakova NO. [Effectiveness of use of omega-3 polyunsaturated fatty acids at physical loads]. *Fiziol Zh*. 2013;59(1):68-77.