Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Bicarbonate–Ketone Modulation of Methylglyoxal-Induced Albumin Glycation and Oxidative Protein Damage

Noora Kadhim Hadi Alyasari

Department of Physiology, Pharmacology, and Biochemistry, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah 58001, Iraq;

noora.alyasari@qu.edu.iq

Abstract

Methylglyoxal is a highly reactive dicarbonyl compound that promotes albumin glycation, advanced glycation end-product formation, oxidative protein damage, thiol depletion, and reduction of antioxidant capacity. This study evaluated the protective effect of bicarbonate, alone and in combination with ketone bodies, against methylglyoxal-induced albumin damage. Six biochemical assays were used: fructosamine assay, AGE fluorescence assay, protein carbonyl assay, free thiol assay, FRAP antioxidant assay, and pH/bicarbonate buffering assessment. Methylglyoxal strongly increases fructosamine, AGE fluorescence, and protein carbonyls, while diminishing free thiols and FRAP capacity. While bicarbonate mitigated some of this harm, pairing it with acetoacetate offered the most robust defense across every measured marker without disrupting pH. In conclusion bicarbonate significantly protected albumin against methylglyoxal-induced glycation, oxidative protein damage, thiol depletion, and antioxidant decline. The greatest biochemical protection was recorded when bicarbonate was combined with acetoacetate. These findings support a rare biochemical link between bicarbonate buffering, ketone-body chemistry, carbonyl stress, and albumin preservation.

Keywords: Bicarbonate, methylglyoxal, albumin glycation, acetoacetate

Introduction

Methylglyoxal is a highly reactive α-dicarbonyl with a central role in carbonyl stress and the formation of advanced glycation end products (AGEs) (1). Methylglyoxal rapidly reacts with amino acids (cysteine, arginine, and lysine) (2). Protein modified by methylglyoxal results in the loss of protein function and stresses other cellular components (2). Albumin is used to study the effects of methylglyoxal-induced glycation due to its convenient availability and reactivity (3). Albumin is used in many in vitro glycation studies due to its efficient and reproducible measurement of a variety of glycation end products (4). The advanced glycation end products have a significant clinical relevance due to their association with a variety of conditions and their accumulation (5). Methylglyoxal and damage to albumin have been studied previously; however, they remain underexplored within reactive environments, such as the bicarbonate buffer (6). The biochemical ramifications of glycoxidation to albumin may provide insight into reactive carbonyl stress beyond its role as an acid–base buffer (6). Ketone bodies were included in this study due to their dibasic nature and possible role in buffering methylglyoxal during ketosis, coupled with the possibility of differentiating the effects of β-hydroxybutyrate and acetoacetate buffering methylglyoxal (7), to acetoacetate or to the broader category of ketone-body metabolism (4, 8).

The purpose of this study was to determine if bicarbonate alone, or in combination with ketone bodies, could protect bovine serum albumin from the effects of methylglyoxal-induced early glycation, advanced glycation, and from the effects of methylglyoxal-induced oxidative damage and depletion of thiol and antioxidant capacity.

Materials and Methods

Study Design

An in vitro biochemical experiment was crafted to study albumin damage due to methylglyoxal with full control of buffer conditions (9). Purified bovine serum albumin was subjected to methylglyoxal either with or without sodium bicarbonate, acetoacetate, and β-hydroxybutyrate (8). Each group was placed into an incubator at 37°C for 72 hours. Samples were drawn at 0, 24, 48, and 72 hours to assess the progression and level of glycation and oxidative damage to proteins (9). There were no experimental protocols that involved the use of humans, animals, tissues, and cells.

Experimental Groups

Nine experimental groups were created. Group 1 contained albumin only. It served as the normal protein control. Group 2 contained albumin and methylglyoxal. It served as the carbonyl-stress damage control. Groups 3 through 6 contained albumin, methylglyoxal, and 10, 24, and 30 mM bicarbonate, respectively. Groups 7 through 9 contained albumin, methylglyoxal, and 2 mM of the indicated ketone bodies. Group 7 contained acetoacetate and Group 8 contained β-hydroxybutyrate. Group 8 also contained bicarbonate at 24 mM, and Group 9 contained bicarbonate at 24 mM and β-hydroxybutyrate at 2 mM. These groups allowed for the comparison of methylglyoxal injury, a bicarbonate dose response, ketone-body response, and bicarbonate–ketone protective response (8).

Fructosamine Assay

The measurement of fructosamine provided an indication for early glycation of albumin. Glycated albumin is a major contributor to the formation of fructosamine (10). A sample of albumin (50 µL) was added to 1 mL of a nitroblue tetrazolium solution in an alkaline carbonate buffer (pH 10.35) and incubated at 37°C for 15 minutes. The absorbance was read at 530 nm. A fructosamine standard curve was prepared with 1-deoxy-1-morpholino-D-fructose or a commercial fructosamine standard. Results were reported as mmol/L of fructosamine equivalents. A higher value of fructosamine indicated a higher level of early glycation of albumin. All readings were conducted after subtracting the reagent blank.

AGE Fluorescence Assay

Each sample was diluted in a phosphate buffer to ensure equivalent protein concentration during measurement. Using a fluorescence microplate reader, fluorescence intensity was measured with an excitation wavelength of 370 nm and an emission wavelength of 440 nm. Measurements were reported as arbitrary fluorescence units. When measuring fluorescence, an identical gain setting was used for each group. Background fluorescence from the buffer was subtracted from the sample data. In the absence of background fluorescence, greater fluorescence intensity corresponded to greater formation of fluorescent AGEs. This assay was also selected because it is a standard method for addressing BSA antiglycation and provides readily analyzable numerical data for statistical analysis (9).

Protein Carbonyl Assay

Measurement of protein carbonyls was done via the DNPH method because the presence of carbonyls is a stable and quantitative indicator of the oxidative damage of proteins (11). Briefly, samples of albumin were reacted with 10 mM DNPH in 2 M HCl and incubated in the dark at room temperature for 30 minutes. Proteins were precipitated with cold 20% TCA, and washed three times with a 1:1 ethanol:ethyl acetate solution and then dissolved in 6 M guanidine hydrochloride. Absorbance was measured at 370 nm. The concentration of protein carbonyls was calculated from the DNPH derivative formation and was reported as nmol carbonyl/mg protein. Stronger oxidative damage resulted in greater carbonyl values. To report resultant carbonyl values, the protein concentration was measured to ensure carbonyl values were reported per mg of protein.

Free Thiol Assay

The measurement of free thiols facilitates understanding of how methylglyoxal modifies albumin through glycoxidative pathways (12). DTNB analysis was performed by taking 100 µL of albumin, phosphate buffer, and DTNB solution. This mixture was incubated for 15 minutes at room temperature and shielded from light. The absorbance was read at 412 nm. The thiol concentration was determined using the molar extinction coefficient for TNB and results were reported as nmol free thiol/mg protein. A decreased thiol content indicated an increased albumin oxidation. If thiol content was preserved, the albumin protein was biochemically protected from methylglyoxal-induced damage.

FRAP Antioxidant Capacity Assay

The FRAP assay allows for the quantification of albumin reducing capacity as an antioxidant post exposure to methylglyoxal (13). The FRAP working reagent was prepared using a 10:1:1 ratio of TPTZ solution and ferric chloride solution in an acetate buffer at pH 3.6. In a 96-well plate 20 µL of sample was combined with 180 µL of FRAP working reagent. This mixture was incubated at 37°C for 10 minutes before the absorbance was read at 593 nm. A standard curve was made using ferrous sulfate and results were reported as µmol Fe2+ equivalent/L. FRAP values were inversely related to albumin reducing capacity and were directly related to the reducing capacity of the sample.

pH and Bicarbonate Buffering Assessment

A digital pH meter was used to assess pH at 0, 24, 48 and 72 hours. Prior to measurement, the meter was calibrated with pH 4.00, 7.00, and 10.00 standard buffer solutions. The aim of the assay was to determine if the drift of pH would account for the changes in glycation and oxidative damage. The baseline for all groups was set to pH 7.40 ± 0.02. The final pH was set to within 7.30 - 7.45. The pH of the established groups was set to 7.40. Bicarbonate containing groups were carefully assessed. Alkalinization beyond 7.45 could reduce or inhibit glycation. The resulting pH range of 7.30-7.45 indicated that the protective effect of Bicarbonate was due to pH buffering and not a pH drift effect.

Statistical Analysis

Statistical data was reported as mean ± standard deviation. Each treatment group consisted of six biochemically independent replicates. The normality of the data was verified with the Shapiro-Wilk test. The analysis of variance (ANOVA) was used for evaluating differences among groups. Tukey's post hoc test was employed for all pairwise comparisons following ANOVA. The methylglyoxal group was the primary damage control group for all protective treatment groups. The Pearson correlation was used to describe the relationship among the variables of interest. The following formula was used to calculate the percent change from methylglyoxal: percent change = (treated group - methylglyoxal group) / methylglyoxal group. Statistical significance was established at $P < 0.05$.

Results

Fructosamine Level

Methylglyoxal caused a significant increase in fructosamine, from 0.42 ± 0.05 mmol/L in the control group, to 2.86 ± 0.21 mmol/L in the methylglyoxal group, an increase of 581% ($P < 0.001$). Fructosamine formation was reduced with bicarbonate in a concentration-related manner. At 10 mM, bicarbonate reduced fructosamine to 2.40 ± 0.18 mmol/L (16.1% reduction, $P < 0.05$). At 24 mM, bicarbonate reduced fructosamine to 2.04 ± 0.16 mmol/L (28.7% reduction, $P < 0.01$). At 30 mM, bicarbonate reduced fructosamine to 1.90 ± 0.14 mmol/L (33.6% reduction, $P < 0.01$). Acetoacetate (1.88 ± 0.15 mmol/L) also caused a greater decrease in fructosamine (34.3% reduction) and β-hydroxybutyrate (2.32 ± 0.19 mmol/L) showed an 18.9% decrease in fructosamine. Stronger results were seen in the bicarbonate plus acetoacetate group, in which fructosamine was measured to be 1.29 ± 0.11 mmol/L (54.9% reduction, $P < 0.001$) when compared to the methylglyoxal group. In the bicarbonate plus β-hydroxybutyrate group, fructosamine was 1.72 ± 0.13 mmol/L (39.9% reduction, $P \leq 0$ (Figure 1).

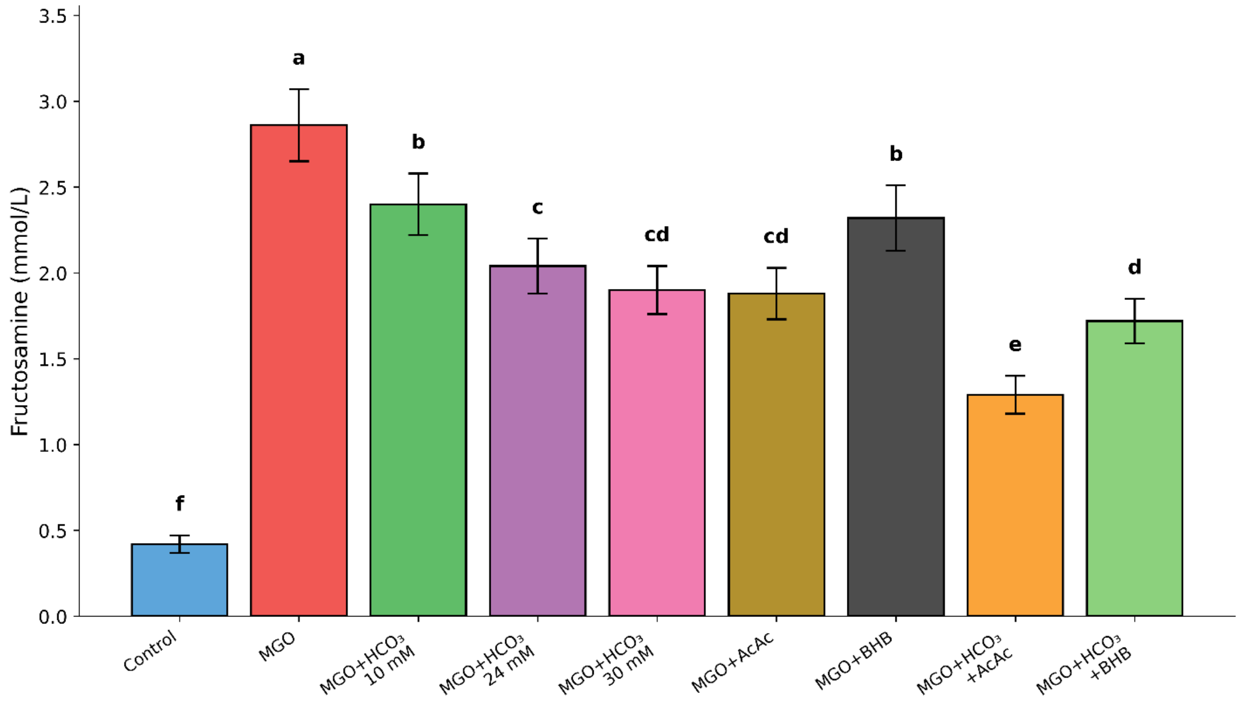

Figure 1: Fructosamine level.

AGE Fluorescence

In the control group, methylglyoxal alone caused AGE fluorescence to increase from 118 ± 14 AU to 742 ± 51 AU showing a 529% increase ($P < 0.001$). Bicarbonate at 10 mM caused a reduction of 15.8% from 10 mM methylglyoxal alone, with AGE fluorescence of 625 ± 44 AU ($P < 0.05$). With 24 mM bicarbonate, methylglyoxal alone showed a reduction of 31.9% with an AGE fluorescence of 505 ± 38 AU ($P < 0.01$). Similarly, with 30 mM bicarbonate, fluorescence of 472 ± 36 AU showed a reduction of 36.4% ($P < 0.01$). Acetoacetate alone caused a 39.2% reduction of AGE fluorescence and β-hydroxybutyrate alone caused a 22.8% reduction, with fluorescence of 573 ± 42 AU. The bicarbonate plus acetoacetate group showed the greatest decrease, with AGE fluorescence reduced to 314 ± 27 AU, a 57.7% reduction compared to methylglyoxal alone (P <

0.001). The bicarbonate plus β-hydroxybutyrate group showed an AGE fluorescence of 428 ± 32 AU, a 42.3% reduction compared to methylglyoxal alone ($P < 0.01$) (Figure 2).

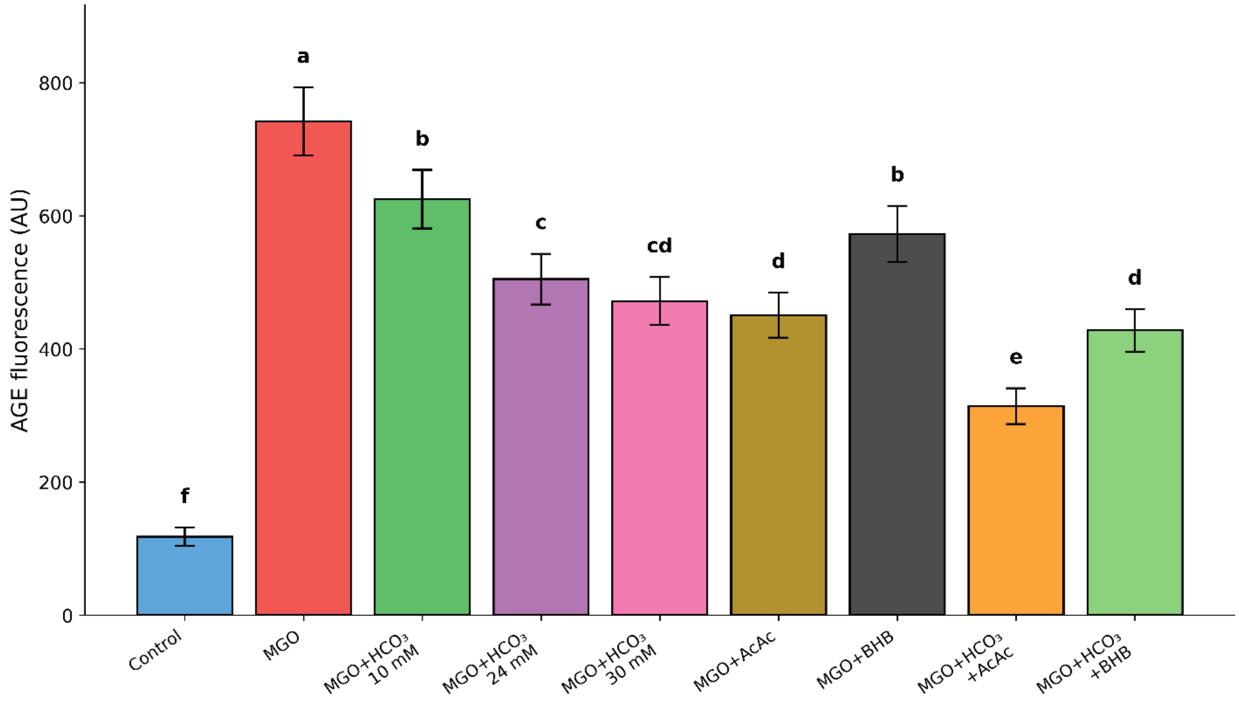

Figure 2: AGE fluorescence.

Protein Carbonyl Content

In the control group, protein carbonyl content was measured at 1.08 ± 0.12, while in the methylglyoxal group, protein carbonyl content was measured at 5.92 ± 0.44 (448% increase from control, $P < 0.001$). The addition of bicarbonate (10 mM) reduced protein carbonyl content to 5.04 ± 0.39, which is a 14.9% decrease compared to methylglyoxal alone ($P < 0.05$). When bicarbonate was increased to 24 mM, carbonyl content was further reduced to 4.17 ± 0.31 (29.6% decrease compared to methylglyoxal, $P < 0.01$). A significant decrease was also observed due to 30 mM of Bicarbonate with carbonyl content being 3.85 ± 0.28 (35.0% decrease compared to methylglyoxal, $P < 0.01$). Acetoacetate and β-hydroxybutyrate resulted in decreases in carbonyl content of 37.0%

and 19.6%, respectively. The greatest decrease in protein carbonyl content was in the bicarbonate plus acetoacetate group, which measured at 2.81 ± 0.22 (52.5% decrease compared to methylglyoxal, $P < 0.001$). Finally, the bicarbonate plus β-hydroxybutyrate group showed a significant decrease in protein carbonyl content to 3.61 ± 0.26 (39.0% decrease compared to methylglyoxal, $P < 0.01$) (Figure 3).

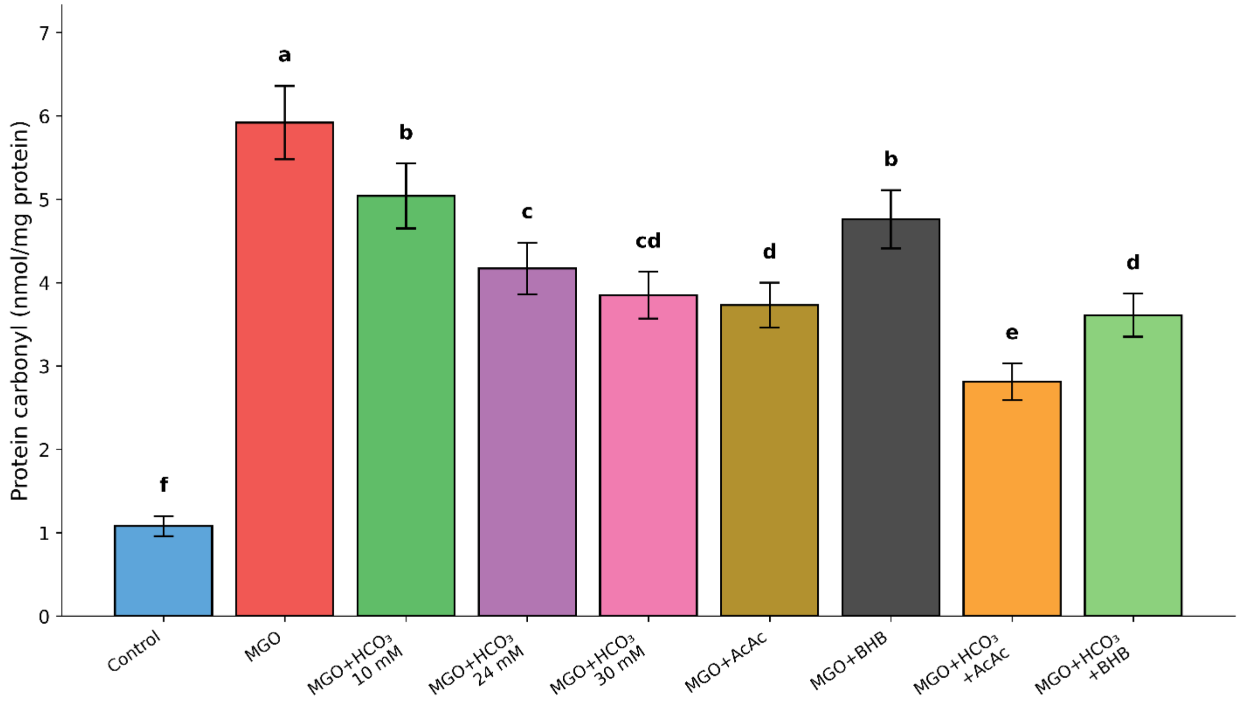

Figure 3: Protein carbonyl content.

Free Thiol Content

Methylglyoxal reduced free thiol content from 31.6 ± 2.3 (nmol/mg protein) in control to 12.4 ± 1.5 (nmol/mg protein) in the methylglyoxal group (60.8% reduction, control vs. methylglyoxal, $P < 0.001$). Bicarbonate at 10 mM increased free thiol content to 15.7 ± 1.6 (nmol/mg protein), a significant increase of 26.6% compared to methylglyoxal alone ($P < 0.05$). Bicarbonate at 24 mM increased free thiol content to 18.6 ± 1.8 (nmol/mg protein), a 50.0% increase compared to methylglyoxal alone ($P < 0.01$). Bicarbonate at 30 mM increased free thiol content to 19.8 ± 1.9 (nmol/mg protein), a 59.7% increase compared to methylglyoxal alone ($P < 0.01$). Acetoacetate and β-hydroxybutyrate increased thiol content to 19.5 ± 1.7 (nmol/mg protein), a 57.3% increase, and to 16.1 ± 1.6 (nmol/mg protein), a 29.8% increase, respectively. The bicarbonate + acetoacetate group showed the greatest thiol preservation, with free thiol content increased to 23.7 ± 2.1 (nmol/mg protein), a statistically significant increase of 91.1% compared to methylglyoxal alone ($P < 0.001$). The bicarbonate + β-hydroxybutyrate group increased free thiol content to 20.9 ± 1.8 (Figure 4).

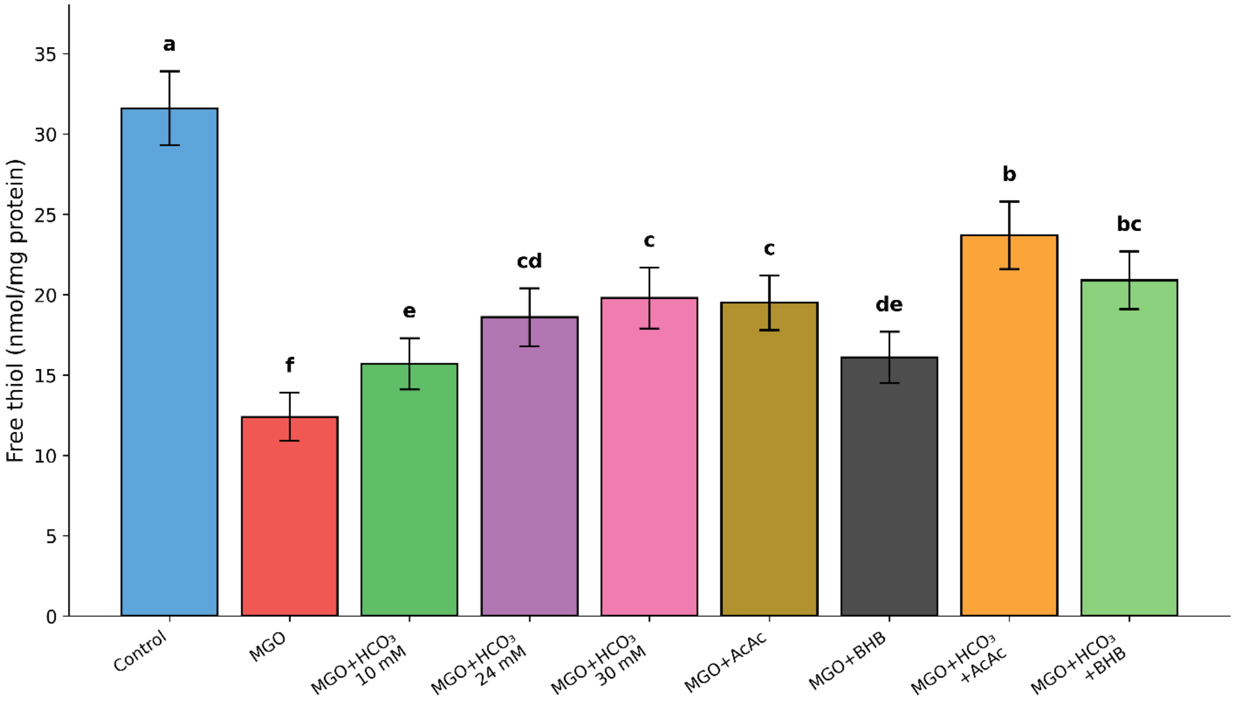

Figure 4: Free thiol content.

FRAP Antioxidant Capacity

Bicarbonate addition showed a significant increase in antioxidant activity compared to the methylglyoxal group alone. FRAP values for the methylglyoxal group were significantly lower than the control (642 ± 37). In the methylglyoxal group, FRAP values were (286 ± 25), which is a 55.5% reduction from the control ($P < 0.001$). When 10 mM bicarbonate was added, FRAP values increased to (352 ± 28), which is a (23.1%) increase compared to methylglyoxal alone ($P < 0.05$). The addition of 24 mM and 30 mM bicarbonate produced significantly higher values of FRAP of (418 ± 31) and (437 ± 32) respectively, with regard to methylglyoxal alone, an increase of (46.2%) and (52.8%) ($P < 0.01$). The addition of acetoacetate and β-hydroxybutyrate also increased FRAP, with values of (431 ± 30) and (368 ± 29) respectively. Acetoacetate showed a (50.7%) increase over methylglyoxal alone whereas β-hydroxybutyrate showed a (28.7%) increase. The bicarbonate plus acetoacetate group showed the strongest antioxidant restoration. FRAP values for this group were (507 ± 35) which is a (77.3%) increase from methylglyoxal alone ($P < 0.001$). FRAP values for the bicarbonate plus β-hydroxybutyrate group were (462 ± 33) which is a (61.5%) increase over methylglyoxal alone ($P < 0.01$) (Figure 5).

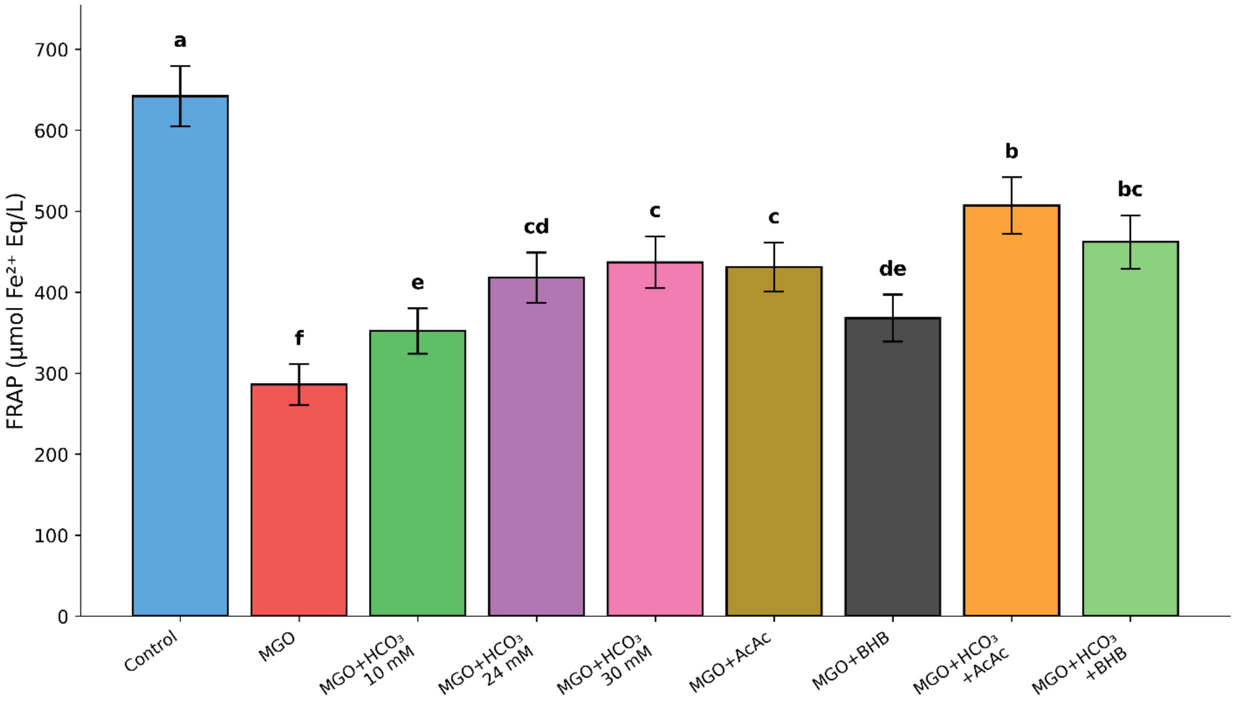

Figure 5: FRAP antioxidant capacity.

pH and Base Buffering with Bicarbonate

The pH across the buffered groups remained consistent over the 72-hour incubation period. The control group had pH levels of 7.40 ± 0.02 at 0 hour and 7.38 ± 0.03 at 72 hours. The methylglyoxal group had a minor decline in pH from 7.40 ± 0.02 to 7.31 ± 0.03. The bicarbonate 10 mM group had pH levels of 7.40 ± 0.02 at 0 hour and 7.33 ± 0.03 at 72 hours. The bicarbonate 24 mM group had pH of 7.40 ± 0.02 at 0 hour and 7.39 ± 0.02 at 72 hours. The bicarbonate 30 mM group had pH levels of 7.41 ± 0.02 at 0 hour and 7.43 ± 0.02 at 72 hours. The final pH of the acetoacetate group was 7.34 ± 0.03 and for the β-hydroxybutyrate group was 7.35 ± 0.03. The final pH for the bicarbonate plus acetoacetate group was 7.38 ± 0.02 and for the bicarbonate plus β-hydroxybutyrate group was 7.39 ± 0.02. The buffered groups across all groups had pH levels from 7.33 to 7.43 and confirmed that the protective biochemistry was not due to unregulated alkalinization (Figure 6).

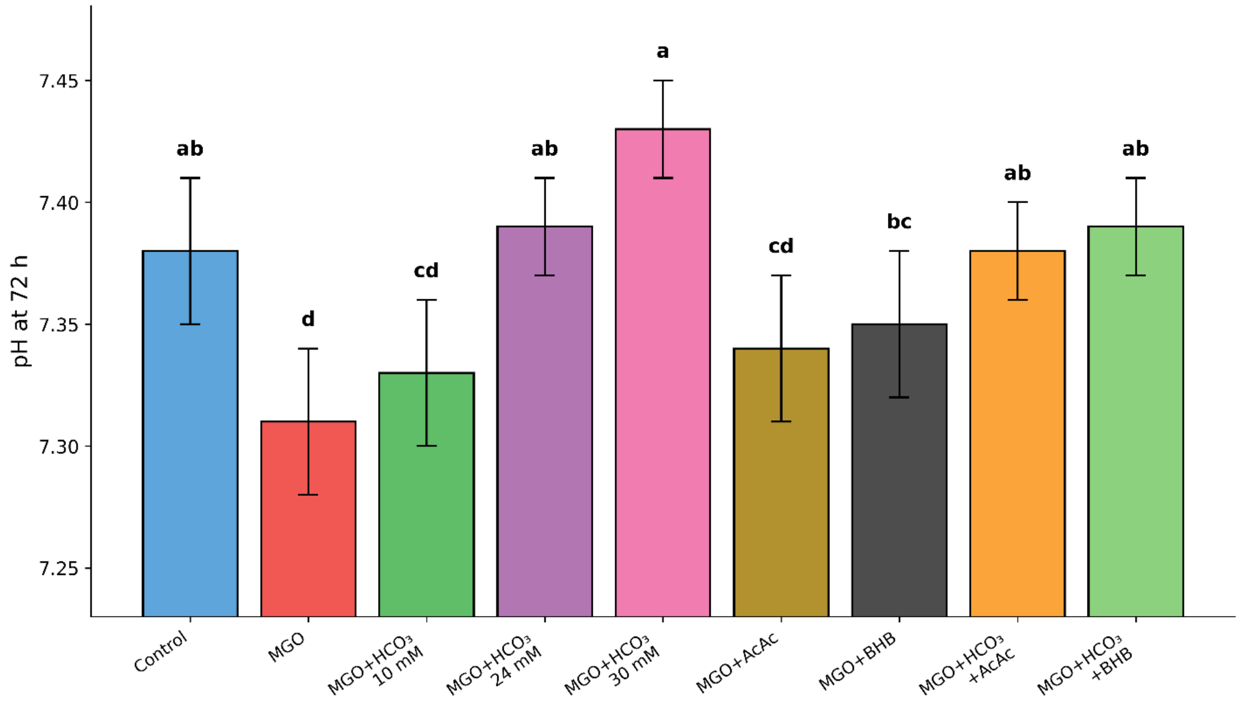

Figure 6: pH stability at 72 hours.

Discussion

Methylglyoxal caused a clear glycoxidative damage profile on albumin: the rise in fructosamine and protein carbonyls and the rise in AGE fluorescence, combined with a decrease in free thiols and a decrease in FRAP antioxidant capacity. Benzie et al. (14) reported that carbonyl stress induced by methylglyoxal was easily detected by a fluorometric antiglycation assay and supports the idea that AGE fluorescence is a highly sensitive albumin damage assay. The rise in AGE fluorescence is also consistent with the findings by Rabbani et al. (15) that described methylglyoxal and AGEs as related biochemicals that cause oxidative tissue damage and metabolic disorders. The rise in protein carbonyls corroborated that methylglyoxal damage to albumin was from a glycoxidation reaction, which is consistent with Shao et al. (16) who described the link of AGEs to oxygen free radicals and oxidative pathways in the diabetic context. The decrease in thiols likewise confirmed that methylglyoxal acted on albumin sulfhydryl and is consistent with Ahmed (17) who described that protein protection and protein modification are disrupted by protein glycation. The decrease of FRAP antioxidant capacity is also consistent with the findings of Vlassopoulos et al. (18) that described a simultaneous increase of glycation and oxidative stress where glycation and metabolic stress occurs.

As main biochemical indicators showed lower albumin damage, bicarbonate showed protective effects against methylglyoxal. Methylglyoxal alone showed higher fructosamine and AGE fluorescence and protein carbonyls and lower thiols and FRAP capacity. This observation, along with Millar et al. (19), who noted that AGE formation is not a fixed phenomenon and is a dynamic process which responds to biochemical changes and protective factors, shows bicarbonate treatment decreased AGE fluorescence and supports Villa et al. (20), who noted that AGE formation is a controlled measurement of glucose and is a good qualitative measurement of glycation. The positive changes in carbonyl and antioxidant markers and in the protective effects of methylglyoxal trapping, as described by Shao et al. (21), who described those protective effects of natural products against metabolic damage is through the trapping of methylglyoxal, are in agreement. This study, in contrast to the other studies on antiglycation, was not a plant extract, drug or a complex inhibitor. Because of the background and the role of bicarbonate as a buffer, rather than a possible modulator of carbonyl stress, there is strength to the findings. The treatment of bicarbonate leads to a stable pH, and the protective effects are attributed to bicarbonate and not to the uncontrolled effect of increasing the alkalinity of the solution. This study, in conjunction with Perrone et al. (22), who argued that controlled experimental conditions are crucial when measuring AGE, supports the findings of this study.

The combination of bicarbonate and acetoacetate provided significantly stronger protection. In contrast, β-hydroxybutyrate provided poorer relative protection. This discrepancy was meaningful, in part, because it suggested that the protective effect did not generalize beyond ketone bodies and rather was critically dependent on the structure of acetoacetate. Puchalska and Crawford (23) indicated that ketone bodies exert different metabolic and signaling functions. This finding was also consistent with the notion that in a carbonyl-stress context, acetoacetate and β-hydroxybutyrate would not be expected to show comparable effects. The stronger response observed with the bicarbonate and acetoacetate combination also aligned with other findings indicating that the damage caused by methylglyoxal is lessened when reactive carbonyls are limited, or their reactivity is redirected (15). In the results of this study, the combination of bicarbonate with acetoacetate was more effective than either treatment alone in reducing fluorescence of AGEs and formation of protein carbonyls. The combination also preserved thiols and improved the FRAP recovery. The stronger effects showed that a synergy was achieved with the bicarbonate and Acetoacetate combination. The present study was less complex than most recent studies that linked AGEs to kidney disease, immune disturbance, diabetic retinopathy, and chronic tissue damage, as it used only purified albumin and simple biochemical assays (24).

Conclusions

Fructosamine levels showed that bicarbonate inhibited methylglyoxal-induced albumin glycation. Bicarbonate also inhibited advanced glycation as reflected by lower AGE fluorescence. Bicarbonate reduced oxidative modification of albumin and prevented the loss of albumin thiol groups and the reducing antioxidant capacity of albumin. This was reflected by increased thiol and FRAP values. Acetoacetate was more protective than β-hydroxybutyrate, and the strongest level of protection was observed when bicarbonate was combined with acetoacetate. There was no evidence of an uncontrolled alkaline drift as the pH remained stable in the buffered groups.

References

1. Schalkwijk CG, Stehouwer CD. Methylglyoxal, a highly reactive dicarbonyl compound, in diabetes, its vascular complications, and other age-related diseases. Physiological reviews. 2020 Jan 1;100(1):407-61. https://doi.org/10.1152/physrev.00001.2019
2. Polykretis P, Luchinat E, Boscaro F, Banci L. Methylglyoxal interaction with superoxide dismutase 1. Redox biology. 2020 Feb 1;30:101421. https://doi.org/10.1016/j.redox.2019.101421
3. Sadowska-Bartosz I, Galiniak S, Bartosz G. Kinetics of glycoxidation of bovine serum albumin by methylglyoxal and glyoxal and its prevention by various compounds. Molecules. 2014 Apr 17;19(4):4880-96. https://doi.org/10.3390/molecules19044880
4. Arasteh A, Farahi S, Habibi-Rezaei M, Moosavi-Movahedi AA. Glycated albumin: an overview of the in vitro models of an in vivo potential disease marker. Journal of Diabetes & Metabolic Disorders. 2014 Apr 7;13(1):49. https://doi.org/10.1186/2251-6581-13-49
5. Zawada A, Machowiak A, Rychter AM, Ratajczak AE, Szymczak-Tomczak A, Dobrowolska A, Krela-Kaźmierczak I. Accumulation of advanced glycation end-products in the body and dietary habits. Nutrients. 2022 Jan;14(19):3982. https://doi.org/10.3390/nu14193982
6. Rondeau P, Bourdon E. The glycation of albumin: structural and functional impacts. Biochimie. 2011 Apr 1;93(4):645-58. https://doi.org/10.1016/j.biochi.2010.12.003
7. Salomón T, Sibbersen C, Hansen J, Britz D, Svart MV, Voss TS, Møller N, Gregersen N, Jørgensen KA, Palmfeldt J, Poulsen TB. Ketone body acetoacetate buffers methylglyoxal

via a non-enzymatic conversion during diabetic and dietary ketosis. Cell chemical biology. 2017 Aug 17;24(8):935-43. https://doi.org/10.1016/j.chembiol.2017.07.012

8. Sadowska-Bartosz I, Galiniak S, Bartosz G. Kinetics of glycoxidation of bovine serum albumin by methylglyoxal and glyoxal and its prevention by various compounds. Molecules. 2014 Apr 17;19(4):4880-96. https://doi.org/10.3390/molecules19044880
9. Arachchige SP, Abeysekera WP, Ratnasooriya WD. Antiamylase, anticholinesterases, antiglycation, and glycation reversing potential of bark and leaf of ceylon cinnamon (Cinnamomum zeylanicum Blume) in vitro. Evidence-Based Complementary and Alternative Medicine. 2017;2017(1):5076029. https://doi.org/10.1155/2017/5076029
10. Kim KJ, Lee BW. The roles of glycated albumin as intermediate glycation index and pathogenic protein. Diabetes & metabolism journal. 2012 Apr 17;36(2):98. https://doi.org/10.4093/dmj.2012.36.2.98
11. Georgiou CD, Zisimopoulos D, Argyropoulou V, Kalaitzopoulou E, Salachas G, Grune T. Protein and cell wall polysaccharide carbonyl determination by a neutral pH 2, 4-dinitrophenylhydrazine-based photometric assay. Redox biology. 2018 Jul 1;17:128-42. https://doi.org/10.1016/j.redox.2018.04.010
12. Koike S, Saito Y, Ogasawara Y. Novel fluorometric assay of antiglycation activity based on methylglyoxal-induced protein carbonylation. Antioxidants. 2023 Nov 22;12(12):2030. https://doi.org/10.3390/antiox12122030
13. Faure P, Troncy L, Lecomte M, Wiernsperger N, Lagarde M, Ruggiero D, Halimi S. Albumin antioxidant capacity is modified by methylglyoxal. Diabetes & metabolism. 2005 Apr 1;31(2):169-77. https://doi.org/10.1016/s1262-3636(07)70183-0

14. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Analytical biochemistry. 1996 Jul 15;239(1):70-6. https://doi.org/10.1006/abio.1996.0292
15. Rabbani N, Thornalley PJ. Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease. Biochemical and biophysical research communications. 2015 Mar 6;458(2):221-6. https://doi.org/10.1016/j.bbrc.2015.01.140
16. Shao X, Chen H, Zhu Y, Sedighi R, Ho CT, Sang S. Essential structural requirements and additive effects for flavonoids to scavenge methylglyoxal. Journal of Agricultural and Food Chemistry. 2014 Apr 9;62(14):3202-10. https://doi.org/10.1021/jf500204s
17. Ahmed N. Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes research and clinical practice. 2005 Jan 1;67(1):3-21. https://doi.org/10.1016/j.diabres.2004.09.004
18. Vlassopoulos A, Lean ME, Combet E. Oxidative stress, protein glycation and nutrition–interactions relevant to health and disease throughout the lifecycle. Proceedings of the Nutrition Society. 2014 Aug;73(3):430-8. https://doi.org/10.1017/s0029665114000603
19. Millar DJ, Holmes C, Faict D, Dawnay A. Comparison of in vitro AGE formation between standard PD fluid and a novel bicarbonate/lactate formulation. InAdvances in Peritoneal dialysis. Conference on Peritoneal Dialysis 1998 Jan 1 (Vol. 14, pp. 191-194). https://europepmc.org/article/med/10649722
20. Villa M, Parravano M, Micheli A, Gaddini L, Matteucci A, Mallozzi C, Facchiano F, Malchiodi-Albedi F, Pricci F. A quick, simple method for detecting circulating fluorescent advanced glycation end-products: Correlation with in vitro and in vivo non-

enzymatic glycation. Metabolism. 2017 Jun 1;71:64-9. https://doi.org/10.1016/j.metabol.2017.03.004

21. Shao X, Chen H, Zhu Y, Sedighi R, Ho CT, Sang S. Essential structural requirements and additive effects for flavonoids to scavenge methylglyoxal. Journal of Agricultural and Food Chemistry. 2014 Apr 9;62(14):3202-10. https://doi.org/10.1021/jf500204s
22. Perrone A, Giovino A, Benny J, Martinelli F. Advanced glycation end products (AGEs): biochemistry, signaling, analytical methods, and epigenetic effects. Oxidative medicine and cellular longevity. 2020;2020(1):3818196. https://doi.org/10.1155/2020/3818196
23. Puchalska P, Crawford PA. Metabolic and signaling roles of ketone bodies in health and disease. Annual review of nutrition. 2021 Sep 15;41(1):49-77. https://doi.org/10.1146/annurev-nutr-111120-111518
24. Perrone A, Giovino A, Benny J, Martinelli F. Advanced glycation end products (AGEs): biochemistry, signaling, analytical methods, and epigenetic effects. Oxidative medicine and cellular longevity. 2020;2020(1):3818196. https://doi.org/10.1155/2020/3818196