2014 International Conference on Food Security and Nutrition
IPCBEE vol.67 (2014) © (2014) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2014. V67. 3

Bovine Beta Casein Variants: Implications to Human Nutrition and Health

Andrew Clarke [1+], Malav Trivedi [2]

[1] A2 Corporation, Auckland, New Zealand.
[2] Northeastern University, Boston, USA.

Abstract. Milk is a whole-food with numerous nutritive components; especially for infants, as milk/milk-based formulas are the only source of nutrition. However, casein free diet is recommended for infants with immunological sensitivities; yet the reason is unknown. It is hard to eliminate milk from diet of sensitive individuals. Several variants of β-casein (~25-30% of cows' milk-proteins) are genetically determined; A1, A2, A3, B, C etc. A2 is the original form of bovine β-casein and is similar to other β-casein in mammals. Additionally, A1 and A2 differ by a single amino acid, resulting in differential secondary structure and enzymatic hydrolysis, i.e. A1 but not A2 β-casein liberates the heptapeptide β-casomorphin-7 (YPFPGPL; BCM7), which acts ~morphine, and is implicated in digestive, immune and brain development changes. Biochemical reports show excess BCM7 in blood and urine samples of patients with neurological defects. Additionally, strong correlation between consumption of BCM7 containing A1 milk and incidences of type-1 diabetes mellitus, autoimmune and cardiovascular diseases is also reported. Anecdotal evidence suggests symptomatic relief in patients with neurological, gastric and immunological problems, after consuming A2 β-casein containing milk. Hence, completely eliminating milk can be avoided by consuming milk containing A2 β-casein, especially for infants' growth and development.

Keywords: A1 casein, beta casomorphin, A2 beta casein, digestive disorders, neurodevelopment.

1. Introduction

Milk is a whole-food with numerous nutritive components exploited by man for many thousands of years. With a balance of protein, carbohydrate and fat coupled with essential minerals dairy milk has been a staple food to many populations, especially for early infant development where milk and/or milk-based formulas are the only source of nutrition. Beneficial components of milk are still being identified, such as whey protein, sphingomyelin and conjugated linoleic acid (CLA), however it is established that the protein component of milk provides significant nutritional contribution itself rationalizing the consumption of milk for growing developing individuals. A major protein component of cow's milk is beta casein, of which there are two primary variants, A1 and A2. Research into the beta casein variants has reported that:

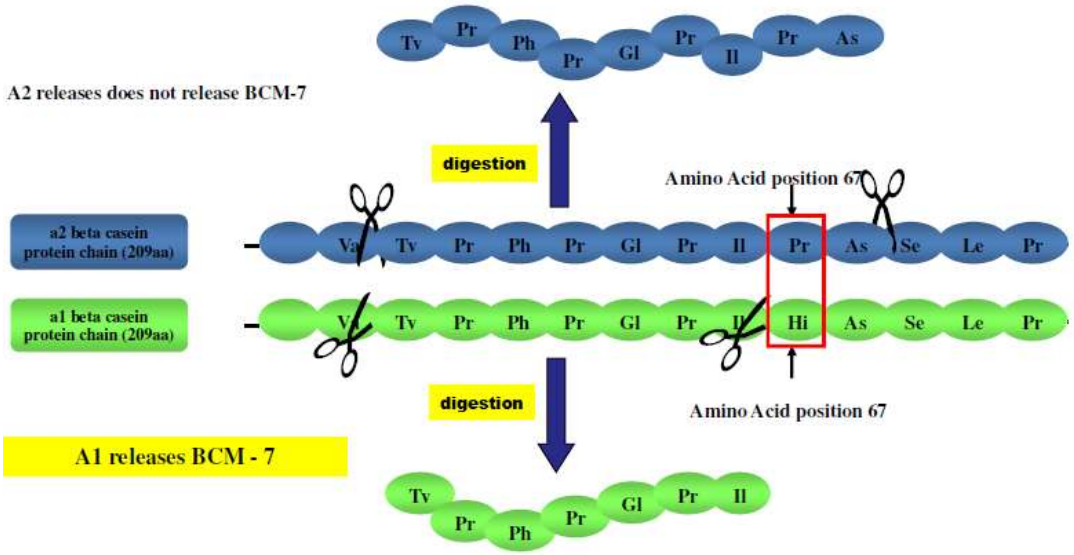
- A2 is the original form of the beta casein protein when cows were domesticated thousands of years ago, the A1 type arose and spread with breeding and migration of man.
- Digestion of A1 beta casein, but not that of A2 beta casein yields β-casomorphin-7 (BCM-7), an exogenous opioid peptide (exorphin) that can potently activate opioid receptors throughout the body
- Opioid receptors are important regulators of signalling processes throughout the body, including the gastrointestinal tract, immune system, and the central nervous system
- A2 is more comparable to the human beta casein than A1 in terms of digestive breakdown.
- Excessive exposure to A1 beta casein or BCM-7 is implicated adverse response, including interference with gastrointestinal function and symptoms of intolerance reactions.

[+] Corresponding author. Tel.: + 006421672521.
E-mail address: andrew@a2corporation.com.

- Reducing or eliminating the consumption of A1 beta casein, and replacing it with another major protein source, such as A2 beta casein, may avoid some of these disorders or improve their symptoms and in the long term reduce the risk of some clinical conditions.

2. Beta Casein and Their Digestion

Casein proteins make up 80% of the total protein in milk and comprise three classes; alpha, beta and kappa which aggregate to form micelles which reflect light to give milk its white colour. Beta caseins are present at a concentration of ~1gram per 100ml, or 2.5grams a standard serving. In cow's milk, two primary variants of beta casein, termed A1 and A2, and several rarer sub-variants have been identified. A1 and A2 beta casein differ in their protein structure by a substitution of the amino acid at position 67. A1 beta casein contains a histidine residue at this position, which allows cleavage of the preceding seven amino acid residues to yield the peptide β-casomorphin-7 (BCM-7) (Figure 1A). A2 beta casein contains a proline residue, which prevents cleavage of this peptide. [1]



A

Tyr[60]-Pro[61]-Phe[62]-Pro[63]-Gly[64]-Pro[65]-Ile[66]-His[67] Bovine β-casein A1
Tyr[60]-Pro[61]-Phe[62]-Pro[63]-Gly[64]-Pro[65]-Ile[66]-Pro[67] Bovine β-casein A2
Tyr[51]-Pro[52]-Phe[53]-Val[54]-Glu[55]-Pro[56]-Ile[57]-Pro[58] Human β-casein

B

Fig. 1: (A) Differential breakdown of A1 vs A2 during digestion and (B) diagram comparing partial sequences of A1 and A2 with that of human beta casein

3. Studies on Beta Casein Variants and A1 Derived BCM-7

BCM-7 and other derivatives of beta casein are potent exogenous agonists of opioid receptors (exorphins), with greatest affinity for μ opioid receptors.[2] The role of opioid receptors in mediating the effects of BCM-7 is supported by the finding that naloxone, a μ opioid receptor-specific competitive antagonist, prevents many of the effects of BCM-7, including effects on gastrointestinal motility[3], [4] and mucus secretion,[5] lymphocyte proliferation,[6] and histamine release from peripheral leukocytes.[7] The affinity of bovine BCM-7 to opioid receptors is approximately 10 times greater than that of human BCM-7, as it requires a 10-fold greater naloxone concentration to prevent receptor binding.[8] Opioid receptors are expressed by many cell types in most organs. Levels of μ opioid receptors are highest in the hypothalamus, cerebral cortex, and spleen, moderate in the cerebellum, intestine, kidney, adrenal, and reproductive organs,

and lowest in the lung and liver. μ opioid receptors are not expressed in the stomach, heart, or endothelium.[9],[10] Opioid receptors are also expressed on inflammatory cells, including lymphocytes and leukocytes.

Experimental studies have shown that BCM peptides and analogues may be able to cross the blood–brain barrier. [11], [12] This was particularly evident in regions with 'leaky' capillaries, such as the pineal gland, the neurohypophysis, and the choroid plexus.[13] An autopsy study revealed BCM immunoreactivity in several brain regions, including the brain stem,[14] while a clinical study detected BCM-8 in the cerebrospinal fluid of pregnant and lactating women.[15] Therefore, BCM peptides may influence central signalling pathways after crossing the blood–brain barrier. BCM-7, and other related peptides with an amino acid sequence of Tyr-Pro-Phe-Pro-Gly-Pro-Ile, including their C-terminally shortened fragments, are primarily degraded by DPP4.[16] DPP4 is a protease that selectively removes the N-terminal dipeptide from peptides bearing a Pro or Ala residue at position 2. It is principally expressed on T lymphocytes and a soluble form exists in plasma.[17], [18] A recent study of infants revealed that infants with apparent life-threatening apnoea had markedly elevated BCM-7 levels but much lower serum DPP4 activity compared with healthy infants of the same age.[19] Those results suggested that individuals with lower DPP4 activity may be more prone to the potential adverse effects of BCM-7; however, the results need to be confirmed in further studies.

Though the clinical relevance of BCM-7 is only beginning to be understood studies have demonstrated that BCM-7 has the potential to cross the gastrointestinal wall and blood–brain barrier, it may be able to influence activities in most systems throughout the body, partly because of the highly ubiquitous expression of opioid receptors (Figure 2).

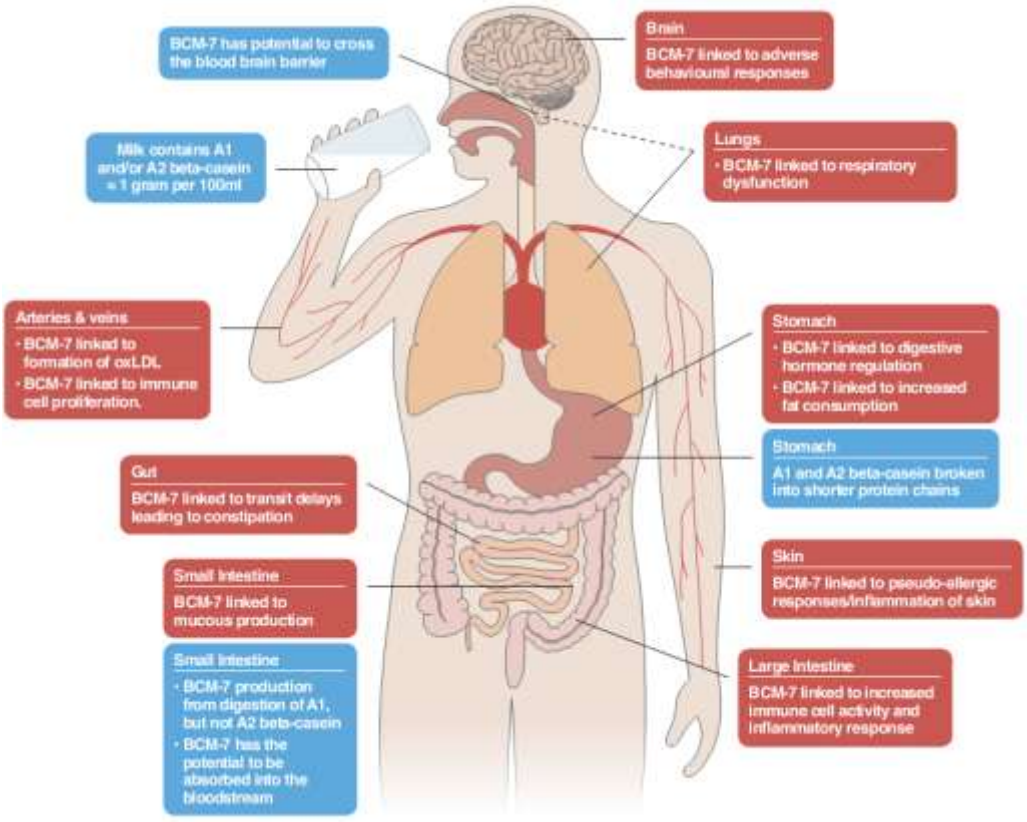


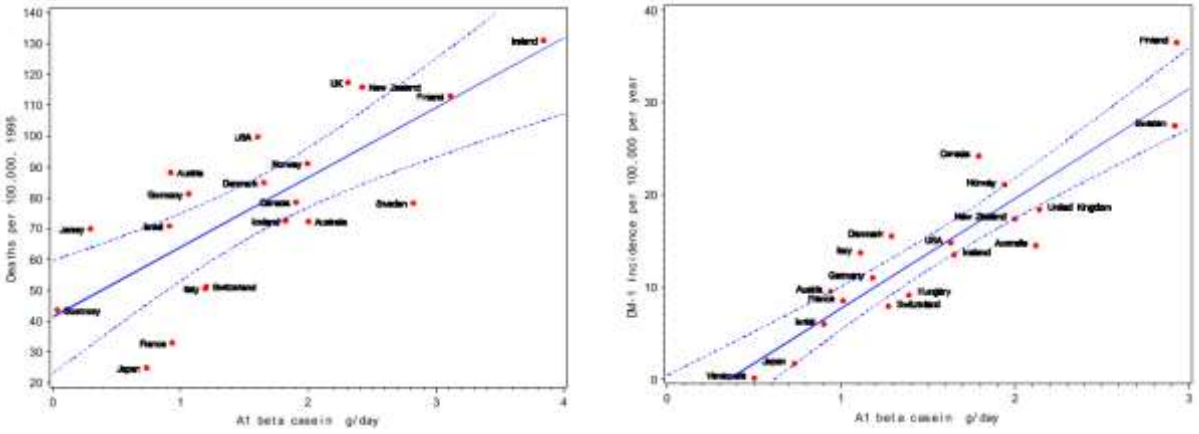
Fig. 2: A1 beta casein and BCM-7: Production, absorption, and potential targets.

Studies have shown direct effects of BCM-7 on several gastrointestinal functions. For example, BCM-7 has been reported to reduce the frequency and amplitude of intestinal contractions, thus slowing gastrointestinal motility,[20]–[22] and to stimulate mucus release.[23], [24] BCM-7 also inhibits lamina propria lymphocyte proliferation, which may affect susceptibility to infection. Recent studies in rodents

report that A1 consumption promotes significant intestinal inflammation and immune activity relative to A2.[25] BCM-7 also has immunomodulatory effects, including earlier noted triggering of histamine release from peripheral leukocytes and suppression of lymphocyte proliferation coupled with secretagogue effects on peritoneal mast cells.[26] Similarly, A1 beta casein has marked pro-atherogenic effects, promoting low-density lipoprotein (LDL) oxidation [27] and the generation of autoantibodies to oxidized LDL, [28]–[30] both of which might contribute to the progression of atherosclerosis.[31]

A1 beta casein and BCM-7 are also implicated in the pathogenesis of type 1 diabetes via two mechanisms. In the first, consumption of A1 beta casein induces the production of autoantibodies that ultimately cause autoimmune-mediated killing of pancreatic β cells. Illustrating the second pathway, A1 beta casein induced diabetes in non-obese diabetogenic mice via opioid receptors, as the diabetogenic effects of A1 beta casein were prevented by the μ-receptor-specific antagonist naloxone.[32] These immunomodulatory and pro-atherogenic effects of A1 beta casein may be responsible for the increased risk of increased risk of heart disease (Figure 3A) and type 1 diabetes (Figure 3B) in populations associated with high per capita A1 beta casein consumption.[33]

The consumption of beta casein has also been linked with the aggravation of symptoms associated with autism and schizophrenia.[34]–[43] These effects were attributed to the opioid activities of BCM-7 [43] and to oxidative stress,[44] causing neurological deficits that manifested as symptoms of schizophrenia and autism.[45]



A B

Fig. 3: Correlations between A1 beta casein supply per capita in 1990 and ischaemic heart disease (A) and type 1 diabetes (B). (A) Correlation between A1 beta casein supply per capita in 1990 and ischaemic heart disease in 1995 in 20 countries. $r = 0.76$ (95% confidence interval: 0.48–0.90; $p \leq 0.0001$). (B) Correlation between A1 beta casein supply per capita in 1990 and incidence of type 1 diabetes (1990–1994) in children aged 0–14 years in 19 countries. $r = 0.92$ (95% confidence interval: 0.72–0.97; $p < 0.0001$). Dotted lines are the 95% confidence limits of the regression line. Reprinted from Laugesen and Elliott (2003).

4. Implications of Research on Beta Basein Bariants

Experimental and small-scale clinical studies, as well as cases in the lay media, have provided evidence supporting the benefits of removing A1 beta casein from the diet of potentially susceptible patients. For example, switching to a gluten- and casein-free diet ameliorates some of the symptoms of autism.[4], [46]–[50] Similarly, some food disorders, such as lactose intolerance, may be wrongly diagnosed and could resolved by replacing A1 beta casein with A2 beta casein.[49], [50] Avoidance of A1 beta casein may also prevent the excess immunomodulatory activity associated with type 1 diabetes, neurological disorders, and ischaemic heart disease. More studies, including randomized controlled trials, observational cohort studies and case reports, are needed to confirm the potential clinical benefits of reducing A1 beta casein consumption.

5. References

[1] Y. Jinsmaa and M. Yoshikawa, “Enzymatic release of neocasomorphin and β-casomorphin from bovine β-casein,” *Peptides*, vol. 20, no. 8, pp. 957–962, 1999.

[2] K. W. G Koch, “Opioid activities of human beta-casomorphins.,” *Naunyn-Schmiedeberg39s Arch. Pharmacol.*, vol. 331, no. 4, pp. 351–4, 1986.

[3] A. Becker, G. Hempel, G. Grecksch, and H. Matthies, “Effects of beta-casomorphin derivatives on gastrointestinal transit in mice,” *Biomed. Biochim. Acta*, vol. 49, no. 11, pp. 1203–1207, 1990.

[4] C. M. Pennesi and L. C. Klein, “Effectiveness of the gluten-free, casein-free diet for children diagnosed with autism spectrum disorder: based on parental report,” *Nutr. Neurosci.*, vol. 15, no. 2, pp. 85–91, Mar. 2012.

[5] A. Trompette, J. Claustre, F. Caillon, G. Jourdan, J. A. Chayvialle, and P. Plaisancié, “Milk bioactive peptides and beta-casomorphins induce mucus release in rat jejunum,” *J. Nutr.*, vol. 133, no. 11, pp. 3499–3503, Nov. 2003.

[6] Y. Elitsur and G. D. Luk, “Beta-casomorphin (BCM) and human colonic lamina propria lymphocyte proliferation.,” *Clin. Exp. Immunol.*, vol. 85, no. 3, pp. 493–497, Sep. 1991.

[7] M. Kurek, B. Przybilla, K. Hermann, and J. Ring, “A naturally occurring opioid peptide from cow’s milk, beta-casomorphine-7, is a direct histamine releaser in man,” *Int. Arch. Allergy Immunol.*, vol. 97, no. 2, pp. 115–120, 1992.

[8] M. Herrera-Marschitz, L. Terenius, L. Grehn, and U. Ungerstedt, “Rotational behaviour produced by intranigral injections of bovine and human beta-casomorphins in rats,” *Psychopharmacology (Berl.)*, vol. 99, no. 3, pp. 357–361, 1989.

[9] M. Satoh and M. Minami, “Molecular pharmacology of the opioid receptors,” *Pharmacol. Ther.*, vol. 68, no. 3, pp. 343–364, 1995.

[10] G. Wittert, P. Hope, and D. Pyle, “Tissue distribution of opioid receptor gene expression in the rat,” *Biochem. Biophys. Res. Commun.*, vol. 218, no. 3, pp. 877–881, Jan. 1996.

[11] H. Stark, J. B. Van Bree, A. G. de Boer, U. Jaehde, and D. D. Breimer, “In vitro penetration of des-tyrosine1-D-phenylalanine3-beta-casomorphin across the blood-brain barrier,” *Peptides*, vol. 13, no. 1, pp. 47–51, Feb. 1992.

[12] A. Ermisch, H. J. Rühle, K. Neubert, B. Hartrodt, and R. Landgraf, “On the blood-brain barrier to peptides: [3H]beta-casomorphin-5 uptake by eighteen brain regions in vivo,” *J. Neurochem.*, vol. 41, no. 5, pp. 1229–1233, Nov. 1983.

[13] A. Ermisch, H.-J. Rühle, R. Landgraf, and J. Hess, “Blood-Brain Barrier and Peptides,” *J. Cereb. Blood Flow Metab.*, vol. 5, no. 3, pp. 350–357, Sep. 1985.

[14] A. Pasi, H. Mahler, N. Lansel, C. Bernasconi, and F. S. Messiha, “beta-Casomorphin-immunoreactivity in the brain stem of the human infant,” *Res. Commun. Chem. Pathol. Pharmacol.*, vol. 80, no. 3, pp. 305–322, Jun. 1993.

[15] F. Nyberg, H. Lieberman, L. H. Lindström, S. Lyrenäs, G. Koch, and L. Terenius, “Immunoreactive beta-casomorphin-8 in cerebrospinal fluid from pregnant and lactating women: correlation with plasma levels,” *J. Clin. Endocrinol. Metab.*, vol. 68, no. 2, pp. 283–289, Feb. 1989.

[16] G. Kreil, M. Umbach, V. Brantl, and H. Teschemacher, “Studies of the enzymatic degradation of β-casomorphins,” *Life Sci.*, vol. 33, Supplement 1, pp. 137–140, 1983.

[17] A.-M. Lambeir, C. Durinx, S. Scharpé, and I. De Meester, “Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV,” *Crit. Rev. Clin. Lab. Sci.*, vol. 40, no. 3, pp. 209–294, Jun. 2003.

[18] E. Boonacker and C. J. F. Van Noorden, “The multifunctional or moonlighting protein CD26/DPPIV,” *Eur. J. Cell Biol.*, vol. 82, no. 2, pp. 53–73, Feb. 2003.

[19] J. Wasilewska, E. Sienkiewicz-Szłapka, E. Kuźbida, B. Jarmołowska, M. Kaczmarski, and E. Kostyra, “The exogenous opioid peptides and DPPIV serum activity in infants with apnoea expressed as apparent life threatening events (ALTE),” *Neuropeptides*, vol. 45, no. 3, pp. 189–195, Jun. 2011.

[20] C. Defilippi, E. Gomez, V. Charlin, and C. Silva, “Inhibition of small intestinal motility by casein: a role of beta casomorphins?,” *Nutr. Burbank Los Angel. Cty. Calif*, vol. 11, no. 6, pp. 751–754, Dec. 1995.

[21] W. A. Mihatsch, A. R. Franz, B. Kuhnt, J. Högel, and F. Pohlandt, “Hydrolysis of casein accelerates

gastrointestinal transit via reduction of opioid receptor agonists released from casein in rats," *Biol. Neonate*, vol. 87, no. 3, pp. 160–163, 2005.

[22] E. Schulte-Frohlinde, R. Schimd, V. Brantl, and V. Schusdziarra, "Effect of bovine b-casomorphin-4-amide on gastrointestinal transit and pancreatic endocrine function in man," in *b casomorphins and related peptides: recent developments*, New York:: VCH Weinheim, 1994, pp. 155–60.

[23] J. Claustre, F. Toumi, A. Trompette, G. Jourdan, H. Guignard, J. A. Chayvialle, and P. Plaisancié, "Effects of peptides derived from dietary proteins on mucus secretion in rat jejunum," *Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 283, no. 3, pp. G521–528, Sep. 2002.

[24] S. Zoghbi, A. Trompette, J. Claustre, M. El Homsi, J. Garzón, G. Jourdan, J.-Y. Scoazec, and P. Plaisancié, "beta-Casomorphin-7 regulates the secretion and expression of gastrointestinal mucins through a mu-opioid pathway," *Am. J. Physiol. Gastrointest. Liver Physiol.*, vol. 290, no. 6, pp. G1105–1113, Jun. 2006.

[25] M. R. U. Haq, R. Kapila, R. Sharma, V. Saliganti, and S. Kapila, "Comparative evaluation of cow β-casein variants (A1/A2) consumption on Th2-mediated inflammatory response in mouse gut," *Eur. J. Nutr.*, Oct. 2013.

[26] M. Stępnik and M. Kurek, "The influence of bovine casein-derived exorphins on mast cells in rodents," *Rev. Fr. Allergol. Immunol. Clin.*, vol. 42, no. 5, pp. 447–453, Sep. 2002.

[27] J. Torreilles and M. C. Guérin, "[Casein-derived peptides can promote human LDL oxidation by a peroxidase-dependent and metal-independent process]," *Comptes Rendus Séances Société Biol. Ses Fil.*, vol. 189, no. 5, pp. 933–942, 1995.

[28] A. Steinerová, M. Korotvicka, J. Racek, D. Rajdl, L. Trefil, F. Stozický, and Z. Rokyta, "Significant increase in antibodies against oxidized LDL particles (IgoxLDL) in three-month old infants who received milk formula," *Atherosclerosis*, vol. 173, no. 1, pp. 147–148, Mar. 2004.

[29] A. Steinerová, J. Racek, F. Stozický, F. Tatzber, and A. Lapin, "Autoantibodies against oxidized LDL in the first phase of life. Low density lipoproteins," *Clin. Chem. Lab. Med. CCLM FESCC*, vol. 37, no. 9, pp. 913–917, Sep. 1999.

[30] A. Steinerová, F. Stožický, J. Racek, F. Tatzber, T. Zima, and R. Šětina, "Antibodies against Oxidized LDL in Infants," *Clin. Chem.*, vol. 47, no. 6, pp. 1137–1138, Jun. 2001.

[31] J. R. Batuca, M. C. Amaral, and J. D. Alves, "Humoral mechanisms of atherogenesis," *Ann. N. Y. Acad. Sci.*, vol. 1173, pp. 401–408, Sep. 2009.

[32] R. Elliott, H. Wasmuth, N. Bibby, and J. Hill, "The role of β-casein variants in the induction of insulin-dependent diabetes in the non-obese diabetic mouse and humans," in *Seminar on milk protein polymorphism*, 1997, vol. IDF special issue no 9702, pp. 445–53.

[33] M. Laugesen and R. Elliott, "Ischaemic heart disease, Type 1 diabetes, and cow milk A1 beta-casein," *N. Z. Med. J.*, vol. 116, no. 1168, p. U295, Jan. 2003.

[34] R. Cade, "Autism and schizophrenia: intestinal disorders.," *Nutr Neurosci*, vol. 2, no. January, pp. 57–72, 2000.

[35] K. Reichelt, J. Ekrem, and H. Scott, "Gluten, milk proteins and autism: dietary intervention effects on behavior and peptide secretion.," *J. Appl. Nutr.*, vol. 42, no. 1, pp. 1–11, 1990.

[36] K. L. Reichelt and A. M. Knivsberg, "Can the pathophysiology of autism be explained by the nature of the discovered urine peptides?," *Nutr. Neurosci.*, vol. 6, no. 1, pp. 19–28, Feb. 2003.

[37] M. I. S. Kawashti, O. R. Amin, and N. G. Rowehy, "Possible immunological disorders in autism: concomitant autoimmunity and immune tolerance," *Egypt. J. Immunol. Egypt. Assoc. Immunol.*, vol. 13, no. 1, pp. 99–104, 2006.

[38] F. C. Dohan, "Genetic hypothesis of idiopathic schizophrenia: its exorphin connection," *Schizophr. Bull.*, vol. 14, no. 4, pp. 489–494, 1988.

[39] D. W. Niebuhr, Y. Li, D. N. Cowan, N. S. Weber, J. A. Fisher, G. M. Ford, and R. Yolken, "Association between bovine casein antibody and new onset schizophrenia among US military personnel," *Schizophr. Res.*, vol. 128, no. 1–3, pp. 51–55, May 2011.

[40] K. L. Reichelt and J. Landmark, "Specific IgA antibody increases in schizophrenia," *Biol. Psychiatry*, vol. 37, no.

6, pp. 410–413, Mar. 1995.

[41] E. G. Severance, F. B. Dickerson, M. Halling, B. Krivogorsky, L. Haile, S. Yang, C. R. Stallings, A. E. Origoni, I. Bossis, J. Xiao, D. Dupont, W. Haasnoot, and R. H. Yolken, "Subunit and whole molecule specificity of the anti-bovine casein immune response in recent onset psychosis and schizophrenia," *Schizophr. Res.*, vol. 118, no. 1–3, pp. 240–247, May 2010.

[42] E. G. Severance, J. Lin, H. A. Sampson, G. Gimenez, F. B. Dickerson, M. Halling, K. Gressitt, L. Haile, C. R. Stallings, A. E. Origoni, D. Dupont, and R. H. Yolken, "Dietary antigens, epitope recognition, and immune complex formation in recent onset psychosis and long-term schizophrenia," *Schizophr. Res.*, vol. 126, no. 1–3, pp. 43–50, Mar. 2011.

[43] Z. Sun and J. R. Cade, "A Peptide Found in Schizophrenia and Autism Causes Behavioral Changes in Rats," *Autism*, vol. 3, no. 1, pp. 85–95, Mar. 1999.

[44] R. M. Adibhatla and J. F. Hatcher, "Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities," *Antioxid. Redox Signal.*, vol. 12, no. 1, pp. 125–169, Jan. 2010.

[45] R. Deth, C. Muratore, J. Benzecry, V.-A. Power-Charnitsky, and M. Waly, "How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis," *Neurotoxicology*, vol. 29, no. 1, pp. 190–201, Jan. 2008.

[46] A. M. Knivsberg, K. L. Reichelt, T. H[illegible]en, and M. N[illegible]land, "A Randomised, Controlled Study of Dietary Intervention in Autistic Syndromes," *Nutr. Neurosci.*, vol. 5, no. 4, pp. 251–261, Jan. 2002.

[47] J. H. Elder, M. Shankar, J. Shuster, D. Theriaque, S. Burns, and L. Sherrill, "The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial," *J. Autism Dev. Disord.*, vol. 36, no. 3, pp. 413–420, Apr. 2006.

[48] E. Sponheim, "[Gluten-free diet in infantile autism. A therapeutic trial]," *Tidsskr. Den Nor. Lægeforen. Tidsskr. Prakt. Med. Ny Række*, vol. 111, no. 6, pp. 704–707, Feb. 1991.

[49] L. Hoffman, "A2 milk stands out from the herd," *TheAustralian*, 17-May-2008.

[50] G. Hammond, "Milk minus mutants boost for the lactose intolerant," *HeraldSun*, 16-Jul-2011.