

β-CASOMORPHIN: AN OPIOID BIOACTIVE PEPTIDE IN BOVINE MILK HAVE IMPACT ON HUMAN HEALTH

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ABSTRACT

Earlier, the research was conducted to enhance the shelf life of milk. There was not much known about the health hazardous substances in the milk found naturally. It was noticed an increased incidence in the cardiovascular diseases due to consumption of bovine milk in humans. In last decade, this opinion was changed because of research advancement regarding the bioactive peptides presence in milk. Casein is chief protein fraction in milk. Bioactive peptides are usually generated in vivo through gastrointestinal procedures from numerous proteins. Bovine A1 and A2 β-casein (β-CN) are 2 alternates differing in an AA sequence i.e.at position, the histidine substituted for proline. Gastric emptying along with transit in intestine can alter peptide presence time at a site in GI tract thus influencing absorption. There are some problems like autism, cardiovascular problems and diabetes etc. have been studied as the cause of opioids bioactive peptides in humans. However, there was not conclusive evidence for the anomalies that clearly shows its role.

Keywords: *Caseine, Milk, Bioactive Peptides, Opioids, Cardiovascular & Diabetes*

BACKGROUND

Milk is known as complete diet except deficient in Iron (Haug, Hostmark & Harstad, 2007). It is indeed a gift from the nature for nourishment of new born. Earlier, the research was conducted to enhance the shelf life of milk. There was not much known about health hazardous substances in milk found naturally. It was noticed an increased incidence in the cardiovascular diseases due to consumption of bovine milk in humans. During the previous century; people were of opinion that use of pasteurization and other methods to improve shelf life may be responsible for these ailments (De Noni, Korhonen, FitzGerald, Livesey, Roux, Tomé, Thorsdottir & Witkamp, 2009).

In last decade, advanced research brought change in opinion regarding bioactive peptides presence in milk. Milk possesses many bioactive peptides. They can play role in changing body physiology of body especially in humans (Alice, Richard & FitzGerald, 2015). β-casomorphin is an opioid considered to be related to some nervous system and cardiovascular system anomalies (De Noni et al., 2009). The current paper is intended to gather the research findings about the role of β-casomorphin in human physiology and how these physiological changes are brought.

Casein protein in Bovine Milk

Bovine milk exhibits amino acids, proteins, lipids, as well as vitamins, minerals and various growth factors. It also has hormones, immunoglobulins and other bioactive compounds etc. Milk composition fluctuates because of factors including age and breed of animal, lactation stage, dietary nutritional value and udder tissues' health condition. The colloidal complexes of casein micelles were formed by protein and calcium. The lipids present in milk are blended in globules in tissues while most of minerals lactose in the solution (Haug et al., 2007). Protein share in milk is 3-3.5%. Casein is chief protein fraction in milk with 76-86% share of whole milk proteins. Whey protein comprises of 14-24% milk proteins. The various casein fractions in bovine milk are:

1. α -Casein (60 %)
 - a. α S1-Casein
 - b. α S2-Casein
 - c. κ -Casein
2. β -Casein (25-35 %)
 - a. β A1-Casein
 - b. β A2-Casein
3. γ -Casein (3-7%)

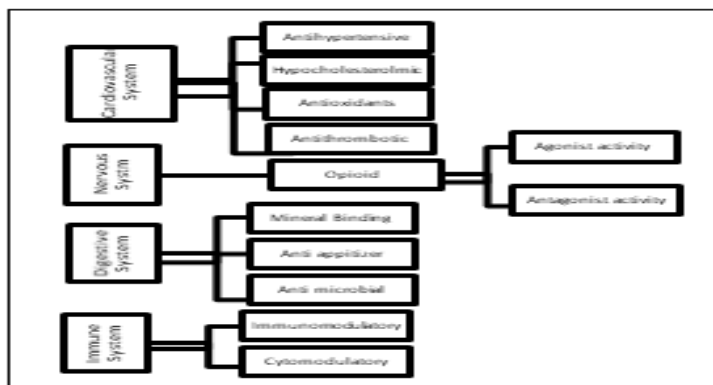
Major portion of the whey protein is consist of β -Lactoglobulin (~50% of whey) followed by α -Lactalbumin (~20 %), Proteose/Pentones (~20 %), immunoglobulins (~8%) and some fractions of blood albumins (Haug et al., 2007).

Protein precursors for Bioactive Peptides

The peptides are produced *in-vivo* through gastrointestinal procedures from numerous proteins. These bioactive peptides could impact various physiological mechanisms as a result of their hormone-like nature (Clare & Swaisgood, 2000). Based on their target site, peptide sequences may or may not necessary for transportation in intestinal mucosa, to facilitate a physiological reaction. Size of bioactive peptides varies from 2-50 amino acids. Peptides perform many physiological functions including immunomodulation, antibacterial as well as antithrombotic etc. Several scientists studied the "milk protein derived bioactive peptides" (Korhonen & Pihlanto, 2003; Korhonen & Pihlanto, 2006; Meisel, 2004; Hartmann & Meisel, 2007; Morris & FitzGerald, 2008). Various bioactive peptides are classifies in Figure 1.

Figure 1: Physiological functions of bioactive peptides derived from food

(Adapted from Korhonen and Pihlanto, 2007)



Opioids Bioactive Peptides in Food Items

Various food proteins have peptide sequences behaving as the opioid receptor ligands (Zioudrou et al., 1979; Paroli 1988; Kostyra et al., 2004; Guesdon et al., 2006). Opioid peptides can be made from milk, meat/poultry proteins, cereal and vegetables (Table 1). Blood proteins i.e. γ -globulins and albumin (Zioudrou et al., 1979) and haemoglobin (Brantl et al., 1986b) may behave as opioid peptides. Likewise egg protein (ovalbumin) also exhibits opioid peptide sequence (Zioudrou et al., 1979).

Table 1: Food Protein Behaving as Opioid Receptor Peptides

Food Group	Foodstuff	Protein precursor	Reference
Dairy	Milk and milk products	Lactoferrin	(Tani et al., 1990)
		α -Lactalbumin	(Yoshikawa et al., 1986)
		β -Lactoglobulin	(Yoshikawa et al., 1986)
		α -Casein	(Zioudrou et al., 1979)
		β -Casein	(Brantl et al., 1979)
		κ -Casein	(Chiba et al., 1989)
Cereals	Wheat	Gluten	(Fukudome & Yoshikawa, 1992)
		Gliadin	(Zioudrou et al., 1979)
	Barley	Hordein	(Zioudrou et al., 1979)
		Avenin	(Zioudrou et al., 1979)
		Secalin	(Zioudrou et al., 1979)
		Zein	(Zioudrou et al., 1979)
	Rice	Albumin	(Takahashi et al., 1994)
Food Group	Foodstuff	Protein precursor	Reference
Vegetable	Soy	α -Protein	(Zioudrou et al., 1979)
	Spinach	Rubisco	(Yang et al., 2003)
Meat/poultry	Blood	Albumin	(Zioudrou et al., 1979)
		Haemoglobin	(Brantl et al., 1986b)
		γ -Globulin	(Zioudrou et al., 1979)
	Egg	Ovalbumin	(Zioudrou et al., 1979)

(Adapted from *Teschemacher, 2003*)

Opioid peptides from food protein are categorized as exogenous while containing tyrosine residue in its sequence, ordinarily at N-terminal or N-terminus point. Other is

endogenous opioid peptides often containing tyrosine-glycine-glycine-phenylalanine as N-terminal region (Teschemacher, 2003). Mostly exogenous peptides are identified and isolated from enzymatic digests of their “parent protein molecules”.

Bioactive Peptides from Bovine and Buffalo Milk

The milk proteins having opioid peptide sequences (Teschemacher et al., 1997; Guesdon et al., 2006; and Pihlanto-Leppzala, 2000). Most of milk proteins have opioid receptor ligand and specifically named as exorphins and casoxin D resulting from α -casein, β -casomorphins. Mostly, the milk peptides identified to date exhibits opioid agonistic properties. Bovine and buffalo milk generated precursor proteins and bioactive peptides are given in Table 2.

Table 2: Precursor milk protein and bioactive peptide

Precursor	Bioactive Peptide
Bovine Milk	
κ - Caseine	Casoxin A,
κ - Caseine	Casoxin B,
κ - Caseine	Casoxin C,
α S1- Caseine	Exorphin,
β - Caseine	β -casomorphin 4
β - Caseine	β -casomorphin 5
β - Caseine	β -casomorphin 7
β - Caseine	β -casomorphin 8
Bovine serum albumin	Serorphin,
α - lactalbumin	α -lactorphin,
β -lactoglobulin	β -lactorphin,
Buffalo Milk	
β - Caseine	β -casomorphin 7

(Adapted from Meisel and FitzGerald, 2000; Yang et al., 2001; Teschemacher, 2003)

β - Caseine variants as source of Opioid like β -casomorphin (BCM)

Bovine A1 and A2 β -casein (β -CN) are 2 variations that vary only because of one AA at position 67 by replacement of histidine for proline (Raynes et al., 2015). β -Casein play its role in casein micelle and oligomeric micelle formation. It also works as the molecular chaperone to avoid accretion of many proteins, comprising the other caseins. Micelle muster of A1 and A2 β -CN was studied by x-rays and the dynamic light scattering. Its “protein functionality was tested by fluorescence” procedures and molecular chaperone methods. The A2 β -CN variant shaped minor micelles than A1 β -CN, with “monomer-micelle equilibrium” of A2 β -CN being lifted toward the monomer. β -casein’s allele frequency distribution is dependent on bovine breed as well as population. In last decade, alteration in selection targets has caused in difference in “bovine breed composition” in European countries.

Polish scientists, Cieslinska et al. (2012) studied cow's raw, hydrolyzed and processed milk to enumerate β -casomorphin-7 and found that processed and hydrolyzed milk was the richest source of β -casomorphin-7. This might be because of β -casein A1 allele. Some traces of β -casomorphin-7 in cow milk were might be due to "acid hydrolysis of β -casein during its digestion with pepsin". Koch et al. (1985) studied the potencies' rank order for BCM and concluded that it was the same for bovine and human BCM β -casomorphins (BCM5 > BCM4 > BCM8 > BCM7). The BCM5 and BCM7 was the most and least potent, respectively.

Causes of Release of BCM from Bovine Milk

Cieslinska et al. (2007) used HPLC/UV methodology and reported the BCM7 presence in unprocessed bovine milk. Presence of somatic cells in milk is the usual spectacle and these cells enhance intensely in clinical and sub-clinical mastitis. The reason of increment in somatic cell count (SCC) was probably due to increased casein's proteolysis. So, increased proteolytic activity, in high SCC animals may lead to BCMs or their precursors' release. The cathepsins and elastase generated from SCCs' activity and specificity has been investigated. The cathepsin B' activity is related to milk SCC (O'Driscoll *et al.*, 1999). In β -casein's *in-vitro* digestion, cathepsin B and plasmin and cell envelope proteinases (CEP) had similar activities (Considine et al., 2004). It is reported that BCM10 (f60–69) could be released from cathepsin B. while studying enzymes activities generated from somatic cell in milk, Wedholm et al. (2008) documented that elastase and cathepsins were associated with proteolysis of β -casein. He did not observe release of BCMs.

Absorption of opioids like bioactive peptides and their fate

Factors affecting opioid peptides absorption in GI tract

The content (intestinal), comprising food has varying composition. The intestinal transit and gastric emptying are the variables that significantly affect absorption by modifying the peptide presence time along the GI tract. The pH microclimate can favor or inhibit the transport of peptide but it depends on peptides' pKa. The peptide absorption having size greater than di-tripeptides is very limited in healthy adults. Intestinal permeability increases in some situations for examples aggression, stress or diseases in both the animal and humans. It is much complex to answer about peptide absorption in human neonate (Vaarala et al. 1998, 2008). In early age of neonates intestinal permeability to proteins is greater but this permeability for proteins and other larger molecules decreases as the neonatal age increases. The permeability of GI tract and its altering situation depends upon neonate's specie. The piglet model is much closer to human neonate and in both species; transport capacity reduces quickly in first few days after natal (Pacha, 2000).

Intestine's absorptive capacity and its maturation degree have close relationship. The protein transport is of importance in early postnatal life because it aids absorption of immunoglobulins and other growth factors present in milk. In ungulates i.e. calves or piglets, this activity is of much importance. Other species such as rats, man or mouse also obtain IgG passively from mother's milk by absorption in the proximal section of small intestine. Kuge et al. (2006) documented that no doubt, intestinal absorption may also take place in adults, for example during stress situations, this group is less vulnerable.

Transfer Mechanisms across the Intestinal Epithelium

Peptides cross the intestinal epithelium by two ways i.e. paracellular and transcellular. In transcellular transport, peptides after metabolized may be subjected to carrier mediated transportation. Another transport mechanism might be the peptide translocation across Peyer's patches (Foltz et al., 2008 and Des Rieux et al., 2006). The permeation of peptide can be modified by different factors including the peptides' physiochemical properties, hydrogen bonding, size of molecule, lipophilicity and hydrophilicity. Opioid peptides are hydrophobic in nature so their transport is not occurred easily across the intestine (Iwan et al., 2008). The active transport mechanism of opioid peptides is not clearly established. In epithelial retinal pigment's cell line, a Na⁺ based active transport (ARPE-19) has been identified however opioid peptide ranging from 4-13 amino acids transport distribution mechanism is not fully clear (Hu et al., 2003). In accessible literature, many active peptides crossing the intestinal via *in-vitro* or *in-vivo* method, the have been reported. These peptide include octreotide (Dorkoosh et al., 2004), tetrapeptide GGYR (Shimizu, 1997), pentapeptide from β -casein HLPLP (Quiros et al., 2008), α hexarelin (Roumi et al., 2001) and s1-casein (residues 1-23, Chabance et al., 1998).

Peptide Transport Mechanism in Blood Stream

There is complex mechanism of peptide transport in blood due to its substantial activities of blood peptidases. Certain peptides in plasma possess very short half-life even with one minute magnitude (Gardner, 1998). Moskowitz (2003) documented that angiotensin II degradation may take place even in seconds. Peptides may be bound weakly with carrier proteins protecting them to be hydrolyzed in blood. An iron-transporting glycoprotein called Transferrin (Tf) is the more suitable object as a peptide carrier (Tuma et al., 2003) and these carrier peptide joined to plasma protein, is not mostly available for binding to the target site.

Transfer across the Blood-Brain Barrier (BBB)

The BBB is metabolic in addition to physical obstacle and it splits microenvironment of "central nervous system" from peripheral circulation. This is present at cerebral micro vessel endothelial cells level, having enzymatic and morphological properties which are

different from capillaries in other sections of body. Some parts of CNS have micro vessels identical to the periphery but these parts do not show classical BBB capillary endothelial cells. These sites are named as circumventricular organs (CVOs) and located near the brain ventricles. These CVOs secrete neurohormones and monitor composition of blood. Egleton and Davis (1997) reported that capillaries are more permeable to solutes, in these areas. Ganapathy et al. (2005) documented the four different peptide transport systems to transport peptides across BBB. These systems are PTS-1, PTS-2, PTS-3 and PTS-4 and “among them only the PTS-1 can make out opioid peptides. Tyr-MIF-1, Met-enkephalin, Leu-enkephalin, BCM7” and dynorphin 1-8 are the substrates of PTS-1. (Ganapathy et al., 2005).

Physiological Changes associated with Opioids in Humans

Opioid peptide sequences contain tyrosine-glycine-glycine-phenylalanine at their N-terminal corner. In case of “atypical opioid peptides”, a “tyrosine residue is present at N-terminus” (Teschemacher et al., 1997) and act like morphine in brain and have found as caseinomorphins and lactorphins in milk proteins. Many studies on laboratory animal are executed on milk protein-derived BAPs with opioid activity however there is need to work on human subject. (Artym & Zimecki, 2013 and Teschemacher, 1997) because no dose-effect relationship of β -casomorphin on various health issues has been studied on human (De Noni et al., 2009). Those who are using dairy products in their diet, opioid peptides have been establish in GIT (Svedberg et al., 1985), brain (Nyberg et al., 1989; Pasi et al., 1993) and diverse body fluids of adults and infants (Renlund et al., 1993; Kost et al., 2009 and Righard et al., 2014). B-casomorphin-8 (β -CN f (51–58) found in lactating female milk suffering from post-partum depression (Renlund et al., 1993).

Similar β -casomorphin-8 was identified in women plasma at pregnancy and post-parturition stage with having sign of mastitis (Koch et al., 1988 and Righard et al., 2014). The level of β -casomorphin-8 was reduced with the reduction of infection planned that there was an association between β -casomorphin-8 application in post-partum depression, mastitis and physiological fluids (Righard et al., 2014). It is also recognized that β -casomorphin-8 creating from the human milk and those using dairy product is suffered hydrophobic character (Renlund et al., 1993; and Pasi et al., 1993), schizophrenia, apnoea and autism, in “sudden infant death syndrome” (Sun et al., 2003; Kaminski et al., 2007). However, to date, the clinical evidence does not appear to be clear in this regard (Sun et al., 2003; Noni et al., 2009). Also, human (β -CN f (51–57) and bovine β -casomorphin-7 can be “found in the plasma of infants” (<1 y) fed “with breast milk or cow’s milk infant” formulae (Kost et al., 2009).

β -Casomorphin-7 fragments were also found in “plasma at pre- and postprandial stage”. An encouraging connection was described with the raised plasma level of human β -casomorphin-7 that result psychomotor development and muscle tone and it was doubled in infant plasma absorption (Kost et al., 2009). β -casomorphin-7 formed from “ β -CN variants A1 and B but not from the A2 variant”. Human and animal nutritionist are trying to mature some relation amid the onset of ischaemic heart disease, type 1 diabetes, autism and schizophrenia with this A1 variant that cause the production of β -casomorphin-7 milk (Elliott et al., 1999; Laugesen and Elliott, 2003). They are also suggested that some other disorders like hypertension and satiety possibly involving opioid receptor agonism may be the cause of milk protein-generated peptides (Froetschel et al., 2001; Nurminen et al., 2000). However, there is need to get more conclusive evidence for these effects in humans (De Noni et al., 2009)

CONCLUSION

Proteins including the dietary proteins are a good source of vast-ranged biologically active peptides. Some of these proteins have affinity for opioid receptors. The BCM7 secretion by bovine β -casein's enzymatic digestion is dictated by the primary amino acid sequence. This sequence depends on protein's genetic variability. β -casein's allele frequency distribution is varied by breed and population of bovine. In last few years, varying selection targets has resulted in altered bovine breed composition. To yet, without much available information, it is believed that breed composition changes impacted the milk composition, including type and concentration of milk protein. The BCM role in hypertension and satiety, possibly having opioid receptor agonism effects, has been documented in literature but no conclusive evidence for these effects in humans are available.

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