S100B Protein, Brain-Derived Neurotrophic Factor, and Glial Cell Line-Derived Neurotrophic Factor in Human Milk

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Abstract

Background: Human milk contains a wide variety of nutrients that contribute to the fulfillment of its functions, which include the regulation of newborn development. However, few studies have investigated the concentrations of S100B protein, brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) in human milk. The associations of the concentrations of S100B protein, BDNF, and GDNF with maternal factors are not well explored.

Methodology/Principal Findings: To investigate the concentrations of S100B protein, BDNF, and GDNF in human milk and characterize the maternal factors associated with their levels in human milk, human milk samples were collected at days 3, 10, 30, and 90 after parturition. Levels of S100B protein, BDNF, and GDNF, and their mRNAs in the samples were detected. Then, these concentrations were compared with lactation and other maternal factors. S100B protein levels in human milk samples collected at 3, 10, 30, and 90 d after parturition were 1249.79±398.10, 1345.05±539.16, 1481.83±573.30, and 1414.39±621.31 ng/L, respectively. On the other hand, the BDNF concentrations in human milk samples were 10.99±4.55, 13.01±5.88, 13.35±6.43, and 2.83±5.47 µg/L, while those of GDNF were 10.90±1.65, 11.38±1.00, 11.29±3.10, and 11.40±2.21 µg/L for the same time periods. Maternal post-pregnancy body mass index was positively associated with S100B levels in human milk (r = 0.335, P = 0.030<0.05). In addition, there was a significant correlation between the levels of S100B protein and BDNF (z = 2.09, P = 0.037<0.05). Delivery modes were negatively associated with the concentration of GDNF in human milk.

Conclusions: S100B protein, BDNF, and GDNF are present in all samples of human milk, and they may be responsible for the long term effects of breast feeding.

Introduction

The S100B protein is a member of the calcium-binding S100 family, which is characterized by a low molecular weight and a special EF-hand structure [1]. Like most members of this family, S100B has a homodimeric structure wherein each beta monomer is approximately 10.5 kDa. Each monomer has two EF hand sites for Ca^{2+} binding and independent sites for Zn^{2+} binding. It has two disulfide bridges, but the dimeric structure is maintained indepedently of this aspect.

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein belonging to the neurotrophin family, which is widely expressed in the mammalian adult brain [2].

Glial cell line-derived neurotrophic factor (GDNF) is a distant member of the transforming growth factor β superfamily that was originally isolated from the rat B49 glial cell line [3]. This protein is a glycosylated, disulfide-bonded homodimer with a molecular weight of 33–45 kDa. Its monomer has a molecular weight of 16 kDa after deamidylation [4].

S100B, BDNF, and GDNF play a critical role in the development and maintenance of the nervous system, and in neuronal survival and proliferation [3,5–19]. These proteins have been implicated in the modulation of learning and memory [20–24]. Human milk protects the infants from infection, modulates their immune function, and affects their overall development [25]. The present study investigates the concentration of S100B, BDNF, and GDNF in the milk of Chinese women after parturition to clarify the function of these cytokines.

Methods

Ethics Statement

Approval from the Ethical Committee of the Harbin Medical University was obtained prior to this study. Written informed consent was obtained before collection of milk samples from donors.

Participants

Samples for ELISA analysis were collected from 42 mothers: 31 of whom had abdominal delivery at term, while 11 delivered vaginally at term. Milk samples were collected at 3, 10, 30, and 90...
parturition. Participants who had gestational hypertension, diabetes, infection, fever, metabolic diseases, breast diseases, central nervous system diseases, malnutrition, maternal allergy, fetal anomaly, and smoking habits were excluded.

Human milk was collected by hand into sterile 5-ml Eppendorf tubes. Upon collection, samples were refrigerated at 4°C in a polystyrene box containing ice. All samples were immediately transferred to the laboratory, where they were stored at −80°C. ELISA analysis

After thawing at room temperature, milk samples were centrifuged at 1000 g for 10 min at 4°C. The supernatant was removed and re-centrifuged at 10,000 g for 30 min at 4°C, and the floating lipid layer and cellular sediments were removed. BDNF and GDNF concentration were measured in all samples by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Inc., United States of America) according to the manufacturer’s instructions. ELISA (Wuhan ELAb Science Co., Ltd., China) was also used to determine the concentration of S100B protein. All samples were tested in duplicate and the averages were reported. Intra-assay and the inter-assay variation coefficients were <5% and <10%, respectively. The assay ranges of the S100B protein, BDNF and GDNF ELISA kits were 15.6–1000 ng/L, 1.5–110 g/L, and 2–60 g/L, respectively.

Western blot analysis

Protein concentrations were determined using the Lowry method of protein assay [26] with bovine serum albumin as standard. About 10 μL of human milk (1000 g supernatant) were separated on 15% SDS–PAGE and transferred to a nitrocellulose membrane. Immunoblotting was performed using rabbit BDNF and GDNF antibodies (Wuhan Boster Biological Technology., LTD, China). The membrane was then incubated with the secondary alkaline phosphatase-conjugated IgG and detected with the Western Blue Stabilized Substrate for alkaline phosphatase secondary alkaline phosphatase-conjugated IgG and detected with GDNF antibodies (Wuhan Boster Biological Technology., LTD, China). Membranes were incubated with 0.05% Tween 20 and 5% skim milk at room temperature for 2 h. After washing membranes, membranes were incubated with rabbit anti-BDNF (1:500 dilution) and rabbit anti-GDNF (1:500 dilution) for 2 h at room temperature. After washing, membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000 dilution) for 2 h at room temperature. Membranes were then washed and incubated with enhanced chemiluminescent kit (ECL). Membranes were developed using a chemiluminescent detection system (Bio-Rad, Hercules, CA) andautoradiography.

RT-PCR analysis

Milk samples for reverse transcription-PCR analysis were collected from a mother at 3, 10, 30, and 90 d after parturition. The milks (15 mL) were centrifuged at 1000 g for 10 min at 4°C, and the RNA was extracted from the cell-pellet using TRIzol reagent (Invitrogen, Carlsbad, CA). RT-PCR (RNA PCR kit, TaKaRa Shuzo Co., Ltd., Japan) was conducted according to the manufacturer’s instructions. The quality of RNA extract was determined using the A260/A280 ratio, and was found to be 1.7–2.0 for all RNA preparations. A 1 μg portion of the total RNA was used for cDNA synthesis by reverse transcription (RT) with a final reaction mixture volume of 20 μL. RT was performed using a thermal program of 25°C for 10 min, 42°C for 30 min, and 95°C for 5 min. The cDNA was stored at −80°C for further use.

A 1.6 μl aliquot of the cDNA solution was used for the PCR assay (20 μl final volume). Samples were subjected to 36 cycles of PCR amplification: each cycle consisting of denaturation at 94°C for 30 s, annealing at a specified temperatures for 30 s, and extension at 72°C for 30 s. A final extension was performed at 72°C for 10 min.

Annealing temperatures (AT) and primer sequences are as follows: β2-actin (forward: 5’-CTGCTGTCGACCCTTTCCA-3’; reverse: 5’-GCTGTGACCTCCTACGTT-3’; size: 256 bp; AT: 56°C), S100B [27] (forward: 5’-CATTTCTTAGAGGAATAC-3’; reverse: 5’-ATGTTCAAAAGAAGCTTGG-3’; size: 147 bp; AT: 46°C), BDNF (forward: 5’-CAAGATCAGGAGCAAAG-3’; reverse: 5’-GGCGTTACCCGACTCTAC-3’; size: 379 bp; AT: 56°C), and GDNF (forward: 5’-ACTTGAGGTCTGGGCTAT-3’; reverse: 5’-TGTGACCTGACGCTTCTATT-3’; size: 132 bp; AT: 53°C). Amplification products were examined by electrophoresis on 1.5% agarose gel stained with ethidium bromide. All assays were performed with at least one replicate. The amplicons were matched with DL500 DNA Marker 100T (TaKaRa Shuzo Co., Ltd., Japan).

Statistical analysis

All data were expressed as the mean ± SD and were analyzed using Stata version 10.0 (StataCorp, United States of America). Statistical analysis was performed using the generalized estimating equation. A linear correlation was conducted to assess the relationship of S100B milk concentrations and the body mass index (BMI) of mothers. Statistical significance was indicated by P values less than 0.05.

Results

Mothers who participated in the study ranged from 19 to 38 years (mean age 25.26 years), with BMIs ranging from 21.7 to 34.8 (mean 27.57 kg/m2) and Gestational Ages between 37 and 42 weeks (mean age 38.98 weeks). All mothers had their first accouche-ments at the time of the study and all showed normal clinical conditions.

Cytokine concentrations in the milk of the mothers, who gave samples at 30 (n = 40) and 90 (n = 24) d after parturition, are presented in Tables 1 and 2.

### Table 1. Cytokines in the human milk from Chinese women during day 3, 10, and 30 after parturition (n = 42).

| Cytokines | day 3 | day 10 | day 30* | P  |
|-----------|-------|--------|---------|----|
| S100B (ng/L) | 1249.79±398.10 | 1345.05±539.16 | 1481.83±573.30 | 0.034 |
| BDNF (μg/L) | 10.99±4.55 | 13.01±5.88 | 13.35±6.43 | 0.205 |
| GDNF (μg/L) | 10.90±1.65 | 11.38±1.78 | 11.29±3.10 | 0.831 |

*number of donors equal to 40.

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### Table 2. Cytokines in human milk from Chinese women during day 3, 10, 30, and 90 after parturition (n = 24).

| Cytokines | day 3 | day 10 | day 30 | day 90 | P  |
|-----------|-------|--------|--------|--------|----|
| S100B (ng/L) | 1221.63±338.27 | 1246.08±542.27 | 1381.26±525.48 | 1414.39±621.31 | 0.434 |
| BDNF (μg/L) | 10.64±5.402811 | 11.69±5.51 | 12.42±6.27 | 12.83±5.47 | 0.293 |
| GDNF (μg/L) | 10.95±1.87 | 11.37±1.93 | 11.47±2.65 | 11.40±2.21 | 0.735 |

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S100B, BDNF, and GDNF were present in all samples of human milk. The levels of S100B protein peaked at 30 d after parturition, while BDNF and GDNF levels did not show variations with time. A significant correlation was found between S100B protein and BDNF levels at the third month after birth (z = 2.09, P = 0.037; Figure 1).

S100B protein levels in milk at 3 d after parturition were positively correlated with the maternal post pregnancy BMI (r = 0.335, P = 0.030; Figure 2). At one month after parturition, the GDNF levels from mothers who delivered vaginally at term were significantly lower than those who delivered abdominally at term (z = 2.19, P = 0.029). This correlation persisted until three months after birth (z = 2.17, P = 0.030). No correlations were found between the levels of other cytokines and age, height, weight, BMI, gestational age, or delivery modes.

Discussion

This is the first study to report on BDNF and GDNF concentrations in the milk of lactating women. This study investigated changes in these concentrations during lactation, in addition to the measurement of S100B protein in the milk of
Chinese women. RT-PCR analysis detected S100B, BDNF, and GDNF mRNA in human milk collected at 3, 10, 30, and 90 d after birth (Figure 4). Western blot analysis was used to confirm the immunoreactivity observed in ELISA assay.

Findings of this study indicate that BDNF and GDNF can be added to the list of bioactive factors (e.g. IL-1β, IL-2, IL-4, IL-5, Lactoferrin, transferrin) [28] present in human milk. S100B protein has been previously documented in human milk at 30 to 929 μg/L, indicating that the lactating human breast secretes S100B protein [29–31]. In view of the broad range of S100B protein concentrations reported and the lack of data on human milk from Chinese women, the S100B protein levels in milk from Chinese subjects were quantified in this study. We found that the S100B protein concentrations in milk collected within three months after giving birth is within 390.7–2623.9 ng/L. At day 3 after birth, the S100B protein concentration in milk was much lower than those in milk from Burkinabe and Sicilian women (204.31±36.25 and 199.42±45.28 μg/L, respectively) [31]. However, this does not mean that Chinese infants consume less S100B protein; the overall amount of milk production is independent of ethnicity [31,32].

S100B, BDNF and GDNF concentrations in milk were much higher than in the serum [33–35]. Although the biological significance of the factors in human milk for breastfeeding infants remains unknown, studies suggest that they may serve potential neurotrophic function that may modulate the function and integrity of the GI tract [36,37] and may exert a stimulating effect on neurodevelopment during breastfeeding or long afterwards [38,39]. Previous studies have shown that these factors are critical molecules that support the process of neuronal growth, development, protection, and repair [3,5–19], and the modulation of learning and memory [20–24]. BDNF plays an important role in the development of the enteric nervous system, defense against intestinal infection, and the modulation of gastrointestinal motility [40,41]. GDNF has been shown to support the development of human enteric nervous system and intestinal epithelial barrier integrity [42,43].

Human milk is known to contain leukocytes expressing BDNF and GDNF [44–46], which may be reasonably supposed to be the sources of BDNF and GDNF mRNA detected by RT-PCR and of the factors detected by ELISA and Western blot assays. There has been no evidence demonstrating that BDNF and GDNF in human milk are derived entirely from the serum or mammary gland cells. Studies have verified that a significant part of S100B protein present in milk is secreted by mammary epithelial cells and that S100B can be expressed by human milk cells [29].
Detailed information on the fate of these cytokines in the gastrointestinal tract is needed, although we can assume that they participate in the nutritional effects of milk because previous studies have shown that human milk proteins are utilized exceptionally well [47]. Several factors may contribute to these nutritional effects. For instance, human milk contains proteins that bind essential nutrients, thus keeping nutrients in solution and facilitating their uptake by the intestinal mucosa. In addition, protease inhibitors limit the activity of proteolytic enzymes, thereby preserving the physiologic function of some relatively stable binding proteins and some enzymes that can affect the digestion and utilization of macronutrients.

In this study, we found a positive correlation between S100B protein levels in human milk and BMI. This result corroborates previous reports of a direct relationship between S100B serum levels and BMI [48]. More direct evidence of the potential role of S100B in fat metabolism comes from animal studies that have demonstrated the presence of S100B in adipose tissue of rats [49] and that serum S100B levels are significantly influenced by adipose tissue [50].

GDNF levels in milk from mothers who delivered vaginally at term were significantly lower than those who delivered abdominally. This could be attributed to the protective role that breast feeding plays in neonates delivered abdominally. Therefore, cesarean deliveries with no labor complications remain at a much higher risk of neonatal mortality than planned vaginal deliveries [51], because emergency and elective cesarean deliveries are similarly associated with a decreased rate of exclusive breastfeeding compared with vaginal delivery [52]. However, no reports have conclusively proven this assumption and it remains to be an important research topic.

This study also found a significant correlation between the levels of S100B protein and BDNF in human milk. At present, no other study had reported this finding, and this association was found in 22 women only. Thus further studies are needed to confirm this relationship.

The present study was constrained by the limited amount of milk samples. Maternal sera were not simultaneously collected due to the peripartum folk customs in China. Furthermore, the absence of previous reports on the basal concentrations of BDNF and GDNF in lactating women prevented comparison of the results. Despite these limitations, the present study was the first to determine basal BDNF and GDNF concentrations in milk from lactating women.

In conclusion, our findings indicate that S100B protein, BDNF, and GDNF are present in human milk. Although their exact functions in human milk are not yet certain, present findings suggest that the physiological function of these cytokines possibly includes a trophic role. The relationship between S100B concentrations in human milk and the BMI of the lactating women has not been described before. This suggests that the S100B protein is a new adipokine. In addition, delivery modes were negatively associated with GDNF concentration in human milk. A positive correlation exists between the levels of S100B protein and BDNF in human milk. Additional investigations are required to clarify the physiologic roles of S100B protein, BDNF, and GDNF in human milk.

Author Contributions

Conceived and designed the experiments: KW RL. Performed the experiments: RL WX ZZ. Analyzed the data: RL. Contributed reagents/materials/analysis tools: RL WX. Wrote the paper: RL.

References

1. Heizman CW, Fritz G, Schäfer BW (2002) S100 proteins: structure, functions and pathology. Front Biosci 7: d1356–68.
2. Hofer M, Paglusi SR, Hohn A, Leibrock J, Barde YA (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 9: 2459–64
3. Lin LF, Doherty DH, Lile JD, Bekesh S, Collins F (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260: 1130–2.
4. Lin LF, Zhang TJ, Collins F, Armes LG (1994) Purification and initial characterization of rat B49 glial cell line-derived neurotrophic factor. J Neurochem 63: 756–68.
5. Kliger M, Marshak DR (1985) Purification and characterization of a neurite extension factor from bovine brain. Proc Natl Acad Sci U S A 82: 7136–41.
6. Winningham-Vagier F, Saeker JL, Burger SW, Coss N, Van Eldik LJ (1989) Neurite extension and neuronal survival activities of recombinant S100 beta proteins that differ in the content and position of cysteine residues. J Cell Biol 109: 3063–71.
7. Haged LG, Yang Q, Hamberger A, Bergman S, Widerberg A, et al. (1997) S-100beta stimulates neurite outgrowth in the rat sciatic nerve grafted with acellular muscle transplants. Brain Res 753: 196–201.
8. Van Eldik LJ, Christie-Pope B, Bolin LM, Shooter EM, Whitfield WO Jr. (1991) Neurotrophic activity of S-100 beta in cultures of dorsal root ganglia from embryonic chick and fetal rat. Brain Res 540: 290–5.
9. Bhattacharyya A, Oppenheim RW, Pretvede D, Moore BW, Brackenbury R, et al. (1992) S100 is present in developing chicken neurons and Schwann cells and promotes motor neuron survival in vivo. J Neurosci 13: 451–66.
10. Nishi M, Whitaker-Azmitia PM, Azmitia EC (1996) Enhanced synaptophysin protein and BDNF in human milk. At present, no other reports have conclusively proven this assumption and it remains to be an important research topic.

Author Contributions

Conceived and designed the experiments: KW RL. Performed the experiments: RL WX ZZ. Analyzed the data: RL. Contributed reagents/materials/analysis tools: RL WX. Wrote the paper: RL.

References

1. Heizman CW, Fritz G, Schäfer BW (2002) S100 proteins: structure, functions and pathology. Front Biosci 7: d1356–68.
2. Hofer M, Paglusi SR, Hohn A, Leibrock J, Barde YA (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 9: 2459–64
3. Lin LF, Doherty DH, Lile JD, Bekesh S, Collins F (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260: 1130–2.
4. Lin LF, Zhang TJ, Collins F, Armes LG (1994) Purification and initial characterization of rat B49 glial cell line-derived neurotrophic factor. J Neurochem 63: 756–68.
5. Kliger M, Marshak DR (1985) Purification and characterization of a neurite extension factor from bovine brain. Proc Natl Acad Sci U S A 82: 7136–41.
6. Winningham-Vagier F, Saeker JL, Burger SW, Coss N, Van Eldik LJ (1989) Neurite extension and neuronal survival activities of recombinant S100 beta proteins that differ in the content and position of cysteine residues. J Cell Biol 109: 3063–71.
7. Haged LG, Yang Q, Hamberger A, Bergman S, Widerberg A, et al. (1997) S-100beta stimulates neurite outgrowth in the rat sciatic nerve grafted with acellular muscle transplants. Brain Res 753: 196–201.
8. Van Eldik LJ, Christie-Pope B, Bolin LM, Shooter EM, Whitfield WO Jr. (1991) Neurotrophic activity of S-100 beta in cultures of dorsal root ganglia from embryonic chick and fetal rat. Brain Res 540: 290–5.
9. Bhattacharyya A, Oppenheim RW, Pretvede D, Moore BW, Brackenbury R, et al. (1992) S100 is present in developing chicken neurons and Schwann cells and promotes motor neuron survival in vivo. J Neurosci 13: 451–66.
10. Nishi M, Whitaker-Azmitia PM, Azmitia EC (1996) Enhanced synaptophysin protein and BDNF in human milk. Additional investigations are required to clarify the physiologic roles of S100B protein, BDNF, and GDNF in human milk.
30. Gazzolo D, Bruschetini M, Lituanis M, Serra G, Santini P, et al. (2004) Levels of S100B protein are higher in mature human milk than in colostrum and milk-formulae milks. Clin Nutr 23: 23–6.
31. Musumeci M, Betta P, Magro E, Iaia T, Simpore J, et al. (2008) S100B concentration in colostrums of Burkinabe and Sicilian women. Nutr Metab (Lond) 22: 5: 15.
32. De Amici D, Gasparoni A, Guala A, Klersy C (2001) Does ethnicity predict lactation? A study of four ethnic communities. Eur J Epidemiol 17: 357–62.
33. Cunha AR, Frey BN, Andreazza AC, Gis JD, Rosa AR, et al. (2006) Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. Neurosci Lett 398: 215–9.
34. Zhang X, Zhang Z, Xie C, Xi G, Zhou H, et al. (2008) Effect of treatment on serum glial cell line-derived neurotrophic factor in depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 32: 886–90.
35. Portela L, Torres A, Schaf DV, Ribeiro L, Nora DB, et al. (2002) The serum S100B concentration is age dependent. Clin Chem 48: 950–952.
36. Schaefer RJ, Schultman RJ, Lai C (1999) Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. Pediatrics 103: 1150–1157.
37. Bhandari N, Babli R, Mazumdar S, Martines J, Black RE, et al. (2003) Effect of community-based promotion of exclusive breastfeeding on diarrhoeal illness and growth: a cluster randomized controlled trial. Infant Feeding Study Group. Lancet 361: 1418–1423.
38. Lucas A, Mezey R, Cole TJ (1998) Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 317: 1481–1487.
39. Horwood LJ, Darlow BA, Mogridge N (2001) Breast milk feeding and cognitive ability at 7–8 years. Arch Dis Child Fetal Neonatal Ed 84: 141–145.
40. Roessmann W, Goerres P, Janssens J, Tack J, Vanden Berghe P (2000) Brain-derived neurotrophic factor amplifies neurotransmitter responses and promotes synaptic communication in the enteric nervous system. Gut 57: 314–322.
41. Delabry L, Gelot A, Archard D, Eschalier A, Bertrand C, et al. (2006) Interactive involvement of brain derived neurotrophic factor, nerve growth factor, and calcitonin gene related peptide in colonic hypersensitivity in the rat. Gut 55: 940–945.
42. Waitesvara K, Sato M, Sainio K, Rintala R, Sariola H (1998) Distribution of glial cell line-derived neurotrophic factor mRNA in human colon suggests roles for muscularis mucosae in innervation. J Pediatr Surg 33: 1501–1506.
43. Zhang DK, He FQ, Li TK, Pang XH, Cui DJ, et al. (2010) Glial-derived neurotrophic factor regulates intestinal epithelial barrier function and inflammation and is therapeutic for murine colitis. J Pathol 222: 213–222.
44. Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Musfeld T, et al. (1999) Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med 194: 865–870.
45. Enstrom A, Omore C, Tatver A, Hertz-Picciotto I, Hansen R, et al. (2008) Periperal blood leukocyte production of bdnf following mitogen stimulation in early onset and regressive autism. Am J Biochem & Biotech 4: 121–129.
46. Hashimoto M, Ito T, Fukiui H, Nomoto H, Furukawa Y, et al. (2005) Stimulation of production of glial cell line-derived neurotrophic factor and nitric oxide by lipopolysaccharide with different dose-responsiveness in cultured rat macrophages. Biomed Res 26: 223–229.
47. Lonnroth B (2003) Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr 77: 1537S–1543S.
48. Stein J, Schiltz K, Walter M, Wunderlich MT, Keilhoff G, et al. (2010) S100B serum levels are closely correlated with body mass index: an important caveat in neuropsychiatric research. Psychoneuroendocrinology 35: 321–34.