

TREATMENT AND PREVENTION OF ACNE

BACKGROUND OF THE INVENTION

ACNE

Acne Vulgaris is a common skin condition affecting all ages, races, and
5 genders. Prevalence peaks at 80-90 percent in adolescents, but continues
beyond the teenage years especially in women. Acne develops due to a
blockage of the hair follicles. Hyperkeratinization and formation of a plug
of keratin and sebum, called microcomedo, is the earliest change. The
microcomedo may enlarge to form an open comedone (blackhead) or closed
10 comedone (whitehead). In these conditions, the largely commensal
bacterium *Propionibacterium acnes* can cause inflammation leading to
inflammatory lesions (papules and pustules). Hormonal activity, such as
puberty and menstrual cycles, which increases sebum production, may
contribute to the formation of acne.

15 Current treatments for acne include topical benzoyl peroxide, topical and
oral retinoids, topical and oral antibiotics, and oral hormones for women.
However, many adverse events are associated with these treatments varying
from dryness, redness and irritation of the skin when using topical retinoids,
to antibiotic resistance when using antibiotics, to birth defects when using
20 oral retinoids, and to weight gain and thrombosis when using hormones.
There is therefore a need for a safer alternative to current treatment
protocols.

LACTOFERRIN

Lactoferrin is a protein that occurs naturally in cow's milk. Lactoferrin is classified as GRAS (Generally Regarded as Safe) by the US Food and Drug Administration and European Food Safety Authority, for use as an
5 ingredient in functional foods. Lactoferrin exhibits antimicrobial, antioxidant and anti-inflammatory properties.

Bovine lactoferrin is obtained commercially from skim milk or from cheese whey. It is important to understand the difference between lactoferrin isolated from skim milk and cheese whey to understand the prior art.

10 Bovine milk protein is broadly classified into casein and whey. Bovine milk protein is 80% casein, which is a name for a family of related phosphoproteins (α s1, α s2, β , κ) with an isoelectric point of 4.6. Casein is relatively hydrophobic, making it poorly soluble in water. Casein is found in milk as a suspension of particles called casein micelles, wrapped in
15 soluble κ -casein molecules. Casein carries a negative charge at the pH of milk of about 6.6-6.8.

Bovine milk protein is 20% whey protein, which is a collection of globular proteins that are soluble independent of pH. Whey proteins include β -lactoglobulin, α -lactalbumin, and serum albumin. These whey proteins have
20 isoelectric points below 6.6 – the pH of milk – which means that they are present as negatively charged proteins in milk.

There are two minor proteins in bovine milk that have isoelectric points above 6.8. One is lactoferrin, which is an 80-kda protein with a reported isoelectric point of 7.8-8.7, and lactoperoxidase with an isoelectric point of 9.6.

- 5 Lactoferrin can be isolated directly from skim milk. First, fat is removed from whole milk by centrifugation to produce skim milk. Skim milk at a natural pH of 6.6-6.8 is filtered and applied to a cationic-exchange chromatography, such as carboxymethyl (CM)-Sephadex, which removes the positively charged proteins lactoferrin and lactoperoxidase. The column
10 is washed with a low concentration (1.6%) NaCl solution, which elutes lactoperoxidase. Then lactoferrin is eluted with a high concentration (5%) NaCl solution. The lactoferrin is concentrated by ultrafiltration and is separated from NaCl by diafiltration. After low heat treatment, lactoferrin is freeze-dried to make a powder. Lactoferrin produced from skim milk is
15 substantially free of other whey proteins. Hereafter, we will refer to lactoferrin derived directly from skim milk as “skim milk-derived lactoferrin”.

Lactoferrin can also be isolated from cheese whey, which is the waste material from cheese making. Hard cheeses, such as cheddar cheese, are
20 produced by adding to whole milk lactic acid-producing bacteria for acidification, and rennet. The combined action of acidification and rennet coagulates milk into solid (curds) and liquid (whey). The curd is mainly fat and casein protein. Lactoferrin ends up in the whey.

Rennet is a complex of enzymes obtained from calf stomach. Rennet contains many enzymes including the protease enzymes chymosin and pepsin, and lipases. Chymosin is an aspartic protease that specifically hydrolyzes the peptide bond in Phe105-106Met of κ -casein, removing the
5 hydrophilic portion of κ -casein that stabilizes the casein micelles, causing the casein to coagulate. The hydrophilic portion of κ -casein is a protein with 64 amino acids called glycomacropeptide that ends up with the whey. Glycomacropeptide has been reported to be antibacterial, antiviral, and anti-inflammatory.

10 Treatment with the lactic acid bacteria and rennet during cheese making also produces a group of proteins, collectively called proteose peptones, derived from proteolysis and hydrolysis of casein and whey proteins. The proteose peptones are heat-stable and acid-soluble proteins that end up in the whey during cheese making.

15 Lactoferrin from whey is purified essentially by the same process described above for skim milk-derived lactoferrin. Because of the additional basic proteins generated from proteolysis and hydrolysis, purification may be more difficult and may require additional steps.

PRIOR ART

20 US 8,147,875B2 discloses a method for the treatment of acne by oral administration of a whey protein fraction containing lactoferrin, and

preferably containing further specific whey proteins. According to this invention, “proteins and peptides as glycomacropeptide, and proteose peptones, which originate from the casein of the milk, are also called whey protein”. Further, “It is preferred that the other whey proteins are basic, i.e. they elute from an acidic ion exchange resin, and have isoelectric points above 6, especially above 7. It is also preferred that the other whey proteins have a molecular weight between 10 kD and 60 kD”. The invention went on to further describe the preferred N-terminal sequences of these basic proteins. Therefore, this patent clearly teaches that for lactoferrin to work, it must be present in a whey protein fraction that contains other whey proteins, preferably basic whey proteins, generated from the action of the lactic acid bacteria and rennet during production of hard cheeses. This patent does not teach that lactoferrin obtained directly from skim milk, which does not contain the other whey proteins, especially basic whey proteins, can be used to treat acne, and further in combination with vitamin E and Zinc.

Our instant invention shows that purified lactoferrin which is not obtained from whey can be used to treat and prevent acne, preferably in combination with Vitamin E and zinc, therefore there is no reason to believe that bovine lactoferrin cannot be replaced with other sources of lactoferrin that are not isolated from whey. Preferred sources of lactoferrin of our instant invention includes bovine lactoferrin isolated directly from skim milk, and recombinant lactoferrin (bovine, human, or others) produced in animals, plants, bacteria, fungi, and mold. In the case of recombinant lactoferrin,

there is no concept of “whey protein” or “whey protein fraction” because only the lactoferrin gene is introduced into the host.

US 8,147,875B2 teaches that “Although native bovine lactoferrin is a preferred component of the whey protein fraction to be used as an active
5 agent, part or all of it may be replaced by other lactoferrins, such as lactoferrin from other mammals...” This teaching again points to the importance of the other whey proteins in the whey protein fraction for lactoferrin to work.

US 8,147,875B2 further states that “altogether, the outcome of the study
10 forms a solid base to position natural, bovine milk-derived bioactive whey composition comprising lactoferrin intake as a new strategy to prevent and treat acne vulgaris. In contrast to current systemic and topical therapies (based on antibiotics, retinoids, and other compounds), lactoferrin/whey composition supplementation is not accompanied by side effects and can be
15 used on an enduring basis.” Clearly, this teaches that lactoferrin must be present with other whey proteins for the bioactive whey composition to work.

The whey protein fraction used in the clinical studies described in Example 1 of US 8,147,875B2, was derived from cheddar whey and contains about
20 80% lactoferrin, the balance being other whey proteins. To date, all published clinical studies of oral lactoferrin for treatment of acne used this crude whey protein fraction containing about 20% other whey proteins.

There is no prior art clinical data that teaches that oral lactoferrin treatment will work unless it is a component of a whey protein fraction, i.e. unless other whey proteins generated from lactic acid bacteria and rennet proteolysis and hydrolysis are present.

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DESCRIPTION OF THE INVENTION

We have surprisingly found that lactoferrin obtained directly from skim milk, without the additional whey proteins, especially basic whey proteins, generated from the lactic acid bacteria and rennet proteolysis/hydrolysis of milk proteins during cheese making, can be used for the oral treatment and
10 prevention of acne. Preferably, the lactoferrin of this instant invention is combined with Vitamin E and Zinc.

This instant invention shows that purified lactoferrin which is not obtained from whey can be used to treat and prevent acne, preferably in combination with Vitamin E and zinc, therefore there is no reason to believe that bovine
15 lactoferrin cannot be replaced with other sources of lactoferrin, as long as they are not isolated from whey. Preferred sources of lactoferrin of this instant invention includes bovine lactoferrin isolated directly from skim milk, and recombinant lactoferrin (bovine, human, or others) produced in animals, plants, bacteria, fungi, and mold.

20 The lactoferrin of this instant invention can be formulated as a tablet, capsule or a liquid for oral administration. The preferred daily dose of

lactoferrin of this instant invention is 100 mg to 500 mg, most preferably 100-300 mg.

Vitamin E is an integral part of the skin's antioxidant defenses, primarily
5 providing protection against UV radiation and other free radicals that may
come in contact with the skin. Deficiency of Vitamin E is associated with
acne (Thiele et al., Molecular Aspects of Medicine, 2007, 28:646-667).
However, it is not known if supplementation with Vitamin E, alone or in
combination with lactoferrin and zinc, can be used to treat and prevent acne.
10 The Vitamin E or its analogues can be selected from, but not limited to, d- α -
tocopherol; dl- α -tocopherol; d- α -tocopheryl esters; dl- α -tocopheryl esters;
natural mixed tocopherols comprising α isomer, β isomer, γ isomer, and δ
isomer; and tocotrienols and tocotrienol esters comprising α isomer, β
isomer, γ isomer, and δ isomer. The daily dose of vitamin E, in
15 combination with lactoferrin and zinc of this instant invention, is preferably
less than 100 IU, most preferably less than 50 IU.

Acne patients have been shown to have reduced levels of zinc in their
serum, hair, nails, and epidermis compared to healthy controls. Zinc
deficiency has also been demonstrated to provoke flare-up of existing acne.
20 The therapeutic role of zinc in acne vulgaris is based on its anti-
inflammatory activity and its role in androgen metabolism. Zinc inhibits the
enzyme 5- α -reductase, responsible for converting testosterone to its
biologically active metabolite dehydrotestosterone, which mediates
stimulation of sebaceous gland activity. Reduction of sebum secretion is

known to result from zinc exposure. Zinc may also have direct activity, inhibiting bacterial lipases from converting serum triglycerides to fatty acids, which are more potent mediators of acne (Amer et al., International Journal of Dermatology, 1982, 21:81-484). However, the prior art does not
5 teach the combination of zinc, Vitamin E and lactoferrin for treatment and prevention of acne. The zinc salt of this instant invention can be selected from, but not limited to, zinc sulfate, zinc gluconate, and zinc citrate. The daily dose of zinc, in combination with Vitamin E and lactoferrin of this instant invention, is preferably less than 50 mg, most preferably less than 25
10 mg.

EXAMPLES

Example 1

Skim milk-derived lactoferrin was obtained commercially. The lactoferrin content was more than 97%. To confirm the absence of other whey
15 proteins, especially basic whey proteins, the purity of this skim milk-derived lactoferrin was assessed using essentially the same procedure used in Example 1 of US 8,147,875 B2 for the preparation of crude whey protein fraction containing 79.6% lactoferrin from Cheddar Whey. Briefly, the skim milk-derived lactoferrin was dissolved in phosphate buffer solution pH
20 6.8, then passed over a CM-Sephadex Fast Flow column. The column was then washed with water, followed by 0.3M NaCl, and finally with 1M NaCl to elute lactoferrin. The 0.3M NaCl and 1M NaCl eluates were analyzed

using SDS-PAGE. The 0.3M NaCl eluate was clean, and the 1M NaCl eluate only showed lactoferrin with no other contaminating proteins. For reference, the whey protein fraction of Example 1 of US 8,147,875B2 showed other basic proteins. This is to be expected since the skim milk-derived lactoferrin should not contain other “whey proteins”, especially basic whey proteins, generated from the proteolytic and hydrolytic action of the lactic-acid bacteria and rennet.

Example 2

Three different formulations of lactoferrin using the commercial skim milk derived lactoferrin of Example 1 were prepared according to Table 1:

Table 1 (per capsule)

	Example 2A	Example 2B	Example 2C
Lactoferrin	100 mg	100 mg	100 mg
Vitamin E Acetate	0	11.4 mg (8 IU)	15.7 mg (11 IU)
Zinc (as zinc gluconate)	0	2.5 mg (17.4 mg)	5 mg (34.8 mg)
Microcrystalline Cellulose	165 mg	151 mg	201 mg
Colloidal Silicon Dioxide	0.3 mg	0.3 mg	0.36 mg
Magnesium Stearate	2.83 mg	2.83 mg	3.56 mg

All the ingredients excluding magnesium stearate were mixed in a sigma blender for 15 minutes. Magnesium stearate was then added, and mixed for

1 minute. The final blend was filled into size 1 gelatin capsule using a GKF 400 encapsulating machine (Bosch, Germany).

Example 3

Ten teenagers (6 males, 4 females) aged 13-20 were enrolled in the study.

- 5 The subjects took one capsule, twice a day, of Example 2A containing only skim milk-derived lactoferrin. The subjects were evaluated by a dermatologist at 0, 2, 4, 8, and 12 weeks. The total number of blackheads, whiteheads, papules, and pustules were counted. The results are given in Table 2:

Table 2

Week	% Reduction
2	62
4	71
8	67
12	60

All reductions were significant versus 0 week. The results show that lactoferrin which is not part of a whey protein fraction can be given orally to treat and prevent acne.

Example 4

40 teenagers (25 males, 15 females) aged 13-20 were enrolled in the study. The subjects took one capsule, twice a day, of Example 2B containing 100 mg of skim milk-derived lactoferrin, 8 IU of Vitamin E, and 2.5 mg of Zinc.

- 5 The subjects were evaluated by a dermatologist at 0, 1, 2, 4, 8, and 12 weeks. The total number of blackheads, whiteheads, papules, and pustules were counted. The results are given in Table 3:

Table 3

Week	% Reduction
1	34
2	62
4	69
8	72
12	82

All reductions were significant versus 0 week. All subjects showed improvement. The results indicate that lactoferrin which is not part of a whey protein fraction, combined with Vitamin E and Zinc, can be given orally to treat and prevent acne.

Example 5

40 females aged 20-40 were enrolled in the study. The subjects took one capsule, twice a day, of Example 2C containing 100 mg of skim milk-derived lactoferrin, 11 IU of Vitamin E, and 5 mg of Zinc. The subjects
5 were evaluated by a dermatologist at 0, 1, 2, 4, 6, 8, 10, and 12 weeks. The study period of 12 weeks included at least three menstrual cycles. Menstrual acne, a flare-up of blemishes every month that coincides with menstruation, is fairly common.

The total number of blackheads, whiteheads, papules, and pustules were
10 counted. The results are given in Table 4:

Table 4

Week	% Reduction
1	47
2	49
4	77
6	72
8	83
10	78
12	94

All reductions were significant versus 0 week. All subjects showed improvement. The above results indicate that lactoferrin which is not part

of a whey protein fraction, combined with Vitamin E and Zinc, can be given orally to treat and prevent acne.

CLAIMS:

1. A method for the treatment and prevention of acne consisting of the oral administration of lactoferrin, without the concomitant topical administration of lactoferrin, and wherein the lactoferrin is not obtained from whey.
- 5 2. The method according to claim 1, wherein the lactoferrin is isolated directly from bovine skim milk.
3. The method according to claim 1, wherein the lactoferrin is a recombinant lactoferrin.
4. The method according to claim 1, wherein the lactoferrin is given orally
10 at 100-500 mg/day.
5. The method according to claim 4, wherein the lactoferrin is given orally at 100-300 mg/day.
6. A method for the treatment and prevention of acne consisting of the oral administration of lactoferrin, Vitamin E, and zinc, without the concomitant
15 topical administration of lactoferrin, and wherein the lactoferrin is not obtained from whey.
7. The method according to claim 6, wherein the lactoferrin is isolated directly from bovine skim milk.

8. The method according to claim 6, wherein the lactoferrin is a recombinant lactoferrin.
9. The method according to claim 6, wherein the lactoferrin is given orally at 100-500 mg/day.
- 5 10. The method according to claim 9, wherein the lactoferrin is given orally at 100-300 mg/day.
11. The method according to claim 6, wherein the Vitamin E is less than 100 IU/day.
12. The method according to claim 11, wherein the Vitamin E is less than
10 50 IU/day.
13. The method according to claim 6, wherein the zinc is less than 50 mg/day.
14. The method according to claim 13, wherein the zinc is less than 25 mg/day.
- 15 15. A method for the treatment and prevention of acne consisting of the daily oral administration of 200 mg of bovine lactoferrin, 16-22 IU of Vitamin E, and 5-10 mg of zinc, without the concomitant topical

administration of lactoferrin, and wherein the bovine lactoferrin is obtained directly from skim milk.