

Lactobionic Acid Anti-Aging Mechanisms: Antioxidant Activity, MMP Inhibition, and Reduction of Melanogenesis

Irina Brouda, MA, Brenda L. Edison, BA, Ronni L. Weinkauff, PhD, Barbara A. Green, RPh, MS
NeoStrata Company, Inc., Princeton, NJ, USA

INTRODUCTION

Lactobionic acid, 4-O-β-galactopyranosyl-D-gluconic acid, is a polyhydroxy bionic acid that was previously reported to provide significant anti-aging and protective effects to human skin. Lactobionic acid can chelate metal ions¹ such as oxidation-promoting iron, protect ischemic organs from breakdown and oxidative stress^{2,3} during reperfusion, and improve stratum corneum barrier structure and function⁴ and prevent inflammation.⁵

Skincare products that contain lactobionic acid can improve skin tone, clarity, and texture as well as reduce lines, wrinkles, and pore size, particularly in photoaged skin.^{6,7}



Before use (Week 0) After 4 weeks of use (Week 4)

This volunteer applied a cream containing a 4% polyhydroxy acid blend of lactobionic acid and gluconolactone plus peptides twice daily for 4 weeks to reduce dark under eye circles.

Note the reductions in fine lines and under eye darkness and improvements in skin texture and clarity.

OBJECTIVE

To investigate how **lactobionic acid may act as an antioxidant and an anti-aging agent** in skincare products in a series of new *in vitro* studies.

IN VITRO ASSAYS

Lactobionic acid was tested for matrix metalloproteinase (MMP) inhibition, lipid peroxidation inhibition, and melanogenesis inhibition in cultured B16 melanocytes.

- ▶ **MMP Inhibition:** MMPs are enzymes (zinc-dependent endopeptidases) that break down and recycle collagen in skin's extracellular matrix as part of the natural process of collagen turnover. Over time, photoaged and naturally aged skin shows upregulation of MMPs and reduced synthesis of new collagen.⁸ Blocking MMP activity can help preserve existing collagen and maintain firmer and tighter skin.
- ▶ **Melanogenesis Inhibition:** Exposure to sunlight stimulates melanin synthesis in melanocytes, which can lead to undesirable tanning, dark spots, uneven pigmentation, and other signs of photoaging. Melanogenesis inhibitors are able to lighten naturally-pigmented skin and/or reduce hyperpigmentation of sun-exposed skin.
- ▶ **Lipid Peroxidation:** UV-induced lipid peroxidation is an oxidative breakdown of polyunsaturated fatty acids by free radicals generated during UV light exposure. Inhibitors of lipid peroxidation scavenge hydroxyl radicals that attack fatty acids.⁹ Inhibition of lipid peroxidation is vital for maintaining cell membranes and mitochondria, protecting cells against sun damage and oxidative stress, and is a measure of a substance's antioxidant capacity.

Together these assays simulate collagen degradation, oxidative stress, and pigmentation changes--processes associated with aging and photodamage¹⁰ in living skin--and are used to assess potential benefit ingredients in skincare products.

TEST MATERIALS

In Vitro Assay	MMP Inhibition	Melanogenesis Inhibition in Cultured B16 Melanocytes	UV-Induced Lipid Peroxidation
-Test Substance	Lactobionic Acid (0.0001% to 0.1% solutions)	Lactobionic Acid (0.0037% to 8% solutions) +/- α-MSH*	Lactobionic Acid (0.0001% to 0.1% solutions)
-Negative Control	Clostridium Collagenase IV	Water +/- α-MSH*	Water
-Positive Control	Phenantroline (0.01%)	Kojic Acid +/- α-MSH*	Vitamin C, Vitamin E

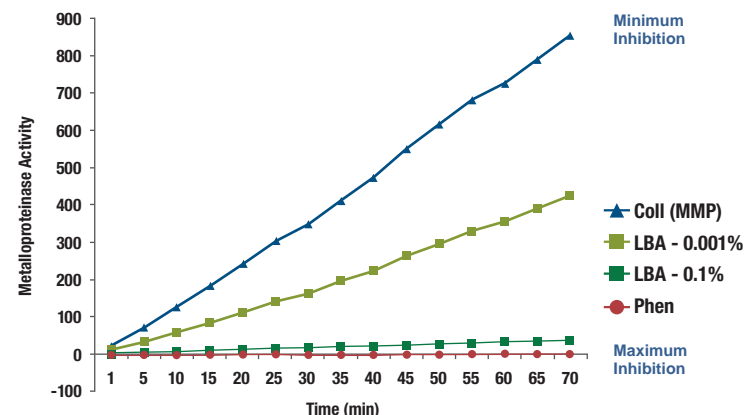
*+/- α-MSH = material was tested in the presence (+) and in the absence (-) of α-melanocyte stimulating hormone (MSH) analog

RESULTS

Inhibition of Matrix Metalloproteinase Activity by Lactobionic Acid *In Vitro*

- ▶ Lactobionic acid was found to be a strong MMP inhibitor *in vitro*.
- ▶ Lactobionic acid blocked the ability of Clostridium Collagenase IV (a matrix metalloproteinase) to break down protein in a dose-dependent manner (Figure 1).
- ▶ Lactobionic acid at 0.1% concentration performed as well as phenantroline—a potent MMP inhibitor.
- ▶ Lactobionic acid demonstrated capacity to block collagen degradation, which can help preserve the skin matrix in naturally aging and photoaged skin.

Figure 1. Inhibition of Matrix Metalloproteinase Activity by Lactobionic Acid *In Vitro*

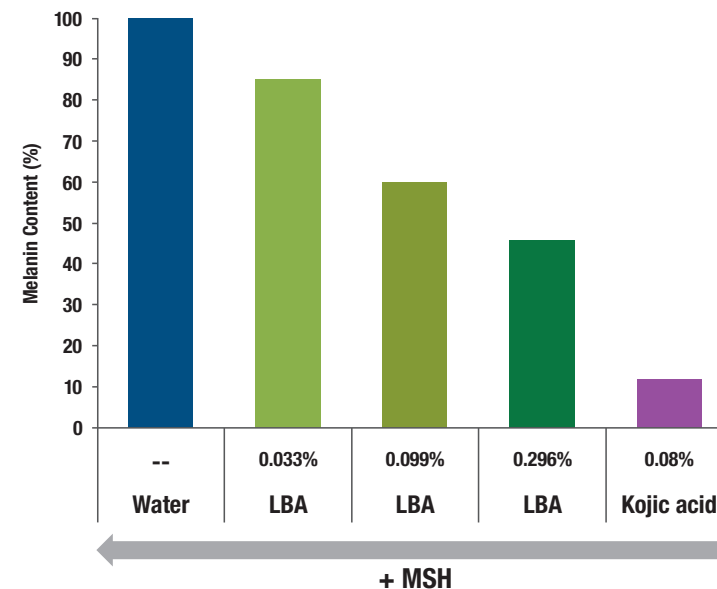


Abbreviations: Coll (MMP) = Clostridium Collagenase IV (a collagen-degrading enzyme—a type of matrix metalloproteinase); LBA = Lactobionic Acid; Phen = Phenantroline (a matrix metalloproteinase inhibitor)

Lactobionic Acid Inhibition of Melanin Synthesis in Cultured B16 Melanocytes

- ▶ Lactobionic acid inhibited melanin synthesis in cultured murine B16 melanocytes in the presence of an analog of α-melanocyte stimulating hormone (α-MSH) in a dose-dependent manner (Figure 2).
- ▶ Lactobionic acid was not toxic to cultured melanocytes.
- ▶ Lactobionic acid exhibited the ability to block melanocyte activity and reduce melanin synthesis induced by an exogenous source such as ultraviolet radiation from daily sun exposure.
- ▶ Lactobionic acid demonstrated capacity to reduce the potential for hyperpigmentation and irregular pigmentation of sun-exposed skin.

Figure 2. Lactobionic Acid Inhibition of Melanin Synthesis in Cultured B16 Melanocytes



Abbreviations: LBA = Lactobionic Acid; +MSH = in the presence of α-melanocyte stimulating hormone analog

Inhibition of UV-Induced Lipid Peroxidation by Lactobionic Acid *In Vitro*

- ▶ Lactobionic acid was found to exhibit antioxidant activity by inhibiting UV-induced lipid peroxidation *in vitro*.
- ▶ Lactobionic acid, Vitamin C (ascorbic acid), and Vitamin E (α-Tocopherol) were shown to reduce the production of malondialdehyde, an oxidative degradation product.
- ▶ Lactobionic acid demonstrated capacity to protect skin against UV-generated free radicals, oxidative stress, and photodamage and thus may help prevent premature aging.

SUMMARY

In vitro studies used to assess a substance's antioxidant and anti-aging activity revealed that:

- ▶ **LACTOBIONIC ACID is a strong matrix metalloproteinase inhibitor:**
 - Can help preserve collagen to maintain healthy structure and function of skin matrix
 - Can help maintain skin firmness, tightness, and suppleness
- ▶ **LACTOBIONIC ACID is an effective inhibitor of UV-triggered melanin synthesis:**
 - Can help prevent pigmentation of sun-exposed skin
 - Can reduce unwanted skin darkening and uneven pigmentation
 - Can improve skin tone and clarity
- ▶ **LACTOBIONIC ACID is an effective inhibitor of UV-induced lipid peroxidation:**
 - Can protect skin cell membranes and mitochondria from sun damage
 - Can act as an antioxidant and fortify skin's natural defenses against free radicals

Lactobionic acid has the capacity to preserve collagen, to protect skin cells against sun damage and oxidative stress, and to help prevent irregular pigmentation. These may be some of the mechanisms through which this skincare ingredient **can help reduce and/or prevent the visible signs of natural aging and photoaging in living human skin.**

REFERENCES

- Upadhyaya GA, Strasberg SM. Glutathione, lactobionate, and histidine: cryptic inhibitors of matrix metalloproteinases contained in University of Wisconsin and histidine/tryptophan/ketoglutarate liver preservation solutions. *Hepatology*. 2000 May;31(5):1115-22.
- Charloux C, Ishii K, Paul M, Loisan D, Astier A. Reduced production of the deleterious hydroxyl free radical during the final reperfusion of isolated rabbit heart with the use of an improved sodium lactobionate-based cardioplegic medium. *J Heart Lung Transplant*. 1994 May-Jun;13(3):481-8.
- Charloux C, Paul M, Loisan D, Astier A. Inhibition of hydroxyl radical production by lactobionate, adenine, and tempol. *Free Radic Biol Med*. 1995 Nov;19(5):699-704.
- Hachem JP, Roelandt T, Schürer N et al. Acute acidification of stratum corneum membrane domains using polyhydroxy acids improves lipid processing and inhibits degradation of corneodesmosomes. *J Invest Dermatol*. 2009 Sep 10; doi:10.1038/jid.2009.249.
- Hatano Y, Man MQ, Uchida Y et al. Maintenance of an acidic stratum corneum prevents emergence of murine atopic dermatitis. *J Invest Dermatol*. 2009 Jul;129(7):1824-35.
- Grimes PE, Green BA, Wildnauer RH, Edison BL. The use of polyhydroxy acids (PHAs) in photoaged skin. *Cutis*. 2004 Feb;73(2 Suppl):3-13.
- Green BA, Edison BL, Sigler ML. Antiaging effects of topical lactobionic acid: results of a controlled usage study. *Cosmet Dermatol*. 2008;21(2):76-82.
- Pinnell SR. Cutaneous photodamage, oxidative stress, and topical antioxidant protection. *J Am Acad Dermatol*. 2003 Jan;48(1):1-19.
- Ogura R, Sugiyama M, Nishi J, Haramaki N. Mechanism of lipid radical formation following exposure of epidermal homogenate to ultraviolet light. *J Invest Dermatol*. 1991 Dec;97(6):1044-7.
- Chung JH, Seo JY, Choi HR et al. Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. *J Invest Dermatol*. 2001 Nov; 117 (5):1218-24.

Poster Exhibit at the *Summer Academy Meeting of the American Academy of Dermatology*, Chicago, IL, August 4-8, 2010.

Lactobionic Acid Anti-Aging Mechanisms: Antioxidant Activity, MMP Inhibition, and Reduction of Melanogenesis

Irina Brouda, MA, Brenda L. Edison, BA, Ronni L. Weinkauf, PhD, Barbara A. Green, RPh, MS
NeoStrata Company, Inc., Princeton, NJ, USA

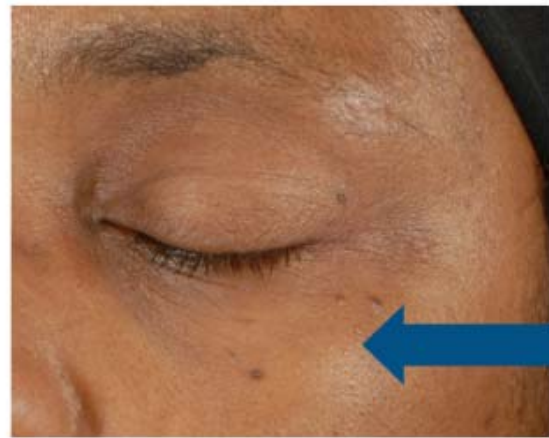
INTRODUCTION

Lactobionic acid, 4-O- β -galactopyranosyl-D-gluconic acid, is a polyhydroxy bionic acid that was previously reported to provide significant anti-aging and protective effects to human skin. Lactobionic acid can chelate metal ions¹ such as oxidation-promoting iron, protect ischemic organs from breakdown and oxidative stress^{2,3} during reperfusion, and improve stratum corneum barrier structure and function⁴ and prevent inflammation.⁵

Skincare products that contain lactobionic acid can improve skin tone, clarity, and texture as well as reduce lines, wrinkles, and pore size, particularly in photoaged skin.^{6,7}



Before use (Week 0)



After 4 weeks of use (Week 4)

This volunteer applied a cream containing a 4% polyhydroxy acid blend of lactobionic acid and gluconolactone plus peptides twice daily for 4 weeks to reduce dark under eye circles.

Note the reductions in fine lines and under eye darkness and improvements in skin texture and clarity.

OBJECTIVE

To investigate how **lactobionic acid** may act as an antioxidant and an anti-aging agent in skincare products in a series of new *in vitro* studies.

Lactobionic Acid Anti-Aging Mechanisms: Antioxidant Activity, MMP Inhibition, and Reduction of Melanogenesis

Irina Brouda, MA, Brenda L. Edison, BA, Ronni L. Weinkauf, PhD, Barbara A. Green, RPh, MS
NeoStrata Company, Inc., Princeton, NJ, USA

IN VITRO ASSAYS

Lactobionic acid was tested for matrix metalloproteinase (MMP) inhibition, lipid peroxidation inhibition, and melanogenesis inhibition in cultured B16 melanocytes.

- ▶ **MMP Inhibition:** MMPs are enzymes (zinc-dependent endopeptidases) that break down and recycle collagen in skin's extracellular matrix as part of the natural process of collagen turnover. Over time, photoaged and naturally aged skin shows upregulation of MMPs and reduced synthesis of new collagen.⁸ Blocking MMP activity can help preserve existing collagen and maintain firmer and tighter skin.
- ▶ **Melanogenesis Inhibition:** Exposure to sunlight stimulates melanin synthesis in melanocytes, which can lead to undesirable tanning, dark spots, uneven pigmentation, and other signs of photoaging. Melanogenesis inhibitors are able to lighten naturally-pigmented skin and/or reduce hyperpigmentation of sun-exposed skin.
- ▶ **Lipid Peroxidation:** UV-induced lipid peroxidation is an oxidative breakdown of polyunsaturated fatty acids by free radicals generated during UV light exposure. Inhibitors of lipid peroxidation scavenge hydroxyl radicals that attack fatty acids.⁹ Inhibition of lipid peroxidation is vital for maintaining cell membranes and mitochondria, protecting cells against sun damage and oxidative stress, and is a measure of a substance's antioxidant capacity.

Together these assays simulate collagen degradation, oxidative stress, and pigmentation changes--processes associated with aging and photodamage¹⁰ in living skin--and are used to assess potential benefit ingredients in skincare products.

TEST MATERIALS

In Vitro Assay	MMP Inhibition	Melanogenesis Inhibition in Cultured B16 Melanocytes	UV-Induced Lipid Peroxidation
-Test Substance	Lactobionic Acid (0.0001% to 0.1% solutions)	Lactobionic Acid (0.0037% to 8% solutions) +/- α -MSH*	Lactobionic Acid (0.0001% to 0.1% solutions)
-Negative Control	Clostridium Collagenase IV	Water +/- α -MSH*	Water
-Positive Control	Phenantroline (0.01%)	Kojic Acid +/- α -MSH*	Vitamin C, Vitamin E

*+/- α -MSH = material was tested in the presence (+) and in the absence (-) of α -melanocyte stimulating hormone (MSH) analog

Lactobionic Acid Anti-Aging Mechanisms: Antioxidant Activity, MMP Inhibition, and Reduction of Melanogenesis

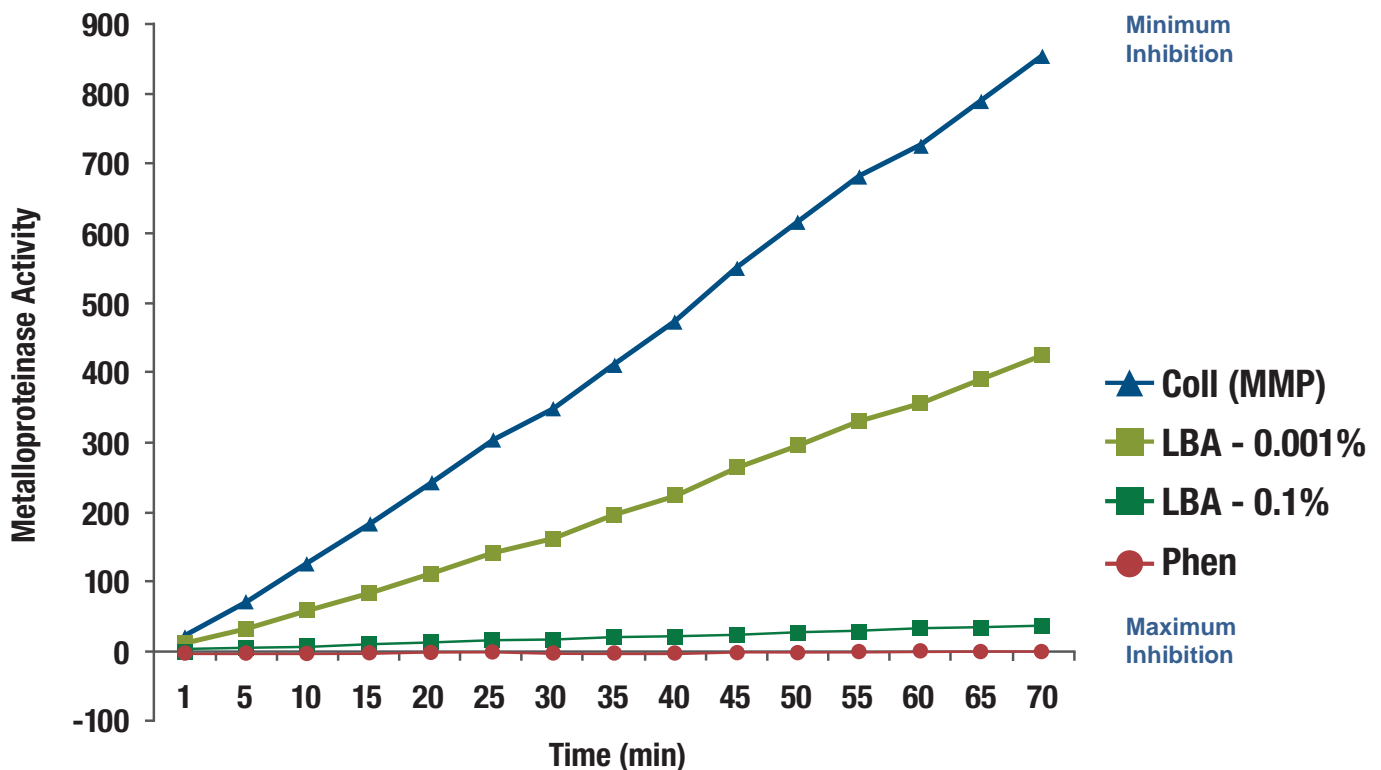
Irina Brouda, MA, Brenda L. Edison, BA, Ronni L. Weinkauf, PhD, Barbara A. Green, RPh, MS
NeoStrata Company, Inc., Princeton, NJ, USA

RESULTS

Inhibition of Matrix Metalloproteinase Activity by Lactobionic Acid *In Vitro*

- Lactobionic acid was found to be a strong MMP inhibitor *in vitro*.
- Lactobionic acid blocked the ability of Clostridium Collagenase IV (a matrix metalloproteinase) to break down protein in a dose-dependent manner (**Figure 1**).
- Lactobionic acid at 0.1% concentration performed as well as phenantroline—a potent MMP inhibitor.
- Lactobionic acid demonstrated capacity to block collagen degradation, which can help preserve the skin matrix in naturally aging and photoaged skin.

Figure 1. Inhibition of Matrix Metalloproteinase Activity by Lactobionic Acid *In Vitro*



Abbreviations: Coll (MMP) = Clostridium Collagenase IV (a collagen-degrading enzyme--a type of matrix metalloproteinase); LBA = Lactobionic Acid; Phen = Phenantroline (a matrix metalloproteinase inhibitor)

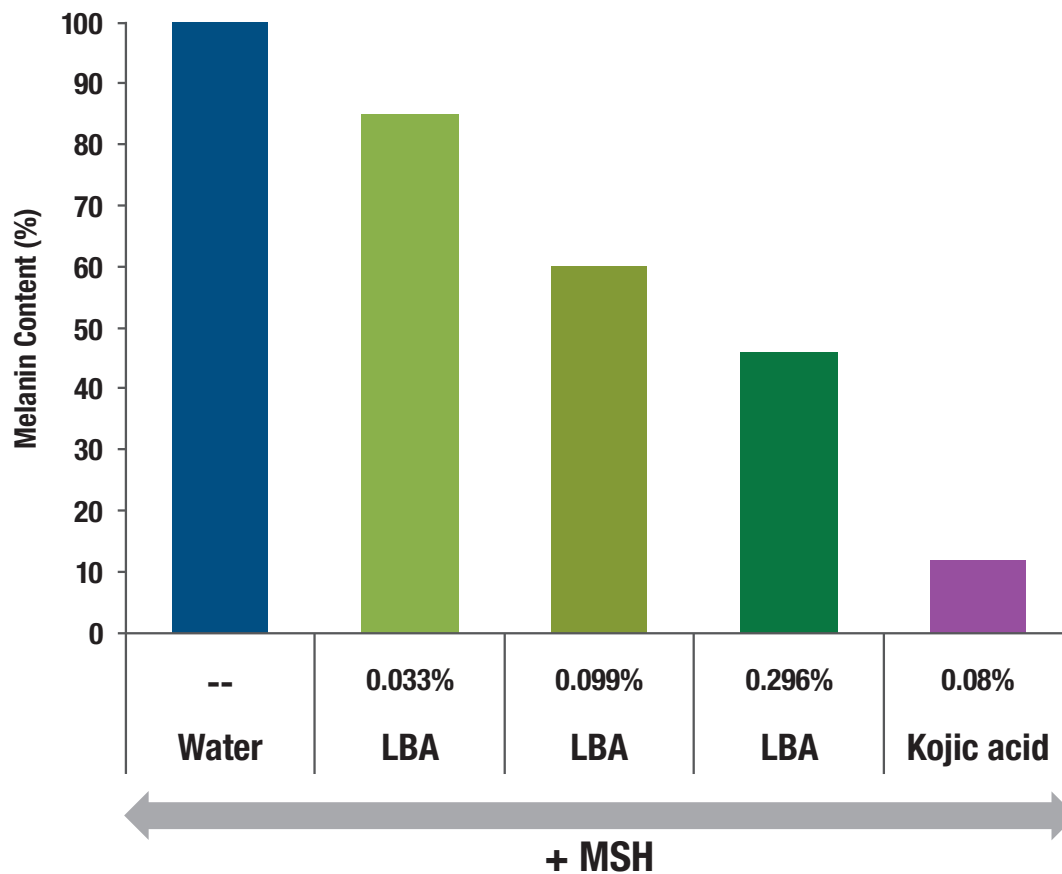
Lactobionic Acid Anti-Aging Mechanisms: Antioxidant Activity, MMP Inhibition, and Reduction of Melanogenesis

Irina Brouda, MA, Brenda L. Edison, BA, Ronni L. Weinkauf, PhD, Barbara A. Green, RPh, MS
NeoStrata Company, Inc., Princeton, NJ, USA

Lactobionic Acid Inhibition of Melanin Synthesis in Cultured B16 Melanocytes

- Lactobionic acid inhibited melanin synthesis in cultured murine B16 melanocytes in the presence of an analog of α -melanocyte stimulating hormone (α -MSH) in a dose-dependent manner (**Figure 2**).
- Lactobionic acid was not toxic to cultured melanocytes.
- Lactobionic acid exhibited the ability to block melanocyte activity and reduce melanin synthesis induced by an exogenous source such as ultraviolet radiation from daily sun exposure.
- Lactobionic acid demonstrated capacity to reduce the potential for hyperpigmentation and irregular pigmentation of sun-exposed skin.

Figure 2. Lactobionic Acid Inhibition of Melanin Synthesis in Cultured B16 Melanocytes



Abbreviations: LBA = Lactobionic Acid; +MSH = in the presence of α -melanocyte stimulating hormone analog

Lactobionic Acid Anti-Aging Mechanisms: Antioxidant Activity, MMP Inhibition, and Reduction of Melanogenesis

Irina Brouda, MA, Brenda L. Edison, BA, Ronni L. Weinkauf, PhD, Barbara A. Green, RPh, MS
NeoStrata Company, Inc., Princeton, NJ, USA

Inhibition of UV-Induced Lipid Peroxidation by Lactobionic Acid *In Vitro*

- ▶ Lactobionic acid was found to exhibit antioxidant activity by inhibiting UV-induced lipid peroxidation *in vitro*.
- ▶ Lactobionic acid, Vitamin C (ascorbic acid), and Vitamin E (α -Tocopherol) were shown to reduce the production of malondialdehyde, an oxidative degradation product.
- ▶ Lactobionic acid demonstrated capacity to protect skin against UV-generated free radicals, oxidative stress, and photodamage and thus may help prevent premature aging.

SUMMARY

In vitro studies used to assess a substance's antioxidant and anti-aging activity revealed that:

- ▶ **LACTOBIONIC ACID is a strong matrix metalloproteinase inhibitor:**
 - Can help preserve collagen to maintain healthy structure and function of skin matrix
 - Can help maintain skin firmness, tightness, and suppleness
- ▶ **LACTOBIONIC ACID is an effective inhibitor of UV-triggered melanin synthesis:**
 - Can help prevent pigmentation of sun-exposed skin
 - Can reduce unwanted skin darkening and uneven pigmentation
 - Can improve skin tone and clarity
- ▶ **LACTOBIONIC ACID is an effective inhibitor of UV-induced lipid peroxidation:**
 - Can protect skin cell membranes and mitochondria from sun damage
 - Can act as an antioxidant and fortify skin's natural defenses against free radicals

Lactobionic acid has the capacity to preserve collagen, to protect skin cells against sun damage and oxidative stress, and to help prevent irregular pigmentation. These may be some of the mechanisms through which this skincare ingredient **can help reduce and/or prevent the visible signs of natural aging and photoaging in living human skin.**

Lactobionic Acid Anti-Aging Mechanisms: Antioxidant Activity, MMP Inhibition, and Reduction of Melanogenesis

Irina Brouda, MA, Brenda L. Edison, BA, Ronni L. Weinkauf, PhD, Barbara A. Green, RPh, MS
NeoStrata Company, Inc., Princeton, NJ, USA

REFERENCES

1. Upadhyya GA, Strasberg SM. Glutathione, lactobionate, and histidine: cryptic inhibitors of matrix metalloproteinases contained in University of Wisconsin and histidine/tryptophan/ketoglutarate liver preservation solutions. *Hepatology*. 2000 May;31(5):1115-22.
2. Charloux C, Ishii K, Paul M, Loisanche D, Astier A. Reduced production of the deleterious hydroxyl free radical during the final reperfusion of isolated rabbit heart with the use of an improved sodium lactobionate-based cardioplegic medium. *J Heart Lung Transplant*. 1994 May-Jun;13(3):481-8.
3. Charloux C, Paul M, Loisanche D, Astier A. Inhibition of hydroxyl radical production by lactobionate, adenine, and tempol. *Free Radic Biol Med*. 1995 Nov;19(5):699-704.
4. Hachem JP, Roelandt T, Schürer N et al. Acute acidification of stratum corneum membrane domains using polyhydroxyl acids improves lipid processing and inhibits degradation of corneodesmosomes. *J Invest Dermatol*. 2009 Sep 10; doi:10.1038/jid.2009.249.
5. Hatano Y, Man MQ, Uchida Y et al. Maintenance of an acidic stratum corneum prevents emergence of murine atopic dermatitis. *J Invest Dermatol*. 2009 Jul;129(7):1824-35.
6. Grimes PE, Green BA, Wildnauer RH, Edison BL. The use of polyhydroxy acids (PHAs) in photoaged skin. *Cutis*. 2004 Feb;73(2 Suppl):3-13.
7. Green BA, Edison BL, Sigler ML. Antiaging effects of topical lactobionic acid: results of a controlled usage study. *Cosmet Dermatol*. 2008;21(2):76-82.
8. Pinnell SR. Cutaneous photodamage, oxidative stress, and topical antioxidant protection. *J Am Acad Dermatol*. 2003 Jan;48(1):1-19.
9. Ogura R, Sugiyama M, Nishi J, Haramaki N. Mechanism of lipid radical formation following exposure of epidermal homogenate to ultraviolet light. *J Invest Dermatol*. 1991 Dec;97(6):1044-7.
10. Chung JH, Seo JY, Choi HR et al. Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. *J Invest Dermatol*. 2001 Nov; 117 (5):1218-24.