Human milk oligosaccharides attenuate solar irradiation induced inflammation and oxidative stress in human skin

Campiche, Remo[1*], Kala, Rishabh[2], Mandary, Madiiha[3], Philibert, Evans[3], Gempeler, Mathias[1], Hueber, Aline[1]

[1] DSM Nutritional Products, Kaiseraugst, Switzerland
[2] Genemarkers LLC, Kalamazoo, United States
[3] Centre International de Développement Pharmaceutique (CIDP), Phoenix, Mauritius

**corresponding author:* Remo Campiche, DSM Nutritional Products, Personal Care & Aroma, Wurmisweg 576, CH-4303 Kaiseraugst, +41618158028, remo.campiche@dsm.com

Abstract

Background: Human milk oligosaccharides (HMOs) are an important part of human breast milk. They regulate infant health via modulation of the gut microbiome, gut epithelial barrier, inflammation, as well as innate immunity. HMOs found their way into infant formula to better mimic the natural composition of human milk. Recently, they came into consideration for cosmetic topical applications.

Methods: In this study, we focused on potential cutaneous benefits in response to solar irradiation. We irradiated reconstructed human skin consisting of an epidermis and a dermis with UVB light. In a gene-expression analysis, we determined the anti-inflammatory potential of the HMOs. Furthermore, we irradiated human skin ex vivo with high energy visible light (HEV) at either 412 nm or 450 nm and investigated the effects on HEV induced oxidative stress by a DCFH-DA assay.

Results: We found anti-inflammatory activity of Lacto-N-neotetraose (LNnT), 3-Sialyllactose (3'SL), 6-Sialyllactose (6'SL), and 3-Fucosyllactose (3'FL). Significantly down-regulated genes comprised e.g. IL-23A, IL-1alpha, IL-1beta and IL-8. In addition, LNnT was able to significantly down-regulate the formation of reactive oxygen species (ROS) when the tissue was irradiated with HEV of 412 nm (-67%, $p<0.05$ vs vehicle). Irradiation with HEV at 450 nm revealed a positive effect for LNnT (-39%, $p<0.05$) and 3'SL (-50%, $p<0.01$).

Conclusion: We provide evidence for beneficial effects of human milk oligosaccharides against solar irradiation induced cutaneous inflammation and oxidative stress. They can thus be used as a novel class of skin care active ingredients.

Keywords: Human milk oligosaccharides, inflammation, solar irradiation, oxidative stress

Introduction.

Human milk oligosaccharides (HMOs) can make up to 15% of total weight of human breast milk and there are about 200 different HMOs identified. In comparison, bovine milk oligosaccharides make up less than 1% of bovine milk and there are only about 40 different species. The amount and complexity of HMOs are unique among mammals. Interestingly, the composition of human breast milk with regard to HMOs changes during breast feeding/lactation time to optimally nurture newborns and infants [1]. HMOs have found their way into infant formula to better mimic the natural composition of human breast milk. HMOs were reported to beneficially modulate the gut microbiome [2], gut epithelial barrier [3], they are anti-inflammatory [4] and good for innate immunity development [5]. Many of the gastro-intestinal effects found for HMOs are also interesting for skin care. Particularly the rise of the skin microbiome topic is prone to be a target for HMOs with their known benefits for the infant gut microbiome. In addition, other topics are of interest, too, as HMOs are implicated in protecting infants from atopic disorders [6]. Furthermore, it was found for example that 2'-Fucosyllactose (2'FL) was able to attenuate particulate matter induced inflammation in keratinocytes [7]. Since recently, it is possible to synthesize HMOs synthetically or via biochemical processes [8]. All this makes HMOs interesting candidates as cosmetic active ingredients.

Our study aimed at testing various HMOs for their anti-inflammatory and anti-oxidative stress activity in skin in response to solar irradiation.

Materials and Methods.

<u>Human milk oligosaccharides</u>

The human milk oligosaccharides use in this study were obtained from Glycom A/S (DSM Early Life Nutrition), Hørsholm, Denmark.

Gene-expression analysis

Human full thickness reconstructed skin (EpidermFT-400, MatTek Life Sciences, Ashland, MA) was maintained at 37°C in a 5% CO_2 atmosphere with 95% relative humidity. Irradiation was done using a Hönle SOL 500 solar simulator with an H2 filter attached. UVB (200 mJ/cm^2 total dose) was monitored using a PMA2106 UVB detector (SolarLight). Tissues were placed in Dulbecco's phosphate buffered saline (DPBS) during irradiation. Cytotoxicity was monitored by lactate dehydrogenase (LDH) assay (Takara MK401). Tissues were first pre-treated topically with 0.5% HMOs in water for 24 hours and then exposed to solar irradiation. After irradiation, test substances were re-applied and incubated for an additional 24 hours before RNA isolation. RNA quality was assessed by the A260/280 method. cDNA was synthesized and amplified by qPCR using Taqman® gene expression assays in an OpenArray format on a QuantStudio 12K Flex instrument (Life Technologies). Data analysis and statistics were run by ThemoFisher Connect Software (Life Technologies). Linear relative quantitation (RQ) values were converted to linear fold-change values to simplify data interpretation.

Intracellular Reactive Oxygen Species (ROS) Scavenging Activity Assay

Full skin explants (Ø 8mm from surgical waste, phototype III-IV according to Fitzpatrick scale) were treated with the test items (20 µl homogeneously spread on the epidermis side with a finger cot) overnight. Afterwards a non-fluorescent probe, 2′, 7′-Dichlorofluorescein diacetate (DCFH-DA) was added. DCFH-DA is taken up by cells by passive diffusion and DCFH-DA is deacetylated by cellular esterases to non-fluorescent DCFH, which was trapped within cells. The fluorescent 2', 7'- dichlorofluorescein (DCF) was generated upon enzymatic reduction and subsequent oxidation by ROS. After treatment with DCFH-DA, the skin explants were exposed to different environmental conditions: either blue light 412 nm 20 J/cm^2, blue light 450 nm 20 J/cm^2 or unexposed in the dark. After exposure, tissues were lysed with 2% Triton X-100. Fluorescence was measured at 465/88 nm excitation and 528/30

nm emission in a Synergy HTX multimode microplate reader. Results were correlated to the test item's ability to scavenge or exacerbate ROS.

Results.

HMOs suppress UV-induced inflammation

We irradiated reconstructed full thickness skin and measured the inflammatory response by gene-expression analysis. We found a robust inflammatory response via significant up-regulation of inflammation markers such as colony stimulating factor (CSF2), various interleukins (IL-8, IL-1beta, IL-1RN, IL-23A), and prostaglandin synthetase (PTGS2) (Fig. 1). Pre-incubation of the tissue with 0.5% HMOs LNnT, 3'SL, 6'SL, and 3'FL reversed this inflammatory response to various degrees with LNnT having the broadest activity and 6'SL the narrowest one (Fig. 2).

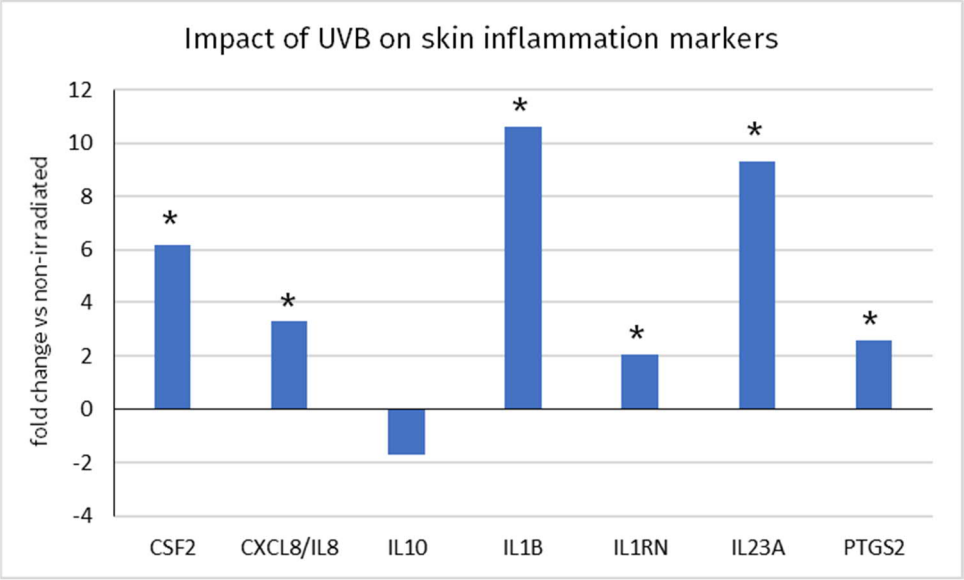

Figure 1: UV-irradiation induced inflammation markers in reconstructed human skin. *p<0.05 vs non-irradiated control.

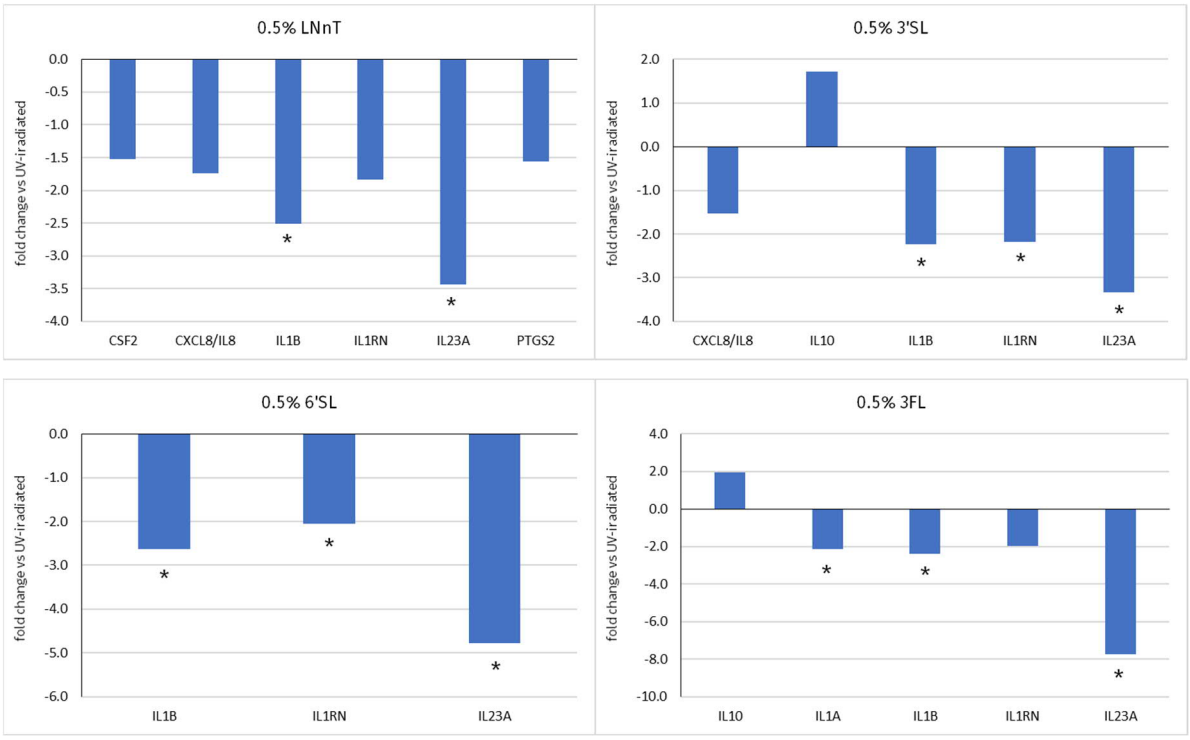

Figure 2: Effect of four HMOs on UV-induced expression of inflammation markers in reconstructed human skin. *p<0.05 vs UV-irradiated control.

Certain HMOs suppress HEV-induced ROS

After confirming the anti-inflammatory activity of HMOs on skin, we wanted to broaden their activity into the visible light spectrum. We therefore irradiated skin ex vivo with high energy visible (HEV) light (also known as blue light) of 412nm and 450nm. According to the literature, an inflammation response with HEV is limited, but one can easily induce oxidative stress via generation of reactive oxygen species (ROS) [9]. We found a significant down-regulation of ROS after irradiation with HEV at 412nm with the reference compound alpha-tocopherol (Vitamin E) and the HMO LNnT, while the HMO 3'FL had no effect (Figure 3).

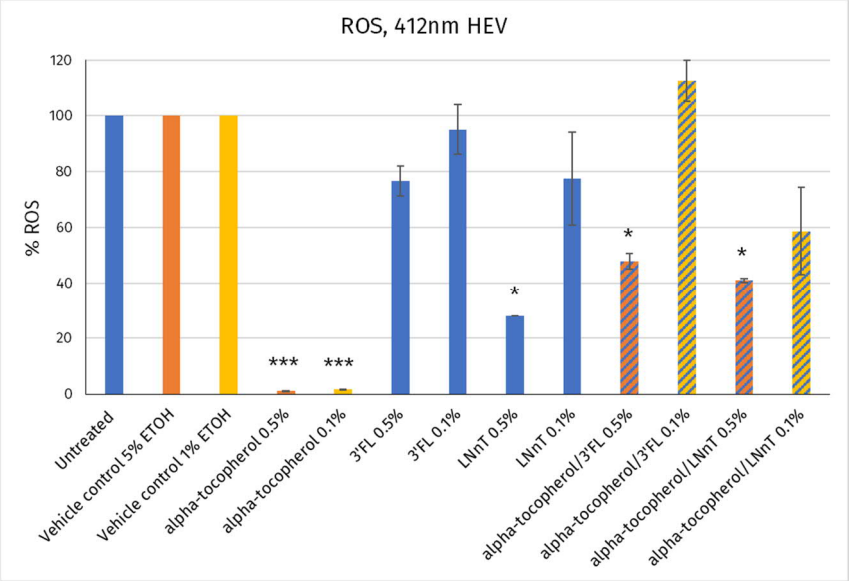

Figure 3: Effect of HMOs on HEV 412nm irradiated skin and ROS formation. ***p<0.001 vs EtOH control, 3p<0.05 vs untreated.

Regarding irradiation of skin with HEV at 450nm we found a positive effect of both 3'SL and again LNnT which were in the range of alpha-tocopherol, while 3'FL again had no effect (Figure 4).

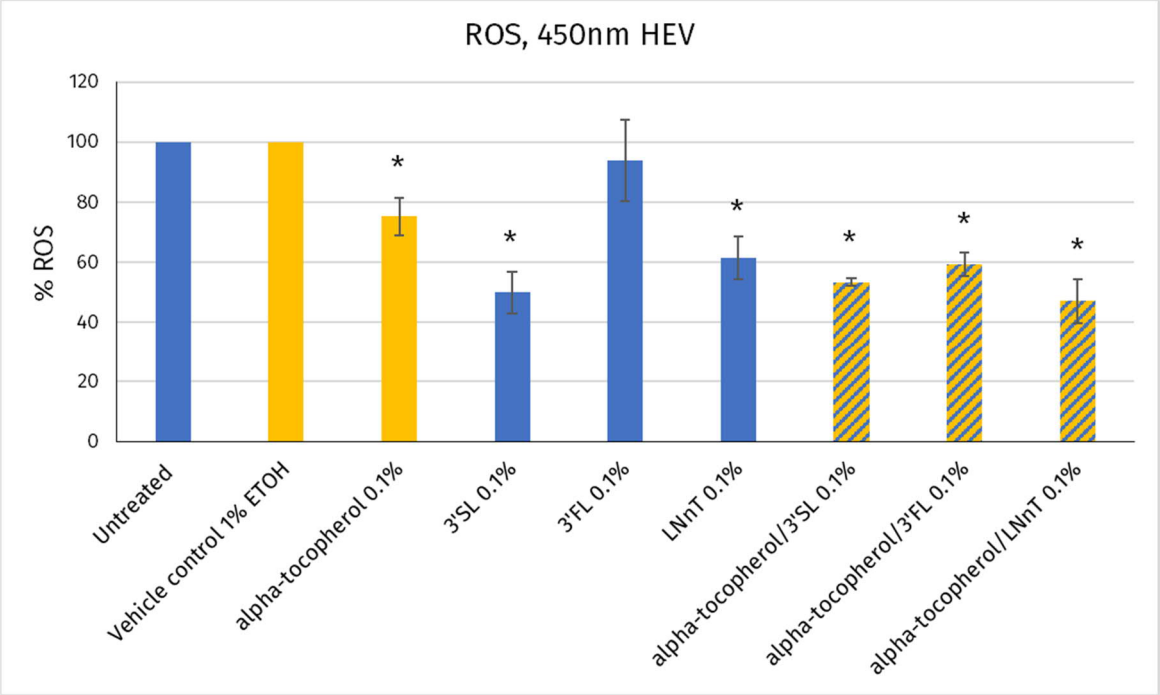

Figure 4: Effect of HMOs on HEV 450nm irradiated skin and ROS formation. *p<0.05 vs untreated or EtOH.

Discussion.

Human milk oligosaccharides are well known for the beneficial effects on gut health, particularly infant gut health [2]. As these beneficial effects are also interesting for skin care applications, HMOs are under evaluation as cosmetic active ingredients. We tested potential skin care activities of a range of HMOs with respect to inflammation and oxidative stress in response to solar irradiation. Our data provide evidence that HMOs are indeed acting in a similar way on skin as in the gut. In line with previously published data, we found anti-inflammatory activity for LNnT which was shown to have anti-inflammatory activity in wounds [10]. In addition, particularly sialylated HMOs prevented intestinal inflammation in rats [11] and promoted inflammation resolution in mice [12]. We also show here that both 3'SL and 6'SL had an anti-inflammatory profile (Fig. 2). The different HMOs tested in this study showed differential anti-inflammatory activity with LNnT havin the broadest activity and 6'SL having the narrowest activity against inflammatory cytokines, highlighting the various specificities of these HMOs. Interestingly, an anti-inflammatory activity was shown

for 2’FL in HaCaT keratinocytes in response to particulate matter [7], but we did not find such an anti-inflammatory effect of 2’FL in our UV-irradiated system (not shown).

It was recently also shown that 2’FL can attenuate the oxidative stress response in the intestine of aging mice, potentially by regulating the sirtuin1 (SIRT1)-related and nuclear factor E2-related factor 2 (Nrf2) pathways [13]. In our case, we found a significant down-regulation of ROS in skin after HEV irradiation at 412 nm and 450 nm by LNnT and 3’SL underlining the potential of HMOs to protect from major skin aging pathways.

Conclusion.

In summary, we provide evidence that HMOs are attractive novel skin care molecules. Our findings highlight anti-inflammatory and anti-oxidative stress activities. As such, HMOs could find their way into anti-aging, photoprotection or after sun care applications to name a few.

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Conflict of Interest Statement. NONE.

References.

1. Rousseaux, A., et al., Human Milk Oligosaccharides: Their Effects on the Host and Their Potential as Therapeutic Agents. Front Immunol, 2021. 12: p. 680911.
2. Zhang, S., et al., Gold standard for nutrition: a review of human milk oligosaccharide and its effects on infant gut microbiota. Microb Cell Fact, 2021. 20(1): p. 108.
3. Natividad, J.M., et al., Blends of Human Milk Oligosaccharides Confer Intestinal Epithelial Barrier Protection in Vitro. Nutrients, 2020. 12(10).
4. Cheng, L., et al., The Human Milk Oligosaccharides 3-FL, Lacto-N-Neotetraose, and LDFT Attenuate Tumor Necrosis Factor-alpha Induced Inflammation in Fetal Intestinal Epithelial Cells In Vitro through Shedding or Interacting with Tumor Necrosis Factor Receptor 1. Mol Nutr Food Res, 2021. 65(7): p. e2000425.
5. Donovan, S.M. and S.S. Comstock, Human Milk Oligosaccharides Influence Neonatal Mucosal and Systemic Immunity. Ann Nutr Metab, 2016. 69 Suppl 2: p. 42-51.
6. Han, S.M., et al., Do Human Milk Oligosaccharides Protect Against Infant Atopic Disorders and Food Allergy? Nutrients, 2020. 12(10).
7. Lee, K.E., et al., 2'-Fucosyllactose Attenuates Particulate Matter-Induced Inflammation via Inhibition of Hypoxia-Inducible Factor in Keratinocytes. Biol Pharm Bull, 2019. 42(10): p. 1620-1627.

8. Patel, S. and A. Goyal, Functional oligosaccharides: production, properties and applications. World Journal of Microbiology and Biotechnology, 2010. 27(5): p. 1119-1128.
9. Liebel, F., et al., Irradiation of Skin with Visible Light Induces Reactive Oxygen Species and Matrix-Degrading Enzymes. J Invest Dermatol, 2012. 132: p. 1901-1907.
10. Farhadihosseinabadi, B., et al., Inducing type 2 immune response, induction of angiogenesis, and anti-bacterial and anti-inflammatory properties make Lacto-n-Neotetraose (LNnT) a therapeutic choice to accelerate the wound healing process. Med Hypotheses, 2020. 134: p. 109389.
11. Zhang, W., et al., Sialylated human milk oligosaccharides prevent intestinal inflammation by inhibiting toll like receptor 4/NLRP3 inflammasome pathway in necrotizing enterocolitis rats. Nutr Metab (Lond), 2021. 18(1): p. 5.
12. Pessentheiner, A.R., et al., The Human Milk Oligosaccharide 3'Sialyllactose Promotes Inflammation Resolution and Reduces Atherosclerosis Development in Mice. bioRxiv, 2021.
13. Wang, J., et al., 2′-Fucosyllactose Ameliorates Oxidative Stress Damage in d-Galactose-Induced Aging Mice by Regulating Gut Microbiota and AMPK/SIRT1/FOXO1 Pathway. Foods, 2022. 11(2): p. 151.