Original Article

Nutrient supplementation influence on keratinocytes' metabolism: an *in vitro* study

Influência da suplementação de nutrientes no metabolismo dos queratinócitos: estudo in vitro

Authors:
Flavia Alvim S Addor [1]

[1] MSc in Dermatology from the Faculdade de Medicina da Universidade de São Paulo (USP) – São Paulo (SP), Brazil;Technical Director, Medcin Instituto da Pele – Osasco (SP), Brazil.

Correspondence:
Dra. Flavia Alvim S Addor
Alameda das Bauhinias 176 - Tamboré 10
06544-540 - Santana de Parnaíba – SP

ABSTRACT

Introdução: Modern lifestyles often lead to eating habits that lack a balance of nutrients. Some of those substances directly interfere with the metabolism of skin appendages.
Objective: To evaluate the combination of vitamins A, C, E, B complex, zinc, magnesium, and iron, and their effects at the cellular level.
Methods: A controlled in vitro study involved culturing keratinocytes, with the incubation of a combination of nutrients containing ascorbic acid, retinol, tocopherol, B complex, zinc, magnesium, and iron at daily recommended doses.
Results: There was a significant increase in cell maturation compared to the control.
Conclusion: The combination of nutrients caused an increase in the expression of keratin synthesis.
Keywords: nutritional policy; hair; nails; keratins.

RESUMO

Introdução: *O estilo de vida moderno muitas vezes leva a alimentação sem balanceamento de nutrientes. Algumas dessas substâncias interferem diretamente no metabolismo dos fâneros.*
Objetivo: *Avaliar a associação de vitaminas A, C, E e complexo B, zinco, magnésio e ferro, e seus efeitos no nível celular.*
Métodos: *Foi desenvolvido estudo experimental in vitro através de cultura de queratinócitos, com incubação de associação de nutrientes contendo ácido ascórbico, retinol, tocoferol, complexo B, zinco, magnésio e ferro nas doses diárias recomendadas.*
Resultados: *Houve aumento significativo da maturação celular em comparação ao controle.*
Conclusão: *a associação de nutrientes aumentou a maior expressão da síntese de queratina.*
Palavras-chave: *recomendações nutricionais; cabelo; unhas; queratinas.*

Received on: 6 March 2011
Approved on: 10 May 2012

This study was carried out at Farmoquímica Laboratórios S/A - São Paulo (SP), Brazil.

Conflict of interest: the *in vitro* study was sponsored by Farmoquímica Laboratórios S/A – São Paulo (SP), Brazil.
Financial support: the *in vitro* study was sponsored by Farmoquímica Laboratórios S/A

INTRODUCTION

There is currently a paradox related to nutrition. On the one hand, there is broad scientific knowledge about nutrients and their importance for maintaining good health, as well as sources and daily requirements. The nutritional value of every food – natural or processed – is widely studied and publicized. [1] Nevertheless, lifestyles that involve eating quick and easy meals, and diets and physical exercises that are conducted without adequate guidance, can lead to nutritional deficiencies that may result in symptoms and clinical signs that are difficult to diagnose and treat. [2]

Therefore individuals with apparently normal nutritional status, or even those considered overweight, can have specific nutritional deficiencies regarding a particular oligoelement or even a few vitamins. [3]

The increase in life expectancy also entails a number of peculiarities regarding degenerative diseases, the use of drugs or physiological conditions that affect the absorption or utilization of nutrients, resulting in deficiencies that are often under-diagnosed. [4]

In order to study the role of nutrients and their actual value in the quality of the proliferation and differentiation of keratinocytes – which affect the structural conditions of hair, such as strength and shine – a combination of vitamins and oligoelements was evaluated in a keratinocytes culture (Figure 1) to assess the influence of this system on cell proliferation and maturation.

OBJECTIVE

The objective of this in vitro experimental study was to evaluate the effect of a combination of nutrients (NC) containing ascorbic acid, retinol, tocopherol, B complex, zinc, magnesium, and iron (in the recommended daily doses) on keratinocytic proliferation and maturation.

METHODS

Ex vivo human keratinocytes were seeded in 75 cm^2 flasks, cultured and expanded in a moist greenhouse at 37°C, in the presence of 5% of CO^2, using a specific standard culture medium. Upon reaching confluency, the cells were seeded in six-well plates for the incubation of the NC and the evaluation of the proposed parameters.

After the seeding, the cells were incubated for 96 hours, with two non-cytotoxic concentrations of the NC that were previously determined using the MTT technique (3-(4,5)-dimetiltialzolil -2.5 diphenyltetrazolium bromide test), which is used to evaluate cellular viability. [5] The concentrations used in this study were 0.006% and 0.003%, in addition to the control.

Cell cultures were photographed before (D0) and after 96 hours of incubation with the NC. An inverted microscope – with a lens system that allows the visualization of thick materials such as culture plates (DMIL, Leica, Germany) – was used, and the images were captured using a DFC300 FX system (Leica, Germany). After having been photographed, the cultures were trypsinized and the cells from each group were counted in a Neubauer chamber so that the maturation could be assessed.

STATISTICAL ANALYSIS

The ANOVA methodology was used in the statistical assessment of variance. The Tukey test was used when the ANOVA detected significant differences between groups. Statistical significance was set at p values below 0.05 for all groups.

ETHIC ASPECTS

This experiment (the use of human cells in optimal culture conditions) was approved by the Ethics in Research Committee of the Faculdade de Ciências Médicas da Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil.

RESULTS

Cellular maturation

In the group of control cells (untreated), no significant increase in the keratinocytes' maturation was observed at 96 hours (Figure 2).

In the group of cells incubated in 0.03% NC, there was an increase in cells presenting maturation stages compatible with spinous and granular cell, characterized by the presence of terminal keratin, as indicated by the arrows in Figure 3. The same findings characterized the group incubated at 0.06% NC, however without a significant difference between groups (Figure 4).

Cellular proliferation

Although there was a higher rate of cellular proliferation in the group with a higher concentration of NC, there were no significant differences between groups, as shown in Graph 1.

DISCUSSION

Changes in hair and nails can occur due to primary nutritional deficits, or they can be secondary to systemic diseases that lead to malnutrition, such as f malabsorption (ulcerative colitis), thyroidopathy, or neoplasias. [6] Recently, some more common conditions, such as deficiencies secondary to bariatric surgery or anorexia, have also been described more frequently. [7,8]

One the most common deficiency states, which is growing in prevalence, is the so-called hidden hunger: the chronic deficiency of one or more micronutrients, with no specific signs or symptoms. It usually occurs in tissues with higher metabolic and mitotic rates. The cutaneous system is therefore often affected by this deficiency. [9] In hidden hunger, although the diet is often rich in carbohydrates, lipids and even proteins, vitamin and mineral reserves decrease gradually.

Hair and nails are mentioned in the literature as frequent areas for the typical signs of deficiency states. [10,11] Primarily, there are changes in the protein synthesis of those structures, which may be reduced or changed in structure.

Hair anda nails's keratin may lose its chemical and physical features if there are nutritional deficiencies. Modifications in the

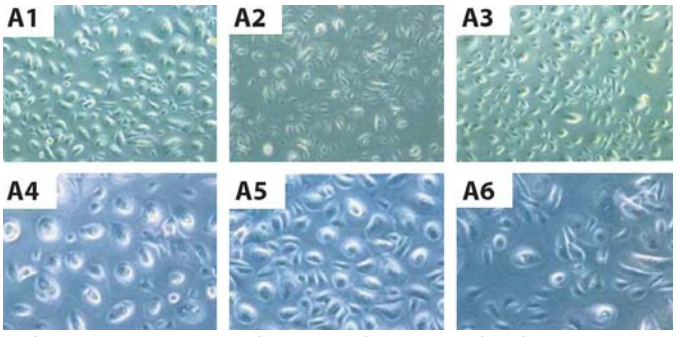


Figure 1: Keratinocytes culture, control group, initial evaluation (A1, A2, and A3 magnified 10X; A4, A5, and A6 magnified 20X)

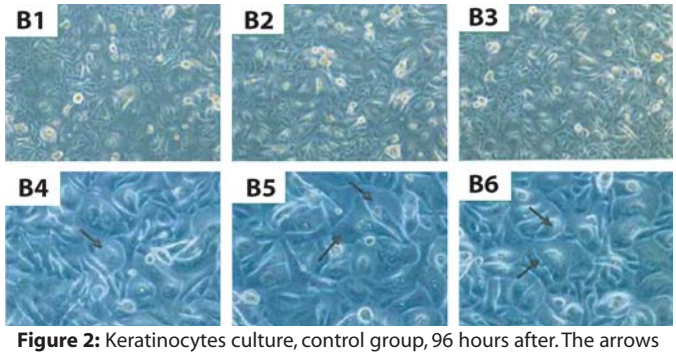


Figure 2: Keratinocytes culture, control group, 96 hours after. The arrows represent terminal keratin (B1, B2, and B3: 10X magnification; B4, B5, and B6: 20X magnification)

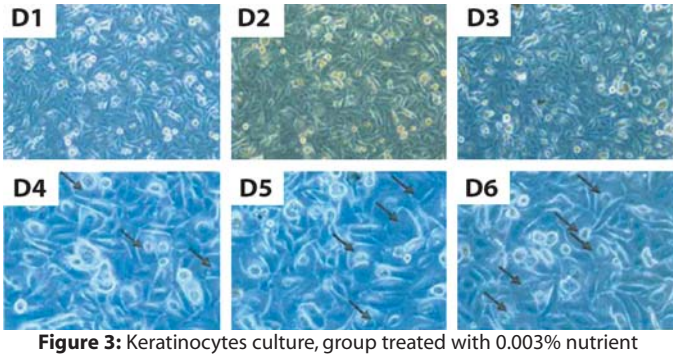


Figure 3: Keratinocytes culture, group treated with 0.003% nutrient solution, 96 hours after. The arrows represent terminal keratin (D1, D2, and D3: 10X magnification; D4, D5, and D6: 20X magnification)

protein spatial structure in turn reduce water retention and result in signs such as dryness and brittleness of the nail and hair shafts.

The chemical profile of normal nails is basically composed of modified keratin with a low water content, in addition to sulfur, magnesium, calcium, iron, zinc, sodium, and copper. [12] Changes in nail consistency and loss of strength with onychoschizia and onychorrhexis can occur. [13] Alterations can include dystrophy (similar to onychomycosis), brittleness, longitudinal grooves, onychocryptosis, and periungueal erythema. [14]

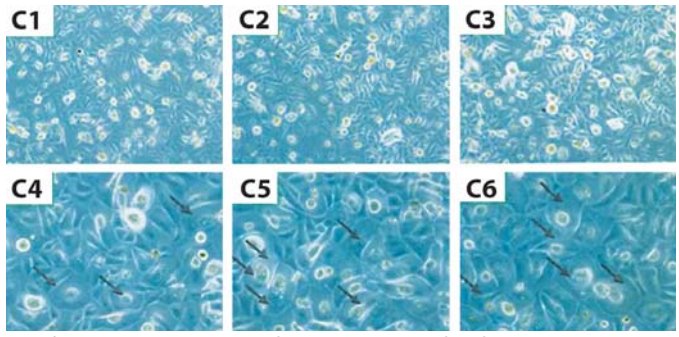


Figure 4: Keratinocytes culture, group treated with 0.006% nutrient solution, after 96 hours. The arrows represent terminal keratin (C1, C2, and C3: 10X magnification; C4, C5, and C6: 20X magnification)

Brittle nail syndrome is a common condition and one of the suggested causes for nutritional deficiency of B complex vitamins and iron. The nails become brittle, and often the problem becomes chronic because there is no precise diagnosis. [15]

Hairs may undergo many modifications according to the degree and type of nutritional deficiency. Commonly, there may be diffuse hair fall following a frontal pattern; hairs become opaque and there is increased telogenization, in addition to a decreased growth rate. [16, 17]

Alterations in the strength of the hair shaft, such as trichorrexis, changes in color and fractures, can also be observed. [18 19]

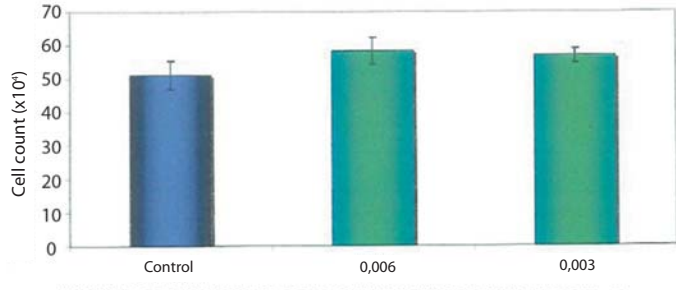
Acute or even chronic telogen effluvium is often caused by nutritional deficiency – especially deficiencies of vitamins or oligoelements. This condition is more common in situations of high physical stress, such as the puerperium, or poor diets or malabsorption syndromes. [20, 21]

Nutrients, hair and nails

Some vitamins and oligoelements interfere directly with the metabolism of hair and nails, which are cofactors of enzymatic reactions, or theirs structural elements. [22,23]

Vitamins

Vitamin A (retinol): in addition to being an antioxidant, retinol regulates the proliferation and differentiation of kerati-



Graph 1: Cell proliferation rate (cell count) by group: control, 0.006%, and 0.003%

nocytes, including also one of the annexes. [24]

Vitamin E (tocopherol): tocopherol's broad antioxidative action is present in most of the synthesis reactions and cellular metabolism of keratinocytes. There is evidence of a positive correlation between an increase in lipid peroxidation and the worsening of diffuse alopecia, however this relationship is still being studied.[25]

Vitamin C (ascorbic acid): vitamin C is also a potent antioxidant, acting as a cofactor in collagenesis and favoring the maintenance of dermal support for the epidermal annexes. Recent studies suggest that L threonate – an ascorbic acid metabolite – helps prevent androgenetic pattern alopecia by inhibiting androgen expression in the dermal papilla. [26]

B Complex: vitamins B1 (thiamine), B3 (Niacin), B5 (pantothenic acid), and B6 (pyridoxine) have an essential role in the energy metabolism of keratinocytes, acting on the speed of cellular proliferation. [27,28] Niacin, in particular, also acts on the synthesis of melanin and collagen. [27] Pyridoxine and vitamin B12 (cyanocobalamin) also have important roles as cofactors in the processes of keratinocytes' cell proliferation. [29]

A deficiency of vitamin B12 can lead to dyschromia in the hair and nails, which is fully reversible with vitamin supplementation. [30] Biotin is an essential cofactor in the synthesis of epidermal,nail and hair keratin. [31] There is evidence of a positive response to the use of biotin supplementation in the treatment of brittle nails. [32] Folic acid acts as a coenzyme in the metabolism of amino acid and nucleic acid synthesis, and is essential in the processes of epidermal differentiation and cell proliferation. [33]

Oligoelements

Iron: in addition to its crucial role in the cell oxygenation process, it acts as a cofactor in many of the keratinocytes' enzymatic processes. Its deficiency can materialize in morphological alterations in the nails (koilonychia). [34]

Zinc: its best-documented role is as a coenzyme in the synthesis and repair of nucleic acids. [35] Its deficiency is associated with diffused hair loss, which is reversible with supplementation. [36]

Selenium: it acts with vitamin E as a cofactor in antioxidative processes.[37]

The extent to which cells use nutrients, especially cells with a higher metabolic rate (proliferation and maturation) depends on the bioavailability and association profile of those molecules. The goal is to promote synergistic effects between vitamins and oligoelements. Thus, combinations of synergistic nutrients can enhance the utilization, and therefore the effects. [38,39]

CONCLUSION

The quality and speed of keratin synthesis is influenced by an individuals' nutritional status and is one of the first tissues to suffer changes due to a deficiency in vitamins or oligoelements. Combining nutrients in those cells creates a positive influence: it increases the expression of keratin synthesis by accelerating the keratinocytes' maturation. ●

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