

Laminine Improves Telomerase Activity to Repair Telomeres and Shows Safety in a Cytotoxicity Study

Cells are often referred to as the building blocks of life because all organisms are composed of one or more cells and all cells making up the variety of tissues in a human, come from preexisting cells. All vital body functions occur within cells and most importantly, cells contain the hereditary information necessary for regulating cell function. Changes in cell function occur based on factors such as nutrition, environment, exercise and stressors. Nutrition is considered of utmost importance.

Nutritional supplements are available to substitute any lack of or absorption of normal daily requirements of nutrients. When nutrients are not absorbed or metabolized correctly homeostasis may be disrupted and the body may not function properly leading to disease or illness. For instance, vitamin C deficiency can lead to disease such as scurvy and higher risk of mortality while doses of vitamin C beyond the minimal amounts have shown heart related benefits (1).

Some naturally occurring polypeptide proteins are involved in cell regeneration and growth. These polypeptide factors are naturally occurring proteins in our body. They deal with many aspects of repair and healing such as cellular growth, proliferation, increased vasculature and changing of stem cells to specific cells in our body (3). Usually they are a protein structure. They normally act as signalers between cells to regulate our body. Hence, they play an important role in maintaining health and wellbeing. Some of these protein factors play an important role in many aspects of maintaining health. Besides being involved in cell growth, they can play a role in development and healing. They also can help maintain stem cells in a growth and unchanged state. Some protein factors may play an important part in wound healing and enhancement of bone and cartilage formation (4). It has been shown that insufficient levels of some protein factors may play a key role in the incidence of excessive anxiety (5).

Telomeres are a part of the DNA material located at the end of the chromosomes and protect the chromosome from deteriorating (like the plastic tips at the end of shoe laces). Recent investigations have shown that telomeres play a major role in healthy cell division and cell ageing. As cell division occurs over time new cells replace older cells, to renew organ systems. Telomere length and telomerase, the enzyme that repairs and helps elongate shortened telomeres, has now been shown to be related to the aging process. Telomere length and telomerase enzyme activity is thus associated with less damage to cell division and helping to sustain healthy cell division. Telomere length has become of interest to scientists as an indicator of cellular integrity and programmed cell "aging" or senescence. It has been found that telomerase can add back lost base pairs to the ends of chromosomes restoring a measure of length and functionality to the chromosome.

They play an essential role in preventing aging of human cells (6,7). Each time a cell divides, telomeres are shortened. Telomere shortening plays a critical role in all aspects of aging and disease (8). However, the enzyme telomerase can lengthen telomeres by adding back lost nucleotide sequences to the end of existing chromosomes. If telomerase is activated in a cell, the cell can continue to divide. Otherwise the cell will age and can become damaged or deteriorate. Hence, greater amounts of telomerase activity in a cell may be better for the cell to maintain proper function and youth (9).

Laminine Helped Maintain Normal Cell Growth

We studied the effects of Laminine on skin cells in order to assess growth and repair (Figure 1). We used various concentrations of Laminine on skin fibroblast cultures to assure the product is safe and does not

impede cell proliferation which is indicative of growth and healing. For this experiment, we grow skin fibroblasts in multiple well dishes with growth medium containing varying concentrations of Laminine. We incubated the cells for 1 week and analyzed cell growth using a neutral red assay, a well-developed and widely used cytotoxicity test that can quantitatively estimate the number of viable cells (11). The data suggest that Laminine is not toxic to cells and can help maintain normal cell growth *in vitro* (Figure 1).

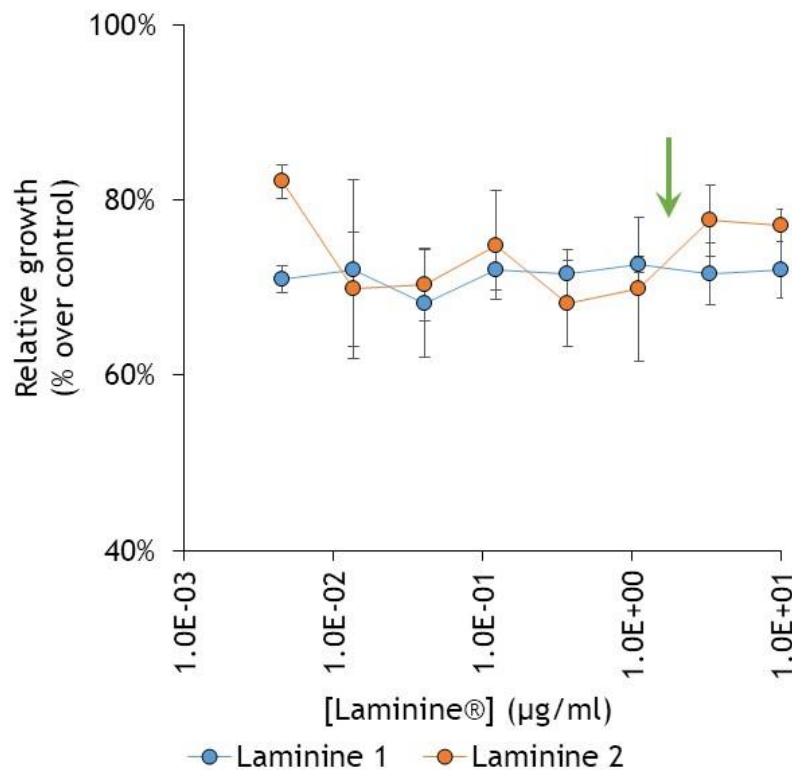


Figure 1: Effects of Laminine on skin fibroblast cells vs standard growth method (control). Laminine 1, Laminine 2: Different lots of Laminine. Arrow denotes the projected physiological concentration of Laminine when a person of average weight (150 lb) takes one Laminine capsule.

Telomerase Activity in Subjects Consuming Four Laminine Daily for Ninety Days

Since telomerase activity may play a critical role in maintaining health and longevity, we next performed a telomerase activity assay on samples of blood from patients that had consumed Laminine. Eight subjects consumed 4 Laminine daily for 90 days. We took blood samples from individuals at day 7 and day 1 prior to Laminine consumption (Pre), and 30, and 90 days post Laminine consumption in order to assess if Laminine changes levels of telomerase activity (Fig 2). The data suggest an increase in

telomerase activity for 50% of the patients and no decrease in telomerase activity during the 3 -month study. An average of the 8 patients suggested an overall increase of telomerase activity.

Individuals Consumed 4 Laminine Capsules Daily for 90 Days. The Results Showed Increased Telomerase Activity, an Indicator of Anti-Aging.

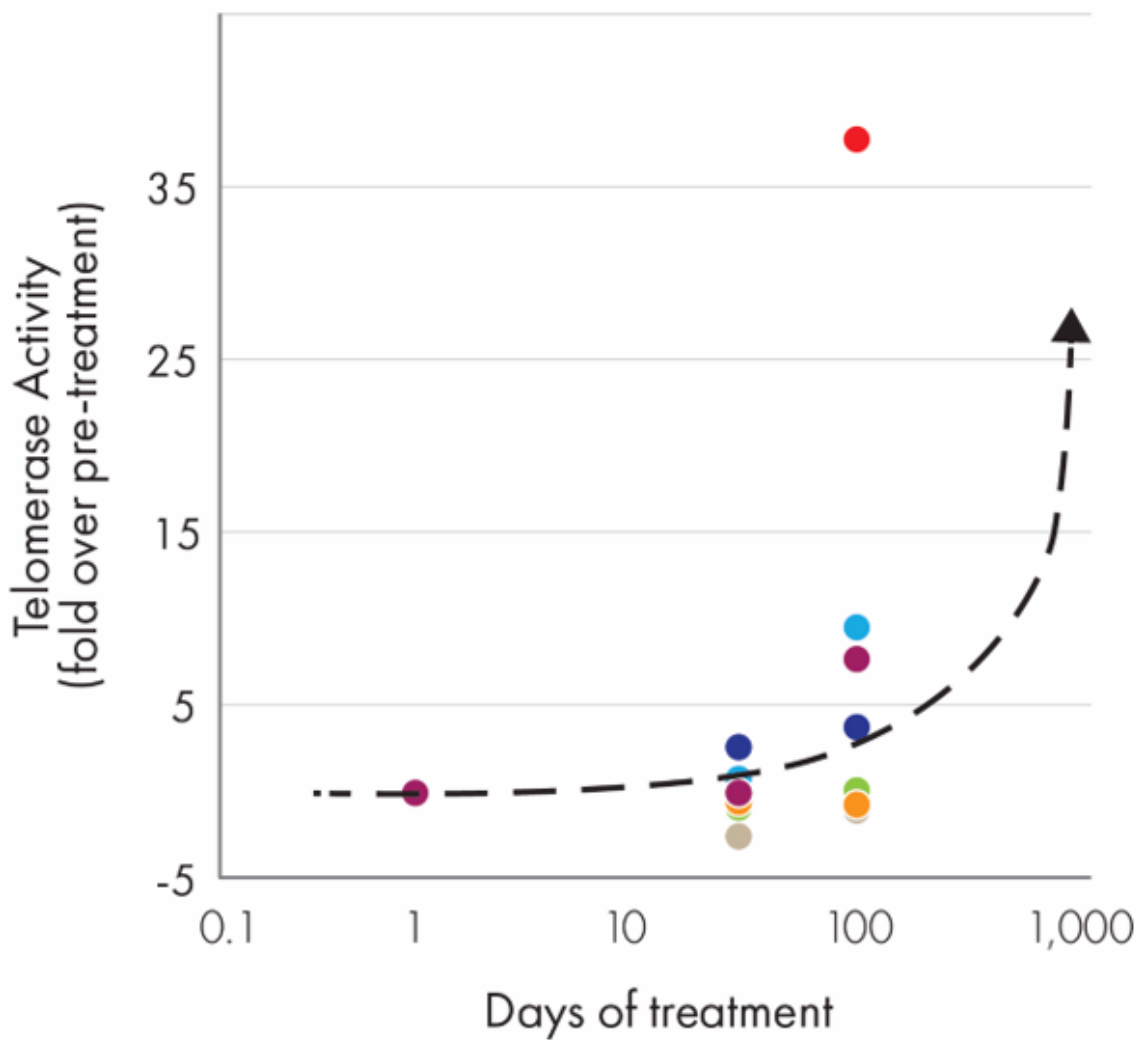


Figure 2. A) Relative telomerase activity of individual samples: Crude lysate from 4.5×10^3 cells of each sample was assayed for telomerase activity. The relative telomerase activity (RTA) was obtained by correction over heat-inactivated lysate; by normalization to an independent internal control of each reaction; and by comparison to the RTA of crude lysate from 4.5×10^3 HeLa cells. The RTA fold-changes of 30 day and 90 day post-treatment over the average of the two pre-treatment RTA were plotted.

Discussion

Supplements have been shown to provide many benefits. Supplements range from standard vitamins i.e. vitamin C, which are required for sustained health and alternative supplements that contain active ingredients that can activate other proteins and cause physiological changes in our bodies. In this article we assess Laminine, a product that is not your standard nutraceutical which may have benefits in cell growth and healing. In order to understand how a supplement works it is necessary to identify active ingredients and key mechanisms of action.

We undertook a study to assure that the product is safe by doing *in vitro* assays using the product Laminine on skin fibroblast cells. The purpose of this is to assure that the product does not impede cell growth and is not cytotoxic (Figure 1) which we verified using a neutral red assay.

Our next step was to look at changes in individuals systemically upon consumption of Laminine. For this we had 8 individuals do blood draws seven and one day prior to commencing recommended doses of Laminine in order to establish a baseline for testing immune cells in the blood. We chose to look at factors that influence cell growth, healing, repair and longevity.

We looked at telomerase activity (Fig 2). The data suggest that 50% of the patients may have improved telomerase activity. It remains to be seen if this caused an increase in telomere length for those individuals. Of note there was no significant decline in telomerase activity during the duration of the 3-month study which illustrated no negative impact on Laminine use.

In summary, our data suggest Laminine is a supplement that is safe to consume daily and may aid in host endogenous repair, healing and growth.

References

1. Moser M.A.; Chun O.K. Vitamin C and Heart Health: A Review Based on Findings from Epidemiologic Studies. *Int J Mol Sci.* 2016; 17(8).
2. Mankowski RT1, Anton SD, Buford TW, Leeuwenburgh C. Dietary Antioxidants as Modifiers of Physiologic Adaptations to Exercise. *Med Sci Sports Exerc.* 2015; 47(9):1857-68.
3. Fioravanti C, et al., Autologous blood preparations rich in platelets, fibrin and growth factors. *Oral Implantol (Rome).* 2016; 8(4):96-113.

4. Lee TJ, et al. Enhancement of osteogenic and chondrogenic differentiation of human embryonic stem cells by mesodermal lineage induction with BMP-4 and FGF2 treatment. *Biochemical and Biophysical Research Communications*. 2013; 430 (2): 793–7.
5. Perez J. A. et al. A new role for FGF2 as an endogenous inhibitor of anxiety. *J. Neurosci*. 2009; 29 (19): 6379–87.
6. Jaskelioff M, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*. 2011; 469:102-107.
7. Sahin E, DePinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*. 2010; 464:520-528.
8. Armanios M, Blackburn EH. The telomere syndromes. *Nature Reviews Genetics*. 2012; 13:693-704.
9. Cong Y, Wright W. E., Shay J. W. Human Telomerase and Its Regulation. *Microbiol Mol Biol Rev*. 2002; 66(3): 407–425.