Aging of lymphoid organs: Can photobiomodulation reverse age-associated thymic involution via stimulation of extrapineal melatonin synthesis and bone marrow stem cells?

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Short title: Can photobiomodulation regenerate the thymus?

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Abstract

Thymic atrophy and the subsequent reduction in T cell production are the most noticeable age-related changes affecting lymphoid organs in the immune system. In fact thymic involution has been described as "programmed aging". New therapeutic approaches such as photobiomodulation (PBM) may reduce or reverse these changes. PBM (also known as low-level laser therapy or LLLT) involves the delivery of non-thermal levels of red or near-infrared light that are absorbed by mitochondrial chromophores, in order to prevent tissue death and stimulate healing and regeneration. PBM may reverse or prevent thymic involution due to its ability to induce extrapineal melatonin biosynthesis via cyclic AMP or NF-kB activation, or alternatively by stimulating bone marrow stem cells that can regenerate the thymus. This perspective puts forward a hypotheses that PBM can alter thymic involution, improve immune functioning in aged people, and even extend lifespan.

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Aging is associated with morphological and physiological changes in various organ systems. These changes include degeneration of lymphoid organs associated with the immune system, including the thymus gland. The thymus is composed of two identical lobes and is located anatomically in front of the heart and behind the sternum (Figure 1). Each lobe of the thymus has two regions: a central medulla and a peripheral cortex, which is surrounded by an outer capsule. The cortex and medulla play different roles in the development of T-lymphocytes (T cells) that are of hematopoietic origin. Cells in the thymus can be divided into thymic stromal cells, and cells of hematopoietic origin (derived from hematopoietic stem cells resident in the bone marrow). The cortex is involved in positive selection and the medulla in negative selection. Stromal cells include epithelial cells of the thymic cortex and medulla, and dendritic cells [1]. The thymus provides a specialized environment for the development of T cells from hematopoietic progenitor cells. Thymic stromal cells allow for the selection of a functional and self-tolerant T cell repertoire.

The thymus is largest and most active during childhood (pre-adolescent period). By the early teens, the thymus begins to atrophy, and the thymic stroma is progressively replaced by adipose (fat) tissue throughout the lifetime. Nevertheless, some residual T lymphopoiesis continues throughout adult life. In age-related thymic atrophy, the maximum decline in the thymic weight occurs just before the start of the mid-phase of life, i.e., at approximately 30–40 years of age in humans [2] and 9–12 months of age in mice [3].

The process of thymic involution involves disruption of the histological structure of the thymus and a marked reduction in the production of naïve T cells [4-7] (Figure 2). These changes can be used as biomarkers of life expectancy [8]. Some commentators have even suggested that age-related thymic involution is evidence of “biologically programmed aging” [9]. Moreover the process is highly conserved amongst all vertebrate species that possess a thymus [7]. It is interesting to note that grafts of thymus tissue from neonatal mice into aged recipients leads to rejuvenation of various immunological and non-immunological functions, and improves the immune system leading to an extension in the lifespan of old mice [10]. There is also a disease state called “acute thymic involution, ATI” [11]. ATI is usually associated with infections (bacteria, viruses, parasites, fungi) but other conditions (stress, pregnancy, malnutrition and chemotherapy) may be responsible. ATI may be only a transient and reversible reduction in the size and weight of the thymus and some depletion of T cells, but severe disruption of the anatomical structure of the organ can be observed in some fungal, parasitic and viral infections.

Some promising therapeutic approaches to postpone or even reverse age-related thymic involution are currently under investigation. For example, administration of exogenous melatonin leads to a marked reduction in signs of thymic aging in mice [12, 13]. Also, the prolongevity ketogenic hormone, fibroblast growth factor 21 (FGF21) (sometimes called the "starvation hormone" protects against immune senescence and thymic involution [14]. The hunger hormone, ghrelin stimulates thymopoiesis [15], human growth hormone (hGH) partially reverses thymic involution [16, 17], upregulation of the transcription factor FoxN1 (forkhead box N1) expression reduces thymus involution [18-21], and some peptides and proteins (e.g., epithalon, thymalin, and interleukin 7, IL-7) regulate thymic cell growth [22-24]. To the best of our knowledge, no study has explored the possibility of using photobiomodulation (PBM) as a method to restore the structure and function of the aged thymus and delay/reverse involution.

Photobiomodulation (PBM) is the application of light in the visible red (600-700 nm) and near-infrared (760-1200 nm) regions of the electromagnetic spectrum. The power density should be between 1–500 mW per cm^2 so that heating of the tissue is avoided. The light is absorbed by chromophores [25] at the cellular, molecular, and tissue levels, [26, 27]. The photon absorption triggers a numbver of signaling pathways leading to activation of transcription factors and changes in protein expression. PBM acts via hormetic mechanisms leading to a biphasic dose response [28], but when the dose of light is appropriate, PBM can positively influence almost every physiological bodily function.

A large number of *in vitro,* i*n vivo* and clinical studies concerning PBM have been published over the last 50 years As of (today, PubMed returns almost 5000 scientific papers when one enters the keywords "low-level laser therapy" (LLLT) and "photobiomodulation"). These papers demonstrate a variety of effects, including improved blood flow [29], increased mitochondrial activity [30], reduced expression of genes encoding inflammatory mediators [31], altered biochemical functions [26], mobilization, proliferation and differentiation of stem cells [32], neuromodulation [27-30], and a reduction in both acute and chronic pain [33], neuromodulation [34-37], and a reduced incidence of acute and chronic pain [38, 39].

The phenomenon was discovered in 1964 by Endre Mester, published in 1967, whoand subsequently termed it "laser biostimulation" [40]. He intended to determine whether laser light applied to the back of shaved mice could induce cancer. The laser-treated mice did not develop cancer; but surprisingly, the hair grew back quicker than that on untreated animals [41].

The absorption of red and NIR light in the cells leads to a range of primary effects and second messengers (ATP, ROS, Ca^{2+}) which in turn leads to increased expression of nuclear transcription factors (e.g., NF-κβ, Nrf2, and AP-1) [27, 42-44]. These induced changes trigger cell proliferation and migration, and modulate the release of cytokines (IL-1α, IL-2, IFN-γ, and TNF-α), growth factors (GH and FGF), and inflammatory mediators in general. Overall there is an increase in tissue oxygenation [45-48] and cellular antioxidant defenses are up-regulated (i.e., glutathione [49], superoxide dismutase [50-52], and catalase [52-55]). In addition, other parts of the visible light spectrum have different physiological effects; for example, blue light regulates vasodilation [56], and induces oxidative stress *in vivo* via ROS generation [57], and inhibits mitosis and cell division [58, 59], and blue, green light and 980 nm light affects stem cell proliferation and differentiation *in vitro* [60-63].

It is becoming increasingly clear that stem cells are highly responsive to light, compared to other types of somatic cells. Stem cells have the capacity for long-term self-renewal without senescence and the ability to differentiate into one or more specialized cell types thus providing an inexhaustible supply of cells for tissue repair. Totipotent stem cells (such as found in the embryo) can generate all different tissue types. Tissue-specific stem cells are found in niches throughout the body, such as bone marrow, brain, liver and skin, as a mechanism for maintenance of tissue homeostasis, and repair in later life. The stem cell niche is hypoxic, which allows stem cells to survive for decades without suffering from any oxidative damage. However when the mitochondria of stem cells absorb light, the phenotype changes, the cells produce many more mitochondria and must exit their hypoxic niche in search or more oxygen. This means that light delivered to the bone marrow can mobilize stem cells into the circulation where they become progenitor cells and are exposed to diverse cues in the bloodstream instructing them where to travel to in order to repair tissue that is damaged or risk of dying. Uri Oron in Israel has shown that by exposing the bone marrow in animal models to PBM either non-invasively or by introducing a fiber optic probe into the bone marrow, a range of diseases and conditions can be treated. These include lessening the infarct size after a heart attack [64], reducing the severity of ischemia-reperfusion-induced kidney injury [65], and ameliorating the severity of Alzheimer's disease in a transgenic mouse model [65, 66]. The improvement in heart attack outcome was also shown to work in a large animal model of myocardial infarction in Yorkshire swine [67]. Another group found that non-invasive PBM to the bone marrow could increase the platelet count and ameliorate thrombocytopenia caused by chemotherapy [68] or by an autoimmune disease [69].

We therefore believe that PBM could treat thymic involution by two different and potentially complementary mechanistic pathways. The first pathway involves stimulation of extrapineal

melatonin production (Figure 3), while the second pathway involves stimulation of bone marrow stem cells that could then replenish the thymus (Figure 4).

Melatonin is biosynthesized from tryptophan via serotonin, and the key enzyme is arylalkylamine N-acetyltransferase (AANAT) or serotonin N-acetyltransferase. Norepinephrine is released from sympathetic nerve fibers exclusively at night, and increases intracellular cAMP concentration via beta-adrenergic receptors, thus activating cAMP-dependent protein kinase A that phosphorylates and activates AANAT. During the daytime in the absence of noradrenergic stimulation, AANAT is immediately destroyed by proteasomal proteolysis. The circadian production of melatonin by the pineal gland is responsible for chronobiotic rhythyms affecting many physiological functions, including both endocrine and non-endocrine systems. Other functions of melatonin, including its antioxidant and anti-inflammatory properties, its genomic effects, and its capacity to modulate mitochondrial homeostasis, have been linked to the redox status of cells and tissues [70]. A recent review covers the effects of melatonin on T-cell biology and T-cell related diseases [71]. Melatonin is highly effective in modulating T-cell activation and differentiation, especially for Th17 and T-reg cells, and also memory T cells. Melatonin interacts with several different cell signaling pathways, including ERK1/2-C/EBPα, and calcineurin that have major functions in T-cell biology. Melatonin exerts beneficial effects in various inflammatory diseases [72]. However all melatonin does not necessarily come from the pineal gland, and the presence of melatonin has been detected in many different extrapineal tissues including the brain, retina, lens, cochlea, Harderian gland, airway epithelium, skin, gastrointestinal tract, liver, kidney, thyroid, pancreas, thymus, spleen, immune system cells, carotid body, reproductive tract, and endothelial cells [73]. In most of these tissues, the melatonin-synthesizing enzymes have been identified. Melatonin is present in essentially all biological fluids including cerebrospinal fluid, saliva, bile, synovial fluid, amniotic fluid, and breast milk. In several of these fluids, melatonin concentrations exceed those measured in the blood. Some studies report that some melatonin function (approximately 20%) persists even after pinealectomy [74], and that it is partially synthesized in the thymus [73, 75-77], the gastrointestinal tract [78], and other organs [79-81]. Melatonin affects numerous biological functions and processes, including (but not limited to) the propensity to sleep, control of circadian rhythms, and inhibition of reproductive processes. It also has an antiproliferative effect on cancer cells, increases immune responses, and has antioxidant as well as anti-aging effects [82].

A review of the literature suggests that not only retinal [83], but also whole body [84] and intranasal [85] irradiation with red light leads to a notable increase in serum melatonin levels in

humans. Moreover, there is growing speculation that melatonin might be an important mediator of many of the physiological changes that occur after photobiomodulation [86].

How could PBM increase extrapineal melatonin production? Two possible routes can be envisaged. The first route involves activation of the pleiotropic transcription factor NF-κβ that has been shown to occur after PBM [44]. NF-kB induces expression of the *AANAT* gene in macrophages, followed by synthesis of extrapineal melatonin [87]. Activation of kappa B elements (aa-nat-κB), localized in the promoter (nat-κB1 and nat-κB2), leads to Aa-nat transcription in RAW 264.7 macrophages. Competitive electrophoretic mobility shift assay (EMSA) with oligonucleotide probes corresponding to each of the two elements, as well as a NF-κB consensus corresponding probe, revealed different specificities for each κB element. In addition, activator protein-1 (AP-1) as well as signal transducers and activator of transcription-1 and 3 (STAT-1; STAT-3) competed with NF-κB for binding to nat-κB1, while only STAT-3 competed with NF-κB for binding to nat-κB2. The expression of RelA and cRel is essential for the induction of AANAT expression and melatonin synthesis. Considering that the expression of cRel is induced by the earlier expressed p50/RelA, the differential effects of NF-κB dimers may be intimately associated with the temporal regulation of inflammatory responses, with the resolution phase being associated with paracrine and autocrine melatonin effects [88]. Macrophages, lymphocytes, and epithelial cells represent a major fraction of thymic cells [89]. These cells may be the primary target of photobiomodulation and, together with cells in the sternum [79], lungs, and other surrounding organs, they contribute to extrapineal melatonin synthesis.

The second route involves the well-known ability of PBM to increase the expression levels of cAMP [90-92]. As mentioned above, cAMP activates protein kinase A which then phopsphorylates AANAT thus preserving its enzyme bioactivity and increasing melatonin synthesis.

Human and animal aging is associated with structural and functional changes in various systems, including deterioration of the immune system (immunosenescence) and reduced plasma melatonin levels [77, 93, 94]. Besides an important role in regulating immune function [95-97], melatonin might also alter age-associated pathological changes in the thymus [12, 13, 98-100], stimulate telomerase activity [101], and even increase the lifespan of rodents [102-105].

What is the best way to deliver light to the body in order to preserve and rejuvenate thymus function in aging humans? It is possible to directly irradiate the thymus with a focused laser spot through the chest wall [106]. Biphasic dose-dependent effects might be expected to be

associated with irradiation of the thymus area, and could vary from immunostimulation to immunosuppression depending on the cumulative dose applied [106-109]. Another possible method of light delivery is whole body illumination using a whole-body light bed, "light pod" or a large area LED array. This would have the advantage of stimulating the bone marrow at the same time bas stimulating extrapineal melatonin synthesis. A whole-body red light device was used in Chinese female basket-ball players to increase serum melatonin, improve sleep quality and improve athletic performance [84]. Thus far, no agreed and defined dosage regimen has yet been devised for administration of photobiomodulation to any partcular part of the body.

Further research is required to test the hypothesis that PBM has potential therapeutic value by restoring thymic morphology and improving immune function in individuals with age-related thymic involution via various mechanisms, including activation of extrapineal melatonin secretion and stimulation of bone-marrow stem cells. This perspective/hypothesis article, which aims to stimulate discussion in the scientific community, may be considered somewhat speculative in nature. However considering the numbers of elderly people who die from a seemingly trivial infection, that a healthy adult would have shrugged off, PBM could in theory make a big difference.

Disclosures

The authors have no conflicts of interest to declare.

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Figures

Figure 1. Structure of the thymus

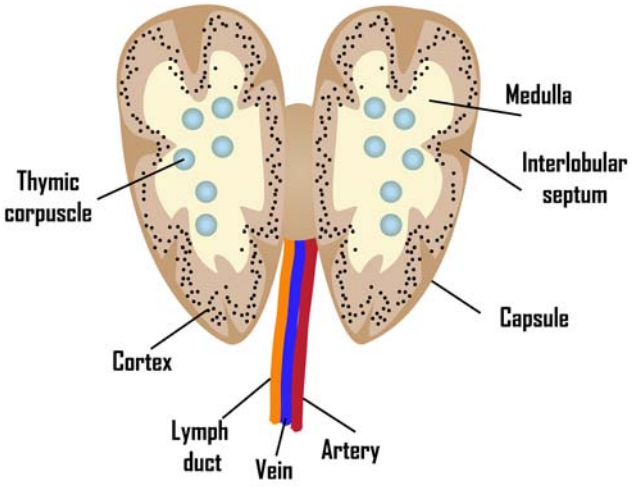

Figure 2. Age-related thymic involution. The perivascular space (PVS) increases dramatically as sex steroid production increases with age, and T-cell production falls with age. ETP = early T cell progenitor cells; TN = CD3, CD4, CD8 triple negative T cells; DP = CD4+, CD8+ double positive T cells; SP = CD4+ or CD8+ single positive T cells.

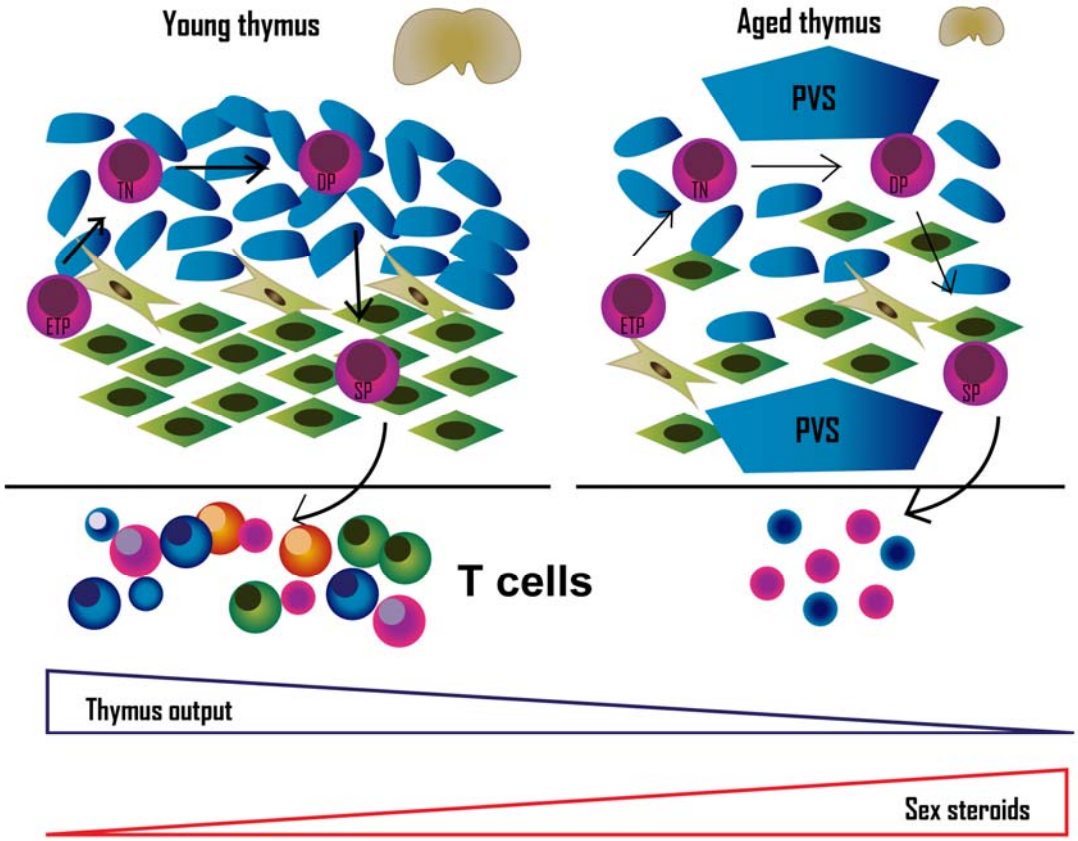

Figure 3. Mechanism of PBM stimulation of extrapineal melatonin biosynthesis.

ATP produced after PBM causes more cAMP leading to activation of protein kinase a and stabiization of AANAT. The burst of ROS from the mitochondria can activate protein kinase d which phosphorylates IkB and leads to proteasomal degradation. The released NF-kB travels to the nucleus where it activates gene transcription of AANAT. The increased enz yme activity of AANAT leads to biosynthesis of more melatonin from tryptophan that can rejuvenate the thymus.

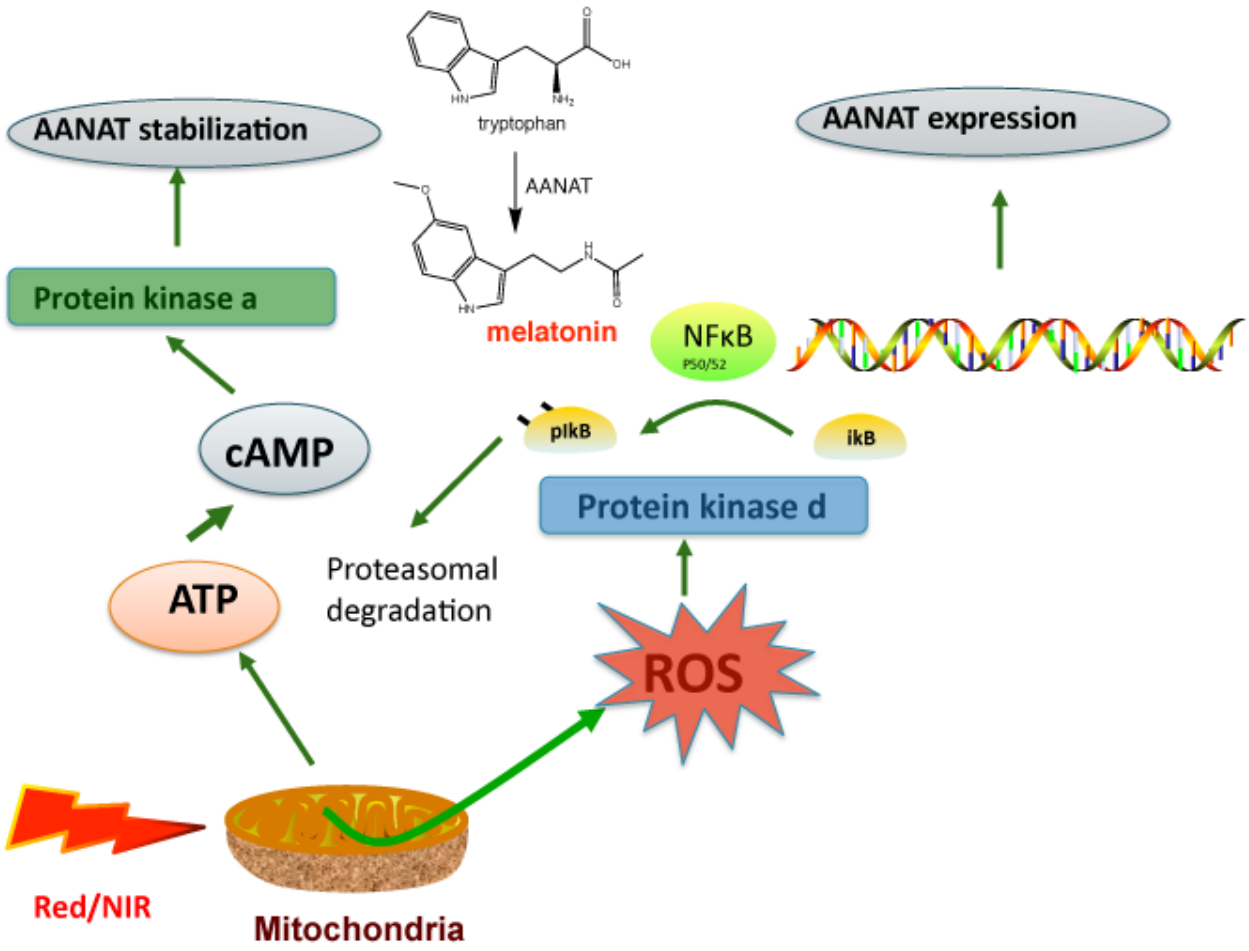

Figure 3. Mechanism of PBM stimulation of melatonin biosynthesis

Figure 4. Mechanism of PBM stimulation of bone marrow stem cells for thymus regeneration

PBM using different wavelengths of light is absorbed by chromophores within the cell, activates secondary messengers (ATP, ROS and Ca^{2+}) that go on to activate signaling pathways in bone marrow stem cells. This causes the cells to leave their hypoxic niche and proliferate. When exposed to cues from the aging thymus, these progenitor cells can replenish the T-cell production.