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The evolving role of the NAD+/nicotinamide metabolome in skin homeostasis, cellular bioenergetics, and aging

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ABSTRACT

Human skin is exposed to daily environmental insults, particularly solar radiation, that triggers a range of molecular responses. These perturbations to the normal homeostatic state can lead to cellular dysfunction and, ultimately, impacts tissue integrity and accelerates skin aging (photoaging). One of the responses is increased oxidative stress which has been shown to disrupt cellular bioenergetics. This can be detected by depletion of the nucleotide energy metabolites NAD+ and ATP as both an acute transient decrease and, over time, a more permanent chronic reduction due in part to cumulative damage of mitochondria. NAD+ and its primary precursor nicotinamide have been known for some time to impact skin homeostasis based on linkages to dietary requirements, treatment of various inflammatory conditions, photoaging, and prevention of cancer. Cellular NAD+ pools are known to be lower in aged skin and treatment with nicotinamide is hypothesized to restore these levels, thereby mitigating cellular bioenergetics dysfunction. In dermal fibroblasts, nicotinamide is able to protect against oxidative stress to glycolysis, oxidative phosphorylation as well as increase mitochondrial efficiency via sirtuin-dependent selective mitophagy. Recent research has found that NAD+ cellular pools are more dynamic than previously thought, oscillating in tandem with free nicotinamide, and serves as a regulatory point and feedback loop in cellular metabolism regulation, maintenance of mitochondrial efficiency, and circadian rhythmicity. Since UV-induced oxidative stress in skin can disrupt these processes, continued molecular understanding of the role of NAD+ and nicotinamide in skin biology is important to identify interventions that would help maintain its normal homeostatic functions and efficient cellular bioenergetics.

1. Impact of dysfunctional cellular bioenergetics on skin homeostasis and premature aging

One of the critical functions of skin in human physiology is to provide protection from damaging environmental stressors that the body experiences on a daily basis. These stressors comprise a broad range of forms that include solar UV radiation, chemicals, tobacco smoke, fossil fuel pollutants, seasonal climate changes, and even internal in the form of cortisol from psychological stress [1]. Of these, solar UV radiation has been most extensively studied since it is known to be such a significant factor in both acute and chronic damage in skin [2,3]. These effects, particularly on more exposed sites such as face and hands, range from transient sunburn to measurable photoaging to the more extreme health risk of skin carcinomas. UV can cause direct macromolecule damage in the form of DNA mutations/deletions, protein modification, and lipid peroxidation. Cellular and molecular responses include inflammatory signaling, altered gene expression patterns, and reactive oxygen species (ROS) production with concomitant oxidative stress and inflammation [2–4]. While cells are able to repair and recover from some of these perturbations [5], long term UV exposure still leads to cumulative structural effects that include erythema and chronic inflammation (inflamm-aging), elevated and inconsistent melanin pigmentation, epidermal thinning, loss in dermal matrix content, and lowered elasticity, all of which can be histochemically and clinically measured [6,7].

The free radical theory of aging was originally proposed based on findings that reactive oxygen species and various free radicals appear to be key drivers of the intrinsic aging process by causing oxidative stress and cumulative damage to cells and tissue [8]. This phenomenon is especially relevant in skin due to the significant elevation in free radicals that can be induced by solar UV radiation, thereby accelerating the free radical damage process via oxidative stress, leading to premature photoaging, UV-induced

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immunosuppression, and skin cancer onset [9–12]. It has been known for some time that oxidative stress related to intrinsic and premature aging can alter cellular metabolism [13] via lower mitochondrial efficiency and overall lowered energy production [14,15]. Over the past decade, there has been a significant increase in research efforts on understanding the direct causative link between lowered mitochondrial efficiency and integrity in such aging-related diseases as cancer, premature aging, obesity, and neurodegenerative and cardiovascular disorders [16–18]. It is hypothesized that this damage to mitochondria is due in part to the accumulation of mtDNA deletions and mutations which cannot be repaired. Since the mitochondrial genome encodes for various subunits that make up the electron transport chain complexes, altered expression patterns would have a direct effect on ATP synthesis efficiency [13]. This phenomenon has been referred to as heteroplasmy in which there is a mixture of wild type mtDNA with mutated copies. If the percentage of mutated mtDNA reaches a threshold, mitochondrial efficiency drops off since mutated mtDNA gene expression is functionally recessive. The relative thresholds have been estimated to range from 50% to 60–70% for deletions and point mutations, respectively [19]. This damage to the mitochondrial genome is further compounded by the fact that dysfunctional mitochondria have been proposed to generate a higher rate of ROS, in what is viewed as a "vicious cycle" that further amplifies the damage [19,20]. This phenomenon supports what has been proposed as the mitochondrial free radical theory of aging [21]. Similar to internal tissue, mtDNA mutations and deletions in epidermal keratinocytes and dermal fibroblasts sourced from skin can function as predictive biomarkers of intrinsic aging as well as an amplified profile in photoaged skin, both from acute and chronic UV exposure [19,22,23]. Interestingly, since the keratinocytes in the epidermis are a renewable source of tissue, mtDNA damage increase is most significantly observed following an acute insult such as sunburn and does not display long term accumulation as a function of aging. In contrast, dermal fibroblasts are much more static in their phenotype and thus are a better indicator of cumulative mtDNA damage due to aging and environmental insults. This damage to mtDNA from either acute damage or chronic accumulation in skin cells strongly supports that there is a direct impact of mitochondrial efficiency, which decreases with age and has been hypothesized to be a key factor in premature aging associated with photoexposure [24–26].

2. Role of the NAD+/nicotinamide metabolome in cellular bioenergetics and skin homeostasis

Nicotinamide is a member of the vitamin B family (B_3, niacinamide) and serves as a precursor for synthesis of the important enzymatic cofactors NAD+ and NADPH [27]. Besides helping maintain cellular redox status, research over the past decade has found that NAD+ serves an important regulatory function as a limiting substrate for several important enzymes. More specifically, NAD+ serves as a key substrate for poly(adenosine diphosphate-ribose) polymerase-1 (PARP) enzymatic activity involved in DNA repair and genome stability [28] as well as for the sirtuin family of deacetylation enzymes that regulate metabolism via selective deacetylation of metabolic enzymes, histones and in an epigenetic manner [29,30]. Additionally, NAD+ and its precursors are thought to play a critical regulatory role as a signal transducer in regulating metabolic programming, circadian rhythmicities, and overall cellular homeostasis [31,32]. This growing body of research has begun to show direct linkages between NAD+ metabolism, sirtuin activity and downstream regulatory points associated with longevity [33,34]. While measurement of cellular NAD+ pools suggests a resting state is overall held constant, the pool actually undergoes a constant dynamic flow between NAD+ and free nicotinamide due to the parent compound being utilized in a destructive manner during catalysis by PARP and sirtuins. To maintain a steady level, free nicotinamide is rapidly incorporated back into NAD+ pools via the salvage pathway involving NAMPT and NMNAT. More recently, it has been shown that NAD+ pools undergo fluctuations under a diurnal pattern that serve as a key regulator of mitochondrial metabolism that links to phases of fasting and feeding [35]. This has been associated with the role that NAD+ plays as a key metabolism "oscillator" or "rheostat" in regulating alterations in gene expression patterns controlled by key clock genes such as BMAL and CLOCK [30,36]. Since there is such a clear dependence of the NAD+ pool facilitating SIRT1 activity during circadian and metabolic regulation, it has been proposed that SIRT1 can also be classified as an "enzymatic rheostat" [37]. It is known that *in vitro* nicotinamide can act as an inhibitor of sirtuins, it is not clear on the physiological implications since the average IC_{50} value of nicotinamide for PARP and sirtuin members has been reported as 50 μM [38]. However, endogenous levels of free nicotinamide have been calculated to be ~1 μM and cellular NAD+ pools to be at 50 μM [39]. Thus, significant elevations of free nicotinamide produced from high sirtuin would lead to a natural feedback loop that reduces the enzymatic activity, allowing for an oscillation back. As nicotinamide reincorporates into NAD+ via the salvage pathway, the oscillation in reverse can occur as the inhibitory pressure on sirtuin activity is removed by nicotinamide as it reincorporates into the NAD+ pool. In the extreme situations where there is significant cellular and DNA damage, depletion of NAD+ pools allows for free nicotinamide to accumulate and thereby inhibiting sirtuin and PARP activity, which one would speculate this would allow an abandonment of repair efforts and allows progression into apoptosis.

One of the original associations between nicotinamide and skin physiology was the discovery that reduced dietary intake has a causative linkage with pellagra, a reversible condition which symptoms include skin dermatitis [40]. A recent hypothesis has used this disease as a basis to propose that alterations in cellular bioenergetics may be a significant causative linkage to aging and many degenerative and inflammatory diseases due to a limitation of NAD+, causing a mismatch and "protonopathies" to develop [41]. Over the past few decades, topical usage of nicotinamide has been shown to provide positive treatment effects on inflammatory conditions such as rosacea, autoimmune bullous dermatoses, and acne [42,43] as well as improving skin barrier integrity, reducing sebum levels, and reducing the appearance of hyperpigmented spots and redness [44–47]. More recently, nicotinamide has been shown to mitigate some of the significant acute and chronic damaging effects of UV exposure on skin [48–50]. It has been reported that nicotinamide can prevent UV-induced gene expression of the inflammatory mediators IL-6 and TNFα [51] as well as DNA damage by enhancing the repair of cyclobutane pyrimidine dimers (CPDs) and 8-oxo-7,8-dihydro-2′-deoxyguanosine [50]. These observations can be mechanistically linked in part to original work that found topical and oral nicotinamide can prevent UV-induced immunosuppression and photocarcinogenesis in rodent models [52]. This effect has been replicated in humans via both topical and oral administration [53,54]. Ongoing clinical studies have found that oral nicotinamide can significantly reduce actinic keratosis incidence compared to placebo-treatment groups, highlighting the potential for oral nicotinamide to provide a certain degree of prevention of non-melanoma skin cancer [55]. Overall, it is clear that both orally and topically administered nicotinamide can provide positive effects on skin biology and homeostasis [49].

Based on established biochemical pathway understanding as described above as well as *in vitro* and *in vivo* studies, it is widely believed that one of nicotinamide's primary mode of

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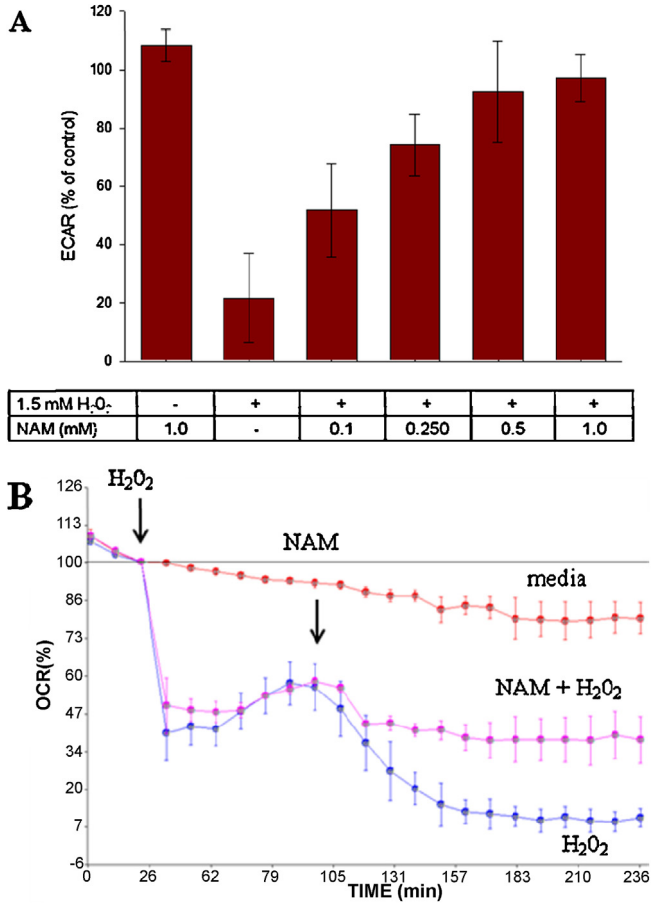

Fig. 1. Impact of nicotinamide (NAM) on protecting cellular bioenergetics under hydrogen peroxide-induced oxidative stress conditions [60]. (A) NAM dose dependent effect on maintaining glycolytic rates (ECAR - extracellular acidification rate) in dermal fibroblasts when exposed to 1.5 mM H_2O_2. (B) Ability of 1 mM NAM to partially restore oxidative phosphorylation (OCR - oxygen consumption rate) rates in dermal fibroblasts after oxidative stress induction by 1.5 mM H_2O_2.

action in human skin is to rapidly incorporate and resupply cellular NAD+ pools, thereby facilitating and/or restoring efficient metabolism and ATP synthesis ([32,56,57,71]). Relative to cellular bioenergetics, nicotinamide has been shown to protect keratinocytes from UV-induced cellular ATP loss as well as against UV-induced glycolytic blockade [58]. This correlates with what we have recently shown in that nicotinamide can restore hydrogen peroxide-induced decreases in glycolytic rates (Fig. 1A) in a dose dependent manner and that FK866, a NAMPT inhibitor [59], can reduce this effect by 60% [60]. While this affect was robust for glycolysis, nicotinamide was only able to partially prevent the reduction in oxidative phosphorylation rates from stress (Fig. 1B). This may be due in part cellular compartmentalization of NAD+ metabolism [57]. Nicotinamide cannot directly incorporate into mitochondrial NAD+ pools because the salvage pathway is absent in mitochondria and requires conversion to the intermediate, nicotinamide mononucleotide, by NAMPT in the cytoplasm [61]. Nicotinamide mononucleotide can subsequently transport into mitochondria where it is incorporated into NAD+ by NMNAT [62]. An important surveillance program called mitophagy exists in cells to monitor mitochondrial efficiency, particularly in the electron transport chain. This sensing selectively drives fusion and fission of functional mitochondrial, thereby increasing overall cellular metabolic output and reducing the number of dysfunctional mitochondria. Extracellular nicotinamide was found to increase mitophagy efficiency in fibroblasts via an increase in NAD+/NADH ratios and activation of SIRT1, suggesting an important role as a metabolic sentinel for mitochondrial quality [63]. Furthermore, it had reported that nicotinamide protective effects on glycolysis and ATP levels from UV damage are due in part to inhibition of PARP [58], supporting previous findings of UV-induction of PARP activity as part of the DNA repair process [64]. However, it was suggested that the effect could not be explained fully by this mechanism and that incorporation into the NAD+ pool could be involved. Since the protective effects were not completely blocked by FK866, it stands to reason that both NAD+ synthesis and PARP inhibition are components of nicotinamide mechanism of action for protecting glycolysis, and to a lesser extent, oxidative phosphorylation. Mechanistically, the protective effect of nicotinamide on cellular bioenergetics under acute stress conditions involves in part rapid incorporation into depleted cytoplasmic NAD+ pools.

Human *in vivo* data is more limiting but what has been published does support the linkage between nicotinamide and incorporation into NAD+ cellular pools. Direct analysis of NAD+ shows in human skin samples collected from the pelvic region that there is a significant depletion in total pool content as a function of age [65]. Interestingly, this correlated with an increase in PARP activity, suggesting an increased requirement of DNA repair in aged skin that would cause the NAD+ pools to remain lower. More recently, usage of two-photon microscopy has allowed for the quantitation of NADH and NADPH in viable epidermal layers. Following arterial occlusion, an increase in NADH fluorescence was noticed, suggesting a reduction of oxidative phosphorylation due to the lowered need for electron donation from NADH to NAD+ [66]. Indirectly supporting this were findings that showed a decrease in fluorescence emission from NADPH in the facial skin of older aged females compared to younger ones [67]. Further indirect evidence is the ability of a niacin ester analog to increase in NAD+ after topical application [68]. Relative to cellular bioenergetics, microarray analysis of human skin samples collected after nicotinamide treatment has led to the mechanistic hypothesis that part of its protective effects involves protection of cellular metabolism [53], including both oxidative phosphorylation and glycolysis [58,69]. These findings are supportive of the more recent *in vitro* bioenergetics studies described above.

3. Summary

Exposure of human skin to environmental stressors leads to a range of changes that includes oxidative stress. Oxidative stress causes changes in cellular metabolism capacity that is hypothesized to be a critical component in causing cellular dysfunction and thereby implicated from a cumulative effect of leading to premature aging as well as the more serious condition of non-melanoma skin cancer induction.

Given the significant increase in research and growing awareness of the role of NAD+ as a signal transducer and regulatory agent [70], continued studies assessing nicotinamide's effects on protecting the skin's cellular bioenergetics from the aging process as well as damage from environmental insults are merited. Mechanistically the evolving role of NAD+ and nicotinamide in cellular metabolic regulation and circadian rhythmicities mediated by master regulators such as sirtuins and AMPK is important to further explore in the context of skin homeostasis and aging. The integration of these various research efforts can be visualized around a central role for nicotinamide and NAD+ with linkages to various mechanisms and processes known to be involved in skin homeostasis. This connectivity could be envisioned as the "NAD+/nicotinamide metabolome" (Fig. 2). This understanding of the molecular changes that occur in skin from both acute exposures as well as the long term cumulative effects is important in order to identify intervention

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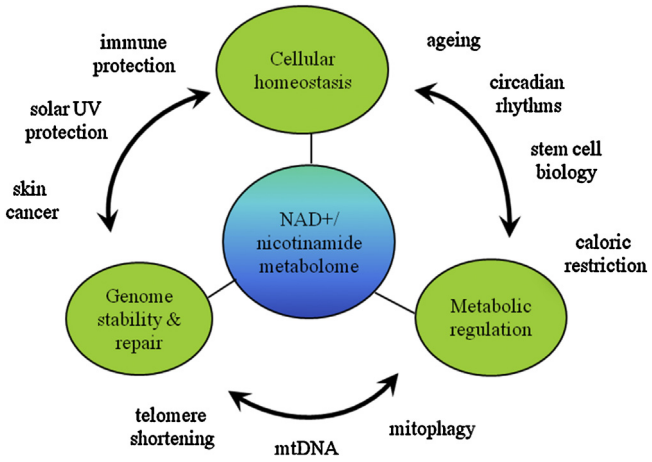

Fig. 2. The evolving central role of NAD+ and its primary precursor nicotinamide as regulatory point and signal transducer in cellular bioenergetics and skin homeostasis.

approaches that would potentially be able to prevent and restore skin to a normal homeostatic state.

Conflict of interest

There is no conflict of interest. The author is a full-time employee of The Procter & Gamble Company (Cincinnati, OH) and any referenced work from the author was funded in its entirety by The Procter & Gamble Company.

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