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Caffeine and Its Pharmacological Benefits in the Management of Androgenetic Alopecia: A Review

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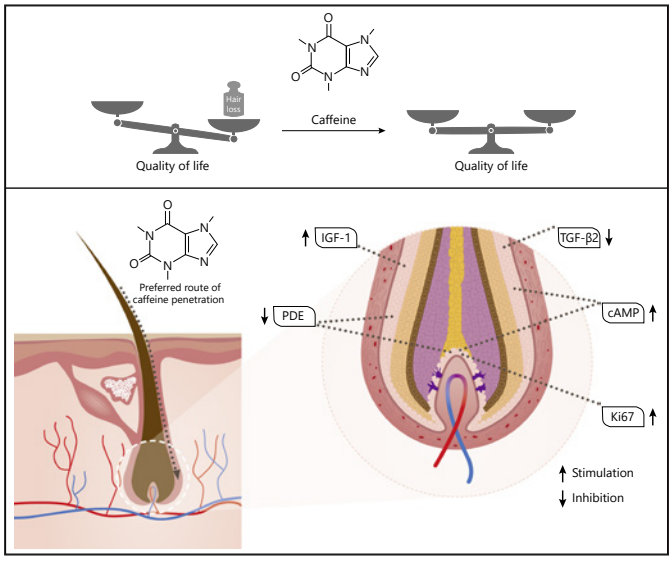
Keywords
Hair loss · Androgenetic alopecia · Caffeine · Skin and hair follicle

Abstract
Caffeine, particularly after ingestion, is well known to exert various pharmacological effects. A growing body of evidence implicates the ingestion of caffeine with beneficial effects on several diseases. The easy penetration of caffeine across the skin barrier and into human skin makes caffeine an ideal compound for topical application. Hair loss is known to negatively affect the quality of life and predispose to depression and anxiety. Androgenetic alopecia (AGA) is the most common type of hair loss in both men and women. To date, only few approved drug-based treatments for AGA exist, and these are inevitably associated with side effects. Therefore, the development of topical treatments based on well-tolerated natural ingredients such as caffeine to alleviate hair loss may provide a much-needed alternative to drug-based approaches.



Importance of hair loss regarding quality of life and caffeine's impact on hair cycle regulation factors. ↑, stimulation; ↓, inhibition; IGF-1, insulin-like growth factor-1 (outer root sheath); cAMP, cyclic adenosine monophosphate (hair matrix and outer root sheath); PDE, phosphodiesterase (hair matrix and outer root sheath); TGF-β2, transforming growth factor beta-2 (outer root sheath); Ki67, antigen KI-67 or MKI67 (Ki-Kiel) (hair matrix).

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Introduction

Caffeine

Caffeine is the most widely consumed psychoactive or central nervous system (CNS) stimulant in the world [1]. This plant secondary metabolite belongs to the group of xanthine alkaloids and is found in more than 60 different plant species but can also be produced synthetically. Caffeine is consumed in beverages and foods. It is a well-known component of coffee and green and black tea as well as chocolate and is used as a food additive, for example, in soft drinks or bottled water. The caffeine content ranges from as little as 2 mg caffeine per 100 mL in beverages based on cocoa powder to as much as 200 mg of caffeine per 100 mL of strong espresso coffee [for an overview of caffeine content in common food products, see 2]. In addition, various pharmaceutical products contain caffeine including over-the-counter pain and weight-loss medications and numerous prescription drugs, including Midol® to treat menstrual symptoms, Vanquish® for headache and general pain relief, orphenadrine as a prescription muscle pain medication, and Fiorinal® and Synalgos®-DC as prescription headache medications [3–6].

Caffeine is rapidly and completely absorbed after ingestion. It crosses cell membranes, including the blood-brain barrier, and distributes throughout the body. Although best known as a CNS stimulant, caffeine also acts on myocardial tissue, respiration, smooth muscles, and kidneys (for a thorough review of the pharmacology of caffeine, the reader is referred to Arnaud [7] and references therein). More recently, antifibrogenic, anti-inflammatory, and antioxidant activities of caffeine have been reported [8]. Further effects of caffeine have been observed in the microvasculature in the skin, including an enhanced microvascular function in cutaneous arterioles and capillaries [9] and improved endothelium-dependent microvascular responses in the forearm skin [10].

Caffeine has been linked to an increased metabolism [11] and is considered to be an ergogenic aid [12]. Several effects of caffeine are thought to enhance performance, particularly for endurance sports (see Pesta et al. [13] for an overview of these effects). In 1984, caffeine was banned by the World Anti-Doping Agency (WADA) after reports of its usage as a doping agent [14, 15]. It was removed from the list in 2004, as tests were not able to distinguish between social caffeine use and abuse [14], but remains on the WADA monitoring list [16].

The stimulating effects of caffeine after ingestion are primarily mediated via adenosine receptors (ARs). ARs are located in the central and peripheral nervous system as well as in various body organs and compartments, such as the heart and blood vessels. The AR-ligand adenosine regulates the release of neurotransmitters in the brain and modulates myocardial oxygen consumption and blood flow in the heart. As caffeine acts as an antagonist of AR, it can, for example, indirectly affect the release of neurotransmitters, including dopamine, acetylcholine, serotonin, and gamma-aminobutyric acid (GABA) [17, 18]. Additionally, caffeine inhibits intracellular phosphodiesterase (PDE) enzymes. PDEs regulate cyclic nucleotide signaling and are coupled to diverse physiological functions: Inhibition of PDE increases intracellular concentrations of cyclic adenosine monophosphate (cAMP), which in turn activates several enzymes and transcription factors linked to fat oxidation and lipolysis. An increase in lipolysis results in a stimulation of cell metabolism due to higher levels of energy [19–21]. Taken together, caffeine can increase metabolic activity and therefore promote cell proliferation via providing higher energy levels to the cells [22].

Benefits and Safety of Caffeine

Over the last decade, more and more publications have emerged that associate ingestion of coffee with beneficial effects in various diseases, covering dementia, liver diseases, diabetes, several types of cancer, and skin conditions as referred to in this paragraph. While it is not always clear if these effects are specifically due to caffeine or due to other substances or combinations of substances contained in coffee, several studies have found a specific association of positive effects of coffee ingestion with caffeine.

The consumption of coffee has been reported to be linked with a decreased dementia risk. Midlife coffee consumption in general was associated with a decreased late life dementia risk, while caffeine intake in particular was associated with a decelerated cognitive decline [23]. A reduction in the frequency of liver disease through consumption of coffee/caffeine has also been observed [24], whereby caffeine consumption from regular coffee above a threshold of approximately 2 coffee cup equivalents per day was accompanied with less severe hepatic fibrosis. Furthermore, coffee intake is associated with a decreased risk of type 2 diabetes (T2DM) [23]. Although epidemiological studies indicate that even decaffeinated coffee can reduce the T2DM risk [23], a reduction of T2DM risk in postmenopausal women was linked to caffeine-containing coffee only as caffeinated coffee increased levels of sex

hormone-binding globulin, which in turn has a strong inverse association with T2DM risk [25]. In various cancer entities, positive effects of coffee or caffeine have been described, for instance, in prostate cancer, breast cancer, and skin cancer. In a population cohort study, patients with newly diagnosed prostate cancer had a lower coffee consumption compared to the disease-free population [26]. Subjects with the highest consumption (>3 cups/day) had a 53% lower prostate cancer risk compared to those with the lowest consumption. Further investigations in prostate cancer cell lines found that caffeine significantly reduced their proliferative and metastatic behavior [26]. Regarding caffeine and breast cancer, protective effects of caffeine may be mediated through a reduced breast volume. Breast volume is associated with breast cancer risk in lean women, and coffee was associated with a reduction in breast volume in women carrying a specific gene variant, the CYP1A2*1F C-allele [27]. For basal cell carcinoma, caffeine intake from coffee and other dietary sources was inversely associated with basal cell carcinoma risk, while decaffeinated coffee consumption was not associated with a similar decrease [28]. Oral administration of caffeine affects the skin due to an inverse association between drinking coffee and the risk of incident rosacea. No decreased association was seen with decaffeinated coffee [29].

In general, oral caffeine consumption is safe for healthy adults at doses typically found in commercially available foods and beverages [6]. However, very high doses of ingested caffeine can produce undesirable effects on mental functions such as fatigue, nervousness, and feelings of anger or depression. The European Food Safety Authority (EFSA) concludes that for healthy adults daily intakes of up to 400 mg per day (about 5.7 mg/kg per day) do not raise safety concerns [2]. Caffeine is well known for its excellent skin penetration properties: In cosmetic formulations, caffeine is currently in use in concentrations up to 30 mg/mL [30, 31], whereby caffeine penetration through the skin is independent of the skin's thickness [32, 33].

The combination of ibuprofen together with caffeine (i.e., Thomapyrin® Tension Duo) has been found to provide better pain relief in acute pain and headache than ibuprofen alone [34]. The additive-drug-specific benefits have been demonstrated especially in postoperative pain and migraine headache for a range of different drug combinations. There is evidence that oral caffeine in combination with ibuprofen can deliver good analgesia at lower doses of ibuprofen. Recently, the effective delivery of caffeine as a hydrophilic model drug out of a topical applied formulation into and through hair follicles was demonstrated [35–37]. Therefore, it was shown that hair follicles and their surrounding regions are a possible treatment route targeted by optimized formulations. In addition to that, the potential of caffeine-catalyzed gels for novel, biocompatible oral drug-delivery systems and biomedical devices has been investigated [38]. In these tailorable systems, caffeine can act as a biocompatible catalyst to create a gel formulation that can be drug-loaded during manufacturing.

These recent investigations strongly emphasize the positive impact the versatile and safe natural active ingredient caffeine can have on human health on a variety of levels. Taken together, there is a growing body of evidence highlighting the positive effects of caffeine in several disease settings, indicating that caffeine is a potent substance associated with multiple beneficial effects. When applied in adequate doses, the ingestion and cosmetic application of caffeine can considered to be safe.

Caffeine and Hair Loss

The caffeine-induced inhibition of PDE enzymes increases intracellular cAMP concentrations, resulting in stimulatory effects on cell metabolism and proliferation. Hence, caffeine has a high potential to be beneficial in subjects suffering from hair loss that originates from premature termination of the hair growth phase [39]. Hair loss can lead to a variety of psychological concerns [40] including the development of depression and anxiety [41] and has a negative impact on the quality of life (QoL) [42]. It affects a person's sense of self and identity [43] as common responses to hair loss include the loss of self-confidence, lowered self-esteem, and heightened self-consciousness [42].

Common hair loss is a general condition related to aging. The most relevant type of hair loss is androgenetic alopecia (AGA). It affects at least 50% of men by the age of 50. AGA is heritable, androgen-dependent, and occurs in a defined pattern [44]. The direct androgen linkage in female AGA is still under current investigation. In this review, both types of hair loss are referred to as AGA. However, female pattern hair loss would be the more adequate term for this characteristic clinical presentation and distribution pattern of scalp hair loss in women. About 40% of women show this specific type of hair loss by the age of 70 [45]. The phenomenon of hair loss in AGA can be explained by the physiology of hair growth (hair follicle cycle).

A single growth period of a hair follicle can be divided into 3 characteristic phases: growth (anagen), involution

(catagen), and rest or rather quasi-quiescent (telogen) [46]. The growth phase of the hair follicle cycle is critical for the generation of new hairs, whereby the hair length (without being cut) is proportional to the length of the anagen phase (scalp hair: 2–8 years) [47]. The transition between the different phases of the hair follicle cycle is a well-regulated and controlled process [47]. The transition from anagen to catagen is regulated by key hair growth regulatory factors including the growth-maintaining insulin-like growth factor 1 (IGF-1) [48] and the catagen-promoting transforming growth factor-β2 (TGF-β2) [49]. Hence, higher expression of IGF-1 and lower expression of TGF-β2 are favorable for maintaining the anagen phase.

In AGA, androgen-stimulated hair follicle miniaturization occurs in genetically predisposed areas of the scalp [50]. In the presence of androgens the anagen cycle of affected hair follicles is shortened, leading to a transition from long anagen and short telogen phases to long telogen and short anagen phases [51]. Along with the reduction of the anagen/telogen ratio, there is a progressive decrease in the size of the hair bulb and hair thickness, which is symptomatically for the miniaturization of the hair follicle [50]. The conversion of testosterone to 5α-dihydrotestosterone by the enzyme 5α-reductase within the dermal papilla plays a central role in AGA [52]. The predisposed scalp exhibits an increased androgen receptor expression and high levels of 5α-dihydrotestosterone, the latter being a ligand of the androgen receptor fivefold more potent than testosterone, which results in thinner and shorter hair [52].

As AGA is associated with hair follicle miniaturization, it may in principle be reversed [47]. For a more detailed overview on this topic, the reader is referred to review articles [53–55].

Until today, caffeine and its pharmacological benefits in the management of AGA have been investigated in numerous studies in vitro/ex vivo and in vivo penetration studies as well as in clinical trials. An overview of the reviewed caffeine studies including type of study, method, gender, mode of action, and a summary of the results is presented in Table 1.

In vitro Studies

The influence of caffeine on hair follicles has been investigated in a hair organ-culture model (HOCM). This ex vivo model was established in the 1990s and enabled in vitro studies on hair follicles [56–58]. Anagen hair follicles are isolated by microdissection from the human scalp skin. These can then be maintained in William's E medium (supplemented with L-glutamine, insulin, hydrocortisone, and penicillin/streptomycin) for up to 10 days, during which they continue to grow at the in vivo rate and produce keratinized hair fibers. The HOCM has, for example, been used to study the influence of regulating factors such as TGF-β and IGF-1 in female hair follicles [56, 57]. Regarding caffeine, HOCMs have been used to investigate the effects of caffeine on testosterone-induced growth suppression in male hair follicles [59] and on the hair follicle cycle with a particular focus on the maintenance of the anagen phase in male and female hair follicles [22].

To assess the effects of testosterone (5 ng/mL–5 μg/mL) and caffeine (10–1,500 μg/mL) on hair follicle growth, scalp biopsies from the vertex area of males with AGA were taken, and the hair follicles together with their root sheaths were microdissected and maintained in the HOCM for up to 8 days [59]. Hair shaft elongation (daily) and keratinocyte proliferation (end of study) were recorded. Hair follicle growth was suppressed by testosterone, and caffeine reversed this testosterone-induced growth suppression already when applied in the lowest investigated concentrations of 10 and 50 μg/mL. In the absence of testosterone, these caffeine concentrations stimulated hair growth as well as keratinocyte proliferation in the dermal papilla, which was quantified by immunocytochemical labeling of the marker for hair matrix keratinocyte proliferation Ki67. In contrast, higher concentrations of caffeine (100, 500, and 1,500 μg/mL) were observed to have inhibitory effects. It is assumed that high caffeine concentrations may cause an overstimulation of hair follicle metabolism, leading to an extensive consumption of energy reserves, an exhaustion of the proliferation capability, and finally a lack of hair shaft elongation. All in all, this study provided clear evidence that caffeine can counteract testosterone-induced growth suppression of hair follicles, while at the same time highlighting the importance of selecting the optimal caffeine dose to produce the best physiological response while avoiding inhibitory effects.

In a more comprehensive study, the response of hair growth parameters and regulatory factors to caffeine was investigated [22]. Hair follicles, microdissected from biopsies taken from the balding vertex region of men affected with AGA and from healthy women undergoing facelift surgery, were cultivated in the HOCM and exposed to caffeine at 10 or 50 μg/mL. At these concentrations, caffeine prolonged the duration of the anagen phase and counteracted testosterone-induced TGF-β2 protein expression in hair follicles from males. In female hair fol-

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Table 1. Overview of the reviewed caffeine studies: from in vitro/ex vivo and in vivo penetration studies towards clinical trials

Study type	Methods	Gender	Mode of action	Results	Reference
Ex vivo/ in vitro	Ex vivo HFs cultivated in vitro (120–192 h) with normal William's E medium (control) or William's E medium + different concentrations of testosterone (5 ng/mL–5 μg/mL) and/or caffeine (10–1,500 μg/mL) Hair shaft elongation measured daily + at end of cultivation Ki67 staining	Male	Stimulation of HFK proliferation Counteraction of growth suppression Cell proliferation increase	HF growth suppression with testosterone (5 μg/mL) Counteraction of growth suppression by caffeine (10 and 50 μg/mL) HF growth stimulation with caffeine (10 and 50 μg/mL) alone Results confirmed immunohistochemically by Ki67 staining	[59]
Ex vivo/ in vitro	Microdissected male and female HFs (120 h) with normal William's E medium (control) or William's E medium + different concentrations of testosterone (0.5 μg/mL) and/or caffeine (10 or 50 μg/mL) Effects on hair shaft elongation evaluated by quantitative (immuno)histomorphometry: HF cycling (anagen–catagen transition); hair matrix keratinocyte proliferation; expression of a key catagen inducer, TGF-β2; expression of anagen-prolonging IGF-1 Caffeine effects in human ORSKs	Male + female	Counteraction of growth suppression Cell proliferation increase Reduced apoptosis Reduced oxidative stress Stimulation of ORS cell proliferation Inhibition of apoptosis and necrosis	Hair shaft elongation (10 and 50 μg/mL) Prolonged anagen duration (10 and 50 μg/mL) Hair matrix keratinocyte proliferation stimulated Female HFs have higher sensitivity to caffeine than male HFs Counteraction of testosterone-enhanced TGF-β2 protein expression in male HFs (10 and 50 μg/mL) No counteraction of testosterone-enhanced TGF-β2 protein expression in female HFs (caffeine: 10 and 50 μg/mL) In female HFs, caffeine reduced TGF-β2 expression (10 and 50 μg/mL) In male and female HFs, caffeine enhanced IGF-1 protein expression (10 and 50 μg/mL) In ORSKs, caffeine (10 and 50 μg/mL) stimulated cell proliferation, inhibited apoptosis/necrosis, and upregulated IGF-1 gene expression and protein secretion, while TGF-β2 protein secretion was downregulated	[22]

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Table 1 (continued)

Study type	Methods	Gender	Mode of action	Results	Reference
Ex vivo	Human scalp skin with terminal HFs was irradiated ex vivo at different UVA and/or UVB doses with and without topically applied caffeine (1 mg/mL) and organ-cultured under serum-free conditions (1–3 days) Effects on toxicity and vitality read-out parameters were measured in defined skin and HF compartments	Male + female	Reduction of UV-induced damage of the epidermis UV protection in the HF epithelium Counteraction of UV-mediated damage (e.g., apoptosis) prominent in the distal HF through caffeine	UVR exerted skin cytotoxicity and epidermal damage Different UVA and/or UVB doses lead to oxidative DNA damage and cytotoxicity in human HFs UVR decreased proliferation and promoted apoptosis of HF outer root sheath (ORS) and hair matrix (HM) keratinocytes, stimulated catagen development, differentially regulated the expression of HF growth factors, and induced perifollicular mast cell degranulation UVR-mediated HF damage was more severe after irradiation with high UVR dose and reached also deeper HF compartments The topical application of caffeine (1 mg/mL) did not induce skin or HF cytotoxicity and stimulated the protein expression of IGF-1 in the proximal HF ORS Caffeine promoted keratinocyte apoptosis in selected HF compartments Caffeine provided protection toward UVR-mediated HF cytotoxicity and dystrophy, keratinocyte apoptosis, and negative modulation of the catagen-promoting growth factor TGF-β2	[62]
In vivo penetration	Penetration measurement of a topical caffeine-containing shampoo formulation (10 mg/mL, application 2 min) into human skin (chest) by SI/MS Detection of transcutaneously absorbed caffeine Distinction between interfollicular and follicular penetration of topically applied caffeine was feasible by selectively blocking the follicular pathway	Male	Interfollicular and follicular penetration of topically applied caffeine	Caffeine penetrated via the HFs and stratum corneum (after 2 min) Penetration via HFs was faster and higher compared with the interfollicular route HFs are the only pathway for fast caffeine absorption during the first 20 min after application Caffeine can be detected up to 24 h after application; therefore, the HF can act as a reservoir	[69]

Table 1 (continued)

Study type	Methods	Gender	Mode of action	Results	Reference
In vivo penetration	Penetration of a fluorescent caffeine-containing topical formulation (10 mg/mL) into the HFs of human scalp skin (application 2 min) Fluorescence (2 mg/mL) was used as a marker for penetration and storage of the formulation into the HFs and was measured by in vivo LSM (limit of detection ~200 μm)	Female	Follicular penetration of a topically applied caffeine-containing shampoo formulation	The fluorescent caffeine-containing formulation penetrated efficiently into the HFs of human scalp skin after 2 min contact time and were detected in 200 μm depths up to 48 h after application	[70]
In vivo penetration	Each HF within a delimited area of human skin was blocked in vivo A caffeine-containing solution (25 mg/mL) was topically applied, transcutaneous absorption was measured by SI/MS Clear distinction could be made between interfollicular and follicular penetration of topically applied caffeine	Male	Interfollicular and follicular penetration of topically applied caffeine through the skin	Caffeine (3.75 ng/mL) was detected (5 min after topical application) when the HFs remained open Caffeine (2.45 ng/mL) was detected (20 min after application) when the HFs were blocked Highest values of caffeine (11.75 ng/mL) were found (1 h after application) when the HFs were open HFs allow a fast delivery of topically applied caffeine	[71]
In vivo penetration	Penetration and storage of a different caffeine-containing shampoo (10 mg/mL, 2 min topical application) into the HFs was investigated on human scalp skin via in vivo LSM (limit of detection ~200 μm)	Female	Follicular penetration of a topically applied caffeine-containing shampoo formulation	Shampoo ingredients were detected in the HFs (after 2 min) Shampoo penetration up to a depth of ~200 μm in the HF, where the network of blood capillaries surrounding the HFs commences, was detected (LSM detection limit) Detection in HFs 24 h later demonstrates the long-term reservoir function of HFs for topically applied substances such as caffeine	[74]

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Table 1 (continued)

Study type	Methods	Gender	Mode of action	Results	Reference
In vivo penetration	Wagner-Nelson modelling or a compartmental model with first-order absorption and elimination was used for caffeine plasma concentration-time profiles (after topical application of a caffeine solution [25 mg/mL]) into skin with or without HF blocking Pharmacokinetic parameters of absorption rate and absorption extent through HFs or stratum corneum were determined and compared	Male	Pharmacokinetic modelling was used for a definition of the underlying relative penetration of caffeine through HFs and through intact stratum corneum with time	Pharmacokinetic parameters obtained by the 2 methods were similar The absorption rate constant of caffeine for HFs was nearly 10 times higher than that for stratum corneum The percentage of absorption from HFs was more than half of that of stratum corneum Absorption from HFs showed no delay whilst absorption from stratum corneum showed an approx. 10 min delay Caffeine absorption by HFs occurs fast (within 30 min) and accounts for 10.5–33.8% of the total absorption amount Caffeine absorption through stratum corneum occurs over several hours	[73]
In vivo	Clinical trial (females with telogen effluvium [TE], phototype [Fitzpatrick] from I to IV) 6 months daily application of a caffeine-containing shampoo (10 mg/mL)	Female	Efficacy of a topical caffeine-containing shampoo in female TE was assessed by objective and subjective parameters	Hair pull test (frontotemporal, parietal, and occipital): Significantly fewer hairs pulled compared to baseline ($p = 0.003$) after 6 months of treatment Significant improvement of hair loss intensity ($p < 0.001$), decrease in hair loss during daily combing ($p < 0.001$), improvement of hair strength ($p < 0.001$) compared to 3 months of treatment in patient assessment Very good skin compatibility and good cosmetic efficacy in treatment of female TE	[83]
In vivo	Double-blind randomized clinical trial (males with AGA, Hamilton-Norwood stages II–IV, positive hair pull test [at least 18 hairs]) of a caffeine shampoo versus placebo shampoo 6 months daily application of a caffeine-containing shampoo	Male	Efficacy of a topical caffeine-containing shampoo in male AGA was assessed by objective and subjective parameters	Significant improvement of hair loss intensity ($p = 0.002$), decrease/normalization in hair loss ($p < 0.001$), decrease in hairs in the basin ($p = 0.002$), improvement of hair strength/thickness ($p < 0.001$) with a caffeine shampoo compared to placebo in patient assessment after 6 months of daily application	[85]

Table 1 (continued)

Study type	Methods	Gender	Mode of action	Results	Reference
In vivo	Double-blind randomized clinical trial (males with AGA, Hamilton-Norwood stages >V, females with AGA, Ludwig stages 2–3) of a topical caffeine (25 mg/mL) + minoxidil (25 mg/mL) lotion versus a topical minoxidil lotion (25 mg/mL) (daily application, 150 days of treatment)	Male + female	Efficacy of a combination of caffeine + minoxidil topical solution against male and female AGA in comparison to a topical minoxidil solution assessed by objective and subjective parameters	Significant increase with caffeine (25 mg/mL) + minoxidil (25 mg/mL) versus minoxidil (25 mg/mL) ($p < 0.05$) in hair density as number of hair strands/cm^2 (frontovertical) after 150 days of application Higher patients' satisfaction with caffeine (25 mg/mL) + minoxidil (25 mg/mL) versus minoxidil (25 mg/mL) alone (58.33 vs. 41.37%)	[96]
In vivo	Double-blind randomized clinical trial (males with AGA) of a topical solution of caffeine (10 mg/mL), minoxidil (50 mg/mL), and azelaic acid (15 mg/mL) versus a topical solution of minoxidil (50 mg/mL) versus a placebo solution	Male	Efficacy of a combined topical solution (caffeine + minoxidil) in male AGA in comparison to a minoxidil solution and placebo	Significant reduction of hair shedding with caffeine (10 mg/mL), minoxidil (50 mg/mL) and azelaic acid (15 mg/mL), and minoxidil (50 mg/mL) alone versus placebo ($p < 0.05$) in hair wash test after 12 weeks of treatment Significantly higher response rate with caffeine (10 mg/mL), minoxidil (50 mg/mL), and azelaic acid (15 mg/mL) versus minoxidil (50 mg/mL) alone ($p < 0.05$) and versus placebo ($p < 0.001$) in dermatologist and patient assessment	[97]
In vivo	Open-label randomized multicenter noninferiority clinical trial (males with AGA, Hamilton-Norwood stages III–V) of a topical caffeine lotion (2 mg/mL) versus minoxidil (50 mg/mL) (daily application)	Male	Efficacy of a topical caffeine-based liquid in male AGA in comparison to a minoxidil solution	No significant difference between both treatment groups in trichogram (frontal and occipital): mean improvement in anagen ratio of 10.59% with caffeine lotion (2 mg/mL) versus 11.68% improvement with minoxidil (50 mg/mL) after 6 months of treatment Significant improvement in intensity of hair loss, number of hairs falling out while combing, and hair thickness compared to baseline with both treatments in patient assessment No significant difference between treatments in patient assessment Significant improvement of hair strength, balding progression, and extent of hair loss in dermatologist assessment No significant difference between treatments in strength, balding progression, and extent of hair loss compared to baseline with both treatments	[86]

Table 1 (continued)

Study type	Methods	Gender	Mode of action	Results	Reference
In vivo	Double-blind randomized clinical trial (females with AGA, Ludwig stages 0–2, positive hair pull test [at least 18 hairs]) of a caffeine shampoo versus placebo (daily application)	Female	Efficacy of a topical caffeine-containing shampoo versus placebo (daily application) in female hair loss was assessed by objective and subjective parameters	Significantly fewer hairs pulled with a caffeine shampoo compared to placebo ($p < 0.001$) in hair pull test (frontotemporal, parietal, and occipital) after 6 months of treatment Significant improvement of hair loss intensity ($p < 0.001$), decrease/normalized hair loss ($p < 0.001$), reduction of number of hairs in the basin ($p < 0.001$) with caffeine shampoo compared to placebo in patient assessment Significant improvement of hair strength/thickness ($p < 0.001$), hair loss ($p < 0.001$) with caffeine shampoo compared to placebo in dermatologist assessment	[84]

AGA, androgenetic alopecia; HF, hair follicle; HFK, hair follicle keratinocyte; HM, hair matrix; HF ORS, hair follicle outer root sheath; IGF-1, insulin-like growth factor 1; LSM, laser scanning microscopy; ORSK, outer root sheath keratinocyte; SI/MS, surface ionization mass spectrometry; TE, telogen effluvium; TGF-β2, transforming growth factor-β2; UV, ultraviolet; UVR, ultraviolet radiation; UVA, ultraviolet radiation A (wavelength 315–380 nm); UVB, ultraviolet radiation B (wavelength 280–315 nm).

licles, testosterone alone had no influence on TGF-β2 protein expression, but the combination with caffeine showed a significant decrease in TGF-β2 expression. These differences of male and female hair follicles found in vitro correspond to the differences of male and female AGA found in vivo regarding severity and pattern [60, 61]. In both males and females, IGF-1 protein expression was enhanced by caffeine, thus promoting anagen maintenance. Further hair cycle analysis of female and male hair follicles revealed that the coincubation of testosterone and caffeine increased the percentage of anagen hair follicles compared to a treatment with testosterone alone, indicating the supportive effect of caffeine to maintain the active growing phase of male and female hair follicles. The beneficial effects of caffeine were also seen in outer root sheath keratinocytes (ORSKs) derived from human plucked eyebrow hair follicles from healthy male donors [22]. Expression and secretion of important hair cycle regulators such as IGF-1 were upregulated while TGF-β2 secretion was downregulated, emphasizing the growth-promoting effects of caffeine on human hair follicles. In addition, caffeine not only stimulated the proliferation of ORSKs but also inhibited apoptosis and necrosis. These findings suggest that protective effects of caffeine seem to be clinically relevant for both men and women, whereby several different mechanisms by which caffeine maintains the anagen state of hair follicles and might counteract AGA progression exist.

Beneficial effects of caffeine were also observed in a study investigating the effects of caffeine on full-thickness human scalp specimens exposed to ultraviolet radiation (UVR) [62]. In a UVA and UVB irradiation experiment, the effect of UVR on hair follicle damage was investigated on female human skin specimens from the temporal or occipital scalp region. High- and low-dose UVR (UVA [340–440 nm]: 10 J/cm^2 [low] and 50 J/cm^2 [high]; UVB [290–320 nm]: 20 mJ/cm^2 [low] and 50 mJ/cm^2 [high]), corresponding to comparable sunlight exposures in Europe in July, induced hair follicle cytotoxicity and dystrophy as well as the downregulation of IGF-1 and the upregulation of TGF-β2 in the proximal (nearest to the scalp) end of the hair follicles. Four-millimeter skin biopsies containing terminal hair follicles, obtained from occipital scalp of male donors undergoing hair transplantation, were cultured in an ex vivo skin organ culture model to investigate the combinatorial effect of topically applied caffeine and UVR. The topically applied caffeine concentration was 1,000 μg/mL, roughly 100-fold higher than in the HOCM as the dose applied topically needs to be higher than the desired effective dose at the hair folli-

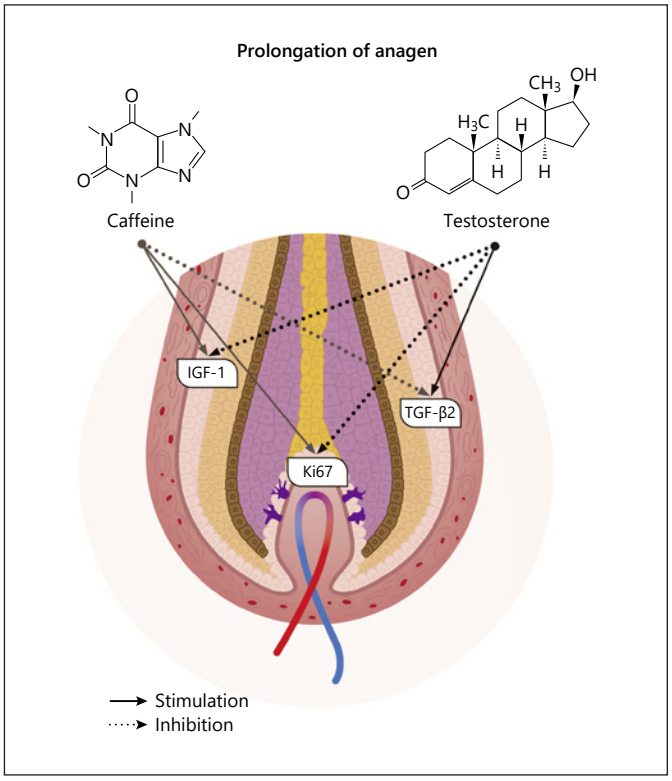

Fig. 1. Mechanism of action of caffeine and testosterone on the anagen duration of hair follicles in vitro. From the outside to the inside of the hair follicle: connective tissue sheath (blush), glassy membrane (light brown), outer root sheath (light beige), inner root sheath (beige), cuticle (brown), cortex (light purple), medulla (yellow), hair matrix with melanocytes (light beige and purple), papilla (light blush), and blood vessel (red/blue). IGF-1, insulin-like growth factor-1 (outer root sheath); TGF-β2, transforming growth factor-β2 (outer root sheath); Ki67, antigen KI-67 or MKI67 (Ki-Kiel) (hair matrix).

cle. The application of caffeine 3 days before and 3 days after irradiation was observed to offer protection from UVR-mediated hair follicle cytotoxicity and damage, such as keratinocyte apoptosis in the distal and central outer root sheath. Furthermore, caffeine induced an increase in IGF-1 protein expression in the hair follicle. Since the applied UVR in these experiments is above the absorption maximum of caffeine at 205 and 273 nm [63], direct UVA and UVB absorption from caffeine can be neglected. These results indicate that caffeine alleviates UVR-induced hair follicle damage and may act as a photoprotectant by triggering specific cellular responses at the hair follicle. The results further substantiate the notion that caffeine can have beneficial effects on the skin in general and hair follicles in particular. Furthermore, this study suggests that the effect of caffeine observed on isolated hair follicles in the HOCM is transferable to hair follicles within the skin. The mechanism of action of caffeine and testosterone on the anagen duration of hair follicles in vitro, affecting the hair cycle regulation factors IGF-1 and TGF-β2 as well as the proliferation nuclear marker protein Ki67 (antigen KI-67 or MKI67 [Ki-Kiel]), is depicted in Figure 1. The protein Ki67 can be assessed by immunocytochemical labeling and is a marker of hair matrix keratinocyte proliferation [22, 59]. All in all, the beneficial effects of caffeine on hair follicles seen in vitro have been recognized by the current European S3 guideline for the treatment of AGA [64], stating that "in male and female human scalp hair follicles caffeine led to an enhanced hair shaft elongation."

In vivo Penetration Studies

Transfer of the promising effects of caffeine on the hair follicle in vitro to an in vivo setting requires that caffeine can reach the desired target tissue. In the case of hair loss, the target can be either the hair bulb or the outer root sheath under the skin surface. However, one important function of the skin is to protect the organism from potentially harmful exogenous substances by acting as a barrier. Therefore, entering the underlying skin compartments is not trivial for most substances. On the other hand, caffeine is a model substance for skin barrier penetration studies [65, 66], as it penetrates well into human skin, resulting in an ample bioavailability of caffeine after topical application on scalp skin.

Several analytic tools are available to determine the in vivo penetration of substances into the different layers of the skin. These include invasive and semi-invasive methods such as tape stripping [67], dermal microdialysis [68], and the detection of substances in blood samples [69]. In vivo laser scanning microscopy (LSM) can also be employed to investigate skin penetration properties. LSM is a noninvasive imaging method that can detect fluorescent reporter molecules in the skin up to a depth of 200 μm [70]. For the investigation of topical treatments, the penetration of these fluorescent reporter molecules, which are added to the topically applied formulation as a marker molecule, reflect the penetration properties of the formulation [67].

An experimental setup was developed that allows for the selective closure of hair follicles to assess the relative importance of follicular penetration in comparison to the transdermal route [69, 71]. This experimental setup has been used to investigate the penetration properties of caffeine in solution and of a shampoo formulation contain-

ing caffeine. Hair follicles from the shaved chest region of 6 healthy male Caucasian volunteers remained open or were closed with a special varnish-wax mixture. After application of caffeine to the test area, blood samples were analyzed at different time points for traces of caffeine. However, detection and quantification of caffeine in blood samples after application of caffeine-containing topical treatments is not straightforward, as only quite small amounts of caffeine may reach the blood when systemic distribution is not the aim. Therefore, a highly sensitive method combining surface ionization with mass spectroscopy was used in order to detect these slight quantities of transcutaneously absorbed caffeine in the blood [69, 71]. Accordingly, after topical application of a caffeine solution (25 mg/mL, leave-on), minimal concentrations of caffeine (in the low ng/ml-range) were detectable already after 5 min when the follicles were left open and after 20 min when the follicles were closed [71]. Similar results were obtained for a caffeine-containing shampoo (10 mg/mL, rinse-off) that was topically applied for 2 min [69]. The highest concentrations of caffeine were observed for open hair follicles [69, 71]. These were around 20 ng/mL and hence substantially lower than the serum caffeine concentrations of 2–4 μg/mL observed after ingestion of one single cup of coffee [72]. Minute amounts of caffeine remained detectable 24 h after a single application [69, 71]. The results of these studies show that transdermal and follicular penetration occur simultaneously. Caffeine penetrates preferentially via the follicular route, providing immediate caffeine availability, while the slower transdermal penetration can provide a caffeine reservoir. Further penetration studies with a similar experimental setup, including the topical application of a caffeine solution (25 mg/mL, leave-on) on the chest area of 6 healthy male Caucasian volunteers, demonstrated that the follicular route contributes significantly to the absorption of caffeine through the skin. Within these experiments, the absorption rate of caffeine was nearly 10 times higher when hair follicles were left open in comparison to penetration through the stratum corneum when the follicles were closed [73]. Taken together, after topical application of caffeine-containing solutions or shampoos, caffeine was available in the skin from very shortly after application up to at least 24 h, even if the duration of the topical application was as short as 2 min.

The penetration and storage behavior of caffeine-containing formulations in the skin has also been investigated using in vivo LSM [70, 74]. Different formulations of a caffeine-containing shampoo with 10 mg/mL caffeine and a fluorescent marker (fluorescein, 2 μg/mL [70] or 1 μg/mL [74]) were applied to the scalp of 10 healthy female volunteers for 2 min. The caffeine-containing shampoo penetrated into the hair follicle up to 200 μm (which corresponds to the maximum measuring depth due to the experimental set up), where the marker was still detectable after 24 h, providing further evidence that the penetration of caffeine-containing formulations across the skin barrier is a fast and efficient process, with a reservoir formation for at least 1 day. Additionally, the results further highlight that a contact time of 2 min is sufficient to detect caffeine-containing shampoos in the skin.

In vivo Analysis of Caffeine-Containing Formulations against Hair Loss

Studies investigating the effects of caffeine on the skin have shown that caffeine is beneficial for the barrier function in male skin [75], has antiviral activity as it is comparable to topical acyclovir for treating herpes simplex virus skin infections at concentrations >1 mg/mL, even in herpes virus resistant to acyclovir [76–78], and has a positive impact on apoptosis in UVB-treated skin when topically applied at 0.062 mmol/mL and therefore has the potential to lower the risk for skin cancer [75, 79–81]. It is conceivable that hair follicles may play a role in these findings, as there is a close relation between the epidermis and the hair follicle [47]. The dermal papilla, for example, controls the size of the hair while cells from the follicle (outer root sheath) can migrate and regenerate the epidermis after injury or loss [47]. The hair follicles also assist in wound healing and skin repigmentation and interact closely with the immune and neuroendocrine systems of the skin, supporting immunosurveillance against pathogens and aiding sensory perception [82].

Based on the observation from in vitro studies that caffeine might counteract AGA progression, and the results of the skin penetration studies showing that caffeine can efficiently penetrate into the hair follicle and is retained for >24 h, the effect of caffeine-containing formulations on hair loss has been investigated in several in vivo studies [83–86]. For the evaluation of hair loss, several diagnostic techniques are available, covering noninvasive, semi-invasive, and invasive methods, as briefly described in the following: both the hair pluck and hair pull tests have been employed in the assessment of caffeine and caffeine-containing shampoos. With the hair pluck test, also known as trichogram, the ratio of anagen to telogen hair follicles can be determined [87, 88]. From a specified site, 60–80 hair shafts including their roots are removed (plucked). The hair bulbs are then immediately placed with their roots on a glass slide in an embedding medium

for microscopic evaluation. Last shampoo application is required to be 5 days prior to testing. The hair pluck test is employed in diagnosis and also for treatment monitoring [89]. The hair pull test on the other hand assesses the severity and location of hair loss [87]. A bundle comprising approximately 20–60 hair shafts is grasped between the thumb, index, and middle fingers from the base of the hairs near the scalp and firmly, but not forcefully, tugged away from the scalp. The percentage of hair shafts pulled that come loose determines the test result. A recent update on the guidelines for the hair pull test recommends to reduce the threshold for the number of pulled hair shafts that constitutes a positive pull test result, indicating active hair shedding, from 10 to <5%, i.e., 2 hair shafts or fewer pulled from a bundle of 60 hairs [90]. While recommendations regarding shampooing prior to a hair pull test varied between 1 and 5 days [87, 88], the recent update on the guidelines provides evidence that hair loss values are uninfluenced by the time since last hair wash and advocates that the 5-day restriction on hair washing prior to the test should be reconsidered to minimize unnecessary inconvenience to patients [90]. A sensitive in situ method to monitor hair loss and treatment response by combining epiluminescence microscopy with an automatic digital image analysis is trichoScan. This optical and noninvasive method is able to determine the amount of hairs, the hair density, and the hair cycle phase (anagen or telogen) [91]. Although other methods for the diagnosis of hair disorders or more preciously for the measurement of hair growth variables exist, including the phototrichogram, the use of the trichogram remains a basic diagnostic tool with widespread use in hair consultation. For an overview of further hair evaluation methods, the reader is referred to review articles [87, 88].

Further characteristics of hair loss are commonly assessed by investigators and self-reported by subjects. As hair loss is known to negatively affect the quality of life and can even give rise to conditions such as depression and anxiety [41] as discussed above, the subjective impact of hair loss on quality of life is frequently assessed with questionnaires. These questionnaires, which are generally easy to comprehend and record, are used in clinical trials for the assessment of therapeutic response [42, 87].

Several studies on caffeine-containing formulations for hair loss have been conducted to date, including single-arm studies, parallel trials, and randomized controlled trials, with placebo and drug-based treatments used as comparators. Caffeine-containing formulations have been studied in men and women with AGA and women with telogen effluvium, a hair-loss condition in which anagen-phase hair follicles prematurely transition to the telogen phase.

A caffeine-containing shampoo (10 mg/mL caffeine) was assessed in females with telogen effluvium ($n = 30$) using the hair pull test to evaluate hair loss as well as investigator and subject questionnaires to evaluate the quality of life [83]. After 6 months of daily application on the scalp with 2 min contact time, a decrease in hair loss was noted in more than half of the participants. In addition, a good cosmetic efficacy was reported including an improvement in the strength of the hair ($p < 0.001$), a decrease in the extent of hairs falling out ($p < 0.001$), and a decrease in the progression of the balding ($p = 0.100$) assessed by the investigator's questionnaire. In females with AGA, a randomized double-blind parallel trial ($n = 140$) compared the efficacy of a caffeine-containing shampoo to a control shampoo without caffeine [84]. The study was conducted in accordance with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice for conducting clinical trials for drugs. Last shampoo application was at least 48 h before application of the test products. Hair loss was evaluated based on the results of a hair pull test and also with subject and investigator questionnaires. The study results showed beneficial effects related to inclusion of caffeine in the shampoo: Fewer hairs were pulled out after 6 months in the hair pull test ($p < 0.001$), hair loss intensity was reduced ($p < 0.001$), and there was a trend to an increase in hair strength ($p = 0.138$) compared to the control shampoo assessed by the investigator's questionnaire. No adverse events were reported for the caffeine-containing shampoo or for the control shampoo, indicating that the addition of caffeine does not change the safety profile of the formulation.

A randomized, controlled, double-blind, parallel group study of a caffeine-containing shampoo was conducted in males with AGA ($n = 66$) [85]. The contentment was significantly higher for the caffeine-containing shampoo compared to a caffeine free control ($p < 0.001$). Further parameters assessed by the subjects themselves as well as from the investigators were also improved significant differences in favor of the caffeine-containing shampoo were observed for the reduction in the intensity of hair loss ($p = 0.002$), the decrease in/normalization of the hair loss ($p < 0.001$), the decrease in the number of hairs in the basin ($p = 0.002$), and the improvement of hair strength and thickness ($p < 0.001$).

More recently, results of a randomized, open-label, multicenter noninferiority study in males with AGA ($n = 210$) were published, reporting on the effects of a caffeine-

containing lotion (2 mg/mL) compared to a drug-based approach (50 mg/mL minoxidil solution) [86]. Minoxidil is one of only 2 drugs in use worldwide for the treatment of hair loss, the other being finasteride [64]. However, topical minoxidil solution has been associated with adverse effects [92], including contact dermatitis [93] and hypertrichosis [94, 95]. On the other hand, caffeine is the most studied natural ingredient with the potential to be a topical multibenefit solution to hair loss and is not known to show any undesired effects in vivo [39]. The primary end point within the noninferiority study was the change in the proportion of anagen hair follicles as assessed by trichogram. Secondary end points were the dermatological assessment and a subject questionnaire. This study showed a similar improvement in the proportion of anagen hair follicles for the caffeine-containing lotion compared to the minoxidil solution ($p = 0.574$). Hence, the results obtained with the caffeine-containing lotion indicate that it was noninferior to the minoxidil solution. No significant differences were observed in the increase from baseline in the proportion of anagen hair follicles after 6 months using either the frontal trichogram or the occipital trichogram alone. The change from baseline in the number of subjects with an increase in anagen hair (and accordingly a decrease in telogen hair) using a frontal or occipital trichogram after 3 and 6 months of application was also similar between groups. Results for the investigator assessment of hair strength, balding progression, and extent of hair loss and the subjective assessment of intensity of hair loss, the number of hair shafts falling out while combing, hair thickness, and treatment satisfaction were all comparable between the caffeine-containing lotion and minoxidil. Furthermore, no safety concerns were observed for the caffeine-containing lotion. In conclusion, this study showed that the caffeine-containing lotion is as effective as an FDA-approved drug in males with hair loss.

Additionally, two further studies have been conducted with topically applied caffeine in combination with conventional hair loss treatments [96, 97]. Within a randomized, double-blind, controlled clinical trial, the topical solution consisting of 25 mg/mL caffeine with 25 mg/mL minoxidil was more effective for male and female patients suffering from AGA than the 25 mg/mL minoxidil alone in terms of patients' satisfaction (58.33% in combined treatment vs. 41.37% in minoxidil alone control group) after 150 days of treatment. Another combined treatment with 10 mg/mL caffeine, 50 mg/mL minoxidil, and 15 mg/mL azelaic acid on male AGA patients showed a higher efficacy for hair regrowth and against hair shedding, evaluated via wash test (hair shedding) as well as patient and dermatologist assessment (hair regrowth), in comparison to minoxidil alone or the placebo after 32 weeks. Both studies emphasize that the combined solutions with caffeine were more effective on male patients with AGA compared to the corresponding control group, further illustrating the efficacy of caffeine on AGA management [98].

The range of studies conducted on the effects of caffeine on hair follicles and caffeine-containing treatments for hair loss closely mirrors the studies performed during development and testing of drugs, even though these products are not pharmaceuticals and hence not subject to the regulatory requirements for drug approval. The current version of the S3 guideline for the treatment of AGA in women and men published in 2017 acknowledges the promising results of caffeine from in vitro experiments [64]. However, the results of the noninferiority study comparing a caffeine-containing lotion with minoxidil in males with AGA as well as the randomized double-blind parallel trial comparing the caffeine-containing shampoo to a control shampoo without caffeine in females with AGA have not been considered by the current guideline, as they were not published at that time. It can be anticipated that these results will be included in the next update of the S3 guidelines. Considering the results from the noninferiority study, one may question whether a drug-based treatment remains appropriate for alleviating a non-life-threatening condition like AGA. The negative impacts of AGA are mainly limited to psychological concerns primarily due to the effects of hair loss on the cosmetic appearance [40]. Hence, special consideration needs to be given to the risk-benefit profile when choosing a suitable treatment option. In particular, the long-term safety aspects need to be considered, since the underlying genetic predisposition entails a lifelong daily administration. While the currently available drug-based approaches minoxidil and finasteride seem to be accompanied by side effects and adverse effects [64], caffeine appears to be an ideal compound for topical treatment, as it is a nontoxic, inexpensive substance that easily penetrates across the skin barrier [75].

Conclusion

The natural ingredient caffeine has proven to be efficient and active against hair loss in topical cosmetic formulations. Caffeine has undergone the important validation steps – from proof of principle in the in vitro setting

to controlled clinical trials in vivo. The caffeine-effect on hair follicles, hair shaft elongation, and key regulatory factors of hair growth in vitro have been found to be dose and gender dependent. Furthermore, caffeine penetrates efficiently via the follicular route compared to the dermal route. In vivo studies in males and females with AGA confirmed that hair loss is reduced using caffeine-containing topical formulations. A topical caffeine-containing lotion was shown to be noninferior to the drug minoxidil in a randomized, controlled, parallel group study in males with AGA.

The results obtained so far have shown that caffeine-containing treatments for hair loss can be as effective as drug-based treatments, while at the same time providing the good safety profile commonly associated with cosmetic products. However, there is a need to dose caffeine adequately to support the biological activity of male and female hair follicles best. Consequently, each formulation requires verification to ensure optimal efficacy against hair loss in men and women.

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