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Caffeine's Mechanisms of Action and Its Cosmetic Use

A. Herman[a] A.P. Herman[b]

[a]Academy of Cosmetics and Health Care, Warsaw, and [b]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

Key Words
Caffeine · Penetration through the skin · Cellulite · Microcirculation · Antioxidant

Abstract
Caffeine is being increasingly used in cosmetics due to its high biological activity and ability to penetrate the skin barrier. This alkaloid is frequently used as a hydrophilic model substance in human and animal skin penetration as well as different synthetic membrane using Franz diffusion cell experiments. The commercially available topical formulations of caffeine normally contain 3% caffeine. As for a cosmetic purpose, caffeine is used as an active compound in anti-cellulite products because it prevents excessive accumulation of fat in cells. This alkaloid stimulates the degradation of fats during lipolysis through inhibition of the phosphodiesterase activity. Caffeine has potent antioxidant properties. It helps protect cells against the UV radiation and slows down the process of photoaging of the skin. Moreover, caffeine contained in cosmetics increases the microcirculation of blood in the skin and also stimulates the growth of hair through inhibition of the 5-α-reductase activity.

Introduction

Caffeine is one of the alkaloids which can be found in coffee, tea and some soft drinks. Caffeine is well known as a mild stimulant of the central nervous system where it is transported with blood after its absorption in the stomach and small intestine. In the liver, caffeine is metabolized by the cytochrome P450 oxidase system into 3 derivative dimethylxanthines: paraxanthine (speeds up lipolysis), theobromine (expands blood vessels), and theophylline (relaxes smooth muscles of the bronchi) [1]. These metabolites of caffeine after demethylation and oxidation pass to derivatives of xanthine and uric acid. Only 10% of the caffeine is excreted from the body by the kidneys in an unchanged form [2]. In the brain, caffeine as a ligand (instead of adenosine) blocks the adenosine A1 and A2 receptors [3]. Both ligands, caffeine and adenosine, show a high similarity of their chemical structures [4]. They can affect the release of neurotransmitters such as acetylcholine, dopamine, noradrenaline, gamma-aminobutyric acid, and serotonin, which enhances mood [5], stimulates the organism, improves concentration and eliminates physical fatigue [6]. Caffeine also inhibits phosphodiesterase (PDE) activity, an enzyme which is re-

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Anna Herman, PhD
Academy of Cosmetics and Health Care
Podwale 13 street
PL–00-252 Warsaw (Poland)
E-Mail anna.herman@wszkipz.pl

sponsible for the degradation of cyclic adenosine monophosphate (cAMP) to the noncyclic form 5′-AMP [3]. The inhibition of PDE increases the cAMP concentration in cells and also elevates blood pressure [3]. Although the impact of caffeine on the human organism is well understood, the mechanism of the cosmetic action of caffeine has not been fully explained. Given that coffee and caffeine are used increasingly for the production of many cosmetics, it seems interesting to clarify whether it is really able to improve the skin's appearance and the hair's condition. The ability of caffeine to penetrate the skin barrier is essential when discussing the mechanism of its action on skin and hair.

Penetration of Caffeine through the Skin Barrier

The ability of active compounds from cosmetics or pharmaceuticals to influence the metabolism of cells and other processes occurring in the skin is largely dependent on the capacity of these molecules to penetrate through the skin barrier. Caffeine is frequently used as a hydrophilic model substance in skin penetration experiments [7–11]. Previous studies found out that caffeine penetration of the skin barrier was unchanged by occlusion [12] and skin thickness [13, 14], but the application of 5% caffeine in a hydroxyethylcellulose gel for 7 days significantly reduced transepidermal water loss in male skin compared with female skin [15]. The maximal absorption rates of caffeine through the human skin were found to be 2.24 ± 1.43 μg/cm^2/h [13], and the maximal absorption was reached at 100 min after local application in vivo [16]. Touitou et al. [17] using quantitative skin autoradiography found out that after 24 h, the greatest concentration of caffeine (280 μg/tissue) was localized in the epidermis, while the lowest amount of this alkaloid (50 μg/tissue) was detected in the dermis.

The penetration of caffeine was also compared in Franz-type diffusion cell experiments with different coated membranes, e.g. skin of humans [8, 18, 19] and animals [9, 10] or synthetic materials [20, 21]. The permeation of different concentrations of caffeine (3% and 1, 3, and 5%) through human skin and synthetic membranes (cellulose acetate impregnated with isopropyl myristate, silicone rubber soaked in isopropyl myristate and polysulfate) using Franz diffusion cells were compared by Dias et al. [20] and Mustapha et al. [21], respectively. Both research groups did not find a correlation between caffeine transfers through the synthetic membranes and those observed through the human skin. Moreover, Mustapha et al. [21] showed that the diffusion flux of caffeine permeation does not depend on the concentration but rather on the quantity of formulation applied. Also Shakeel and Ramadan [10] confirmed that the type of emulsion affects the transdermal delivery of caffeine. A significant increase in the permeability of caffeine in Franz diffusion cells using rat skin as permeation membrane was observed in water-in-oil nanoemulsion formulations as compared to aqueous solutions of caffeine [10].

Other studies concerned the permeation of caffeine from microspheres applied in aqueous suspension (diameter of the microspheres: 2.8 μm, caffeine loading: 2.3 mg/g of particles) and from solution through in vitro diffusion measurements with Franz-type diffusion cells over 24 or 72 h [22]. After 24 h, the total amount of caffeine from microspheres carried out on full-thickness skin without hypodermis was twice as high as that from aqueous solution (22.6 vs. 9.99%). The results suggested that microspheres could easily penetrate through the skin and accumulate in the receptor compartment, ensuring continuous caffeine release. After 72 h of exposure, the receptor fluid contained 15.3% free caffeine and 27.8% encapsulated fractions. For the cosmetic use of caffeine, its penetration through the skin is the key.

Anti-Cellulite Properties of Caffeine

Cellulite (gynoid lipodystrophy), commonly called 'orange peel effect', is a typical women's problem, which mainly appears on the thighs and buttocks. Cellulite is a complex disorder involving the microcirculatory and lymphatic systems, the extracellular matrix and the presence of excess subcutaneous fat that bulges into the dermis [23]. Lipolysis is the degradation process of triglycerides from adipocytes by lipoprotein lipases, leading to the formation of fatty acids and glycerol. Lipases located on the fat cell membrane can be activated or inhibited by catecholamines (noradrenaline and adrenaline) and hormones (insulin, glucagon, and adrenocorticotropin). Adrenaline, noradrenaline, glucagon, and adrenocorticotropin activate the lipases, while insulin inhibits the activity of these enzymes. Depending on the hormones and type of adrenergic receptor (α or β) in adipocytes, the lipolysis process can be activated or inhibited. Insulin binding to the α adrenergic receptor stimulates the collection of fat in adipocytes, while the second receptor (β adrenergic) binds to adrenaline, noradrenaline, glucagon, or adrenocorticotropin and stimulates the degradation of fats during the lipolysis process. The biological

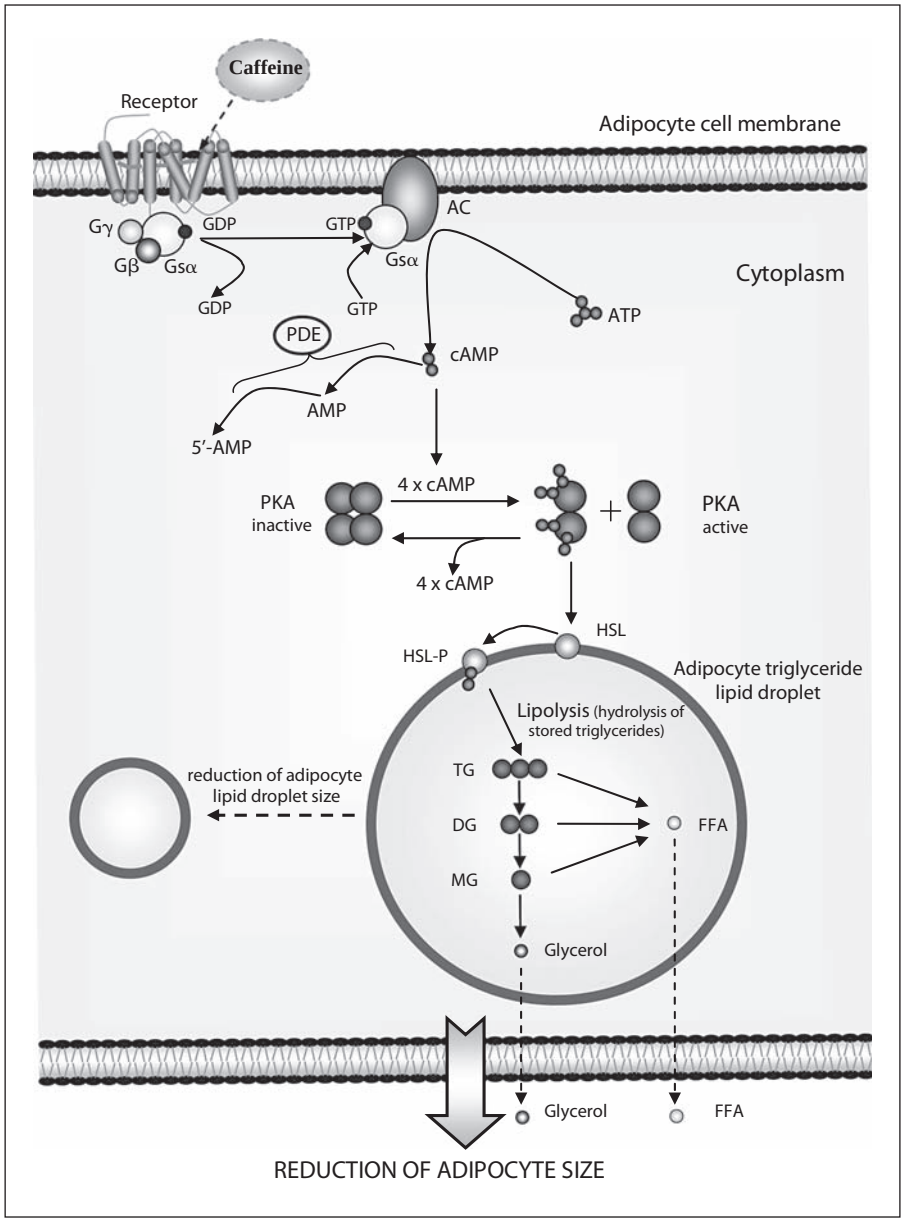

Fig. 1. Caffeine's mechanism of action during lipolysis in adipocyte. GDP = Guanosine diphosphate; GTP = guanosine triphosphate; AC = adenylate cyclase; ATP = adenosine triphosphate; PKA = protein kinase A; HSL-P = phosphorylated hormone-sensitive lipase; TG = triglyceride; DG = diglyceride; MG = monoglyceride; FFA = free fatty acid.

compounds activating the lipolysis pathway induce the conformational change in the structure of the G protein-coupled receptor and stimulate the adenylate cyclase to synthesize the cytosolic cAMP. An increasing level of cAMP stimulates protein kinase A to activate hormone-sensitive lipase (HSL) by phosphorylation. Phosphorylated HSL hydrolyzes triglycerides into diglycerides, monoglycerides, free fatty acids, and glycerol. The lipolysis process can be inhibited by a decreasing level of cAMP. PDE activity is responsible for the degradation of cAMP into its noncyclic form 5′-AMP [24, 25].

Caffeine can affect the above-mentioned intracellular signaling pathways in several ways. It may affect the secretion of catecholamine, which activates β adrenergic receptors, increases the concentration of cAMP in cells and activates HSL in the lipolysis process [26]. This alkaloid also blocks α adrenergic receptors, preventing an excessive accumulation of fats, and speeds up the lipolysis process [27, 28]. Caffeine also stimulates lipolysis via the inhibition of PDE activity and by increasing the cAMP levels in adipocytes [29]. Then caffeine activates HSL, which leads to the degradation of triglycerides in

the lipolysis process, and takes part in the reduction of cellulite (fig. 1). Caffeine also stimulates the draining lymph systems in fatty tissue by removing accumulated fat, toxin and unnecessary substances arising during the lipolysis process, which all together may impede the microcirculation in blood vessels and foster the emergence of cellulite.

Pires-de-Campos et al. [30] explored the effect of gel application on swine hypodermis (dorsal area): gel with ultrasound treatment (3 MHz, intensity: 0.2 W/cm^2, rate: 1 min/cm^2), gel with caffeine (5%, water-in-water), and gel with caffeine and ultrasound, daily for 15 days. A prespecified (fifth) area received no topical application and was used as control. Among all the experimental groups, only caffeine treatment associated with ultrasound therapy was effective. The results showed a significant reduction in the thickness of the subcutaneous adipose tissue, as well as damage of the adipocytes, consequently decreasing the number of cells. Velasco et al. [31] also examined the effect of emulsion with caffeine, caffeine and sodium benzoate, and siloxanetriol alginate caffeine (SAC) on the diameter and number of fatty cells with a light microscope. Emulsion with caffeine and its derivatives was applied topically for 21 days on Wistar female mice. Emulsion with caffeine and SAC provoked a reduction of the diameter of fatty cells compared with controls of 17 and 16%, respectively, while the emulsion with caffeine and sodium benzoate did not cause alterations of the cell diameter; moreover, sodium benzoate inhibited the efficiency of caffeine. Caffeine also had a significant anticellulite effect versus baseline, shown by its superiority versus placebo in the skin's macrorelief (it decreased the 'orange peel' effect), and increased the cutaneous microcirculation [32].

Caffeine's Effect on Microcirculation of Blood Vessels

Caffeine can improve the microcirculation of blood vessels. Some research carried out using computed tomography showed that a dose of 250 mg caffeine given orally increased the circulation of blood in the human brain by 30% [33] and a dose of 100 mg caffeine given orally increased the microcirculation of blood in the human ocular fundus [34]. The study performed by Lupi et al. [35] showed that a 7% caffeine solution affected the reduction of cellulite and improved the microcirculatory blood flow in all women taking part in the experiment. For observing the changes in the blood microcirculation, a noninvasive method was used – orthogonal polarization spectral imaging, which determined capillary density (number of flowing capillaries per unit area of the skin) and centimetrical measurements of thighs and hips. In the same study, the influence of tobacco, alcohol and physical activity on the effectiveness of the cellulite treatment was also examined. A significant reduction of thigh circumferences in more than 80% of the cases and a reduction of hip circumference in 67.7% were observed after 1 month of treatment. The capillary density after 1 month of caffeine administration was slightly increased, but the changes were not statistically significant. The results of Lupi et al.'s study [35] showed that smoking, alcohol consumption and regular physical activity do not affect the reduction of hip circuit in women.

Caffeine can effectively reduce swelling of the tissue around the eyes. Amnuaikit et al. [18] compared the impact of both a 3% caffeine gel and a gel base on the decrease of puffy eyes among Thai volunteers (18 women and 16 men, aged 19–24 years). An insignificant difference between the efficacy of caffeine gel and gel base to reduce puffy eyes was found as only 23.5% of the volunteers responded to the caffeine activity.

Antioxidant Properties of Caffeine

It is well known that UV rays accelerate photoaging of the skin, reduce the synthesis of procollagen, affect the fiber of collagen, reduce skin elasticity, cause expansion and cracking of skin blood vessels, stimulate the formation of wrinkles, spots and discoloration, and in extreme cases they can lead to cancer of the skin, e.g. melanoma [36]. UV radiation also increases the production of free radicals, leading in consequence to cell damage. Adding caffeine to the formula of sunscreen cosmetics raises its protective effect against UV radiation, reduces the formation of free radicals in skin cells and could be useful in preventing UV-induced skin cancers [37, 38]. Leon-Carmona and Galano [39] revealed that caffeine is an effective scavenger of hydroxyl radicals (·OH) and alkoxyl radicals (·OCH_3), a poor scavenger of HOO(·) radicals, inefficient for directly scavenging O_2(·–) and (·)$OOCH_3$ radicals and most likely other alkyl peroxyl radicals. There are also reports concerning the impact of caffeine on the protection of skin cells from cancer caused by UV radiation.

Caffeine affects the UV-damaged cells of human skin causing cellular divisions and apoptosis before they

begin to transform into cancer cells. Kerzendorfer and O'Driscoll [36] demonstrated that caffeine administered orally or topically accelerated apoptosis of mice keratinocytes damaged by UVB radiation. The study performed by Abel et al. [40] also demonstrated that daily intake of coffee (>6 cups) caused a 30% reduction in the prevalence of nonmelanoma skin cancer in Caucasian women. Others found that caffeine has the ability to selectively induce apoptosis in vitro in UV-damaged keratinocytes [41]. Studies were carried out on p53-mutated keratinocytes, isolated from the human skin. This cell line contains about 4% mutated keratinocytes from all isolated keratinocytes of the human skin. In comparison with the other keratinocytes isolated from the skin, p53-mutated keratinocytes are more resistant to UV-induced apoptosis. Sunlight also acts as a tumor promoter by favoring the clonal expansion of p53-mutated cells over the other keratinocytes of the skin. Caffeine induced apoptosis in p53-defective cells. Lu et al. [42] demonstrated that topical application of caffeine to mice enhanced the elimination of p53-mutated keratinocytes from the skin. Immunohistochemical analyses showed that topical applications of caffeine increased apoptosis in nonmalignant skin tumors and in squamous cell carcinomas, but there was no effect on apoptosis in tumor-free areas of the epidermis. Also Kramata et al. [43] reported that mice orally treated with caffeine or green tea during chronic UVB irradiation showed changes in the mutation profile of the p53 gene in early mutant p53-positive epidermal patches. Topical applications of caffeine after discontinuing the chronic UVB irradiation specifically eliminated patches harboring homozygous p53 mutations. Studies carried out by Heffernan et al. [44] explained the caffeine's mechanism of action for these important observations. Caffeine at a concentration of 2 mM inhibits cAMP PDE, increases the intracellular levels of cAMP and promotes apoptosis in UV-damaged primary human keratinocytes [44]. Also Conney et al. [45] showed that the oral administration of green tea or caffeine to hairless SKH-1 mice for 2 weeks stimulated apoptosis in UV-induced sunburnt cells in the epidermis. A similar effect was observed when caffeine was applied topically immediately after UV exposure. In mice pretreated with UV radiation for 22 weeks (high-risk mice without tumors), topical applications of caffeine 5 days a week for 18 weeks with no further UV treatment inhibited carcinogenesis and stimulated apoptosis in the tumors. In another next experiment, Conney et al. [46] showed that caffeine and caffeine and sodium benzoate may be useful as novel inhibitors of sunlight-induced skin cancer. They found out that the application of caffeine and sodium benzoate was more active as sunscreen than caffeine alone. Also caffeine and sodium benzoate stimulated apoptosis of UVB-induced carcinogenesis more than caffeine and they were also highly active in inhibiting carcinogenesis in UVB-pretreated high-risk mice.

Caffeine's Effects on the Growth of Hair

5-α-reductase is an enzyme that converts testosterone into the more active dihydrotestosterone (DHT), which is responsible for baldness. Particularly sensitive to the action of DHT are hair follicles. Its application results in a shortened anagen phase of the hair growth cycle, most of the hair goes into the telogen phase with fallowing hair follicle miniaturization and reduction of hair roots. The newly growing hair is thinner and shorter, and after several cycles of hair growth, the hair ceases to grow. Caffeine inhibits the activity of the 5-α-reductase enzyme and allows a renewed growth phase of the hair [47]. Caffeine in concentrations of 0.001 and 0.005% led to a significant stimulation of human hair follicle growth in vitro [47]. The stimulating effects of caffeine on the growth of the hair can also be explained by its ability to inhibit PDE enzymes. Inhibiting PDE activity increases the intracellular concentration of cAMP, stimulating cellular metabolism in a multitude of new cells [47]. Caffeine reduces smooth muscle tension near the hair follicle causing an easier delivery of nutrients through the blood vessels of the papillae of the hair [47]. Caffeine also arouses capillary vessel microcirculation in the skin of the head, thereby contributing to nurture hair bulbs. It strengthens and stimulates rapid growth by regularly providing nutrients with blood to the hair [46]. Teichmann et al. [48] and Lademann et al. [49] demonstrated that a 2-min contact of a shampoo with caffeine was sufficient for the formulation to penetrate deeply into the hair follicles and remain there for up to 48 h, even after washing of the hair. Otberg et al. [50] showed that there is a quantitative distinction between follicular penetration and interfollicular diffusion of a formulation containing 2.5% caffeine applied to the chest of male Caucasian volunteers aged 26–39 with normal body mass indices. Caffeine (3.75 ng/ml) was detected in blood samples 5 min after topical application, when the follicles remained open. When the follicles were blocked, caffeine was detectable after 20 min (2.45 ng/ml). The highest values (11.75 ng caffeine/ml) were found 1 h after application, when the follicles were open. The ability of caffeine to penetrate

the hair follicles and to stimulate the human hair growth in vitro may have an important clinical impact on the management of androgenetic alopecia, a common problem in men of all ages.

Summary

Extracts from coffee have a wide spectrum of actions; therefore, they are used in many kinds of cosmetics. Caffeine stimulates the metabolism, contributes to the removal of deposits of toxins from the organism, reduces puffy eyes, accelerates the drainage of the lymph system from fatty tissue, improves the microcirculation of the blood in the capillary vessels, exhibits anti-cellulite properties, activates lipolysis, and releases the excess of fat from adipocyte cells by reducing their size. Polyphenol compounds contained in coffee have antioxidant properties (protecting against the UVB radiation), neutralize free radicals, and therefore are used as sunscreen in anti-wrinkle and anti-aging cosmetics. Caffeine is also used in hair-care products because it reduces and slows down the process of baldness and also stimulates hair growth. All these properties make caffeine an important biologically active compound which can be used in different cosmetic products. New findings concerning the biological properties of this alkaloid will make that the spectrum of caffeine applications in the field of cosmetology and dermatology in the future may be even wider. For this purpose, more research is necessary to determine the appropriate doses and delivery systems for caffeine penetration through the skin. There is little scientific data based on clinical trials and none about the side effects of the cosmetic use of caffeine. More research is needed to confirm or deny the cosmetic application of caffeine.

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