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REVIEWS: CURRENT TOPICS

Phytochemicals and regulation of the adipocyte life cycle☆

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Abstract

Natural products have potential for inducing apoptosis, inhibiting adipogenesis and stimulating lipolysis in adipocytes. The objective of this review is to discuss the adipocyte life cycle and various dietary bioactives that target different stages of adipocyte life cycle. Different stages of adipocyte development include preadipocytes, maturing preadipocytes and mature adipocytes. Various dietary bioactives like genistein, conjugated linoleic acid (CLA), docosahexaenoic acid, epigallocatechin gallate, quercetin, resveratrol and ajoene affect adipocytes during specific stages of development, resulting in either inhibition of adipogenesis or induction of apoptosis. Although numerous molecular targets that can be used for both treatment and prevention of obesity have been identified, targeted monotherapy has resulted in lack of success. Thus, targeting several signal transduction pathways simultaneously with multiple natural products to achieve additive or synergistic effects might be an appropriate approach to address obesity. We have previously reported two such combinations, namely, ajoene+CLA and vitamin D+genistein. CLA enhanced ajoene-induced apoptosis in mature 3T3-L1 adipocytes by synergistically increasing the expression of several proapoptotic factors. Similarly, genistein potentiated vitamin D's inhibition of adipogenesis and induction of apoptosis in maturing preadipocytes by an enhanced expression of VDR (vitamin D receptor) protein. These two examples indicate that combination therapy employing compounds that target different stages of the adipocyte life cycle might prove beneficial for decreasing adipose tissue volume by inducing apoptosis or by inhibiting adipogenesis or both.

Keywords: Adipogenesis; Lipolysis; Adipocyte apoptosis; Genistein; Resveratrol; Ajoene

1. Introduction

Obesity is no longer considered to be only a cosmetic problem. Studies indicate that higher levels of body fat are associated with an increased risk for the development of numerous adverse health conditions [1]. Weight loss is increasingly recognized to have major health benefits for overweight people [2] and also increases life expectancy in people having obesity-related complications. While reducing dietary fat content combined with increased physical exercise was shown to be effective in preventing obesity [3], only one third of those trying to lose weight reported eating fewer calories and exercising more [4]. Although weight loss and weight control drugs are becoming extremely common in today's society, the remedies provided by the diet industry have failed in the long-term maintenance of weight loss in obese patients [5]. Moreover, it has been estimated that more than 90% of the people who lose weight by dieting return to their original weight within 2–5 years [6]. Adipose tissue growth involves formation of new adipocytes from precursor cells, further leading to an increase in adipocyte size. The transition from undifferentiated fibroblast-like preadipocytes into mature adipocytes constitutes the adipocyte life cycle, and treatments that regulate both size and number of adipocytes may provide a better therapeutic approach for treating obesity.

The decrease of adipose tissue mass that occurs with weight loss may involve the mobilization of lipids through lipolysis or the loss of mature fat cells through apoptosis [7,8]. While development of obesity is a greater problem

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during middle age, elderly people can have a relative increase in body fat content accompanied by an accumulation of adipocytes in nonadipose tissues, such as muscle and bone marrow. Since marrow adipocytes inhibit osteoblast proliferation [9] and disrupt the normal blood supply to bone tissue [10], treatments that inhibit marrow adipogenesis and decrease bone marrow adipocyte populations would have positive consequences for bone health. Furthermore, loss of weight in the elderly is associated with acceleration of both muscle tissue loss [11] and bone loss [12], and hence, treatments that selectively remove adipocytes while sparing muscle and bone tissue could be of tremendous benefit for prevention of sarcopenia, osteoporosis and adiposity in the elderly.

Medicinal plants and plant extracts represent the oldest and most widespread form of medication. At least 25% of the active compounds in currently prescribed synthetic drugs were first identified in plant sources [13]. Dissatisfaction with the high costs and potentially hazardous side effects of pharmaceuticals have resulted in a larger percentage of people in the United States purchasing and exploring the applications of medicinal plants than before [14]. Several plants like willow, poppy, foxglove, cinchona, aloe and garlic have been verified as medicinally beneficial through repeated clinical testing and laboratory analyses [15,16], and a number of plant extracts like green tea [17], garlic compounds [18] and conjugated linoleic acid (CLA) [19] were shown to possess either antidiabetic effects or have direct effects on adipose tissue.

A large body of literature indicates that substantial progress has been made concerning our knowledge of bioactive components in plant foods and their links to obesity. Polyphenols constitute one of the ubiquitous groups of plant metabolites [20] widely found in fruits, vegetables, cereals, legumes and wine [21,22]. A number of studies have been carried out to investigate the antiobesity effects of polyphenols like apigenin and luteolin [23], kaempferol [24], myricetin and quercetin [25], genistein and diadzein [26–28], cyanidin [29], grape seed proanthocyanidin extract (GSPE) [30], xanthohumol [31] and epigallocatechin gallate (EGCG) [32]. Likewise, studies involving the effects on lipid metabolism have been carried out with carotenoids like fucoxanthin [33], coumarin derivatives like esculetin [34] and phytoalexins like resveratrol [35]. Other bioactive components of food with antiobesity effects include phytosterols, polyunsaturated fatty acids and organosulfur compounds.

2. Natural compounds used for the treatment of obesity

2.1. Metabolic stimulants

Caffeine and ephedrine have been proposed as treatments for weight loss and weight maintenance for a long time. Caffeine increases energy expenditure by inhibiting the phosphodiesterase (PDE)-induced degradation of intracellular cyclic adenosine monophosphate (cAMP) [36] and decreases energy intake by reducing food intake [37]. Ephedrine, an alkaloid, mediates thermogenic effects by enhancement of sympathetic neuronal release of norepinephrine (NE) and epinephrine [36]. Although, the thermogenic effect of ephedrine was shown to be markedly potentiated by caffeine [38], owing to adverse cardiovascular side effects, the Food and Drug Administration has banned the sale of ephedra-containing dietary supplements [39]. EGCG, a flavonoid [40,41] and capsaicin, an alkaloid [42] were also shown to increase energy expenditure and thermogenesis in humans. Capsaicin dose-dependently enhanced catecholamine secretion from the adrenal medulla [43] to exert its thermogenic effect, whereas EGCG stimulated thermogenesis by inhibition of catechol *O*-methyl-transferase, an enzyme that degrades NE [44].

2.2. Appetite suppressants

Better understanding of the endogenous mechanisms involved in appetite and appetite suppression has dramatically increased interest in appetite suppressants. Extract of *Hoodia gordoni* is one of the most popular herbal supplements claimed to possess appetite suppressant properties. An oxypregnane steroidal glycoside, known as P57, is the only reported active constituent from hoodia [45]. This compound increased the adenosine triphosphate (ATP) content in hypothalamic neurons that regulate food intake after intracerebroventricular injection in rats [46]. Several other herbal supplements and plant extracts like ephedra [47], *Citrus aurantium* [48], hydroxycitric acid [49], *Caralluma fimbriata* [50] and *Phaseolus vulgaris* isolectins [51] have also been reported to possess appetite-suppressing properties.

2.3. Starch blockers

It has been well established that certain plant foods, such as *P. vulgaris* extract (derived from white kidney beans) and wheat, contain a substance that inhibits the activity of salivary and pancreatic amylase, and therefore, they are called starch blockers [52]. The plant extracts or herbal supplements that act as starch blockers promote weight loss by either interfering with the breakdown of complex carbohydrates or by providing resistant starches to the lower gastrointestinal tract [53]. Starch blockers show potential promise in the treatment of obesity, but further studies are warranted to conclusively demonstrate the effectiveness.

2.4. Glucose/insulin metabolism

Metabolism of glucose is a complex process regulated by peptides and steroid hormones and is highly influenced by diet. Hypoglycemic effects of several plant extracts like *Siraitia grosvenori* [54], *Stachytarpheta cayennensis* [55], *Platycodon grandiflorum* [56], *Gynostemma pentaphyllum* [57], *Cichorium intybus* [58], *Oryza sativa* [59], *Cucurbita ficifolia* [60], *Allium sativum* [61], *Vitex megapotamica* [62] and cinnamon bark [63] have been investigated. Soy protein

was shown to significantly improve insulin sensitivity and glucose effectiveness compared with casein [64]. Dietary fiber was also shown to significantly improve blood glucose control but the mechanisms by which dietary fiber exerts its hypoglycemic activities are unknown [65].

2.5. Lipid metabolism

Obesity is generally linked to complications in lipid metabolism and oxidative stress. The effects of several plant extracts like *Cissus quadrangularis* [66], *Aralia mandshurica* (aralax) [67], Kochujang (Korean fermented red pepper paste) [68], psyllium [69], *Salix matsudana* leaves [23,70] and *Arachis hypogaea* [71] on lipid metabolism revealed a reduction in serum triglyceride levels. However, there are no major long-term studies demonstrating harm or benefit in using lipid-lowering drugs compared to low-fat diets in children [72]. Phytosterols have been widely studied for their cholesterol-lowering effects. One such phytochemical, guggulsterone [4,17(20)-pregnadiene-3,16-dione], has been used to treat a variety of ailments, including obesity, arthritis and lipid disorders [73]. Several other plant sterols like diosgenin, campesterol, sitosterol, stigmasterol and brassicasterol were shown to possess cholesterol lowering effects [74–76]. Since turnover of cholesterol was shown to bear a relationship to body fat mass [77], phytosterols may also decrease body fat. A number of studies have demonstrated the beneficial effects of polyunsaturated fatty acids (PUFAs) on lipid-related disorders in humans [78].

2.6. Adipocyte-specific effects

Adipose tissue mass can be reduced by both inhibiting adipogenesis and inducing apoptosis of adipocytes. Natural products that specifically target both these pathways therefore will have better potential for treatment and prevention of obesity. Polyphenolic compounds are widely found in fruits and vegetables [21], among which flavonoids and several classes of nonflavonoids are usually distinguished [22]. The antiobesity effects and also adipocyte-specific effects of several polyphenols have been investigated, as discussed below. PUFAs are vital components of the phospholipids of cell membranes and serve as important mediators of the nuclear events regulating the adipocyte-specific gene expression involved in lipid metabolism and adipogenesis [79]. Although most commonly used dietary supplements like CLA showed an effect on glucose and lipid metabolism, these effects are also likely secondary effects mediated through adipocyte-specific transcription factors and their nuclear receptors [80]. Likewise, although the beneficial effects of organosulfur compounds present in natural food are due to their antioxidant and anticarcinogenic properties [81], recently, the adipocyte specific effects of ajoene, a garlic derivative, were reported [82]. This study indicates that garlic extracts may influence fat cell number, thereby suggesting a therapeutic possibility for obesity. The adipocyte-specific effects of natural products are described in detail in the following sections.

3. The adipocyte life cycle

The biologic events leading to obesity are characterized by changes in cell properties of adipocytes and may include an increase in the number or size or both [83]. Adipocytes are derived from mesenchymal stem cells, which have the potential to differentiate into myoblasts, chondroblasts, osteoblasts or adipocytes. The adipocyte life cycle includes alteration of cell shape and growth arrest, clonal expansion and a complex sequence of changes in gene expression leading to storage of lipid and finally cell death (Fig. 1) [84].

During the growth phase, preadipocytes resemble fibroblasts morphologically. Pref-1, a preadipocyte-secreted factor serves as a marker for preadipocytes and is extinguished during adipocyte differentiation [85]. At confluence, preadipocytes enter a resting phase called growth arrest before undergoing the differentiation process. Two transcription factors, CCAAT/enhancer binding protein (C/EBPα) and peroxisome proliferator-activated receptor (PPAR) γ were shown to be involved in the preadipocyte growth arrest that is required for adipocyte differentiation [86]. Following growth arrest, preadipocytes must receive an appropriate combination of mitogenic and adipogenic signals to continue through the subsequent differentiation steps. During the process of differentiation, preadipocytes undergo one round of DNA replication leading to clonal amplification of committed cells [87]. The induction of differentiation also results in drastic change in cell shape as the cells convert from fibroblastic to spherical shape.

Following induction, a dramatic decrease in Pref-1 expression accompanies a rapid increase in the expression of C/EBPβ, followed by expression of C/EBPα and PPARγ [88]. During the terminal stages of differentiation, the mRNA levels for enzymes involved in triacylglycerol metabolism like glycerol-3-phosphate dehydrogenase, fatty acid synthase and glyceraldehyde-3-phosphate dehydrogenase, increase to a great extent [89,90]. Finally, although it was once believed that the total number of adipocytes does not change throughout life, it is now recognized that new adipocytes can be formed or can be removed by the process of apoptosis [7].

4. Targeting the adipocyte life cycle

4.1. Preadipocytes

Preadipocytes can proliferate throughout life to increase fat mass. A number of natural products were shown to inhibit preadipocyte proliferation and induce apoptosis. Polyphenols are powerful antioxidants [91], and induction of apoptosis in preadipocytes by flavonoids was shown to be associated with their antioxidant activity [92]. Quercetin, one of the most abundant flavonoids present in various common fruits and vegetables, induced apoptosis in 3T3-L1 preadipocytes by decreasing mitochondria membrane potential,

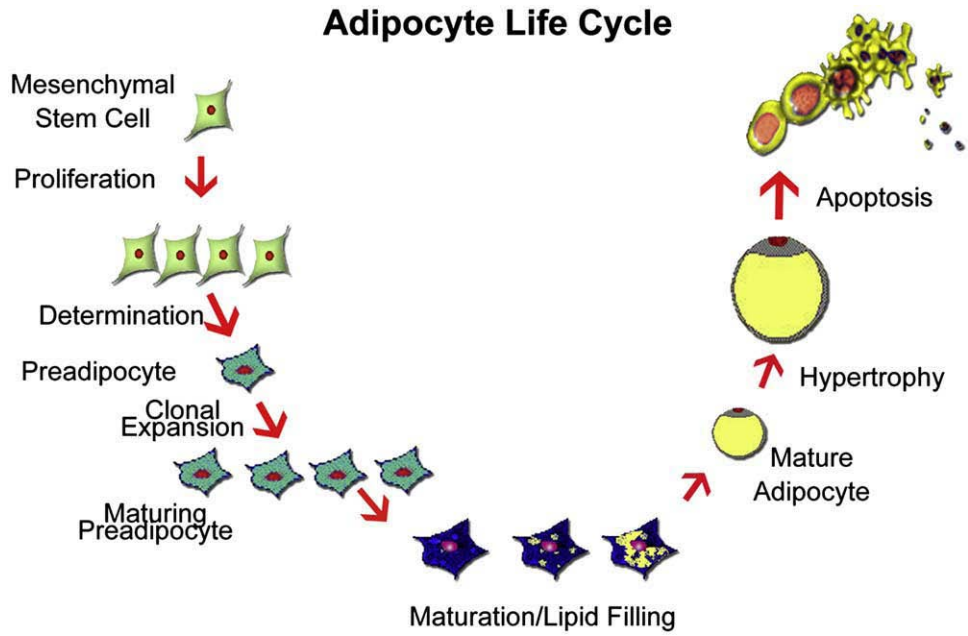

Fig. 1. Mesenchymal stem cells are the precursors of several different types of cells, including myoblasts, chondroblasts, osteoblasts and preadipocytes. Once preadipocytes are triggered to mature, they begin to change shape and undergo a round of cell division known as clonal expansion, followed by initiation of the genetic program that allows them to synthesize and store triglycerides. Mature adipocytes can continue storing lipid when energy intake exceeds output, and they can mobilize and oxidize lipid when energy output exceeds input. Mature adipocytes can also undergo apoptotic cell death under certain conditions.

down-regulating poly (ADP-ribose) polymerase (PARP) and Bcl-2 and activating caspase 3, Bax and Bak. Several other flavonoids like naringenin, rutin, hesperidin, resveratrol, naringin and genistein also decreased preadipocyte proliferation [93–95]. The green tea polyphenol EGCG also induced apoptosis in preadipocytes. The apoptotic effects were Cdk2- and caspase 3-dependent and could be attributed to inhibition of cell mitogenesis [96]. The induction of apoptosis in 3T3-L1 preadipocytes by capsaicin was mediated through the activation of caspase 3, Bax and Bak, and then through the cleavage of PARP and the down-regulation of Bcl-2 [97].

Dividing cells when exposed to stress will undergo cell cycle arrest to either repair the DNA or to initiate apoptosis [98]. Natural antioxidants were reported to cause G_1 phase arrest in prostatic carcinoma cells [99]. Phenolic acids like *o*-coumaric acid, *m*-coumaric acid and chlorogenic acid caused cell cycle arrest at the G_1 phase in a time- and dose-dependent manner in preadipocytes [93], and another coumarin derivative, esculetin, also induced apoptosis in 3T3-L1 preadipocytes [94]. More recently, CLAs were shown to promote human preadipocyte apoptosis [100]. However, docosahexaenoic acid (DHA), an omega-3 fatty acid, showed no effect on the proliferation of preconfluent preadipocytes [101].

4.2. Maturing preadipocytes

Adipocyte number increases not only as a result of increased preadipocyte proliferation but also due to differentiation [102]. Induction of differentiation stimulates clonal expansion resulting in doubling of the cell number [87]. Two critical events occur during the early stage of differentiation, namely, mitotic clonal expansion and an irreversible commitment to differentiation [103]. Genistein inhibited mitotic clonal expansion of 2-day postconfluent 3T3-L1 preadipocytes, whereas naringenin, a flavonoid structurally similar to genistein, failed to exert antiproliferative effects on maturing preadipocytes [95] (Fig. 2). Esculetin induced apoptosis in maturing preadipocytes during the late differentiation stage [94]. DHA-induced apoptosis in 3T3-L1 cells during postconfluent mitotic expansion was accompanied by increased LDH release [104]. Postconfluent preadipocytes treated with CLA had more apoptotic cells than control cultures and also had fewer cells in the S-phase than control cultures [105].

EGCG also induced apoptosis in postconfluent maturing preadipocytes during treatment with insulin, but the biochemical mechanisms involved are not known [106]. Cell cycle and growth-related genes in maturing preadipocytes were down-regulated after treatment with GSPE during the early stage of differentiation [107]. Since irreversibly committed preadipocytes undergo several rounds of replication during the first 2 days of differentiation, the induction of apoptosis in postconfluent differentiating cells will lead to fewer adipocytes. Therefore, maturing preadipocytes could be an important target for natural products in regulating the adipocyte life cycle.

4.3. Adipogenesis

The first hallmark of the adipogenesis process is alteration in cell shape paralleled by changes in the type and expression levels of extracellular matrix components and cytoskeletal components [108]. These events further promote the expression of adipogenic transcription factors, including C/EBPα and PPARγ. C/EBP and PPAR are the central transcriptional regulators of adipogenesis and are required for the synthesis of many adipocyte functional proteins. C/EBP up-regulation is a very early event and mediates the downstream up-regulation of PPAR and C/EBP expression [109]. A number of studies have demonstrated that natural compounds like EGCG, genistein, esculetin, DHA, berberine, resveratrol, guggulsterone, CLA, capsaicin, baicalein and procyanidins inhibited adipogenesis [35,94,97,104,110–115]. The protein expression of PPAR and C/EBP was decreased in adipocytes treated with capsaicin, genistein, berberine and EGCG [106,107,95,97,111,116]. PUFAs were shown to suppress lipogenesis by down-regulating the expression of the sterol regulatory element-binding proteins [117] and also by down-regulating the late phase of adipocyte differentiation [118]. The decrease in adipogenesis by resveratrol was associated with increase in the expression of Sirt1, which promotes fat mobilization by repressing PPARγ [35]. The anti-adipogenic effect of baicalein was due to its ability to enhance the expression of cyclooxygenase-2 (COX-2), which is normally down-regulated during adipogenesis [115]. AMP-activated protein kinase (AMPK) is another target molecule for antiobesity treatments, and genistein, EGCG and capsaicin were shown to inhibit adipocyte differentiation by activating AMPK [119].

4.4. Lipolysis

Breakdown of triglycerides in adipocytes and the release of glycerol and fatty acids are important for the regulation of energy homeostasis [120]. Hormone-sensitive lipase (HSL) is the most important lipase that catalyses the process of lipolysis, and HSL is subject to hormonal regulation [121]. Lipolysis is stimulated by protein kinase A (PKA) activation, which phosphorylates HSL, or by phosphorylation of HSL by G protein-coupled receptors and cyclic AMP-activated extracellular signal-regulated kinase (ERK) [122]. Preadipocytes do not have lipolytic activity until they are differentiated to mature adipocytes [123]. The cytokine tumor necrosis factor alpha (TNFα) has been shown to increase the lipolysis rate in humans in vivo [124] and in primary cultures of newly differentiated human preadipocytes [125].

Apart from inhibiting adipogenesis, several natural compounds stimulate lipolysis in adipocytes. Flavonoids genistein, diadzein, coumestrol and zearalenone stimulated a dose-dependent increase in lipolysis in rat adipocytes [126,127]. Quercetin, luteolin and fisetin caused a dose- and time-dependent increase in lipolysis in rat adipocytes, which was synergistic with epinephrine, and these effective lipolytic flavonoids were also reported to be potent PDE inhibitors [128]. Grape seed proanthocyanidins stimulated

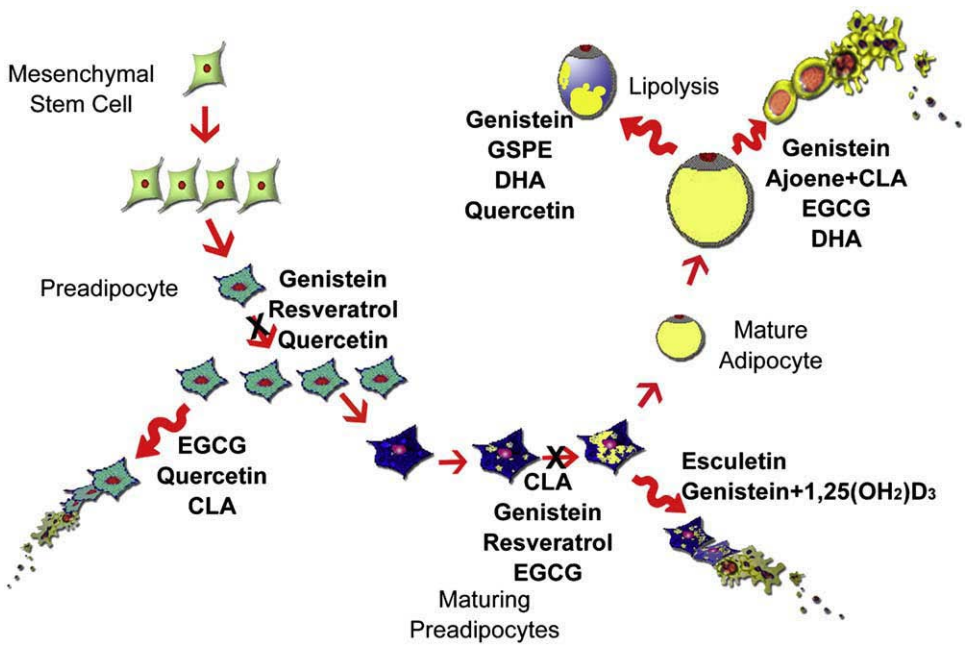

Fig. 2. Examples of individual natural compounds and combinations of compounds that affect specific stages of the adipocyte life cycle. Genistein inhibits preadipocyte proliferation and suppresses lipid accumulation in maturing preadipocytes. It also triggers lipolysis and induces apoptosis in mature adipocytes, and in combination with $1,25(OH)_2D_3$, it can induce apoptosis in maturing preadipocytes. EGCG induces apoptosis in both preadipocytes and mature adipocytes, and it can inhibit lipid accumulation in maturing preadipocytes. Quercetin also has multiple effects — it can inhibit preadipocyte proliferation, induce preadipocyte apoptosis and stimulate lipolysis in mature adipocytes. Ajoene+CLA are especially potent in inducing apoptosis in mature adipocytes.

long-term lipolysis by increasing cAMP and PKA in 3T3-L1 adipocytes [129]. CLA increased basal lipolysis in 3T3-L1 preadipocytes [130,131] and human adipocytes [132]. The mechanism of induction of lipolysis by CLA is not mediated by HSL activation via classic cAMP or PKA pathways but via an ERK-dependent activation of HSL [132]. DHA also stimulated lipolysis when added to mature adipocytes; however, the cellular mechanisms involved in DHA's effects on lipid metabolism have not yet been investigated [104]. In contrast, EGCG did not induce lipolysis, indicating that the anti-obesity effects of EGCG are not mediated via increased lipid mobilization [133].

4.5. Mature adipocyte apoptosis

Compounds that induce adipocyte apoptosis can reduce body fat content, and the effect has the potential to last much longer than body fat reduction caused by lipid mobilization alone. Apoptosis is a form of cell suicide that plays an important role in maintaining cellular homeostasis, and at times, it is necessary to eliminate excessive cells and cells that hinder development. Although a number of stimuli trigger apoptosis, there are two major signaling pathways: the death receptor pathway and the mitochondrial pathway [134]. A series of molecular steps leads to activation of caspases in both of these pathways. Finally this results in cleavage of a number of nuclear and cytoplasmic substrates resulting in cell death [135].

Several natural compounds were reported to induce apoptosis primarily in cancer cells, but relatively little research exists on investigating the various natural compounds that induce apoptosis in adipose tissue. Green tea extracts [17], soy isoflavones [26,27], CLA [130,136,137] and garlic compounds [18] were shown to reduce body fat in experimental animals, but the mechanisms of action in inducing adipocyte apoptosis with these compounds has been investigated only recently. Although the effect of CLA on body fat is not completely understood, it is thought that a marked increase of TNFα mRNA with an increase of uncoupling protein 2 (UCP2) in adipocytes caused CLA-induced apoptosis [137]. In contrast, EGCG-induced apoptosis is mediated by activator protein-1, nuclear factor kappa B and p53 [138] and increased caspase 3 activity [139].

Reactive oxygen species (ROS) were reported to play a key role in cell signaling, and the role of ROS generation in the proliferation of various cells has been investigated [140,141]. In leukemic cells, increased ROS generation leads to the activation of mitogen-activated protein kinases, resulting in cell death [142]. Genistein, EGCG and capsaicin stimulated intracellular ROS release, which activated AMPK rapidly leading to apoptosis [119]. Ajoene also induced apoptosis in leukemic cells through the generation of ROS [143], and more recently, ajoene was shown to induce ROS-mediated apoptosis in adipocytes as well [82]. The cellular mechanisms involved in DHA-mediated apoptosis in mature adipocytes has not been investigated yet [104].

4.6. Dietary bioactive entities targeting multiple signaling pathways

Synergistic interactions with combinations of phytochemicals such as quercetin, tea catechins, curcumin, genistein and resveratrol for the treatment of cancer has been investigated [144]. The apoptosis-inducing activity of EGCG on lung cancer cells was found to be synergistically enhanced by other chemopreventive agents, such as sulindac and tamoxifen [145]. Additionally, curcumin, a component of the culinary spice turmeric, was shown to potentiate the antitumor and apoptotic effects of cisplatin in ovarian carcinoma cells [146]. While all the above studies were performed in cancer cells, such synergistic interactions among dietary bioactives on adipocytes have not been investigated in detail.

Recently we have reported that t10,c12CLA potentiates ajoene-induced apoptosis in 3T3-L1 adipocytes [147]. Cytochrome *c* release is regulated by Bcl-2 family proteins, and these proteins are associated with the mitochondrial membrane and regulate its integrity. Bax, a member of Bcl-2 family proteins, exerts proapoptotic activity by translocation from the cytosol to the mitochondria and inducing cytochrome *c* release [148]. CLA and ajoene as individual compounds showed no effect on cytochrome *c*, whereas ajoene increased and CLA had no effect on Bax expression. However, the combination of ajoene and CLA caused a synergistic increase in both cytochrome *c* and Bax expression.

Similarly, we have reported that 1,25 dihydroxy vitamin D_3 ($1,25(OH)_2D_3$ [calcitriol]), potentiates the effects of genistein in inducing apoptosis and inhibiting adipogenesis in maturing 3T3-L1 preadipocytes. An interesting feature about this study is that the synergistic effect was observed only in maturing preadipocytes and not in either mature adipocytes or preadipocytes, the reasons for which are not clearly understood. The combination of genistein and $1,25(OH)_2D_3$ caused a significant increase in vitamin D receptor (VDR) mRNA expression in human prostate cancer cells [149], and we found that in maturing 3T3-L1 adipocytes, genistein+$1,25(OH)_2D_3$ increased VDR protein levels by more than 100%, whereas $1,25(OH)_2D_3$ by itself increased VDR protein levels by only 40%, and genistein alone at the tested concentration had no effect. This effect on VDR correlated with an increase in apoptosis of about 200% with the combination treatment. The VDR, a member of the nuclear receptor superfamily, plays a key role in adipocyte biology when bound to its ligand, $1,25(OH)_2D_3$ [150], and these results indicate that the potentiation of both the increase in apoptosis and suppression of adipogenesis with the combination treatment might be mediated in part through the VDR.

Such studies of synergistic activity suggest that the desired effects on adipocytes could be achieved by using lower doses of two or more compounds, thereby decreasing potential toxic effects. Although results from in vitro experiments cannot be directly extrapolated to clinical effects, such studies will help in elucidating various molecular pathways by which

selected natural products, either as individual treatments or in combination, might be effective in regulating adipose tissue volume through adipocyte apoptosis and inhibition of adipogenesis.

5. Conclusions

Obesity is a risk factor for diseases like non-insulin-dependent diabetes mellitus, atherosclerosis and certain cancers [151]. Adipose mass can be decreased by removing adipocytes, and it is becoming evident that fat cells have a finite life span and can be eliminated by apoptosis [7,152,153]. Since adipogenesis is intricately related to adipocyte differentiation and maturation, inducing apoptosis and inhibiting adipogenesis at various stages of the adipocyte life cycle may be target pathways for treating obesity. In cancer cells, phytochemicals tend to increase the therapeutic effect by either blocking one or more targets of the signal transduction pathway or by increasing the bioavailability of the other drug in the system [144]. Dietary bioactives derived from natural products have shown interesting effects on adipose tissue like inducing apoptosis, decreasing lipid accumulation and inducing lipolysis. Since a number of complex interconnected cell signaling pathways are involved in regulating all the abovementioned processes, treating adipocytes with multiple natural products can result in enhanced effects. This strategy can be achieved by exerting beneficial effects through additive or synergistic actions of several natural compounds acting at single or multiple target sites in the adipocyte life cycle associated with physiological processes like apoptosis, adipogenesis and lipolysis.

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