

REVIEWS: CURRENT TOPICS

Phytochemicals and regulation of the adipocyte life cycle[☆]Srujana Rayalam^a, Mary Anne Della-Fera^a, Clifton A. Baile^{a,b,*}^a*Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602, USA*^b*Department of Foods and Nutrition, University of Georgia, Athens, GA 30602, USA*

Received 9 July 2007; accepted 14 December 2007

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.

© 2008 Elsevier Inc. All rights reserved.

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

[☆] This work was supported in part by grants from AptoTec and the Georgia Research Alliance and by the Georgia Research Alliance Eminent Scholar endowment held by C.A.B.

* Corresponding author. 444 Edgar L. Rhodes Center for Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771, USA. Tel.: +1 706 542 2771; fax: +1 706 542 7925.

E-mail address: cbaile@uga.edu (C.A. Baile).

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 intracellu-

lar 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 gordonii* 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/EBP α) 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/EBP β , followed by expression of C/EBP α and PPAR γ [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,

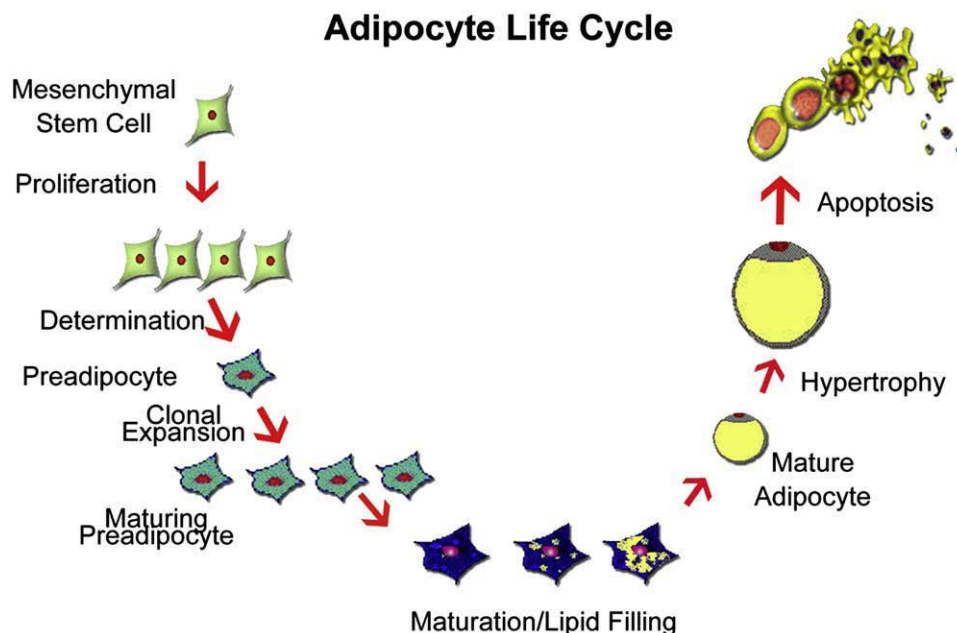


Fig. 1. Mesenchymal stem cells are the precursors of several different types of cells, including myoblasts, chondroblasts, osteoblasts and preadipocytes. Once preadipocytes are triggered to mature, they begin to change shape and undergo a round of cell division known as clonal expansion, followed by 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 G₁ 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 G₁ 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 differ-

entiation [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/EBP α and PPAR γ . 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 PPAR γ [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 (TNF α) 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 zeaxenone 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

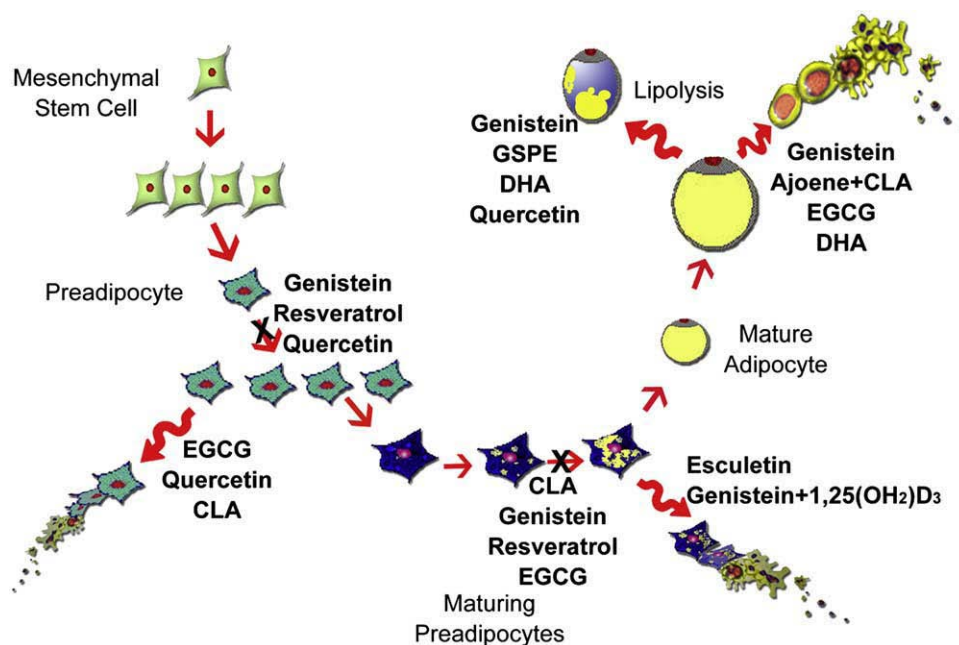


Fig. 2. Examples of individual natural compounds and combinations of compounds that affect specific stages of the adipocyte life cycle. Genistein inhibits preadipocyte proliferation and suppresses lipid accumulation in maturing preadipocytes. It also triggers lipolysis and induces apoptosis in mature adipocytes, and in combination with 1,25(OH) $_2$ D $_3$, 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 TNF α 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 D₃ (1,25(OH)₂D₃ [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)₂D₃ 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)₂D₃ increased VDR protein levels by more than 100%, whereas 1,25(OH)₂D₃ 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)₂D₃ [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.

References

- [1] Visscher TL, Seidell JC. The public health impact of obesity. *Annu Rev Public Health* 2001;22:355–75.
- [2] Goldstein DJ. Beneficial health effects of modest weight loss. *Int J Obes Relat Metab Disord* 1992;16:397–415.
- [3] Astrup A. Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. *Public Health Nutr* 2001;4: 499–515.
- [4] Kruger J, Galuska DA, Serdula MK, Jones DA. Attempting to lose weight: specific practices among U.S. adults. *Am J Prevent Med* 2004;26:402–6.
- [5] Wadden TA. Treatment of obesity by moderate and severe caloric restriction. Results of clinical research trials. *Ann Intern Med* 1993; 119:688–93.
- [6] Stern JS, Hirsch J, Blair SN, Foreyt JP, Frank A, Kumanyika SK, et al. Weighing the options: criteria for evaluating weight-management programs. The Committee to Develop Criteria for Evaluating the Outcomes of Approaches to Prevent and Treat Obesity. *Obes Res* 1995;3:591–604.
- [7] Prins JB, O'Rahilly S. Regulation of adipose cell number in man. *Clin Sci (Lond)* 1997;92:3–11.
- [8] Sorisky A, Magun R, Gagnon AM. Adipose cell apoptosis: death in the energy depot. *Int J Obes Relat Metab Disord* 2000;24(Suppl 4): S3–S7.
- [9] Jilka RL. Osteoblast progenitor fate and age-related bone loss. *J Musculoskelet Neuronal Interact* 2002;2:581–3.
- [10] Laroche M. Intraosseous circulation from physiology to disease. *Joint Bone Spine* 2002;69:262–9.
- [11] Newman AB, Lee JS, Visser M, Goodpaster BH, Kritchevsky SB, Tyllavsky FA, et al. Weight change and the conservation of lean mass in old age: the Health, Aging and Body Composition Study. *Am J Clin Nutr* 2005;82:872–8 [915–6].
- [12] Knoke JD, Barrett-Connor E. Weight loss: a determinant of hip bone loss in older men and women. The Rancho Bernardo Study. *Am J Epidemiol* 2003;158:1132–8.
- [13] Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: sources of industrial and medicinal materials. *Science (New York, NY)* 1985;228:1154–60.
- [14] Kessler RC, Davis RB, Foster DF, Van Rompay MI, Walters EE, Wilkey SA, et al. Long-term trends in the use of complementary and alternative medical therapies in the United States. *Ann Intern Med* 2001;135:262–8.
- [15] Youngkin EQ, Israel DS. A review and critique of common herbal alternative therapies. *The Nurse practitioner*. 1996; 21:39, 43–6, 9–52 passim.
- [16] O'Hara M, Kiefer D, Farrell K, Kemper K. A review of 12 commonly used medicinal herbs. *Arch Fam Med* 1998;7:523–36.
- [17] Hasegawa N, Yamada N, Mori M. Powdered green tea has antilipogenic effect on Zucker rats fed a high-fat diet. *Phytother Res* 2003;17:477–80.
- [18] Elkayam A, Mirelman D, Peleg E, Wilchek M, Miron T, Rabinkov A, et al. The effects of allicin on weight in fructose-induced hyperinsulinemic, hyperlipidemic, hypertensive rats. *Am J Hypertens* 2003;16:1053–6.
- [19] Hargrave KM, Li C, Meyer BJ, Kachman SD, Hartzell DL, Della-Fera MA, et al. Adipose depletion and apoptosis induced by *trans*-10, *cis*-12 conjugated linoleic acid in mice. *Obes Res* 2002;10:1284–90.
- [20] Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev* 1998;56:317–33.
- [21] Aherne SA, O'Brien NM. Dietary flavonols: chemistry, food content, and metabolism. *Nutrition* 2002;18:75–81.
- [22] Harborne JB. Methods in plant biochemistry. I. Plant phenolics. London: Academic Press; 1989.
- [23] Han LK, Sumiyoshi M, Zheng YN, Okuda H, Kimura Y. Anti-obesity action of *Salix matsudana* leaves (Part 2). Isolation of anti-obesity effectors from polyphenol fractions of *Salix matsudana*. *Phytother Res* 2003;17:1195–8.
- [24] Yu SF, Shun CT, Chen TM, Chen YH. 3-*O*-beta-D-glucosyl-(1→6)-beta-D-glucosyl-kaempferol isolated from *Sauropus androgenus* reduces body weight gain in Wistar rats. *Biol Pharm Bull* 2006;29: 2510–3.
- [25] Kwon O, Eck P, Chen S, Corpe CP, Lee JH, Kruhlak M, et al. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. *FASEB J* 2007;21:366–77.
- [26] Kim HK, Nelson-Dooley C, Della-Fera MA, Yang JY, Zhang W, Duan J, et al. Genistein decreases food intake, body weight, and fat pad weight and causes adipose tissue apoptosis in ovariectomized female mice. *J Nutr* 2006;136:409–14.
- [27] Naaz A, Yellayi S, Zakroczymski MA, Bunick D, Doerge DR, Lubahn DB, et al. The soy isoflavone genistein decreases adipose deposition in mice. *Endocrinology* 2003;144:3315–20.
- [28] Dang Z, Lowik CW. The balance between concurrent activation of ERs and PPARs determines diadzein-induced osteogenesis and adipogenesis. *J Bone Miner Res* 2004;19:853–61.
- [29] Tsuda T, Ueno Y, Kojo H, Yoshikawa T, Osawa T. Gene expression profile of isolated rat adipocytes treated with anthocyanins. *Biochim Biophys Acta* 2005;1733:137–47.
- [30] Preuss HG, Wallerstedt D, Talpur N, Tutuncuoglu SO, Echard B, Myers A, et al. Effects of niacin-bound chromium and grape seed proanthocyanidin extract on the lipid profile of hypercholesterolemic subjects: a pilot study. *J Med* 2000;31:227–46.
- [31] Nakagawa Y, Iinuma M, Matsuura N, Yi K, Naoi M, Nakayama T, et al. A potent apoptosis-inducing activity of a sesquiterpene lactone,

- arucanolid, in HL60 cells: a crucial role of apoptosis-inducing factor. *J Pharmacol Sci* 2005;97:242–52.
- [32] Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Riegger C, et al. Epigallocatechin gallate supplementation alleviates diabetes in rodents. *J Nutr* 2006;136:2512–8.
- [33] Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K. Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem Biophys Res Commun* 2005;332:392–7.
- [34] Yang JY, Della-Fera MA, Hartzell DL, Nelson-Dooley C, Hausman DB, Baile CA. Esculetin induces apoptosis and inhibits adipogenesis in 3T3-L1 cells. *Obesity (Silver Spring, Md)* 2006;14:1691–9.
- [35] Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR- γ . *Nature* 2004;429:771–6.
- [36] Dulloo AG. Ephedrine, xanthines and prostaglandin-inhibitors: actions and interactions in the stimulation of thermogenesis. *Int J Obes Relat Metab Disord* 1993;17(Suppl 1):S35–S40.
- [37] Racotta IS, Leblanc J, Richard D. The effect of caffeine on food-intake in rats — involvement of corticotropin-releasing factor and the sympathoadrenal system. *Pharmacol Biochem Behav* 1994;48:887–92.
- [38] Astrup A, Breum L, Toubro S, Hein P, Quaade F. The effect and safety of an ephedrine caffeine compound compared to ephedrine, caffeine and placebo in obese subjects on an energy restricted diet — a double-blind trial. *Int J Obes* 1992;16:269–77.
- [39] Diepvens K, Westerterp KR, Westerterp-Plantenga MS. Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. *Am J Physiol Regul Integr Comp Physiol* 2007;292:R77–R85.
- [40] Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, et al. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr* 1999;70:1040–5.
- [41] Dulloo AG, Seydoux J, Girardier L, Chantre P, Vandermander J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int J Obes Relat Metab Disord* 2000;24:252–8.
- [42] Yoshioka M, Lim K, Kikuzato S, Kiyonaga A, Tanaka H, Shindo M, et al. Effects of red-pepper diet on the energy metabolism in men. *J Nutr Sci Vitaminol* 1995;41:647–56.
- [43] Kawada T, Watanabe T, Takaishi T, Tanaka T, Iwai K. Capsaicin-induced beta-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. *Proc Soc Exp Biol Med* 1986;183:250–6.
- [44] Borchardt RT, Huber JA. Catechol *O*-methyltransferase. 5. Structure-activity relationships for inhibition by flavonoids. *J Med Chem* 1975;18:120–2.
- [45] Avula B, Wang YH, Pawar RS, Shukla YJ, Schaneberg B, Khan IA. Determination of the appetite suppressant P57 in *Hoodia gordonii* plant extracts and dietary supplements by liquid chromatography/electrospray ionization mass spectrometry (LC-MSD-TOF) and LC-UV methods. *J AOAC Int* 2006;89:606–11.
- [46] MacLean DB, Luo LG. Increased ATP content/production in the hypothalamus may be a signal for energy-sensing of satiety: studies of the anorectic mechanism of a plant steroidal glycoside. *Brain Res* 2004;1020:1–11.
- [47] Fleming RM. The effect of ephedra and high fat dieting: a cause for concern! A case report. *Angiology* 2007;58:102–5.
- [48] Klontz KC, Timbo BB, Street D. Consumption of dietary supplements containing *Citrus aurantium* (bitter orange) — 2004 California Behavioral Risk Factor Surveillance Survey (BRFSS). *Ann Pharmacother* 2006;40:1747–51.
- [49] Ohia SE, Opere CA, LeDay AM, Bagchi M, Bagchi D, Stohs SJ. Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). *Mol Cell Biochem* 2002;238:89–103.
- [50] Kuriyan R, Raj T, Srinivas SK, Vaz M, Rajendran R, Kurpad AV. Effect of *Caralluma fimbriata* extract on appetite, food intake and anthropometry in adult Indian men and women. *Appetite* 2007;48:338–44.
- [51] Baintner K, Kiss P, Pfeller U, Bardocz S, Pusztai A. Effect of orally and intraperitoneally administered plant lectins on food consumption of rats. *Acta Physiol Hung* 2003;90:97–107.
- [52] Bo-Linn GW, Santa Ana CA, Morawski SG, Fordtran JS. Starch blockers — their effect on calorie absorption from a high-starch meal. *N Engl J Med* 1982;307:1413–6.
- [53] Celleno L, Tolaini MV, D'Amore A, Perricone NV, Preuss HG. A dietary supplement containing standardized *Phaseolus vulgaris* extract influences body composition of overweight men and women. *Int J Med Sci* 2007;4:45–52.
- [54] Suzuki K, Konno R, Shimzu T, Nagashima T, Kimura A. A fermentation product of phytosterol including campestenone reduces body fat storage and body weight gain in mice. *J Nutr Sci Vitaminol (Tokyo)* 2007;53:63–7.
- [55] Adebajo AC, Olawode EO, Omobuwajo OR, Adesanya SA, Begrow F, Elkhawad A, et al. Hypoglycaemic constituents of *Stachytarpheta cayennensis* leaf. *Planta Med* 2007;73:241–50.
- [56] Zheng J, He J, Ji B, Li Y, Zhang X. Antihyperglycemic effects of *Platycodon grandiflorum* (Jacq.) A. DC. extract on streptozotocin-induced diabetic mice. *Plant Foods Hum Nutr* 2007;62:7–11.
- [57] Megalli S, Davies NM, Roufogalis BD. Anti-hyperlipidemic and hypoglycemic effects of *Gynostemma pentaphyllum* in the Zucker fatty rat. *J Pharm Sci* 2006;9:281–91.
- [58] Pushparaj PN, Low HK, Manikandan J, Tan BK, Tan CH. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 2007;111:430–4.
- [59] Guo H, Ling W, Wang Q, Liu C, Hu Y, Xia M, et al. Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. indica) on hyperlipidemia and insulin resistance in fructose-fed rats. *Plant Foods Hum Nutr* 2007;62:1–6.
- [60] Xia T, Wang Q. D-Chiro-inositol found in *Cucurbita ficifolia* (Cucurbitaceae) fruit extracts plays the hypoglycaemic role in streptozotocin-diabetic rats. *J Pharma Pharmacol* 2006;58:1527–32.
- [61] Eidi A, Eidi M, Esmaeili E. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine* 2006;13:624–9.
- [62] Zanatta L, de Sousa E, Cazarolli LH, Junior AC, Pizzolatti MG, Szpoganicz B, et al. Effect of crude extract and fractions from *Vitex megapota mica* leaves on hyperglycemia in alloxan-diabetic rats. *J Ethnopharmacol* 2007;109:151–5.
- [63] Kannappan S, Jayaraman T, Rajasekar P, Ravichandran MK, Anuradha CV. Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rat. *Singapore Med J* 2006;47:858–63.
- [64] Wagner JD, Cefalu WT, Anthony MS, Litwak KN, Zhang L, Clarkson TB. Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesterol ester content in ovariectomized cynomolgus monkeys. *Metabolism* 1997;46:698–705.
- [65] Riccardi G, Rivellese AA. Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. *Diabetes care* 1991;14:1115–25.
- [66] Oben JE, Enyegue DM, Fomekong GI, Soukontoua YB, Agbor GA. The effect of *Cissus quadrangularis* (CQR-300) and a *Cissus* formulation (CORE) on obesity and obesity-induced oxidative stress. *Lipids Health Dis* 2007;6:4.
- [67] Abidov MT, del Rio MJ, Ramazanov TZ, Klimenov AL, Dzhamirze S, Kalyuzhin OV. Effects of *Aralia mandshurica* and *Engelhardtia chrysolepis* extracts on some parameters of lipid metabolism in women with nondiabetic obesity. *Bull Exp Biol Med* 2006;141:343–6.

- [68] Ahn IS, Do MS, Kim SO, Jung HS, Kim YI, Kim HJ, et al. Antiobesity effect of Kochujang (Korean fermented red pepper paste) extract in 3T3-L1 adipocytes. *J Med Food* 2006;9:15–21.
- [69] Moreno LA, Tresaco B, Bueno G, Fleta J, Rodriguez G, Garagorri JM, et al. Psyllium fibre and the metabolic control of obese children and adolescents. *J Physiol Biochem* 2003;59:235–42.
- [70] Han LK, Sumiyoshi M, Zhang J, Liu MX, Zhang XF, Zheng YN, et al. Anti-obesity action of *Salix matsudana* leaves (Part 1). Anti-obesity action by polyphenols of *Salix matsudana* in high fat-diet treated rodent animals. *Phytother Res* 2003;17:1188–94.
- [71] Moreno DA, Ilic N, Poulev A, Raskin I. Effects of *Arachis hypogaea* nutshell extract on lipid metabolic enzymes and obesity parameters. *Life Sci* 2006;78:2797–803.
- [72] Bhatnagar D. Should pediatric patients with hyperlipidemia receive drug therapy? *Paediatric drugs* 2002;4:223–30.
- [73] Urizar NL, Moore DD. GUGULIPID: a natural cholesterol-lowering agent. *Annu Rev Nutr* 2003;23:303–13.
- [74] Kwon CS, Sohn HY, Kim SH, Kim JH, Son KH, Lee JS, et al. Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. *Biosci Biotechnol Biochem* 2003;67:1451–6.
- [75] Vaskonen T, Mervaala E, Seppanen-Laakso T, Karppanen H. Diet enrichment with calcium and magnesium enhances the cholesterol-lowering effect of plant sterols in obese Zucker rats. *Nutr Metab Cardiovasc Dis* 2001;11:158–67.
- [76] Fernandez C, Suarez Y, Ferruelo AJ, Gomez-Coronado D, Lasuncion MA. Inhibition of cholesterol biosynthesis by Delta22-unsaturated phytosterols via competitive inhibition of sterol Delta24-reductase in mammalian cells. *Biochem J* 2002;366:109–19.
- [77] Miettinen TA. Cholesterol production in obesity. *Circulation* 1971;44:842–50.
- [78] Hirafuji M, Machida T, Hamaue N, Minami M. Cardiovascular protective effects of n-3 polyunsaturated fatty acids with special emphasis on docosahexaenoic acid. *J Pharmacol Sci* 2003;92:308–16.
- [79] Lombardo YB, Chicco AG. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. *J Nutr Biochem* 2006;17:1–13.
- [80] Taylor CG, Zahradka P. Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. *Am J Clin Nutr* 2004;79:1164S–8S.
- [81] Sahu SC. Dual role of organosulfur compounds in foods: a review. *J Environ Sci Health* 2002;20:61–76.
- [82] Yang JY, Della-Fera MA, Nelson-Dooley C, Baile CA. Molecular mechanisms of apoptosis induced by ajoene in 3T3-L1 adipocytes. *Obes Res* 2006;14:388–97.
- [83] Flier JS. The adipocyte: storage depot or node on the energy information superhighway? *Cell* 1995;80:15–8.
- [84] Gregoire FM. Adipocyte differentiation: from fibroblast to endocrine cell. *Exp Biol Med* (Maywood) 2001;226:997–1002.
- [85] Wang Y, Kim KA, Kim JH, Sul HS. Pref-1, a preadipocyte secreted factor that inhibits adipogenesis. *J Nutr* 2006;136:2953–6.
- [86] Umek RM, Friedman AD, McKnight SL. CCAAT-enhancer binding protein: a component of a differentiation switch. *Science* 1991;251:288–92.
- [87] Pairault J, Green H. A study of the adipose conversion of suspended 3T3 cells by using glycerophosphate dehydrogenase as differentiation marker. *Proc Natl Acad Sci U S A* 1979;76:5138–42.
- [88] Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, et al. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. *Genes Dev* 2002;16:22–6.
- [89] Paulauskis JD, Sul HS. Cloning and expression of mouse fatty acid synthase and other specific mRNAs. Developmental and hormonal regulation in 3T3-L1 cells. *J Biol Chem* 1988;263:7049–54.
- [90] Spiegelman BM, Frank M, Green H. Molecular cloning of mRNA from 3T3 adipocytes. Regulation of mRNA content for glycerophosphate dehydrogenase and other differentiation-dependent proteins during adipocyte development. *J Biol Chem* 1983;258:10083–9.
- [91] Robak J, Gryglewski RJ. Bioactivity of flavonoids. *Polish J Pharmacol* 1996;48:555–64.
- [92] Hsu CL, Yen GC. Induction of cell apoptosis in 3T3-L1 preadipocytes by flavonoids is associated with their antioxidant activity. *Mol Nutr Food Res* 2006.
- [93] Hsu CL, Huang SL, Yen GC. Inhibitory effect of phenolic acids on the proliferation of 3T3-L1 preadipocytes in relation to their antioxidant activity. *J Agric Food Chem* 2006;54:4191–7.
- [94] Yang JY, Della-Fera MA, Hartzell DL, Nelson-Dooley C, Hausman DB, Baile CA. Esculetin induces apoptosis and inhibits adipogenesis in 3T3-L1 cells. *Obes Res* 2006;14:1691–9.
- [95] Harmon A, Harp J. Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. *Am J Physiol Cell Physiol* 2001;280:C807–13.
- [96] Kao YH, Hiipakka RA, Liao S. Modulation of obesity by a green tea catechin. *Am J Clin Nutr* 2000;72:1232–4.
- [97] Hsu CL, Yen GC. Effects of capsaicin on induction of apoptosis and inhibition of adipogenesis in 3T3-L1 cells. *J Agric Food Chem* 2007;55:1730–6.
- [98] Iliakis G, Wang Y, Guan J, Wang H. DNA damage checkpoint control in cells exposed to ionizing radiation. *Oncogene* 2003;22:5834–47.
- [99] Nyska A, Suttie A, Bakshi S, Lomnitski L, Grossman S, Bergman M, et al. Slowing tumorigenic progression in TRAMP mice and prostatic carcinoma cell lines using natural anti-oxidant from spinach, NAO — a comparative study of three anti-oxidants. *Toxicol Pathol* 2003;31:39–51.
- [100] Fischer-Posovszky P, Kukulus V, Zulet MA, Debatin KM, Wabitsch M. Conjugated linoleic acids promote human fat cell apoptosis. *Horm Metab Res* 2007;39:186–91.
- [101] Kim HK, Della Fera MA, Lin J, Baile CA. Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes. *J Nutr* 2006;36.
- [102] Roncari DA, Lau DC, Kindler S. Exaggerated replication in culture of adipocyte precursors from massively obese persons. *Metabolism* 1981;30:425–7.
- [103] Scott RE, Florine DL, Wille Jr JJ, Yun K. Coupling of growth arrest and differentiation at a distinct state in the G1 phase of the cell cycle: GD. *Proc Natl Acad Sci U S A* 1982;79:845–9.
- [104] Kim HK, Della-Fera MA, Lin J, Baile CA. Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes. *J Nutr* 2006;136:2965–9.
- [105] Evans M, Geigerman C, Cook J, Curtis L, Kuebler B, McIntosh M. Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. *Lipids* 2000;35:899–910.
- [106] Lin J, Della-Fera MA, Baile CA. Green tea polyphenol epigallocatechin gallate inhibits adipogenesis and induces apoptosis in 3T3-L1 adipocytes. *Obes Res* 2005;13:982–90.
- [107] Pinent M, Blade MC, Salvado MJ, Arola L, Hackl H, Quackenbush J, et al. Grape-seed derived procyanidins interfere with adipogenesis of 3T3-L1 cells at the onset of differentiation. *Int J Obes (Lond)* 2005;29:934–41.
- [108] Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. *Physiol Rev* 1998;78:783–809.
- [109] Wu Z, Bucher NL, Farmer SR. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. *Mol Cell Biol* 1996;16:4128–36.
- [110] Kim HK, Nelson-Dooley C, Della-Fera MA, Yang JY, Zhang W, Hartzell DL, et al. Genistein decreases food intake, body weight and fat pad weight and causes adipose tissue apoptosis in ovariectomized female mice. *J Nutr* 2006;136:409–14.
- [111] Huang C, Zhang Y, Gong Z, Sheng X, Li Z, Zhang W, et al. Berberine inhibits 3T3-L1 adipocyte differentiation through the PPARgamma pathway. *Biochem Biophys Res Commun* 2006;348:571–8.
- [112] Rizzo G, Disante M, Mencarelli A, Renga B, Gioiello A, Pellicciari R, et al. FXR promotes adipocyte differentiation and regulates adipose cell function in vivo. *Mol Pharmacol* 2006.

- [113] Brown JM, Boysen MS, Jensen SS, Morrison RF, Storkson J, Currie RL, et al. *trans*-10, *cis*-12 conjugated linoleic acid decreases glucose and fatty acid uptake and oxidation and inhibits PPARgamma-dependent gene expression in human preadipocytes. *J Lipid Res* 2003.
- [114] Sisk MB, Hausman DB, Martin RJ, Azain MJ. Dietary conjugated linoleic acid reduces adiposity in lean but not obese Zucker rats. *J Nutr* 2001;131:1668–74.
- [115] Cha MH, Kim IC, Lee BH, Yoon Y. Baicalein inhibits adipocyte differentiation by enhancing COX-2 expression. *J Med Food* 2006;9:145–53.
- [116] Moon HS, Lee HG, Choi YJ, Kim TG, Cho CS. Proposed mechanisms of (-)-epigallocatechin-3-gallate for anti-obesity. *Chem Biol Interact* 2007.
- [117] Worgall TS, Sturley SL, Seo T, Osborne TF, Deckelbaum RJ. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. *J Biol Chem* 1998;273:25537–40.
- [118] Okuno M, Kajiwaru K, Imai S, Kobayashi T, Honma N, Maki T, et al. Perilla oil prevents the excessive growth of visceral adipose tissue in rats by down-regulating adipocyte differentiation. *J Nutr* 1997;127:1752–7.
- [119] Hwang JT, Park IJ, Shin JI, Lee YK, Lee SK, Baik HW, et al. Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process via activating AMP-activated protein kinase. *Biochem Biophys Res Commun* 2005;338:694–9.
- [120] Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW. Integrative physiology of human adipose tissue. *Int J Obes Relat Metab Disord* 2003;27:875–88.
- [121] Holm C. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Biochem Soc Trans* 2003;31:1120–4.
- [122] Greenberg AS, Shen WJ, Muliro K, Patel S, Souza SC, Roth RA, et al. Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J Biol Chem* 2001;276:45456–61.
- [123] Hauner H, Skurk T, Wabitsch M. Cultures of human adipose precursor cells. *Methods Mol Biol* 2001;155:239–47.
- [124] Starnes Jr HF, Warren RS, Jeevanandam M, Gabrilove JL, Larchian W, Oettgen HF, et al. Tumor necrosis factor and the acute metabolic response to tissue injury in man. *J Clin Invest* 1988;82:1321–5.
- [125] Hauner H, Petruschke T, Russ M, Rohrig K, Eckel J. Effects of tumour necrosis factor alpha (TNF alpha) on glucose transport and lipid metabolism of newly-differentiated human fat cells in cell culture. *Diabetologia* 1995;38:764–71.
- [126] Kandulska K, Nogowski L, Szkudelski T. Effect of some phytoestrogens on metabolism of rat adipocytes. *Reprod Nutr Dev* 1999;39:497–501.
- [127] Szkudelska K, Szkudelski T, Nogowski L. Daidzein, coumestrol and zeaxanthone affect lipogenesis and lipolysis in rat adipocytes. *Phytomedicine* 2002;9:338–45.
- [128] Kuppusamy UR, Das NP. Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes. *Biochem Pharmacol* 1992;44:1307–15.
- [129] Pinet M, Blade MC, Salvado MJ, Arola L, Ardevol A. Intracellular mediators of procyanidin-induced lipolysis in 3T3-L1 adipocytes. *J Agric Food Chem* 2005;53:262–6.
- [130] Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 1997;32:853–8.
- [131] Evans M, Lin X, Odle J, McIntosh M. *trans*-10, *cis*-12 conjugated linoleic acid increases fatty acid oxidation in 3T3-L1 preadipocytes. *J Nutr* 2002;132:450–5.
- [132] Chung S, Brown JM, Sandberg MB, McIntosh M. *trans*-10, *cis*-12 CLA increases adipocyte lipolysis and alters lipid droplet-associated proteins: role of mTOR and ERK signaling. *J Lipid Res* 2005;46:885–95.
- [133] Wolfram S, Wang Y, Thielecke F. Anti-obesity effects of green tea: from bedside to bench. *Mol Nutr Food Res* 2006;50:176–87.
- [134] Gupta S. Molecular steps of death receptor and mitochondrial pathways of apoptosis. *Life Sci* 2001;69:2957–64.
- [135] Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407:770–6.
- [136] West DB, Delany JP, Camet PM, Blohm F, Truett AA, Scimeca J. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol* 1998;275:R667–72.
- [137] Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, et al. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* 2000;49:1534–42.
- [138] Hastak K, Gupta S, Ahmad N, Agarwal MK, Agarwal ML, Mukhtar H. Role of p53 and NF-kappaB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. *Oncogene* 2003;22:4851–9.
- [139] Hsu S, Lewis J, Singh B, Schoenlein P, Osaki T, Athar M, et al. Green tea polyphenol targets the mitochondria in tumor cells inducing caspase 3-dependent apoptosis. *Anticancer research* 2003;23:1533–9.
- [140] Burdon RH, Rice-Evans C. Free radicals and the regulation of mammalian cell proliferation. *Free Radic Res Commun* 1989;6:345–58.
- [141] Carriere A, Fernandez Y, Rigoulet M, Penicaud L, Casteilla L. Inhibition of preadipocyte proliferation by mitochondrial reactive oxygen species. *FEBS Lett* 2003;550:163–7.
- [142] Chen YR, Wang W, Kong AN, Tan TH. Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. *J Biol Chem* 1998;273:1769–75.
- [143] Dirsch VM, Gerbes AL, Vollmar AM. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB. *Mol Pharmacol* 1998;53:402–7.
- [144] Hemalswarya S, Doble M. Potential synergism of natural products in the treatment of cancer. *Phytother Res* 2006;20:239–49.
- [145] Suganuma M, Okabe S, Kai Y, Sueoka N, Sueoka E, Fujiki H. Synergistic effects of (-)-epigallocatechin gallate with (-)-epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. *Cancer Res* 1999;59:44–7.
- [146] Chan MM, Fong D, Soprano KJ, Holmes WF, Heverling H. Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. *J Cell Physiol* 2003;194:63–70.
- [147] Yang JY, Della-Fera MA, Hausman DB, Baile CA. Enhancement of ajoene-induced apoptosis by conjugated linoleic acid in 3T3-L1 adipocytes. *Apoptosis* 2007.
- [148] Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science* 1998;281:1322–6.
- [149] Swami S, Krishnan AV, Peehl DM, Feldman D. Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D3 in DU145 human prostate cancer cells: role of the direct inhibition of CYP24 enzyme activity. *Mol Cell Endocrinol* 2005;241:49–61.
- [150] Blumberg JM, Tzamelis I, Astapova I, Lam FS, Flier JS, Hollenberg AN. Complex role of the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. *J Biol Chem* 2006;281:11205–13.
- [151] Saltiel AR. You are what you secrete. *Nat Med* 2001;7:887–8.
- [152] Prins JB, Walker NI, Winterford CM, Cameron DP. Human adipocyte apoptosis occurs in malignancy. *Biochem Biophys Res Commun* 1994;205:625–30.
- [153] Commons GW, Halperin B, Chang CC. Large-volume liposuction: a review of 631 consecutive cases over 12 years. *Plast Reconstr Surg* 2001;108(1753–63) [discussion 64–7].