

1 **PHYTOCHEMICALS AND REGULATION OF THE ADIPOCYTE LIFE**

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3 **ABSTRACT**

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

23
24 **Key Words: adipogenesis, adipocyte apoptosis, ajoene, genistein, lipolysis, resveratrol,**
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28 Obesity is no longer considered to be only a cosmetic problem. Studies indicate that higher
29 levels of body fat are associated with an increased risk for the development of numerous
30 adverse health conditions (Visscher and Seidell, 2001). Weight loss is increasingly
31 recognized to have major health benefits for overweight people (Goldstein, 1992) and also
32 increases life expectancy in people having obesity-related complications. While reducing
33 dietary fat content combined with increased physical exercise was shown to be effective in
34 preventing obesity (Astrup, 2001), only one third of those trying to lose weight reported
35 eating fewer calories and exercising more (Kruger et al., 2004). Although weight loss and
36 weight control drugs are becoming extremely common in today's society, the remedies
37 provided by the diet industry have failed in the long term maintenance of weight loss in
38 obese patients (Wadden, 1993). Moreover, it has been estimated that more than 90% of the
39 people who lose weight by dieting return to their original weight within 2 to 5 years (Stern et
40 al., 1995). Adipose tissue growth involves formation of new adipocytes from precursor cells,
41 further leading to an increase in adipocyte size. The transition from undifferentiated
42 fibroblast-like preadipocytes into mature adipocytes constitutes the adipocyte life cycle and
43 treatments that regulate both size and number of adipocytes may provide a better therapeutic
44 approach for treating obesity.

45 The decrease of adipose tissue mass that occurs with weight loss may involve the
46 mobilization of lipids through lipolysis or the loss of mature fat cells through apoptosis (Prins
47 and O'Rahilly, 1997; Sorisky et al., 2000). While development of obesity is a greater problem
48 during middle age, elderly people can have a relative increase in body fat content
49 accompanied by an accumulation of adipocytes in non-adipose tissues, such as muscle and
50 bone marrow. Since marrow adipocytes inhibit osteoblast proliferation (Jilka, 2002) and
51 disrupt the normal blood supply to bone tissue (Laroche, 2002), treatments that inhibit
52 marrow adipogenesis and decrease bone marrow adipocyte populations would have positive
53 consequences for bone health. Furthermore, loss of weight in the elderly is associated with
54 acceleration of both muscle tissue loss (Newman et al., 2005) and bone loss (Knoke and
55 Barrett-Connor, 2003) and hence treatments that selectively remove adipocytes while sparing
56 muscle and bone tissue could be of tremendous benefit for prevention of sarcopenia,
57 osteoporosis, and adiposity in the elderly.

58 Medicinal plants and plant extracts represent the oldest and most widespread form of
59 medication. At least 25% of the active compounds in currently prescribed synthetic drugs
60 were first identified in plant sources (Balandrin et al., 1985). Dissatisfaction with the high
61 costs and potentially hazardous side effects of pharmaceuticals have resulted in a larger
62 percentage of people in U.S purchasing and exploring the applications of medicinal plants
63 than before (Kessler et al., 2001). Several plants like willow, poppy, foxglove, cinchona, aloe
64 and garlic have been verified as medicinally beneficial through repeated clinical testing and
65 laboratory analyses (O'Hara et al., 1998; Youngkin and Israel, 1996) and a number of plant
66 extracts like green tea (Hasegawa et al., 2003), garlic compounds (Elkayam et al., 2003) and
67 CLA (Hargrave et al., 2002) were shown to possess either antidiabetic effects or have direct
68 effects on adipose tissue.

69 A large body of literature indicates that substantial progress has been made concerning our
70 knowledge of bioactive components in plant foods and their links to obesity. Polyphenols
71 constitute one of the ubiquitous groups of plant metabolites (Bravo, 1998) widely found in

72 fruits, vegetables, cereals, legumes, and wine (Aherne and O'Brien, 2002) (Harborne, 1989).
73 A number of studies have been carried out to investigate the anti-obesity effects of
74 polyphenols like apigenin and luteolin (Han et al., 2003b), kaempferol (Yu et al., 2006),
75 myricetin and quercetin (Kwon et al., 2007), genistein and diadzein (Dang and Lowik, 2004;
76 Kim et al., 2006d; Naaz et al., 2003), cyanidin (Tsuda et al., 2005), grape seed
77 proanthocyanidin extract (GSPE) (Preuss et al., 2000), xanthohumol (Nakagawa et al., 2005),
78 and epigallocatechin gallate (EGCG) (Wolfram et al., 2006a). Likewise, studies involving the
79 effects on lipid metabolism have been carried out with carotenoids like fucoxanthin (Maeda
80 et al., 2005), coumarin derivatives like esculetin (Yang et al., 2006b) and phytoalexins like
81 resveratrol (Picard et al., 2004). Other bioactive components of food with anti-obesity effects
82 include phytosterols, polyunsaturated fatty acids and organosulfur compounds.
83

84 **NATURAL COMPOUNDS USED FOR THE TREATMENT OF OBESITY**

85 *Metabolic Stimulants*

86 Caffeine and ephedrine have been proposed as treatments for weight loss and weight
87 maintenance for a long time. Caffeine increases energy expenditure by inhibiting the
88 phosphodiesterase-induced degradation of intracellular cyclic AMP (cAMP) (Dulloo, 1993),
89 and decreases energy intake by reducing food intake (Racotta et al., 1994). Ephedrine, an
90 alkaloid, mediates thermogenic effects by enhancement of sympathetic neuronal release of
91 norepinephrine (NE) and epinephrine (Dulloo, 1993). Although, the thermogenic effect of
92 ephedrine was shown to be markedly potentiated by caffeine (Astrup et al., 1992), owing to
93 adverse cardiovascular side effects, the FDA has banned the sale of ephedra-containing
94 dietary supplements (Diepvens et al., 2007). EGCG (epigallocatechin gallate), a flavonoid
95 (Dulloo et al., 1999), (Dulloo et al., 2000) and capsaicin, an alkaloid (Yoshioka et al., 1995)
96 were also shown to increase energy expenditure and thermogenesis in humans. Capsaicin
97 dose-dependently enhanced catecholamine secretion from the adrenal medulla (Kawada et al.,
98 1986) to exert its thermogenic effect, whereas EGCG stimulated thermogenesis by inhibition
99 of catechol O-methyl-transferase (COMT), an enzyme that degrades norepinephrine (NE)
100 (Borchardt and Huber, 1975).
101

102 *Appetite Suppressants*

103 Better understanding of the endogenous mechanisms involved in appetite and appetite
104 suppression has dramatically increased interest in appetite suppressants. Extract of Hoodia
105 gordonii is one of the most popular herbal supplements claimed to possess appetite
106 suppressant properties. An oxypregnane steroidal glycoside, known as P57, is the only
107 reported active constituent from hoodia (Avula et al., 2006). This compound increased the
108 ATP content in hypothalamic neurons that regulate food intake after intracerebroventricular
109 injection in rats (MacLean and Luo, 2004). Several other herbal supplements and plant
110 extracts like ephedra (Fleming, 2007), Citrus aurantium (Klontz et al., 2006), hydroxycitric
111 acid (Ohia et al., 2002), caralluma fimbriata (Kuriyan et al., 2007) and Phaseolus vulgaris

112 isoelectins (Baintner et al., 2003) have also been reported to possess appetite suppressing
113 properties.
114

115 ***Starch Blockers***

116 It has been well established that certain plant foods, such as *Phaseolus vulgaris* extract
117 (derived from white kidney beans) and wheat, contain a substance that inhibits the activity of
118 salivary and pancreatic amylase, and therefore they are called starch blockers (Bo-Linn et al.,
119 1982). The plant extracts or herbal supplements that act as starch blockers promote weight
120 loss by either interfering with the breakdown of complex carbohydrates or by providing
121 resistant starches to the lower gastrointestinal tract. (Celleno et al., 2007). Starch blockers
122 show potential promise in the treatment of obesity but further studies are warranted to
123 conclusively demonstrate the effectiveness.
124

125 ***Glucose/Insulin Metabolism***

126 Metabolism of glucose is a complex process regulated by peptides and steroid hormones and
127 is highly influenced by diet. Hypoglycemic effects of several plant extracts like *Siraitia*
128 *grosvenori* (Suzuki et al., 2007), *Stachytarpheta cayennensis* (Adebajo et al., 2007),
129 *Platycodon grandiflorum* (Zheng et al., 2007), *Gynostemma pentaphyllum* (Megalli et al.,
130 2006), *Cichorium intybus* (Pushparaj et al., 2007), *Oryza sativa* (Guo et al., 2007), *Cucurbita*
131 *ficifolia* (Xia and Wang, 2006), *Allium sativum* (Eidi et al., 2006), *Vitex megapotamica*
132 (Zanatta et al., 2007) and cinnamon bark (Kannappan et al., 2006) have been investigated.
133 Soy protein was shown to significantly improve insulin sensitivity and glucose effectiveness
134 compared with casein (Wagner et al., 1997). Dietary fiber was also shown to significantly
135 improve blood glucose control but the mechanisms by which dietary fiber exerts its
136 hypoglycemic activities are unknown (Riccardi and Rivellese, 1991).
137

138 ***Lipid Metabolism***

139 Obesity is generally linked to complications in lipid metabolism and oxidative stress. The
140 effects of several plant extracts like *Cissus quadrangularis* (Oben et al., 2007), *Aralia*
141 *mandshurica* (aralax) (Abidov et al., 2006), Kochujang (Korean fermented red pepper paste)
142 (Ahn et al., 2006), psyllium (Moreno et al., 2003), *Salix matsudana* leaves (Han et al., 2003a;
143 Han et al., 2003b) and *Arachis hypogaea* (Moreno et al., 2006) on lipid metabolism revealed
144 a reduction in serum triglyceride levels. However, there are no major long-term studies
145 demonstrating harm or benefit in using lipid-lowering drugs compared to low fat diets in
146 children (Bhatnagar, 2002). Phytosterols have been widely studied for their cholesterol
147 lowering effects. One such phytochemical, guggulsterone (4,17(20)-pregnadiene-3,16-dione),
148 has been used to treat a variety of ailments, including obesity, arthritis and lipid disorders
149 (Urizar and Moore, 2003). Several other plant sterols like diosgenin, campesterol, sitosterol,
150 stigmasterol and brassicasterol were shown to possess cholesterol lowering effects
151 (Fernandez et al., 2002; Kwon et al., 2003; Vaskonen et al., 2001). Since turnover of

152 cholesterol was shown to bear a relationship to body fat mass (Miettinen, 1971), phytosterols
153 may also decrease body fat. A number of studies have demonstrated the beneficial effects of
154 polyunsaturated fatty acids (PUFAs) on lipid-related disorders in humans (Hirafuji et al.,
155 2003).
156

157 *Adipocyte Specific Effects*

158 Adipose tissue mass can be reduced by both inhibiting adipogenesis and inducing apoptosis
159 of adipocytes. Natural products that specifically target both these pathways therefore will
160 have better potential for treatment and prevention of obesity. Polyphenolic compounds are
161 widely found in fruits and vegetables (Aherne and O'Brien, 2002), among which flavonoids
162 and several classes of nonflavonoids are usually distinguished (Harborne, 1989). The anti-
163 obesity effects and also adipocyte specific effects of several polyphenols have been
164 investigated, as discussed below. PUFAs are vital components of the phospholipids of cell
165 membranes and serve as important mediators of the nuclear events regulating the adipocyte
166 specific gene expression involved in lipid metabolism and adipogenesis (Lombardo and
167 Chicco, 2006). Although most commonly used dietary supplements like Conjugated Linoleic
168 Acid (CLA) showed an effect on glucose and lipid metabolism, these effects are also likely
169 secondary effects mediated through adipocyte specific transcription factors and their nuclear
170 receptors (Taylor and Zahradka, 2004). Likewise, although the beneficial effects of
171 organosulfur compounds present in natural food are due to their antioxidant and
172 anticarcinogenic properties (Sahu, 2002), recently the adipocyte specific effects of ajoene, a
173 garlic derivative, were reported (Yang et al., 2006c). This study indicates that garlic extracts
174 may influence fat cell number, thereby suggesting a therapeutic possibility for obesity. The
175 adipocyte specific effects of natural products are described in detail in the following sections.
176

177 **THE ADIPOCYTE LIFE CYCLE**

178 The biologic events leading to obesity are characterized by changes in cell properties of
179 adipocytes and may include an increase in the number or size, or both (Flier, 1995).
180 Adipocytes are derived from mesenchymal stem cells, which have the potential to
181 differentiate into myoblasts, chondroblasts, osteoblasts, or adipocytes. The adipocyte life
182 cycle includes alteration of cell shape and growth arrest, clonal expansion, and a complex
183 sequence of changes in gene expression leading to storage of lipid and finally cell death
184 (Figure 1) (Gregoire, 2001).

185 During the growth phase, preadipocytes resemble fibroblasts morphologically. Pref-1, a
186 preadipocyte secreted factor serves as a marker for preadipocytes and is extinguished during
187 adipocyte differentiation (Wang et al., 2006). At confluence, preadipocytes enter a resting
188 phase called growth arrest before undergoing the differentiation process. Two transcription
189 factors, C/EBP- α and PPAR- γ were shown to be involved in the preadipocyte growth arrest
190 that is required for adipocyte differentiation (Umek et al., 1991). Following growth arrest,
191 preadipocytes must receive an appropriate combination of mitogenic and adipogenic signals
192 to continue through the subsequent differentiation steps. During the process of differentiation,
193 preadipocytes undergo one round of DNA replication leading to clonal amplification of

194 committed cells (Pairault and Green, 1979). The induction of differentiation also results in
195 drastic change in cell shape as the cells convert from fibroblastic to spherical shape.
196 Following induction, a dramatic decrease in pref-1 expression accompanies a rapid increase
197 in the expression of C/EBP β , followed by expression of C/EBP α and peroxisome proliferator
198 activated receptor γ (PPAR γ) (Rosen et al., 2002). During the terminal stages of
199 differentiation, the mRNA levels for enzymes involved in triacylglycerol metabolism like
200 glycerol-3-phosphate dehydrogenase, fatty acid synthase, and glyceraldehyde-3-phosphate
201 dehydrogenase, increase to a great extent (Paulauskis and Sul, 1988; Spiegelman et al., 1983).
202 Finally, although it was once believed that the total number of adipocytes does not change
203 throughout life, it is now recognized that new adipocytes can be formed or can be removed
204 by the process of apoptosis (Prins and O'Rahilly, 1997).
205

206 **TARGETING THE ADIPOCYTE LIFE CYCLE**

207 *Preadipocytes*

208 Preadipocytes can proliferate throughout life to increase fat mass. A number of natural
209 products were shown to inhibit preadipocyte proliferation and induce apoptosis. Polyphenols
210 are powerful antioxidants (Robak and Gryglewski, 1996) and induction of apoptosis in
211 preadipocytes by flavonoids was shown to be associated with their antioxidant activity (Hsu
212 and Yen, 2006). Quercetin, one of the most abundant flavonoids present in various common
213 fruit and vegetables induced apoptosis in 3T3-L1 preadipocytes by decreasing mitochondria
214 membrane potential, down-regulating PARP and Bcl-2, and activating caspase-3, Bax, and
215 Bak. Several other flavonoids like naringenin, rutin, hesperidin, resveratrol, naringin and
216 genistein also decreased preadipocyte proliferation (Harmon and Harp, 2001; Hsu et al.,
217 2006; Yang et al., 2006a). The green tea polyphenol EGCG, also induced apoptosis in
218 preadipocytes. The apoptotic effects were Cdk2 and caspase-3 dependent and could be
219 attributed to inhibition of cell mitogenesis (Kao et al., 2000). The induction of apoptosis in
220 3T3-L1 preadipocytes by capsaicin was mediated through the activation of caspase-3, Bax,
221 and Bak, and then through the cleavage of PARP and the down-regulation of Bcl-2 (Hsu and
222 Yen, 2007).

223 Dividing cells, when exposed to stress will undergo cell cycle arrest to either repair the DNA
224 or to initiate apoptosis (Iliakis et al., 2003). Natural antioxidants were reported to cause G₁
225 phase arrest in prostatic carcinoma cells (Nyska et al., 2003). Phenolic acids like *o*-coumaric
226 acid, *m*-coumaric acid, and chlorogenic acid caused cell cycle arrest at the G₁ phase in a
227 time- and dose-dependent manner in preadipocytes (Hsu et al., 2006) and another coumarin
228 derivative, esculetin, also induced apoptosis in 3T3-L1 preadipocytes (Yang et al., 2006a).
229 More recently, conjugated linoleic acids (CLA) were shown to promote human preadipocyte
230 apoptosis (Fischer-Posovszky et al., 2007). However, docosahexaenoic acid (DHA), an
231 omega-3 fatty acid, showed no effect on the proliferation of preconfluent preadipocytes (Kim
232 et al., 2006b).
233

234 *Maturing Preadipocytes*

235 Adipocyte number increases not only as a result of increased preadipocyte proliferation but
236 also due to differentiation (Roncari et al., 1981). Induction of differentiation stimulates clonal
237 expansion resulting in doubling of the cell number (Pairault and Green, 1979). Two critical
238 events occur during the early stage of differentiation, namely, mitotic clonal expansion and
239 an irreversible commitment to differentiation (Scott et al., 1982). Genistein inhibited mitotic
240 clonal expansion of 2-day postconfluent 3T3-L1 preadipocytes, whereas naringenin, a
241 flavonoid structurally similar to genistein, failed to exert antiproliferative effects on maturing
242 preadipocytes (Harmon and Harp, 2001). Esculetin induced apoptosis in maturing
243 preadipocytes during the late differentiation stage (Yang et al., 2006a). DHA-induced
244 apoptosis in 3T3-L1 cells during postconfluent mitotic expansion was accompanied by
245 increased LDH release (Kim et al., 2006a). Postconfluent preadipocytes treated with CLA
246 had more apoptotic cells than control cultures and also had fewer cells in the S-phase than
247 control cultures (Evans et al., 2000).
248 EGCG also induced apoptosis in postconfluent maturing preadipocytes during treatment with
249 insulin, but the biochemical mechanisms involved are not known (Lin et al., 2005). Cell
250 cycle and growth-related genes in maturing preadipocytes were downregulated after
251 treatment with GSPE during the early stage of differentiation (Pinent et al., 2005b). Since
252 irreversibly committed preadipocytes undergo several rounds of replication during the first 2
253 days of differentiation, the induction of apoptosis in postconfluent differentiating cells will
254 lead to fewer adipocytes. Therefore, maturing preadipocytes could be an important target for
255 natural products in regulating the adipocyte life cycle.
256

257 *Adipogenesis*

258 The first hallmark of the adipogenesis process is alteration in cell shape paralleled by changes
259 in the type and expression levels of extracellular matrix (ECM) components and cytoskeletal
260 components (Gregoire et al., 1998). These events further promote the expression of
261 adipogenic transcription factors, including C/EBP α and PPAR γ . C/EBP α and PPAR γ are the
262 central transcriptional regulators of adipogenesis and are required for the synthesis of many
263 adipocyte functional proteins. C/EBP β upregulation is a very early event and mediates the
264 downstream upregulation of PPAR γ and C/EBP α expression (Wu et al., 1996). A number of
265 studies have demonstrated that natural compounds like EGCG, genistein, esculetin, DHA,
266 berberine, resveratrol, guggulsterone, CLA, capsaicin, baicalein and procyanidins inhibited
267 adipogenesis (Brown et al., 2003; Cha et al., 2006; Hsu and Yen, 2007; Huang et al., 2006;
268 Kim et al., 2006a; Kim et al., 2006c; Lin et al., 2005; Picard et al., 2004; Pinent et al., 2005b;
269 Rizzo et al., 2006; Sisk et al., 2001; Yang et al., 2006a). The protein expression of PPAR γ ,
270 and C/EBP α were decreased in adipocytes treated with capsaicin, genistein, berberine, and
271 EGCG (Harmon and Harp, 2001; Hsu and Yen, 2007; Huang et al., 2006; Moon et al., 2007).
272 PUFAs were shown to suppress lipogenesis by downregulating the expression of the sterol
273 regulatory element-binding proteins (Worgall et al., 1998) and also by downregulating the
274 late phase of adipocyte differentiation (Okuno et al., 1997). The decrease in adipogenesis by
275 resveratrol was associated with increase in the expression of Sirt1, which promotes fat
276 mobilization by repressing PPAR γ (Picard et al., 2004). The anti-adipogenic effect of
277 baicalein was due to its ability to enhance the expression of COX-2, which is normally down-
278 regulated during adipogenesis (Cha et al., 2006). AMP-activated protein kinase (AMPK) is

279 another target molecule for anti-obesity treatments, and genistein, EGCG and capsaicin were
280 shown to inhibit adipocyte differentiation by activating AMPK (Hwang et al., 2005).
281

282 *Lipolysis*

283 Breakdown of triglycerides in adipocytes and the release of glycerol and fatty acids are
284 important for the regulation of energy homeostasis (Frayn et al., 2003). Hormone sensitive
285 lipase (HSL) is the most important lipase that catalyses the process of lipolysis, and HSL is
286 subject to hormonal regulation (Holm, 2003). Lipolysis is stimulated by protein kinase A
287 (PKA) activation, which phosphorylates HSL, or by phosphorylation of HSL by G protein-
288 coupled receptors and cyclic AMP - activated extracellular signal-regulated kinase (ERK)
289 (Greenberg et al., 2001). Preadipocytes do not have lipolytic activity until they are
290 differentiated to mature adipocytes (Hauner et al., 2001). The cytokine tumor necrosis factor
291 alpha (TNF α) has been shown to increase the lipolysis rate in humans *in vivo* (Starnes et al.,
292 1988) and in primary cultures of newly differentiated human preadipocytes (Hauner et al.,
293 1995).

294 Apart from inhibiting adipogenesis several natural compounds stimulate lipolysis in
295 adipocytes. Flavonoids genistein, diadzein, coumestrol and zearalenone stimulated a dose-
296 dependent increase in lipolysis in rat adipocytes (Kandulska et al., 1999; Szkudelska et al.,
297 2002). Quercetin, luteolin and fisetin caused a dose- and time-dependent increase in lipolysis
298 in rat adipocytes which was synergistic with epinephrine, and these effective lipolytic
299 flavonoids were also reported to be potent phosphodiesterase (PDE) inhibitors (Kuppusamy
300 and Das, 1992). Grape seed proanthocyanidins stimulated long-term lipolysis by increasing
301 cAMP and PKA in 3T3-L1 adipocytes (Pinent et al., 2005a). CLA increased basal lipolysis
302 in 3T3-L1 preadipocytes (Evans et al., 2002; Park et al., 1997) and human adipocytes (Chung
303 et al., 2005). The mechanism of induction of lipolysis by CLA is not mediated by HSL
304 activation via classic cAMP or PKA pathways but via an ERK-dependent activation of HSL
305 (Chung et al., 2005). DHA also stimulated lipolysis when added to mature adipocytes;
306 however, the cellular mechanisms involved in DHA's effects on lipid metabolism have not
307 yet been investigated (Kim et al., 2006a). In contrast, EGCG did not induce lipolysis,
308 indicating that the anti-obesity effects of EGCG are not mediated via increased lipid
309 mobilization (Wolfram et al., 2006b).

310

311 *Mature Adipocyte Apoptosis*

312 Compounds that induce adipocyte apoptosis can reduce body fat content and the effect has
313 the potential to last much longer than body fat reduction caused by lipid mobilization alone.
314 Apoptosis is a form of cell suicide that plays an important role in maintaining cellular
315 homeostasis, and at times it is necessary to eliminate excessive cells and cells that hinder
316 development. Although a number of stimuli trigger apoptosis, there are two major signaling
317 pathways: the death receptor pathway and the mitochondrial pathway (Gupta, 2001). A series
318 of molecular steps leads to activation of caspases in both of these pathways. Finally this
319 results in cleavage of a number of nuclear and cytoplasmic substrates resulting in cell death
320 (Hengartner, 2000).

321 Several natural compounds were reported to induce apoptosis primarily in cancer cells, but
322 relatively little research exists on investigating the various natural compounds that induce
323 apoptosis in adipose tissue. Green tea extracts (Hasegawa et al., 2003), soy isoflavones (Kim
324 et al., 2006d; Naaz et al., 2003), CLA (Park et al., 1997; Tsuboyama-Kasaoka et al., 2000;
325 West et al., 1998) and garlic compounds (Elkayam et al., 2003) were shown to reduce body
326 fat in experimental animals but the mechanisms of action in inducing adipocyte apoptosis
327 with these compounds has been investigated only recently. Although the effect of CLA on
328 body fat is not completely understood, it is thought that a marked increase of TNF-alpha
329 mRNA with an increase of UCP2 in adipocytes caused CLA-induced apoptosis (Tsuboyama-
330 Kasaoka et al., 2000). In contrast, EGCG-induced apoptosis is mediated by activator protein-
331 1 (AP-1), nuclear factor kappa B (NF-kappa B), p53 (Hastak et al., 2003) and increased
332 caspase 3 activity (Hsu et al., 2003).
333 Reactive oxygen species (ROS) were reported to play a key role in cell signaling, and the
334 role of ROS generation in the proliferation of various cells has been investigated (Burdon and
335 Rice-Evans, 1989; Carriere et al., 2003). In leukemic cells increased ROS generation leads to
336 the activation of mitogen-activated protein kinases (MAPK) resulting in cell death (Chen et
337 al., 1998). Genistein, EGCG, and capsaicin stimulated intracellular ROS release, which
338 activated AMPK rapidly leading to apoptosis (Hwang et al., 2005). Ajoene also induced
339 apoptosis in leukemic cells through the generation of ROS (Dirsch et al., 1998) and more
340 recently ajoene was shown to induce ROS-mediated apoptosis in adipocytes, as well (Yang et
341 al., 2006c). The cellular mechanisms involved in DHA-mediated apoptosis in mature
342 adipocytes has not been investigated yet (Kim et al., 2006a).
343

344 **DIETARY BIOACTIVE ENTITIES TARGETING MULTIPLE SIGNALING**

345 **PATHWAYS**

346 Synergistic interactions with combinations of phytochemicals such as quercetin, tea catechins,
347 curcumin, genistein, and resveratrol for the treatment of cancer has been investigated
348 (Hemalswarya and Doble, 2006). The apoptosis inducing activity of epigallocatechin-3-
349 gallate (EGCG) on lung cancer cells was found to be synergistically enhanced by other
350 chemopreventive agents, such as sulindac and tamoxifen (Suganuma et al., 1999).
351 Additionally, curcumin, a component of the culinary spice turmeric, was shown to potentiate
352 the antitumor and apoptotic effects of cisplatin in ovarian carcinoma cells (Chan et al., 2003).
353 While all the above studies were performed in cancer cells, such synergistic interactions
354 among dietary bioactives on adipocytes have not been investigated in detail.
355 Recently we have reported that t10,c12CLA potentiates ajoene-induced apoptosis in 3T3-L1
356 adipocytes (Yang et al., 2007). Cytochrome *c* release is regulated by bcl-2 family proteins,
357 and these proteins are associated with the mitochondrial membrane and regulate its integrity.
358 Bax, a member of bcl-2 family proteins, exerts proapoptotic activity by translocation from
359 the cytosol to the mitochondria and inducing cytochrome *c* release (Adams and Cory, 1998).
360 CLA and ajoene as individual compounds showed no effect on cytochrome *c*, whereas ajoene
361 increased and CLA had no effect on bax expression. However, the combination of ajoene and
362 CLA caused a synergistic increase in both cytochrome *c* and bax expression.

363 Similarly, we have reported that 1,25(OH)₂D₃ (1,25 dihydroxy vitamin D₃, calcitriol),
364 potentiates the effects of genistein in inducing apoptosis and inhibiting adipogenesis in
365 maturing 3T3-L1 preadipocytes. An interesting feature about this study is that the synergistic
366 effect was observed only in maturing preadipocytes and not in either mature adipocytes or
367 preadipocytes, the reasons for which are not clearly understood. The combination of
368 genistein and 1,25(OH)₂D₃ caused a significant increase in VDR mRNA expression in
369 human prostate cancer cells (Swami et al., 2005) and we found that in maturing 3T3-L1
370 adipocytes, genistein + 1,25(OH)₂D₃ increased VDR protein levels by more than 100 %,
371 whereas 1,25(OH)₂D₃ by itself increased VDR protein levels by only 40 % and genistein
372 alone at the tested concentration had no effect. This effect on VDR correlated with an
373 increase in apoptosis of about 200% with the combination treatment. The VDR, a member of
374 the nuclear receptor superfamily, plays a key role in adipocyte biology when bound to its
375 ligand, 1,25(OH)₂D₃ (Blumberg et al., 2006), and these results indicate that the potentiation
376 of both the increase in apoptosis and suppression of adipogenesis with the combination
377 treatment might be mediated in part through the VDR.
378 Such studies of synergistic activity suggest that the desired effects on adipocytes could be
379 achieved by using lower doses of two or more compounds, thereby decreasing potential toxic
380 effects. Although results from in vitro experiments cannot be directly extrapolated to clinical
381 effects, such studies will help in elucidating various molecular pathways by which selected
382 natural products, either as individual treatments or in combination, might be effective in
383 regulating adipose tissue volume through adipocyte apoptosis and inhibition of adipogenesis.
384

385

CONCLUSIONS

386 Obesity is a risk factor for diseases like non-insulin dependent diabetes mellitus,
387 atherosclerosis, and certain cancers (Saltiel, 2001). Adipose mass can be decreased by
388 removing adipocytes, and it is becoming evident that fat cells have a finite life span and can
389 be eliminated by apoptosis (Commons et al., 2001; Prins and O'Rahilly, 1997; Prins et al.,
390 1994). Since adipogenesis is intricately related to adipocyte differentiation and maturation,
391 inducing apoptosis and inhibiting adipogenesis at various stages of the adipocyte life cycle
392 may be target pathways for treating obesity. In cancer cells, phytochemicals tend to increase
393 the therapeutic effect by either blocking one or more targets of the signal transduction
394 pathway or by increasing the bioavailability of the other drug in the system (Hemalswarya
395 and Doble, 2006). Dietary bioactives derived from natural products have shown interesting
396 effects on adipose tissue like inducing apoptosis, decreasing lipid accumulation and inducing
397 lipolysis. Since a number of complex interconnected cell signaling pathways are involved in
398 regulating all the above mentioned processes, treating adipocytes with multiple natural
399 products can result in enhanced effects. This strategy can be achieved by exerting beneficial
400 effects through additive or synergistic actions of several natural compounds acting at single
401 or multiple target sites in the adipocyte life cycle associated with physiological processes like
402 apoptosis, adipogenesis and lipolysis.
403

404

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785 **Figure Captions**

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

793
794 Figure 2. Examples of individual natural compounds and combinations of compounds that
795 affect specific stages of the adipocyte life cycle. Genistein inhibits preadipocyte proliferation
796 and suppresses lipid accumulation in maturing preadipocytes. It also triggers lipolysis and
797 induces apoptosis in mature adipocytes, and in combination with $1,25(\text{OH})_2\text{D}_3$, it can induce
798 apoptosis in maturing preadipocytes. EGCG induces apoptosis in both preadipocytes and
799 mature adipocytes, and it can inhibit lipid accumulation in maturing preadipocytes. Quercetin
800 also has multiple effects—it can inhibit preadipocyte proliferation, induce preadipocyte
801 apoptosis and stimulate lipolysis in mature adipocytes. Ajoene + CLA are especially potent
802 in inducing apoptosis in mature adipocytes. EGCG, epigallocatechin gallate; $1,25(\text{OH})_2\text{D}_3$,
803 $1,25$ dihydroxy vitamin D_3 (calcitriol); DHA, docosahexaenoic acid; GSPE, grape seed
804 proanthocyanidin extract; CLA, conjugated linoleic acid.