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Curcumin Inhibits Adipogenesis in 3T3-L1 Adipocytes and Angiogenesis and Obesity in C57/BL Mice[1–3]

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Abstract

Angiogenesis is necessary for the growth of adipose tissue. Dietary polyphenols may suppress growth of adipose tissue through their antiangiogenic activity and by modulating adipocyte metabolism. We investigated the effect of curcumin, the major polyphenol in turmeric spice, on angiogenesis, adipogenesis, differentiation, apoptosis, and gene expression involved in lipid and energy metabolism in 3T3-L1 adipocyte in cell culture systems and on body weight gain and adiposity in mice fed a high-fat diet (22%) supplemented with 500 mg curcumin/kg diet for 12 wk. Curcumin (5–20 μmol/L) suppressed 3T3-L1 differentiation, caused apoptosis, and inhibited adipokine-induced angiogenesis of human umbilical vein endothelial cells. Supplementing the high-fat diet of mice with curcumin did not affect food intake but reduced body weight gain, adiposity, and microvessel density in adipose tissue, which coincided with reduced expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2. Curcumin increased 5′AMP-activated protein kinase phosphorylation, reduced glycerol-3-phosphate acyl transferase-1, and increased carnitine palmitoyltransferase-1 expression, which led to increased oxidation and decreased fatty acid esterification. The in vivo effect of curcumin on the expression of these enzymes was also confirmed by real-time RT-PCR in subcutaneous adipose tissue. In addition, curcumin significantly lowered serum cholesterol and expression of PPARγ and CCAAT/enhancer binding protein α, 2 key transcription factors in adipogenesis and lipogenesis. The curcumin suppression of angiogenesis in adipose tissue together with its effect on lipid metabolism in adipocytes may contribute to lower body fat and body weight gain. Our findings suggest that dietary curcumin may have a potential benefit in preventing obesity. J. Nutr. 139: 919–925, 2009.

Introduction

The prevalence of being overweight, obesity, type-II diabetes, and metabolic syndrome has increased dramatically in the past 2 decades (1). Obesity is a multi-factorial disorder resulting from various causes, including genetic, medicinal, metabolic rate, endocrine function, nutritional, and other environmental factors (2). Consequently, the prevention and treatment of obesity is critical to curtail the increasing incidences of morbidity and mortality.

Obesity results from the growth and expansion of adipose tissue in which lipid storage and energy metabolism are tightly controlled. 5′AMP-activated protein kinase (AMPK)[6] is a major regulator of energy metabolism that is activated in response to an increase in the AMP:ATP ratio within the cell and therefore acts as a sensor for cellular energy regulation. Binding of AMP with AMPK allosterically phosphorylates and activates AMPK (3), which in turn shuts down anabolic pathways and supports catabolic pathways through regulating the activity of several key enzymes of energy metabolism (Fig. 7); in adipocytes, this inhibits lipid accumulation (3). Activation of AMPK downregulates acetyl CoA carboxylase (ACC) through phosphorylation of this enzyme. Carnitine palmitoyltransferase-1 (CPT-1) and glycerol-3-phosphate acyl transferase-1 (GPAT-1) are the enzymes involved in fatty acid metabolism. CPT-1 transfers cytosolic long-chain fatty acyl CoA into the mitochondria for oxidation. It is allosterically inhibited by malonyl CoA. In contrast, GPAT-1 esterifies fatty acids to glycerol to form triglyceride for storage.

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[3] Supplemental Table 1 and Figures 1–6 are available with the online posting of this paper at jn.nutrition.org.
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[6] Abbreviations used: ACC, acetyl CoA carboxylase; ACM, adipocyte-conditioned medium; AMPK, 5′AMP-activated protein kinase; C/EBPα, CCAAT/enhancer binding protein α; CPT-1, carnitine palmitoyltransferase-1; GPAT-1, glycerol-3-phosphate acyl transferase-1; HF, high-fat diet group; HF+curcumin, high-fat diet supplemented with curcumin diet group; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor-receptor 2.

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Like ACC, the activation of AMPK also phosphorylates GPAT-1 and inhibits activity of this enzyme. AMPK also regulates the ligand-activated transcriptional factors PPARγ and CCAAT/enhancer binding protein α (C/EBP), which are the central regulators of adipogenesis and lipid storage in adipocytes (4).

The growth and expansion of adipose tissues, similar to the growth of cancerous tumors, requires recruitment of new blood vessels or angiogenesis (5). In adipose tissue, this is mediated by adipose tissue secretion of adipokines, including leptin, adiponectin, resistin, visfatin, tumor necrosis factor-α, interleukin-6, interleukin-1, and vascular endothelial growth factor (VEGF) (6). Therefore, in addition to reducing energy intake, the inhibition of angiogenesis in adipose tissue can be a strategy to prevent adipose tissue growth and obesity. For this effect, an antiangiogenic drug, TNP-470, has recently been shown to suppress obesity through suppression of angiogenesis in the adipose tissue of mice (7). Because curcumin is known to have antiangiogenic activity and suppress tumor growth (8) and an earlier study suggested that it increases catabolic activity in rat liver (9), we therefore hypothesized that dietary curcumin prevents adipose tissue growth and expansion through inhibition of angiogenesis and modulation of energy metabolism in adipose tissues. Curcumin is the major polyphenol in turmeric spice, which has been consumed daily throughout Asian countries over centuries without reported toxicity (10). Therefore, to test our hypothesis, we investigated the effect of curcumin supplementation on angiogenesis, adipocyte differentiation, apoptosis, and gene expression of several key enzymes and transcription factors involved in energy and lipid metabolism in cell culture system and on body weight gain, adiposity, adipose tissue vascularity, liver steatosis, and expression of genes and transcription factors that are involved in energy/lipid metabolism in a mouse model of dietary high-fat–induced obesity.

Materials and Methods

Adipokine and in vitro angiogenesis. The formation of capillary-like structures by human umbilical vein endothelial cells on Matrigel (BD Biosciences) was studied as previously described (11). To stimulate angiogenesis, 500 μL adipocyte-conditioned medium (ACM) containing adipokines was added for 24 h. The ACM was a supernatant of adipocytes isolated from gonadal adipose tissue of mice, which was incubated in DMEM for 24 h. The images of tubes formed on Matrigel were captured after 24 h.

3T3-L1 cell culture and differentiation. 3T3-L1 mouse embryonic fibroblasts were grown. Curcumin was dissolved in dimethylsulfoxide and added to the cell culture medium at concentrations of 0, 5, 10, or 20 μmol/L and incubated for 24 h. The final concentration of dimethylsulfoxide was 100 mg/L or 0.01%. Adipogenesis was induced as described previously (12) and fat droplets were stained with Oil Red O stain (13). All experiments, unless otherwise indicated, were performed at least in triplicate.

Mice and diets. Eighteen 4-wk-old male C57BL/6 mice (Jackson Laboratory) were randomly assigned to 3 groups of 6 mice each after 1 wk of acclimation. The control group was fed with the purified diet (AIN-93) containing 4% fat by weight (TD.06432 Harlan Teklad); the high fat-fed group (HF) was fed an AIN-93 diet containing 22% fat by weight (TD.06433) (for complete diet composition, see **Supplemental Table 1**) and another group was fed the same HF diet supplemented with 500 mg of curcumin (Sigma, 98% purity)/kg of diet (HF+curcumin). We recorded the body weights and food consumption of mice twice per week. After 12 wk of dietary treatments, the mice were killed by CO_2. Subcutaneous, visceral, and perigonadal adipose tissues were dissected and weighed and total body fat, percent adiposity, and distribution were determined. Serum, liver, and adipose tissue were collected and stored at −80°C until analysis. The study protocol was reviewed and approved by the Animal Care and Use Committee of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University.

Histological analysis. Subcutaneous adipose tissue samples following fixation with paraformaldehyde were washed with 30% sucrose and embedded in paraffin. The standard immunohistochemical protocol was followed by using primary polyclonal rabbit anti-mouse CD31 antibody (Abcam). Expression of CD31 was detected with fluorescein isothiocyanate-labeled anti-rabbit-IgG (Abcam). The signals were examined by fluorescence microscopy (Zeiss, Axiovert 200). Digital images at 20× magnification were obtained and microvessel densities were counted as pixels. Formaldehyde-fixed liver samples were embedded in paraplast and sections were stained with hematoxylin and eosin.

Quantitative real-time PCR. RNA was isolated from 3T3L cells, which were incubated with different concentrations of curcumin for 24 h and from subcutaneous adipose tissues. The expressions of the genes listed below were analyzed using the primers as below. After an initial incubation for 2 min at 50°C, the cDNA was denatured at 95°C for 10 min followed by 40 cycles of PCR (95°C, 15 s, 55°C, 60 s). The mRNA levels of all genes were normalized using glyceraldehyde 3-phosphate dehydrogenase as internal control. VEGFα: sense, 5′-TCTCACCGGAAAGACCGAT, anti-sense, 5′- CCCAAAGTGCTCCTCGAA-3′; VEGF receptor-2 (VEGFR-2): sense, 5′-GATGCAGGAAACTACACGGTCA, anti-sense, 5′-GAGATCAAGGCTTTCTCACCGA-3′; CPT-1: sense, 5′- ACCCTGAGGCATCTATTGACAG-3′, anti-sense, 5′-ATGACATACTCCCACAGATGGC-3′; GPAT: sense, 5′-GGCATC TCGTATGAT CGCAT-3′, anti-sense 5′- GCAAAATCCACTCGGACGTA-3′; C/EBPα: sense, 5′-GGAACTTGAAGCACAATCGATC-3′, anti-sense, 5′-TGGTTTAGCATAGACGTGCACA-3′; PPARγ: sense, 5′- ACGTGCAGCT ACTGCATGTGA-3′, anti-sense, 5′-AGAAGGAACACGTTGTCAGCG-3′; glyceraldehyde 3-phosphate dehydrogenase: sense, 5′-TGAGCAT CTCCCTCACAATTTC-3′, anti-sense, 5′-GTGCAGCGAACTTTAT TGATGG-3′;

Protein extraction and Western blotting. After incubation with different levels of curcumin, 3T3-L1 cells were rinsed twice with ice-cold PBS and scraped with lysis buffer (Cell Signaling Technology). For subcutaneous adipose tissue, 100 mg was crushed in lysis buffer, the homogenate was centrifuged at 18,300 × *g*; 30 min at 4°C and the supernatant was collected and stored at −80°C. The protein concentrations were measured using the bicinchoninic acid method (Pierce Biotech). Western blotting was performed using antibodies against phosphorylated AMPK and phosphorylated ACC (Cell Signaling Technology) following the manufacturer's protocols. The antibody for β-actin, internal control, was purchased from Sigma.

Serum lipids and glucose. Cholesterol (14), triglycerides (15), and glucose (16) concentrations in serum were measured using the Roche Diagnostics system and FFA (17) were measured according to the kit manufacturer's instructions (Wako Chemicals).

Fatty acid oxidation. To determine the effect of curcumin on fatty acid oxidation by adipocytes, oxidation of [9, 10-^{3}H] palmitic acid by 3T3-L1 adipocyte in the absence and presence of different concentrations of curcumin was assayed as described (18).

Apoptosis. To examine the apoptotic activity of curcumin on adipocytes, 3T3-L1 cells were incubated with 0, 5, 10, and 20 μmol/L of curcumin for 24 h and analyzed using a TUNEL assay kit (Molecular Probes). Fluorescence intensity was visualized under a fluorescent microscope.

Statistical analysis. Data were analyzed with ANOVA using SAS for Windows, version 9.13 (SAS Institute). Adjustment for multiple comparisons among controls, curcumin treatments, and high fat was established using Tukey's test. Significance was set at $P < 0.05$.

Results

In vitro studies

Effect of curcumin on adipokine-induced angiogenesis. The growing adipose tissue stimulates angiogenesis through

secretion of several mediators collectively called adipokines (6). We hypothesized that curcumin inhibits adipokine-induced angiogenesis and thus the growth of adipose tissue. Curcumin suppressed ACM-induced angiogenesis (**Supplemental Fig. 1**). To determine whether the inhibitory effect of curcumin on angiogenesis was in part due to suppressing expression of VEGFα by adipocytes, we examined the expression of VEGFα mRNA. The mRNA expression of VEGFα by 3T3-L1 adipocytes was significantly reduced by curcumin (**Table 1**).

Effect of curcumin on preadipocyte differentiation. Following incubation of preadipocytes (3T3-L1) with different concentrations of curcumin, we examined cell differentiation after 6 d. With increasing the concentration curcumin in culture media, a significant inhibition of differentiation of preadipocytes to mature adipocytes was observed and it was evident from less accumulation of fat in the cells (**Supplemental Fig. 2**).

Effect of curcumin on adipocyte apoptosis. The TUNEL assay showed that increasing doses of curcumin remarkably induced apoptosis of adipocytes (**Supplemental Fig. 3**).

Curcumin modulation of energy- and lipid metabolism-related enzymes and transcription factors in adipocytes. Western blot analysis revealed that curcumin increased the activation of AMPK in 3T3-L1 adipocytes by phosphorylating at the α-subunit of AMPK and suppressed the expression of ACC by phosphorylation (**Fig. 1**). Curcumin also upregulated the expression of CPT-1 and downregulated the expression of GPAT-1. However, curcumin did not affect adipogenesis regulatory transcription factor PPARγ and C/EBPα in 3T3L-1 adipocytes ($P = 0.075$) (Table 1). Treatment of adipocytes with curcumin increased ($P < 0.05$) fatty acid oxidation as measured by the production of ^3H_2O from oxidation of [9, 10-^{3}H] palmitic acid (**Fig. 2**), which was further confirmed by Oil Red O staining that showed that curcumin at a 20-μmol/L concentration significantly reduced the accumulation fat in adipocytes (**Fig. 3**; **Supplemental Fig. 4**).

In vivo studies

Effect of dietary curcumin on body weight and adiposity. The HF diet increased body weight gain in mice, but supplementing the HF diet with 500 mg curcumin/kg suppressed the body weight gain (**Fig. 4**) without affecting food intake ($P = 0.09$) (data not shown). This effect of curcumin supplementation

TABLE 1 Effect of curcumin on mRNA expression of genes regulating angiogenesis and fatty acid and energy metabolism in 3T3-L1 adipocytes[1]

	Curcumin, μmol/L			
	0	5	10	20
Angiogenesis	*Fold of control*			
VEGF-α	1.0 ± 0.7^a	0.8 ± 0.5^{ab}	0.5 ± 0.1^{ab}	0.4 ± 0.1^b
Fatty acid and energy metabolism				
CPT-1	1.0 ± 0.6^c	1.8 ± 0.1^c	3.4 ± 0.2^{ab}	3.8 ± 0.3^a
GPAT	1.0 ± 0.1^a	0.8 ± 0.1^a	0.5 ± 0.1^{ab}	0.1 ± 0.1^b
C/EBPα	1.0 ± 0.4	0.9 ± 0.1	0.6 ± 0.2	0.6 ± 0.1
PPARγ	1.0 ± 0.5	1.1 ± 0.3	0.9 ± 0.1	0.8 ± 0.2

[1] Values are means ± SEM, $n = 3$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

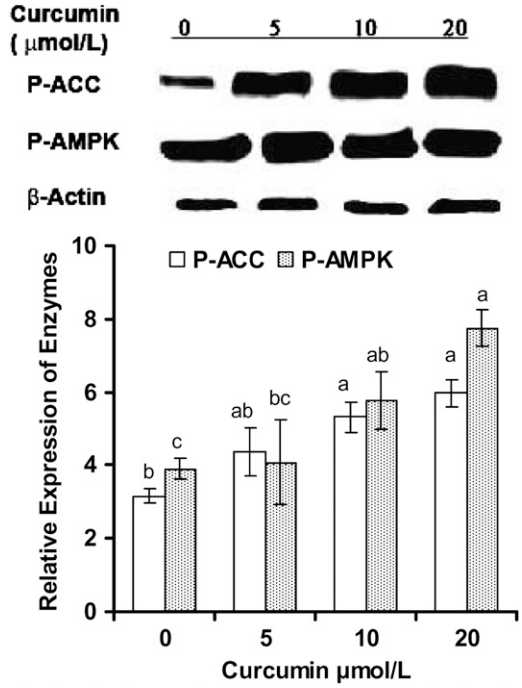

FIGURE 1 Effect of curcumin on phosphorylation of ACC and AMPK in 3T3-L1 adipocytes. Adipocytes incubated with curcumin. Western blot of P-ACC and P-AMPK (*A*) and their quantitative analysis (*B*). Bars represent mean ± SEM, $n = 3$. For each enzyme, means without a common letter differ, $P < 0.05$.

became apparent from wk 3 to wk 9 of the dietary treatment ($P = 0.1$) and was more pronounced and significant ($P < 0.05$) at wk 10 and 11 of dietary treatment (Fig. 4). Reduction in body weight gain with the HF+curcumin diet (**Table 2**) can be attributed to a lower total body fat (Table 2). Furthermore, the percent adiposity (total body fat/body weight) was higher in the HF-fed mice compared with mice fed the HF+curcumin diet (Table 2). However, the percent distribution of fat did not

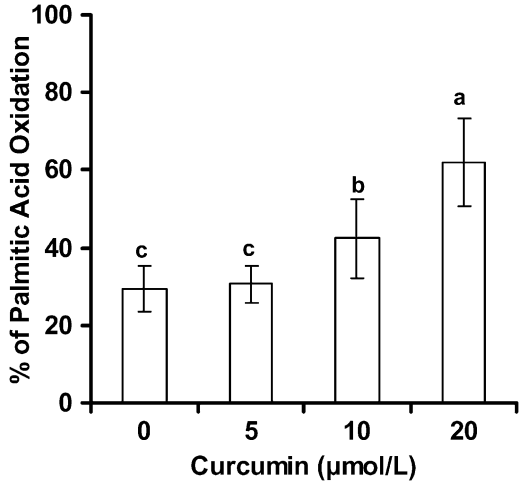

FIGURE 2 Increased fatty acid oxidation in 3T3-L1 adipocytes by curcumin treatment. Cells were preincubated with curcumin and [9,10-^{3}H] palmitic acid was then added for 2 h. Oxidation of palmitic acid was determined by measuring ^3H_2O released into the aqueous phase. Bars represent mean ± SEM, $n = 3$. Means without a common letter differ, $P < 0.05$.

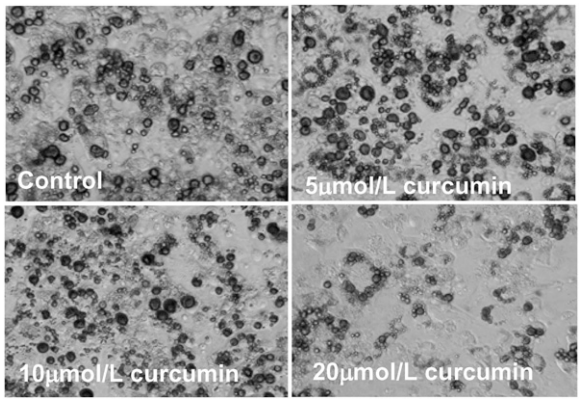

FIGURE 3 The effect of different doses of curcumin on the inhibition of fat accumulation in 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated with curcumin for 24 h and lipid droplets were stained with Oil Red O stain (magnification: 400×).

change with curcumin supplementation (data not shown). The HF diet increased the liver weights and its ratio to body weight (Table 2). In contrast, curcumin supplementation significantly reduced liver weights and its ratio to body weight (Table 2). Although the HF diet increased the accumulation of lipids in hepatocytes (hepatic steatosis), the curcumin-supplemented diet prevented hepatic steatosis (**Supplemental Fig. 5**).

Effect of curcumin on angiogenesis in subcutaneous adipose tissue. The curcumin-treated mice had a lower expression of the angiogenic factor, VEGF mRNA, than those of mice fed a HF diet ($P < 0.05$), implying less production of this growth factor (**Table 3**). Furthermore, gene expression of VEGFR-2 was lower ($P < 0.05$) in the adipose tissue of mice

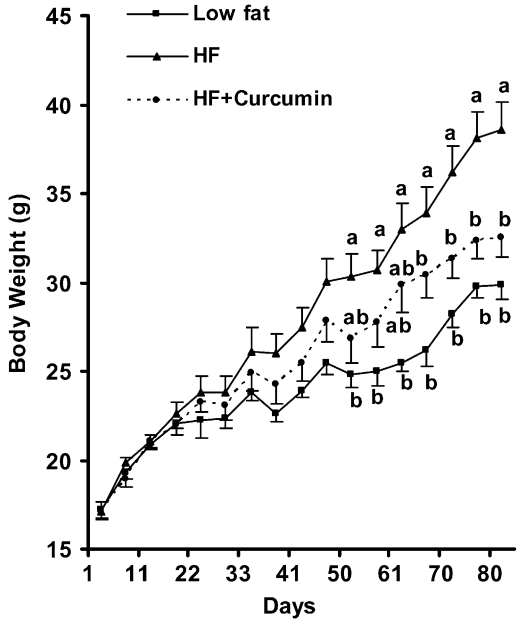

FIGURE 4 Effect of dietary curcumin supplementation on diet-induced obesity in mice. Mice were fed control, HF, or a HF+curcumin diet for 12 wk. Body weight was measured twice per week. Bars represent mean ± SEM, $n = 6$. Means at a time without a common letter differ, $P < 0.05$.

TABLE 2 Effect of dietary curcumin supplementation on body weight, adiposity, liver weight, serum glucose, FFA, cholesterol, and triglycerides in mice fed a HF diet[1]

	Control	HF	HF+curcumin
Body weight, *g*	30.0 ± 2.1[b]	37.0 ± 2.9[a]	33.1 ± 2.1[b]
Total body fat, *g*	10.4 ± 1.2[b]	20.9 ± 1.3[a]	11.8 ± 1.9[b]
Body fat, *g/100 g body*	7.7 ± 1.9[b]	14.1 ± 1.9[a]	10.7 ± 2.1[b]
Liver weight, *g*	4.0 ± 1.1[b]	5.7 ± 1.1[a]	4.5 ± 1.1[b]
Glucose, *mmol/L*	13.1 ± 3.5	16.4 ± 2.1	15.4 ± 1.5
FFA, *mmol/L*	2.6 ± 0.9	2.5 ± 0.2	1.9 ± 0.4
Cholesterol, *mmol/L*	5.5 ± 1.3[a]	5.8 ± 0.2[a]	3.6 ± 0.3[b]
Triglyceride, *mmol/L*	1.7 ± 0.3	1.5 ± 0.1	0.9 ± 0.1

[1] Values are means ± SEM, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

fed a curcumin-supplemented diet (Table 3). To further determine whether the antiangiogenic effect of curcumin might lead to the reduction of obesity in mice, we examined the microvessel density in subcutaneous adipose tissue. Using an anti-CD31 antibody in tissue section, we found that the adipose tissue of mice fed a HF diet was highly vascularized, whereas curcumin-treated mice had significantly lower microvessel density (**Fig. 5**). (For color photomicrographs, please see **Supplemental Fig. 6**).

In vivo modulation of enzymes and transcription factors involved in energy and lipid metabolism by curcumin. We used subcutaneous fat to evaluate the effect of dietary curcumin on metabolic pathways associated with energy metabolism. In agreement with our in vitro cell culture studies, dietary curcumin supplementation increased the phosphorylation of AMPK and ACC (**Fig. 6**) and upregulated the mRNA expression of CPT-1 by 10-fold ($P < 0.05$) and downregulated the mRNA expression of GPAT-1 compared with those of HF-fed mice. In contrast to our observation in vitro, dietary curcumin supplementation significantly lowered PPARγ and C/EBPα expression in subcutaneous adipose tissue (Table 3).

Effect of dietary curcumin on serum lipids and glucose. Mice fed the HF+curcumin diet had a significantly lower serum total cholesterol concentration than those fed the HF diet (Table 2). Curcumin-supplemented mice tended to have lower serum concentrations of glucose ($P = 0.09$), triglycerides ($P = 0.07$), and FFA ($P = 0.058$) than unsupplemented mice (Table 2).

TABLE 3 Effect of dietary curcumin supplementation on mRNA expression of genes regulating angiogenesis and fatty acid metabolism in subcutaneous adipose tissue of mice fed a HF diet[1]

	Control	HF	HF+curcumin
Angiogenesis		*Fold of control*	
VEGFα	1.0 ± 0.0.6[c]	5.5 ± 0.1[a]	3.8 ± 1.0[b]
VEGFR-2	1.0 ± 0.9[c]	17.8 ± 5.6[a]	1.9 ± 0.8[b]
Fatty acid and energy metabolism			
CPT-1	1.0 ± 0.5[b]	1.0 ± 0.5[b]	10.5 ± 0.5[a]
GPAT	1.0 ± 0.6[b]	7.6 ± 0.9[a]	0.6 ± 0.2[b]
C/EBPα	1.0 ± 0.7[b]	5.4 ± 0.4[a]	0.5 ± 0.3[b]
PPARγ	1.0 ± 0.9[b]	3.9 ± 1.4[a]	0.1 ± 0.1[c]

[1] Values are means ± SEM, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

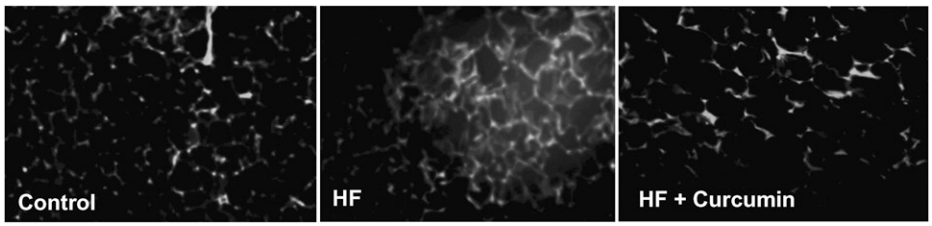

FIGURE 5 The effect of dietary curcumin supplementation on microvessel density in subcutaneous adipose tissue of mice fed a HF diet. Paraffin-embedded subcutaneous adipose tissues were incubated with CD31 antibody. The immunopositive microvessels were photomicrographed.

Discussion

Development of obesity is characterized by the growth of adipose tissue mass. Like growing tumors, growth and expansion of adipose tissue require the formation of new blood vessels or angiogenesis to provide oxygen and nutrients to adipocytes, which are expanding in both size and numbers. Thus, one strategy to reduce adiposity would be to inhibit angiogenesis along with reducing adipocyte numbers and fat content of adipocytes. To this end, using in vitro cell culture systems and in vivo animal models of dietary-induced obesity, we have made interesting observations that curcumin has the ability to inhibit angiogenesis in adipose tissue, decrease differentiation of preadipocytes, and reduce accumulation of lipids in adipocytes and liver, all of which contribute to a lower growth of adipose tissue, lower body weight gain and obesity. We clearly demonstrate that curcumin at concentrations of 5 μmol/L is capable of suppressing angiogenesis when human umbilical vein endothelial cells are stimulated with ACM that is rich in adipokines. These adipokines are constantly produced and play a pivotal role in the growth of adipose tissue. In this study, we did not identify the composition of adipokines in ACM, which might have contained a mixture of various proinflammatory cytokines, growth factors, and angiogenic factors. We also found that supplementing a HF diet with 500 mg curcumin/kg diet for 12 wk markedly reduced microvessel density in adipose tissue in mice along with reduced expression of VEGF and VEGF-R2, 2 important factors in angiogenesis progression. These alterations in adipose tissue vascularity, together with the effect of curcumin on lipid and energy metabolism in vitro and in vivo and curcumin's ability to suppress growth factors (19), collectively appear to be responsible for the lower body weight gain and lower total body fat in the treated mice.

PPARγ and C/EBPα, which are mainly found in adipose tissue, are the key transcription factors in adipogenesis and lipogenesis (20). In this study, dietary curcumin significantly suppressed the expression of these transcription factors in subcutaneous adipose tissue, which may have contributed to a lower adiposity and lower weight gain in HF-fed mice. However, the suppressive effect of curcumin on these transcription factors did not reach significance in our cell culture system despite curcumin suppression of differentiation of preadipocyte 3T3-L1 fibroblasts to mature adipocytes. A PPARγ agonist such as thiazolidinedione, which is used to treat type 2 diabetes, induces differentiation of human preadipocytes and increases subcutaneous adiposity (21). Thus, reduction in adiposity by curcumin in part mediated by curcumin suppression of these transcription factors.

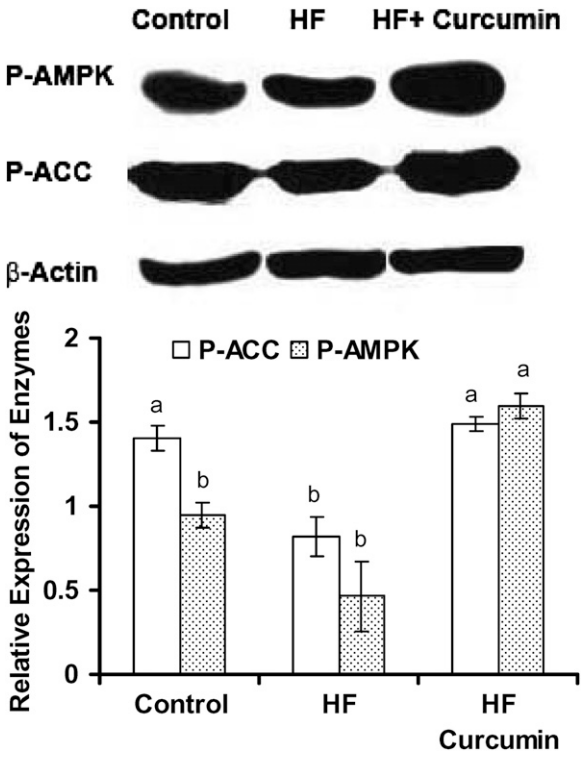

FIGURE 6 Effect of dietary curcumin supplementation on expression of enzymes involved in energy and lipid metabolism in subcutaneous adipose tissues of mice fed a HF diet. Western blot analysis of P-ACC and P-AMPK (*A*) and their quantitative analysis in subcutaneous adipose tissue (*B*). Bars represent mean ± SEM, $n = 6$. For each enzyme, means without a common letter differ, $P < 0.05$.

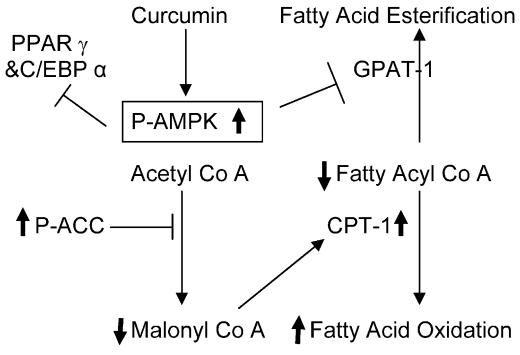

FIGURE 7 Proposed model for the effect of curcumin on lipid and energy metabolism. Curcumin increases AMPK and ACC activities by increasing their phosphorylation. This results in suppression of acetyl CoA conversion to malonyl CoA. A lower level of malonyl CoA increases CPT-1 expression, which increases fatty acid oxidation. The phosphorylated AMPK also suppresses expression of GPAT-1, which results in reduced fatty acid esterification. The phosphorylated AMPK inhibits PPARγ and C/EBPα transcription factors.

Activation of AMPK attenuates the synthesis of glycerol lipids and augments fatty acid oxidation by downregulating GPAT-1 and upregulating CPT-1 expression, respectively, which lead to diminution of the cytosolic long-chain fatty acyl CoA (3,22). Our cell culture studies also revealed that curcumin in a dose-dependent manner suppressed accumulation of lipids in adipocytes by decreasing the GPAT-1 mRNA expression and enhanced fatty acid oxidation through increasing the mRNA expression of CPT-1. Furthermore, we found that activation of AMPK by curcumin downregulated ACC through phosphorylation of this enzyme, which in turn downregulated the flow of acetyl CoA to malonyl CoA (23) (**Fig. 7**). These effects of curcumin on the activation and expression of enzymes involved in adipocytes energy metabolism were verified in vivo by examining the subcutaneous adipose tissue from mice fed a HF+curcumin diet. Although we did not investigate the effect of curcumin on body temperature and physical activity, our data from in vitro and in vivo studies on the effect of curcumin on energy metabolism revealed that curcumin supplementation does not affect food intake, it may suppress anabolic pathways and stimulate catabolic pathways, resulting in a higher energy expenditure, probably a higher basal metabolism, and a higher body temperature in HF-fed mice. Furthermore, in vivo effects of dietary curcumin on lipid metabolism were partly reflected in a significant reduction in serum cholesterol and to some extent (due to low numbers of animals per group) a reduced trend in serum triglyceride, FFA, and glucose concentrations in mice fed a HF diet. These factors are the major players in metabolic syndrome and associated disorders. We also found that a HF diet in C57BL/6 mice in addition to increasing adipose tissue mass in 12 wk also resulted in hepatic macrosteatosis. Our observation is consistent with the recent report by Strissel et al. (24), who showed that chronic high fat feeding in C57BL/6 mice resulted in hepatic macrosteatosis within 12 wk. They suggested that loss of epididymal fat due to continuous remodeling during high fat feeding may have directed the lipid overflow to the liver contributed to the hepatic steatosis, which potentially lead to steatohepatitis. In line with other anti-lipogenic activities of curcumin in our study, dietary curcumin supplementation remarkably prevented formation of hepatic steatosis in mice fed a HF diet.

Our study is partly in agreement with the recent report showing supplementation of a HF diet of mice with 3% curcumin reduced body weight and adiposity in C57BL/6J mice (25). However, the dose of curcumin used in that study (30 g/kg diet) was 60-fold higher than that of our study (0.5 g/kg diet) and according to the authors it was a nonphysiological and impractical dose. The dose of 0.5 g curcumin/kg diet in the present study corresponds to about three-quarters of a teaspoon of curcumin or 3–4 commercially available 1-g curcumin capsules. Furthermore, in our study, curcumin did not affect food intake, but we did observe reduction in body weight and adiposity, which is supported by our data suggesting that curcumin supplementation at a physiological dose increases fatty acid oxidation and reduces fatty acid esterification, resulting in net catabolism in adipose tissue (Fig. 7). In contrast, a very high dose of curcumin supplementation in a study by Weisberg et al. (25) resulted in a significant increase in food intake, yet the mice had a small but significant decrease in body weight and fat content. We suspect that a very high dose of curcumin may block fat absorption from the gut, which has been observed with other polyphenols such as green tea epigallocatechin gallate (26).

In conclusion, dietary curcumin is the major polyphenol found in turmeric with no known toxicity (10). In addition to several reported pharmacological activities (27), our results clearly demonstrate that curcumin at cellular and whole organism levels displays remarkable potential health benefits for prevention of obesity and associated metabolic disorders by suppressing angiogenesis in adipose tissue, upregulating adipocyte energy metabolism and apoptosis, and downregulating preadipocyte differentiation. The mechanism(s) by which curcumin modulates these processes need to be further investigated.

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