Curcumin may induce lipolysis via proteo-stress in Huh7 human hepatoma cells

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Curcumin has been shown to have anti-obesity effects in animal studies. Although several molecular mechanisms of action have been reported, the initial or upstream molecular events remain to be revealed. In this study, we found that curcumin or heat shock treatment up-regulated the expression of adipose triglyceride lipase (ATGL) in Huh7 hepatoma cells, which resulted in acceleration of lipolysis. Interestingly, perturbation of protein homeostasis was seen in curcumin-treated cells, as detected by formation of numerous ubiquitinated proteins and conjugated proteins with p62 (SQSTM). Curcumin activated the protein expression of molecular chaperones, such as heat shock protein (HSP)40 and HSP70. Pre-treatment of the cells with 4-phenylbutyric acid, a chemical chaperone, suppressed proteo-stress induced by curcumin and reduced its lipolysis effect. Importantly, the cytotoxicity of curcumin was markedly alleviated when intracellular triglyceride was consumed by the polyphenol. Thus, energy supplementation from lipolysis may play substantial roles in adaptation and survival of curcumin-exposed cells. To support this notion, the cytotoxicity of curcumin was aggravated in ATGL-knockdown cells. Curcumin decreased intracellular ATP for activating AMP-activated protein kinase, which initiates catabolic pathways including ATGL-dependent lipolysis. Taken together, we propose a hypothesis that curcumin induces lipolysis to compensate for ATP reduction due to its proteo-stress effects.

Key Words: curcumin, lipolysis, heat shock protein, AMP-activated protein kinase, proteo-stress

The phytochemical curcumin is a polyphenol found in the rhizome of turmeric (Curcuma longa), used as food colorant and spice, and also known to possess versatile biological and physiological activities, such as anti-oxidative and anti-inflammatory activities. In addition, curcumin exhibits anti-obesity effects and rodents treated with it decreased body weight, triglyceride (TG), cholesterol, free fatty acids, and low-density lipoprotein levels in blood and tissues. Several molecular mechanisms underlying its anti-obesity effect have been proposed, e.g., suppression of peroxisome proliferator-activated receptor (PPAR)γ, a key transcription factor in adipogenesis and lipogenesis. Curcumin has also been shown to inhibit differentiation of mouse adipocytes by activating the AMP-activated protein kinase (AMPK) pathway and suppressing downstream PPARγ expression, resulting in suppressed adipogenesis. This polyphenol may also stimulate lipolysis of adipose tissue by increasing hormone-sensitive lipase, while another study found that curcumin enhanced lipid elimination by increasing fatty acid oxidation via activation of AMPK in adipocytes and liver tissues.

Exposure of biological proteins to chemical and physical stresses may result in disruption of their three-dimensional structures, causing dysfunction. However, ubiquitous organisms possess both constitutive and inducible defense mechanisms to cope with such protein stresses, termed ‘proteo-stress’. These mechanisms are collectively termed protein quality control (PQC) systems, and are indispensable for maintaining cellular protein homeostasis. An essential group of proteins involved in PQC systems is comprised of molecular chaperones, i.e., heat shock proteins (HSPs), which are ubiquitously expressed in a large variety of cells and participate in folding, activation and reactivation of non-native proteins (misfolded and unfolded proteins). Induction of HSPs is mainly regulated by the transcription factor heat shock factor 1 (HSF1) and the process of HSP induction is termed heat shock response (HSR).

As the most abundant and essential energy carrier in cells, ATP functions to drive a variety of biochemical reactions. Stresses encountered by an organism can be classified into physical, chemical and biological stimuli. Self-defense systems, which are generally specific though occasionally non-specific to a stimulus for adaptation and survival. Regulation of defense mechanisms greatly depends on cellular ATP availability, affirming the crucial role of ATP in stress responses. Lipolysis has been well described as a catabolic pathway, initiated by activation of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), as well as others. Based on the background information, we examined our hypothesis that curcumin induces proteo-stress to consume cellular ATP, leading to lipolysis which compensates for ATP reduction, and the present results propose a new mechanism underlying the anti-obesity effects of curcumin.

Materials and Methods

Reagents. High (4.5 g/L) and low (1.0 g/L) glucose Dulbecco’s modified eagle medium (DMEM), and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY). Antibodies were obtained from the following sources: rabbit anti-ATGL, mouse anti-ubiquitin, rabbit anti-p62, rabbit anti-anti-carboxyl-terminus of HSP70 interacting protein (CHIP), rabbit anti-AMPK, rabbit anti-pAMPK and horseradish peroxidase-conjugated anti-rabbit IgG were purchased from Cell Signaling Technologies.

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Technology (Beverly, MA); mouse anti-α-tubulin was from Calbiochem (La Jolla, CA); and HRP-conjugated anti-mouse IgG was purchased from Dako (Tokyo, Japan). Control siRNA, ATGL siRNA and Lipofectamine RNAiMAX were purchased from Invitrogen. Curcumin (purity >98%) and other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan) unless specified otherwise.

**Cell culture.** Human hepatoma HuH7 cells were purchased from American Type Culture Collection (Manassas, VA) and maintained in high-glucose DMEM supplemented with heat-inactivated (56°C for 30 min) 10% FBS, streptomycin (100 μg/ml) and penicillin (100 U/ml) at 37°C under a humidified atmosphere of 95% O2 and 5% CO2.

**Sample treatment.** HuH7 cells were seeded onto well plates or dishes at the density of 6–9×104 cells/ml with 10% FBS DMEM. Unless otherwise stated, cells were pre-incubated for 24 h. For treatment, samples diluted in low-glucose DMEM (0.5% FBS) were added to cells. DMSO was used as the vehicle for curcumin and 3-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), while water was used for 4-phenylbutyric acid (PBA).

The concentration of each vehicle was 0.2% (v/v). TG assay. TG was assayed using an Adipogenesis Colorimetric/Fluorometric Assay Kit (BioVision, Milnitas, CA) according to the manufacturer’s protocol. HuH7 cells (3×104 cells/0.2 ml/96-well plate) were treated with sample or vehicle (0.2%, v/v) for specified times, and then lysed with 75 μl of Lipid Extraction Solution from the kit. Lysates were transferred to microtubes and placed in heating block at 90°C for 30 min. Each sample was then cooled at room temperature and then 25 μl of each sample was transferred to a 96-well black plate and assayed. Lipase (1.5 μl) was added to each sample, which was mixed and incubated for 10 min at room temperature to convert TG to glycerol and free fatty acid. Glycerol was subsequently oxidized by an enzyme reaction to convert the probe to generate visible absorption at 570nm measuring ADP. ADP was then converted to ATP through an enzyme reaction measuring luminescence again. That measurement was used as the residual ATP signal that provides the background prior to measuring ADP. ADP was then converted to ATP through an enzyme reaction and luminescence was again measured. The value derived from this newly formed ATP was determined as intracellular ATP.

**Cell viability.** Cell viability was determined by using a Cell Counting Kit-8 (DOJINDO, Kumamoto, Japan). After incubation, cells were washed with PBS, then 200 μl of FBS and phenol red-free low-glucose DMEM (1.0 g/L) containing 7.5% of WST-8 solution were added, which was followed by incubation at 37°C for 20–30 min. Absorbance at 630 nm due to turbidity of the cell culture was measured using a microplate reader MULTISKAN JX (Promega, Fitchburg, WI), with the value subtracted from that at 450 nm, which was correlated with the reduction of tetrazolium salt WST-8 to show cell viability.

**Western blotting.** HuH7 cells (6.8×105 cells/2 ml/dish) were seeded onto 35-mm dishes and treated with a sample or the vehicle (0.2%, v/v) for specified times, then lysed in BioPlex cell lysis buffer (Bio-Rad Laboratories, Hercules, CA) containing 1% protease inhibitor (Sigma Aldrich, St. Louis, MO), followed by centrifugation at 10,000 × g for 10 min at 4°C. Proteins were separated using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) on a 10% polyacrylamide gel, then transferred to Immobilon-P membranes (Millipore, Billerica, MA). After blocking with 2% Block Ace (GE Healthcare, Buckinghamshire, UK) at room temperature for 1 h, each membrane was treated with a specific primary Ab (1:1,000 for pAMPK, 1:10,000 for ubiquitin, 1:20,000 for α-tubulin, 1:2,000 dilution for other proteins), followed by the corresponding HRP-conjugated secondary Ab (1:2,000). The blots were developed using ECL western blot detection reagents (GE Healthcare, Buckinghamshire, UK).

**Protein fractionation.** Lysis buffers for fractionation of water soluble and insoluble proteins were prepared as previously described.7 Soluble lysis buffer contains 30 mM phosphate, 1% Tween-20, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM ethylene glycol tetraacetic acid and 1% protease inhibitor (pH 7.4), while insoluble lysis buffer contains 65 mM Tris-HCl, 2% SDS, 50 mM dithiothreitol and 150 mM NaCl (pH 8.0). HuH7 cells (1.8×105 cells/ml/24-well plate) were treated with a sample or the vehicle (0.2%, v/v) for specified times, then trypsinized to collect cell pellets. Cells were lysed on ice with 40 μl soluble lysis buffer followed by sonication with a Bioruptor (COSMO BIO, Carlsbad, CA) for 30 s. After centrifugation (10,000 × g, 10 min), the supernatant thus obtained was designated as a soluble fraction. On the other hand, the pellets were washed with PBS before extraction with 30 μl of insoluble lysis buffer. The resulting sample was then briefly vortexed to yield an insoluble fraction.

**HS treatment.** Following pre-incubation, cells were washed with PBS and incubated in low-glucose, serum-free DMEM in 96-well plates. Next, the plate was sealed with Parafilm and placed into a water bath, and incubated at 37°C or 43°C for specified times. The cells were then immediately treated for western blotting or ATP measurement, or further incubated at 37°C for specified times in a CO2 incubator.

**ADP/ATP ratio.** The ADP/ATP ratio was determined by using an EnzLight ADP/ATP Ratio Assay Kit (BioAssay Systems, Hayward, CA) according to the manufacturer’s protocol. HuH7 cells (2×104 cells/0.2 ml/96-well plate) were treated with a sample or the vehicle (0.2%, v/v) for specified times, then lysed to determine intracellular ATP, which was reacted with luciferase in the presence of D-luciferin to produce luminescence. Luminescence intensity was measured using a Veritas Microplate Luminometer (Promega, Fitchburg, WI). After determination of ATP, samples were incubated at room temperature for 10 min before measuring luminescence again. That measurement was used as the residual ATP signal that provides the background prior to measuring ADP. ADP was then converted to ATP through an enzyme reaction and luminescence was again measured. The value derived from this newly formed ATP was determined as intracellular ADP.

**RNA interference of ATGL.** Transfection of control or ATGL siRNA was performed using Lipofectamine RNAiMAX, according to the manufacturer’s specifications. Control or ATGL siRNA solutions (25 nM final concentration) were added to Lipofectamine RNAiMAX solution (1:35), and incubated for 20 min. This transfection mixture was diluted 5 times with serum-free Opti-MEM, and used to treat cells for 6 h. The medium was replaced with low-glucose medium containing 10% FBS and cells were incubated for 24 h before treatment with a sample.

**Statistical analyses.** Each experiment was performed at least 3 times and values are shown as the mean ± SE where applicable. Statistically significant differences between groups for each assay were determined using Student’s t test. Differences were considered to be significant at p<0.05.

**Results**

**Curcumin and HS treatments induced lipolysis.** Treatment of HuH7 cells with curcumin at a concentration of 5 μM, but not 25 μM, for 24 h significantly decreased the level of intracellular TG (Fig. 1A). The reason why no concentration-dependent decrease remains to be elucidated. Next, we attempted to examine the effects of HS treatment on lipolysis, as that is a well-known proteo-stress inducer. Interestingly, HS treatment at 43°C for 2 h, followed by incubation at 37°C for 24 h, decreased the concentration of TG, as compared to treatment at 37°C (Fig. 1B). In addition, curcumin (5 and 25 μM) up-regulated the protein expression of ATGL after 24 h in a concentration-dependent manner, while HS treatment at 43°C for 2 h showed a similar effect (Fig. 1C). On the other hand, expression levels of HSL were not significantly altered by these treatments (data not shown).
Curcumin induced proteo-stress and activated PQC systems. Denatured proteins expose hydrophobic regions to initiate a cascade of aberrant protein-protein hydrophobic interactions, which may lead to formation of insoluble protein aggregates. Hence, denatured proteins and their aggregates exhibit poor water solubility. Previous studies have reported that HS increased accumulation of insoluble proteins with poly-ubiquitin chains as well as other proteins that lose solubility.\(^{(18)}\) Additionally, we previously found that proteo-stress induced by zerumbone led to the formation of conjugated proteins with p62 (SQSTM), which plays essential roles in sequestration of aggregated proteins.\(^{(19)}\) Thus, we examined the formation of both ubiquitinated and p62/SQSTM1-conjugated proteins using western blotting (Fig. 2A). Treatment of Huh7 cells with curcumin (25 \(\mu M\)) for both 1 and 3 h markedly increased ubiquitinated proteins (Fig. 2A), while curcumin did not increase those proteins (data not shown). However, it was intriguing to note that exposure to 50 \(\mu M\) curcumin for 3 h resulted in abolishment of ubiquitinated proteins, which might have been due to a dysfunction of the protein ubiquitination system caused by excessive exposure to curcumin. On the other hand, curcumin increased p62 (SQSTM1)-conjugated proteins, whose bands appeared in upper molecular-weight regions (more than 200 kDa), under all of the experimental conditions utilized. We also examined the expression level of CHIP, a chaperone-dependent E3 ligase, that binds ubiquitin to denatured proteins. Treatment with curcumin decreased the CHIP protein level, suggesting consumption of CHIP by degradation of curcumin-ubiquitinated proteins in the proteasome.\(^{(20)}\) On the other hand, curcumin (25 \(\mu M\)) dramatically increased expression of HSP70 and HSP40 proteins, but not of HSP90, at 12 and 24 h, indicating that it is capable of inducing HSR (Fig. 2B).

To further assess the effect of curcumin on protein homeostasis, we attempted to divide cellular proteins into water-soluble and -insoluble fractions, since proteo-stress is considered to increase water-insoluble proteins. The amounts of insoluble protein were divided by those of soluble proteins to yield the I/S ratio. Meanwhile, chemical chaperones are a group of low-molecular weight compounds that function to prevent or repair unfolded and/or aggregated proteins. For example, glycerol, trimethylamine N-oxide and PBA, representative chemical chaperones, have been reported to decrease protein aggregation.\(^{(21)}\) As shown in Fig. 2C, curcumin increased the I/S ratio when used at a concentration of 50 \(\mu M\) for 3 h, whereas pre-treatment with PBA (2 mM) for 6 h abolished that increase in I/S ratio induced by curcumin. On
Fig. 2. (A) Curcumin increased p62 conjugates and ubiquitinated proteins. Huh7 cells treated with CUR (0, 25, 50 μM) for 1 or 3 h were lysed and subjected to western blotting analysis. This experiment was performed in duplicates. CHIP, carboxy-terminus of Hsc70 interacting protein; CUR, curcumin; NT, non-treated; Ub, ubiquitinated. (B) CUR activated HSR. Huh7 cells treated with CUR (0, 5, 25 μM) for 3, 6, 12 or 24 h were lysed and subjected to western blotting analysis. CUR, curcumin; HSP, heat shock protein. (C) Huh7 cells were pre-treated with PBA (0, 2 mM) for 6 h, washed, and treated with curcumin (0, 50 μM) for 3 h. Soluble and insoluble proteins resulting from protein fractionation were quantified and the I/S ratio was calculated. This experiment was performed 6 times. (D) Cells were pre-treated with PBA (0, 2 mM) for 6 h, then washed, and treated with curcumin (0, 5 μM) for 24 h. Then, the TG concentration of was determined and corrected based on protein amount. This experiment was performed 4 times. *p<0.05 vs control; **p<0.05 vs CUR. CUR, curcumin; I/S, insoluble/soluble protein ratio; PBA, 4-phenylbutyric acid; TG, triglyceride.
the other hand, curcumin (5 µM), which significantly decreased TG level (Fig. 2D), did not increase the I/S ratio (data not shown). This discrepancy may be due to low sensitivity of assay for evaluating proteo-stress employed in the present study. Importantly, pre-treatment with PBA significantly decreased the lipolysis effect (Fig. 2D), supporting our hypothesis that curcumin induces lipolysis via proteo-stress.

**Lipolysis attenuated curcumin-induced cytotoxicity.** Lipolysis is presumably more accelerative for cellular energy homeostasis in low- compared with high-glucose medium, which may result in higher ATP levels when low-glucose medium is used. Therefore, we examined whether the cytotoxicity of curcumin showed a decline in cells incubated in low-glucose medium. Huh7 cells were pre-incubated for 24 or 48 h in low-glucose (1.0 g/L) or high-glucose (4.5 g/L) medium, then treated with curcumin (0, 5, 25 µM) for additional 18 h in low-glucose medium to assess cell viability. Treatment with curcumin for 24 h in low-, as well as high-glucose medium, resulted in cytotoxicity (Fig. 3A). In contrast, even though curcumin exhibited substantial growth inhibition activity in high-glucose medium, cell viability was not decreased, but rather significantly increased in the low-glucose environment.

The relationship between the curcumin cytotoxicity and intracellular TG content was also evaluated. Cells were pre-incubated in low-glucose medium for 24 h and then treated with curcumin (0, 5, 25 µM) for 48 h. After a total incubation time of 72 h in low-glucose medium, the viability of non-treated cells was decreased to 27% as compared with the initial condition (Fig. 3B). However, it was interesting to note that curcumin rescued the cells from glucose starvation-induced death in a concentration-dependent manner. Furthermore, as the concentration of TG was decreased, the cytotoxicity of curcumin was evidently mitigated. This inverse relationship suggests that lipid catabolism is promoted to supply intracellular ATP, which may result in counteraction of xenobiotic stress by curcumin. To examine this notion, we down-regulated the expression of ATGL by 68% using siRNA (Fig. 3C) and found that cytotoxicity induced by curcumin was significantly increased, as compared with the control cells (Fig. 3D). This raised the possibility that ATGL-dependent lipolysis has an important role in conferring stress resistance against curcumin.

**Proteo-stress decreased intracellular ATP level.** Next, the levels of intracellular ATP were measured after treatment with curcumin (0, 25, 50 µM for 1 h) or HS (37°C, 43°C for 15 min), along with antimycin A (0, 10 µM for 1 h), an electron transport inhibitor, using a chemiluminescence method to yield the ADP/ATP ratio. As shown in Fig. 4A, that ratio was significantly increased by each treatment as compared to the controls. Cellular ATP level is tightly regulated by the major energy sensor enzyme AMPK. Although AMPK, and its activity is sensitive to an increase in AMP and/or decrease in ATP. Therefore, we utilized western blotting to determine whether curcumin increases phosphorylation of AMPK for its activation, with AICAR, a known AMPK activator, used as a positive control. As shown in Fig. 4B, curcumin (25, 50 µM) and AICAR (5 mM) increased the level of the phosphorylated active form of AMPK protein (pAMPK) after 3 h as compared with the vehicle control, while resulted in a marked increase. Finally, we examined whether AMPK is involved in ATGL activation for promoting lipolysis. Interestingly, treatment with AICAR for 24 h induced showed a pronounced increase in ATGL protein expression (Fig. 4C).

**Discussion**

In the present study, we attempted to reveal the initial mechanism or the primary cause of lipolysis caused by curcumin. Curcumin was found to induce proteo-stress in Huh7 cells, which was detected by significant increases in ubiquitinated- and p62/SQSTM1-conjugated abnormal proteins (Fig. 2A). Previously reported evidence indicates that ubiquitin binds to unfolded proteins that are subjected to proteasome-dependent degradation while p62/SQSTM1 binds to ubiquitinated proteins and the resulting protein complex is involved in degradation through selective autophagy. Therefore, these modified proteins may be validated as proteo-stress markers by the present results. On the other hand, proteo-stress induced by curcumin was shown to be adaptable in our study since both HSP40 and HSP70 were markedly up-regulated for repairing denatured proteins (Fig. 2B).

These results are in line with our previously reported findings of 8 phytochemicals, which demonstrated curcumin as the most potent inducer of HSP70 in Hepa1c1c7 mouse hepatoma cells. However, at this time, we do not have data showing how curcumin interacts with and/or binds to cellular proteins to perturb their 3-dimensional structures. On the other hand, our previously reported findings indicate that phytochemical hydrophobicity and electrophilicity may be determinants of non-specific protein interactions. In this context, it is notable that the molecular hydrophobicity of curcumin (logP: 3.85) may be associated with its proteo-stress function to expose hydrophobic regions of proteins. Also, curcumin, which contains an α,β-unsaturated carbonyl group, is electrophilic and has also been demonstrated to bind nucophile amino acid residues of cellular proteins via the Michael reaction. In addition, 4-vinyl guaiacol, vanillin, and trans-6(4’-hydroxy-3’-methoxyphenyl)-2,4-dioxo-5-hexenal, degradation products of curcumin with nucophile properties, may have critical roles in proteo-stress. We intend to address these issues in the near future.

It is important to note that ATP is indispensable, and largely consumed for operation and maintenance of PQC systems. In the present study, treatment of Huh7 cells with curcumin resulted in a marked increase in abnormal proteins (Fig. 2A) and significant decrease in intracellular ATP level (Fig. 4A), leading us to speculate that the decrease in ATP by curcumin is responsible for adaptation to proteo-stress through the PQC systems. However, the possibility that ATP is consumed by curcumin for activation of xenobiotics metabolizing systems, known to be an ATP-dependent event, cannot be ruled out. In addition, curcumin is an uncoupler of oxidative phosphorylation to perturb ATP biogenesis, as previously demonstrated in an isolated rat liver mitochondria model. Thus, the mechanism of ATP decrease by curcumin treatment remains to be fully elucidated.

Decreased intracellular ATP by curcumin is critical and may be lethal for cells unless it is resupplied from an endogenous or exogenous energy source. When cells are starved, energy sensor proteins are activated in turn to promote glucose uptake and/or lipolysis to compensate for ATP reduction. AMPK, described as a ‘fuel gauge’, is a major energy sensor, which is able to activate several different catabolic pathways and concomitantly suppress anabolic ones. Importantly, AMPK activation is correlated with lower ATP level and several reports have shown that curcumin is an effective AMPK inducer in different types of cells, though the precise activation mechanisms remain unknown. Furthermore, others have reported that activated AMPK induces phosphorylation of ATGL at Ser406 to increase its lipase activity, leading to promotion of fatty acid oxidation for ATP production. Taken together with the present data (Fig. 4B and C), ATP depletion due to proteo-stress by curcumin is likely involved in AMPK activation and resultant ATGL activation and lipolysis.

Interestingly, we found that treatment of Huh7 cells by HS, characterized as a typical stimulus causing proteo-stress in cellular proteins, increased both ATGL expression and lipolysis (Fig. 1B and C). As support for those findings, it was previously demonstrated in ex vivo experiments that HS treatment of white adipose tissue from male Wister rats promoted free fatty acid release from TG. Lu et al. also reported that chronic heat stress increased mRNA expressions related to fatty acid oxidation and promoted...
lipolysis in experiments with broiler chickens. In addition, the level of TG in human leg muscle tissues was shown to decrease during exercise under a heated condition. Therefore, proteo-stress can be described as a physical stimulus that promotes lipolysis, as long as its potency remains at a level below the stress capacity. To the best of our knowledge, the present study is the first to show the possibility that curcumin induces lipolysis through proteo-stress (Fig. 2C and D).

It has been widely reported that curcumin and other phytochemicals, such as (−)-epigallocatechin-3-gallate, resveratrol and sulforaphane, are beneficial agents that show anti-obesity activities. However, as mentioned above, because these phytochemicals have no nutritional values and are thus recognized as xenobiotics, cells activate the ATP-dependent self-defense mechanisms for survival. Therefore, it is quite reasonable to

**Fig. 3.** (A) Lipolysis decreased curcumin-induced cytotoxicity. Huh7 cells were incubated for 24 or 48 h under a high-glucose (4.5 g/L) or low-glucose (1 g/L) condition, and then treated with CUR (0, 5, 25 μM) for 18 h. Cell viability was determined using a WST-8 test. This experiment was performed 3 times. *p<0.01 vs control (0 μM curcumin). Closed bars, high-glucose medium; open bars, low-glucose medium. (B) Cells were incubated for 48 h with curcumin (0, 5, 25 μM) after pre-incubation for 24 h using low-glucose medium. Non-treated cells were collected following pre-incubation. TG concentration was corrected based on protein amount. *p<0.01 vs control (0 μM curcumin at 0 h), **p<0.01 vs 0 μM curcumin (at 48 h). CUR, curcumin, TG, triglyceride. (C, D) Reduced ATGL protein expression aggravated curcumin-induced cytotoxicity. Huh7 cells were treated with a Lipofectamine RNAiMAX and siRNA solution (control or ATGL, 25 nM) for 6 h. After gaining recovery with low-glucose medium containing 10% FBS for another 24 h, RNA was collected and the cells were treated with curcumin (0, 25 μM) for 48 h. Cell viability was determined using a WST-8 test. The experiments shown in (C) were performed 3 times (*p<0.01 vs control) and that in (D) 8 times (*p<0.01 vs 0 μM curcumin in ATGL-silenced cells). ATGL, adipose triglyceride lipase; CTL, control, CUR, curcumin.
speculate that curcumin-induced lipid catabolism is a homeostatic process, in which cells produce ATP from stored lipids to cope with curcumin-induced stress stimuli. In the present experiments, lipolysis was found to play a significant role in conferring stress resistance against the cytotoxicity of curcumin (Fig. 3A and B), and yet decreased ATGL expression resulted in increased cytotoxicity (Fig. 3D). In conjunction with these observations, Chandak et al. found that phagocytosis in ATGL-deficient mice was impaired by challenge from bacterial particles, implying that lipids are an essential energy source to fight against foreign organisms. Moreover, another study noted that prominent accumulation of ubiquitinated proteins was observed in ATGL-deficient mice, which was accompanied by dysfunction of the ubiquitin-proteasome system and activation of NF-κB signaling under pro-inflammatory and pro-oxidative conditions. Furthermore, ATGL-knockout, but not HSL-knockout, mice showed exacerbated hepatic steatosis and inflammation. Taken together, catabolic pathways involved in lipid metabolism are presumably necessary for the processes of self-protection and disease prevention by supplying ATP.

In conclusion, based on the present findings, we propose a unique mechanism underlying the lipolysis effects of curcumin, i.e., this phytochemical induces proteo-stress to cause decrease in ATP for AMPK activation, which leads to lipolysis via ATGL activation. Interestingly, we recently found that proteo-stress induced by zerumbone plays an important role in its anti-inflammatory functions through HSF1 activation. In addition to target molecule-mediated mechanisms, xenobiotic stress-mediated mechanisms may also be described as an intrinsic property to phytochemicals, which are secondary metabolites and have no nutritional value to animals. In fact, curcumin exhibits quite a low level of bioavailability and rapid excretion from the body, common characteristics of phytochemicals. Therefore, the process by which curcumin exhibits beneficial health effects for animals is intriguing, and in line with the concept of hormesis, in which mild stress can produce self-protective effects.

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Abbreviations
AICAR 5-aminimidazole-4-carboxamide-1-β-D-ribofuranoside
AMPK AMP-activated protein kinase
ATGL adipose triglyceride lipase
CHIP the carboxyl-terminus of HSP70 interacting protein
DMEM Dulbecco’s modified eagle medium
FBS fetal bovine serum
HS heat shock
HSF1 heat shock factor 1
HSL hormone-sensitive lipase
HSP heat shock protein
HSP heat shock response
PAGE polyacrylamide gel electrophoresis
PBA 4-phenylbutyric acid
PPAR peroxisome proliferator-activated receptor
PQC protein quality control
SDS sodium dodecyl sulfate
TG triglyceride

Conflict of Interest
No potential conflicts of interest were disclosed.

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