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Regulatory Effects of Curcumin on Lipid Accumulation in Monocytes/Macrophages

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ABSTRACT

Recent evidence suggests potential benefits from phytochemicals and micronutrients in protecting against atherosclerosis and inflammation, but the molecular mechanisms of these actions are still unclear. Here, we investigated whether the dietary polyphenol curcumin can modulate the accumulation of lipids in monocytes/macrophages. Curcumin increased the expression of two lipid transport genes, the fatty acids transporter CD36/FAT and the fatty acids binding protein 4 (FABP4/aP2; $P < 0.05$), leading to increased lipid levels in THP-1 and RAW264.7 monocytes and macrophages ($P < 0.05$). To investigate the molecular mechanisms involved, we assessed the activity of Forkhead box O3a (FOXO3a), a transcription factor centrally involved in regulating several stress resistance and lipid transport genes. Curcumin increased FOXO3a-mediated gene expression by twofold ($P < 0.05$), possibly as a result of influencing FOXO3a phosphorylation and nuclear translocation. The curcumin derivative, tetrahydrocurcumin (THC), with similar chemical antioxidant activity as curcumin, did not show any measurable effects. In contrast to the in vitro results, curcumin showed a trend for reduction of lipid levels in peritoneal macrophages in LDL receptor knockout mice fed a high fat diet for 4 months, suggesting additional regulatory mechanisms in vivo. Thus, the up-regulation of FOXO3a activity by curcumin could be a mechanism to protect against oxidant- and lipid-induced damage in the inflammatory cells of the vascular system. J. Cell. Biochem. 113: 833–840, 2012.

KEY WORDS: CURCUMIN; TETRAHYDROCURCUMIN; FOXO3a; LIPIDS; MONOCYTES; MACROPHAGES

Monocytes/macrophages are central to the development of atherosclerosis since they release inflammatory cytokines and accumulate lipids leading to foam cells formation and fatty streaks in the vascular wall. Recent evidence suggests potential benefits from phytochemicals such as curcumin, a polyphenol in turmeric spice, for reducing oxidative and lipid-mediated damage, but the molecular mechanisms of these actions are still unclear [Shehzad et al., 2011]. These compounds may either scavenge reactive oxygen or nitrogen species directly or they may modulate the activity of signal transduction enzymes, which may lead to changes in gene expression, for example, of proteins with an antioxidant function.

A number of stress resistance genes with antioxidant activity, such as catalase, MnSOD, and eNOS are known to be regulated by the transcription factor Forkhead box O3a (FOXO3a) [Murakami, 2006; Calnan and Brunet, 2008]. At the molecular level, FOXO3a activity is modulated through phosphorylation by protein kinase B (PKB/Akt) [Miyauchi et al., 2004], leading to translocation of FOXO3a out of the nucleus and its consequent inactivation [Van Der Heide et al., 2004]. We and others have shown that Akt is modulated by phytochemicals such as resveratrol [Reiter et al., 2007], vitamin E [Kempna et al., 2004], curcumin [Hussain et al., 2006], or by catechins from green tea, such as epigallocatechin-3-gallate (EGCG) [Lin and Lin-Shiau, 2006], but not much is known about FOXO3a regulation by these compounds in general and in particular, in monocytes/macrophages.

In addition to antioxidant genes, a number of genes involved in energy storage, energy expenditure, and lipid homeostasis are regulated by FOXO proteins [Nakae et al., 2008], including the fatty acid binding protein 4 (FABP4/aP2) [Song et al., 2010], sterol carrier

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protein [Dansen et al., 2004], and the 3-hydroxy-3-methylglutaryl-CoA synthase [Nadal et al., 2002]. Free fatty acids can increase lipid-mediated oxidative and endoplasmic stress [Brookheart et al., 2009] by inducing aP2 [Grimaldi et al., 1992] via increasing FOXO3a activity [Barreyro et al., 2007], or by activating CD36 [Okamura et al., 2009] and various other mechanisms [de Kreutzenberg et al., 2010]. The aP2 mediates endoplasmic stress triggered by accumulation of lipids [Erbay et al., 2009]; therefore, restoring correct aP2 and CD36 expression by specific phytochemicals or micronutrients could alleviate lipid-mediated stress.

We and others have shown that curcumin can reduce lipid levels in adipocytes [Asai and Miyazawa, 2001; Ejaz et al., 2009; Ahn et al., 2010], but the molecular mechanisms of influencing lipid accumulation in monocytes/macrophages are not yet resolved. Recently, it was observed that curcumin induced the expression of LDL receptor in mouse macrophages (ANA-1), leading to increased uptake of LDL [Fan et al., 2006]. Similar to that, an increase of lipids was observed in hepatic stellate cells (HSC) [Tang and Chen, 2010], whereas in activated HSC, curcumin suppressed LDL receptor expression via a sterol regulatory element (SRE) [Kang and Chen, 2009]. However, in HepG2 cells, curcumin activated LDL receptor expression which has been proposed to contribute its cholesterol lowering and anti-atherosclerotic effects [Dou et al., 2008]. In skeletal muscle, curcumin increased the expression and membrane exposition of CD36 [Bastie et al., 2005; Na et al., 2011], possibly via activation of FOXO1 [Nahle et al., 2008], whereas in HepG2 cells, CD36 expression was reduced by curcumin [Peschel et al., 2007].

To our knowledge, the ability of curcumin to modulate the expression of lipid transporters and lipid accumulation in monocyte/macrophages has not been investigated. Here, we sought to define whether curcumin can influence lipid accumulation in cultured human THP-1 and mouse RAW264.7 monocytes/macrophages. To understand whether curcumin influences lipid accumulation by regulating the expression of lipid transporters, we investigated whether curcumin can regulate aP2 and CD36 expression, and whether such regulation occurs by affecting FOXO3a expression and activity. In addition, to establish whether dietary curcumin can affect macrophage lipid uptake in a LDL receptor-independent manner, we used peritoneal macrophages isolated from LDL receptor-knockout (LDL-$R^{-/-}$) mice fed a high fat diet (HFD).

MATERIALS AND METHODS

Gene expression was measured by quantitative RT-PCR using primers and conditions of the TaqMan two steps RT-PCR protocol (Applied Biosystems, Foster City, CA). Amplification of GAPDH was used as endogenous control. Protein expression and phosphorylation was assessed using Western blots according to standard methods using monoclonal mouse anti-human β-actin as primary control antibody. Promoter activity was measured after transfection of promoter-firefly luciferase reporter plasmids using the Dual-Luciferase assay kit and a GLOmax luminometer (Promega, Madison, WI). CD36 surface exposition was analyzed by FACS using a monoclonal anti-CD36-FITC antibody. LDL-$R^{-/-}$ in the background of C57BL/6 male mice were individually housed at the Jean Mayer Human Nutrition Research Center on Aging (HNRCA) Comparative Biology Unit at Tufts University. One group of mice was fed an AIN-93G diet for 8 weeks and served as "low fat diet (LFD) negative controls." The other four groups of mice were fed a Western style high fat/cholesterol AIN-93G diet (HFD) formulated to contain 0.1% cholesterol and 21% fat by weight for 4 months. From the HFD-fed mice, 1 group of mice was designated as positive HFD control, and the diets of the other 3 groups were either supplemented with 500 mg curcumin per kg of diet (low curcumin), 1,000 mg of curcumin per kg of diet (medium curcumin), or 1,500 mg curcumin per kg of diet (high curcumin) for 4 months. All procedures adhered to the HNRCA Institutional Animal Care and Use Committee Guidelines at Tufts University. Peritoneal macrophages were collected from peritoneal lavage, fixed, and cellular lipid accumulation was assessed using Nile red staining and FACS, or oil red O staining, extraction of accumulated oil red O, and measurement of absorbance at OD_{490}. All values are expressed as the mean ± standard errors (SEM) as inscribed in the figure legends. Student's *t*-test was used to analyze the significant differences between two conditions. A $P < 0.05$ was considered as significant and indicated by * in the graphs. A detailed expanded Materials and Methods Section is available in the online Supplemental Information.

RESULTS

CURCUMIN INDUCES LIPID ACCUMULATION IN THP-1 MONOCYTES AND MACROPHAGES

Recently, it was observed that curcumin induced the expression of LDL receptor in mouse macrophages (ANA-1), leading to increased uptake of LDL [Fan et al., 2006]. To investigate whether curcumin influences lipid uptake in human monocytes/macrophages, THP-1 monocytes and macrophages (differentiated with 100 nM PMA for 24 h) were treated with curcumin and the cellular accumulation of lipids measured. Curcumin significantly increased the lipid levels in THP-1 monocytes, as assessed by Nile red staining and FACS (Fig. 1A). Curcumin also significantly increased lipid accumulation in THP-1 macrophages, as assessed by oil red O staining (Fig. 1B). In both cases, lipid accumulation became significant at 5 μM curcumin, whereas no effect was observed with the curcumin metabolite, tetrahydrocurcumin (THC; Supplemental Fig. 1).

CURCUMIN UP-REGULATES CD36 AND AP2 EXPRESSION IN THP-1 MONOCYTES/MACROPHAGES AND RAW264.7 MACROPHAGES

In addition to the LDL receptor, lipids are transported into monocytes/macrophages by two major transport proteins, the CD36 scavenger receptor/fatty acid transporter (FAT) and the fatty acid-binding protein 4 (FABP4/aP2) [Fu et al., 2002, 2006; Su and Abumrad, 2009]. In THP-1 macrophages, the formation of foam cells is facilitated [Fu et al., 2002] by up-regulation of aP2 and CD36 by oxLDL [Fu et al., 2000; Ricciarelli et al., 2000] via acceleration of cholesterol and triglyceride accumulation [Fu et al., 2006]. To investigate whether the observed lipid accumulation by curcumin involves the up-regulation of CD36 or aP2, PMA-differentiated THP-1 macrophages were treated with curcumin and the protein level of CD36 and aP2 was assessed by Western blotting. Both, CD36 and aP2 were up-regulated by treatment with 5 and 10 μM curcumin

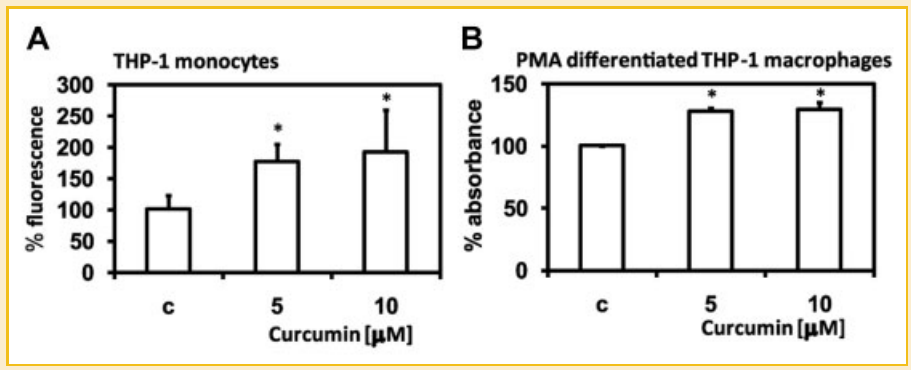

Fig. 1. Curcumin increases lipid accumulation in THP-1 monocytes and macrophages. A: Treatment of THP-1 monocytes with 5 and 10 μM curcumin for 24 h increases lipid accumulation as measured by Nile red staining and FACS (n = 2, ±SEM, $P < 0.05$, with control (c) set to 100%). B: Treatment of THP-1 macrophages (differentiated with 100 nM PMA for 24 h) with 5 and 10 μM curcumin for 24 h increases lipid accumulation as measured by oil red staining (n = 2, ±SEM, $P < 0.05$, with control (c) set to 100%).

for 24 h (Fig. 2A,B). For CD36, an increase was observed in particular in the higher immunoreactive band, representing the highly glycosylated form of CD36, mainly expressed at the surface of the cell [Alessio et al., 1996]. Consistently, CD36 surface exposition was induced by curcumin but not by THC (Fig. 2C,D) [Alessio et al., 1996].

To exclude a PMA-dependent effect, we assessed whether a similar regulation also occurs in mouse RAW264.7 macrophages, which do not need differentiation by PMA. Cells were treated with curcumin or THC, and the expression of CD36 and aP2 was measured by quantitative RT-PCR. Curcumin at 5 μM increased both CD36 and aP2 mRNA expression, whereas THC did not have any effect (Supplemental Fig. 2).

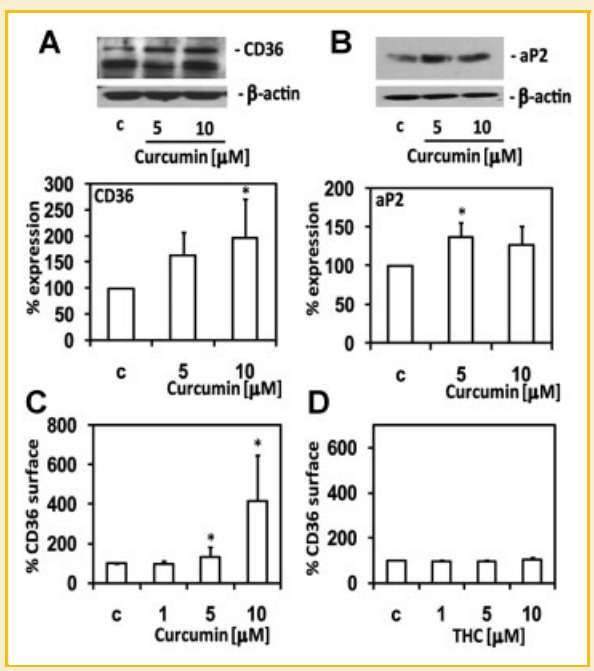

Fig. 2. Curcumin increases CD36 and aP2 protein expression in THP-1 monocytes/macrophages. A: Treatment of THP-1 macrophages with 5 and 10 μM curcumin for 24 h increases expression of highly glycosylated CD36 expression (n = 2, ±SEM, $P < 0.05$, with control (c) set to 100%). B: Treatment of THP-1 macrophages with 5 and 10 μM curcumin for 24 h increases aP2 expression (n = 2, ±SEM, $P < 0.05$, with control (c) set to 100%). C: Treatment of THP-1 monocytes with increasing concentration of curcumin for 6 h increases CD36 surface expression, (D) whereas THC treatment has no effects (n = 4, ±SEM, $P < 0.05$, with control (c) set to 100%).

CURCUMIN UP-REGULATES CD36 AND AP2 PROMOTER ACTIVITIES

To investigate the mechanisms of CD36 and aP2 regulation by curcumin, CD36-promoter-luciferase constructs and aP2-promoter-luciferase constructs were transfected into HEK293 cells, and the effects of curcumin and THC assessed. These cells were chosen since they can be efficiently transfected, thus offering more reliable results. A significant induction of CD36 promoter activity was seen only at high curcumin concentrations (20 μM) using a CD36 construct (pCD36b) containing a relatively short piece of the CD36 promoter (491 bp) [Zingg et al., 2002] (Fig. 3A). However, a stronger induction was detected using an extended CD36-promoter-luciferase construct containing 4557 bp of the human CD36 promoter (pCD36extpro; Fig. 3B), indicating the presence of curcumin responsive elements in the distant part of the CD36 promoter. As expected, THC did not have any effect on this construct (Fig. 3C). Similar to CD36, aP2 promoter activity was induced by curcumin in HEK293 cells in a concentration-dependent manner, whereas THC had only a very weak effect (Fig. 3D,E). A weaker effect of curcumin was observed when these constructs were transfected into THP-1 monocytes, in which transfection efficiency is known to be much lower (Fig. 3F,G).

REGULATION OF aP2 AND CD36 BY FOXO3A OVER-EXPRESSION

CD36 and aP2 are known to be regulated by NF-κB and PPARγ [Tontonoz et al., 1998; Rival et al., 2004]. To assess whether these factors are involved in the effects of curcumin, luciferase-reporter gene constructs containing NF-κB and PPAR response elements (pNF-κB-RE-Luc and pPPAR-RE-Luc, respectively) were transfected into HEK293 cells and the effects of curcumin assessed. Curcumin inhibited pNF-κB-RE-Luc and activated pPPAR-RE-Luc significantly only at high concentration (20 μM; Supplemental Fig. 3). The weak effect on pPPAR-RE-Luc could also be due to low expression of PPARγ. Thus, an expression vector of PPARγ was co-transfected

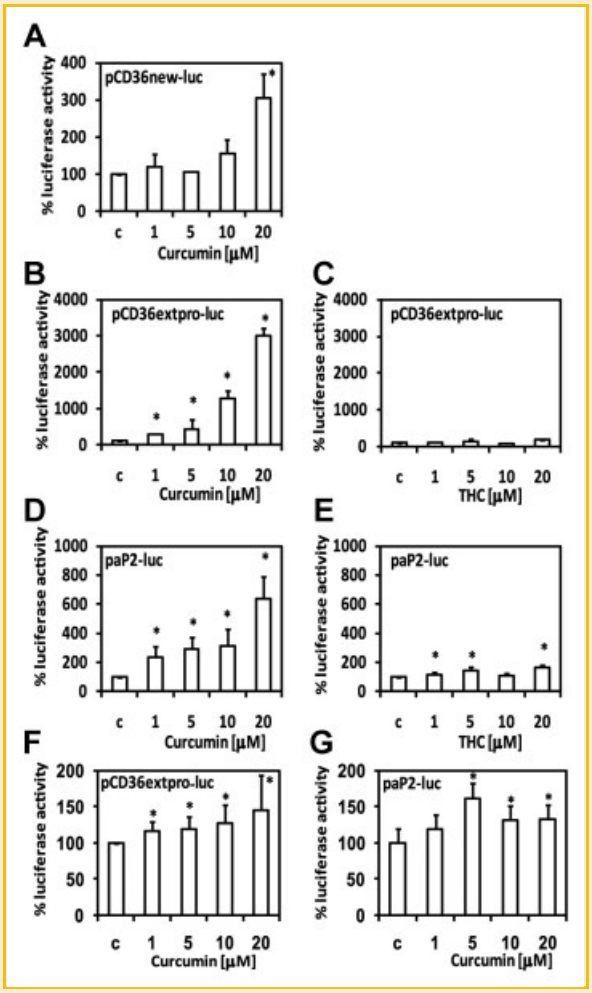

Fig. 3. Curcumin increases CD36 and aP2 promoter activity. A: Treatment of HEK293 cells with increasing concentration of curcumin for 24 h only weakly increases the activity of a short CD36 promoter (n = 2, ±SEM, $P < 0.05$, with control (c) set to 100%). B: Treatment of HEK293 cells with increasing concentration of curcumin for 24 h increases the activity of an extended CD36 promoter (n = 2, ±SEM, $P < 0.05$, with control (c) set to 100%), (C) whereas THC treatment has no effects (n = 2, ±SEM, $P < 0.05$, with control (c) set to 100%). D: Treatment of HEK293 cells with increasing concentration of curcumin for 24 h increases the activity of the aP2 promoter (n = 4, ±SEM, $P < 0.05$, with control (c) set to 100%), (E) whereas THC treatment has only weak effects (n = 4, ±SEM, $P < 0.05$, with control (c) set to 100%). F: Treatment of THP-1 monocytes with increasing dosage of curcumin for 24 h weakly increases the activity of an extended CD36 promoter (n = 4, ±SEM, $P < 0.05$, with control (c) set to 100%), and (G) of the aP2 promoter (n = 4, ±SEM, $P < 0.05$, with control (c) set to 100%).

with pPPAR-RE-Luc; even then, curcumin did not affect pPPAR-RE-Luc (Supplemental Fig. 3) suggesting that the observed up-regulation of CD36 and aP2 by curcumin involves other transcription factors.

The FOXO transcription factors have been previously involved in up-regulating CD36 in skeletal muscle, in which increased expression and membrane exposition of CD36 [Bastie et al., 2005; Na et al., 2011] after treatment with curcumin may result from activation of FOXO1 [Nahle et al., 2008]. To investigate a possible involvement of FOXO transcription factors in the modulation of CD36 and aP2 by curcumin, the expression levels of FOXO1, FOXO3a, and FOXO4 were assessed in THP-1 monocytes and in PMA-differentiated THP-1 macrophages. The expression of FOXO1 and FOXO4 was very low and difficult to detect by Western blotting both in monocytes and macrophages; however, FOXO3a was expressed in THP-1 monocytes and up-regulated in PMA-differentiated THP-1 macrophages (data not shown), in line with previously published data (reviewed by Dejean et al. [2011]). Over-expression of FOXO3a in THP-1 monocytes up-regulated aP2 but not CD36 promoter activity (Fig. 4A,B). A similar regulation by FOXO3a also occurred in HEK293 cells (data not shown).

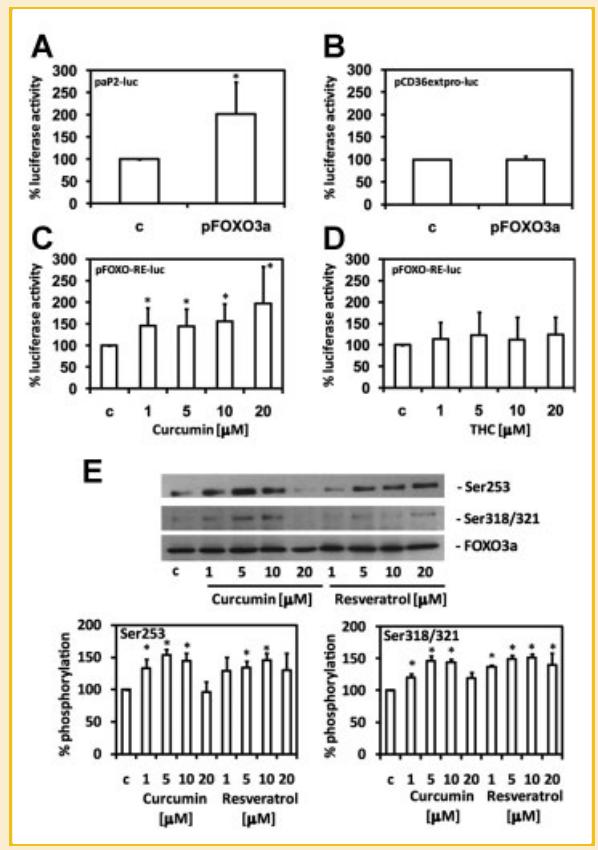

Fig. 4. Curcumin increases FOXO3a activity. A: Over-expression of FOXO3a in THP-1 monocytes for 24 h increases aP2 promoter activity, (B) but not the activity of the extended CD36 promoter (n = 4, ±SEM, $P < 0.05$, with control (c) set to 100%). C: Treatment of THP-1 monocytes with increasing concentrations of curcumin increases promoter activity derived from a FOXO-response element-SV40 promoter construct, (D) whereas treatment with THC has no effects (n = 4, ±SEM, $P < 0.05$, with control (c) set to 100%). E: THP-1 cells were treated for 24 h with increasing concentrations of curcumin or resveratrol and the FOXO3a phosphorylation level was assessed by Western blotting using anti-phospho-FOXO3a(Ser253), anti-FOXO3a(Ser318/321), and anti-FOXO3a antibodies. The level of phospho-FOXO3a was calculated relative to unphosphorylated FOXO3a and plotted (n = 3, ±SEM, $P < 0.05$, with control (c) set to 100%).

CURCUMIN INCREASES FOXO3A TRANSCRIPTIONAL ACTIVITY

FOXO3a activity is regulated by protein modifications at several sites (phosphorylation, acetylation, ubiquitination) [Calnan and Brunet, 2008]. To determine whether curcumin affects overall FOXO3a transcription factor activity, a FOXO-element luciferase-reporter vector (pFOXO-RE-Luc) was transfected into THP-1 cells and the effect of curcumin assessed. Curcumin, but not THC, increased the luciferase response of the pFOXO3-RE-Luc construct, indicating that curcumin can increase FOXO3a transcriptional activity (Fig. 4C,D, respectively). Curcumin treatment of THP-1 monocytes also led to rapid translocation of FOXO3a to the nucleus where the transcriptional activity occurs (Supplemental Fig. 4).

CURCUMIN AFFECTS FOXO3A PHOSPHORYLATION IN THP-1 MONOCYTES IN A TIME- AND CONCENTRATION-DEPENDENT MANNER

Since Akt activity is influenced by curcumin [Hussain et al., 2006], it was interesting to see whether this translates into changes in FOXO3a phosphorylation and activity. Changes in FOXO3a phosphorylation upon treatment with curcumin were assessed in THP-1 monocytes using anti-phospho-FOXO3a and anti-FOXO3a antibodies. Phosphorylation of FOXO3a (Ser253) and (Ser318/321) increased in a concentration-dependent manner up to 10 μM curcumin, whereas 20 μM curcumin decreased it (Fig. 4E). A similar increase of phosphorylation was observed by treating with resveratrol as a control (Fig. 4E). In THP-1 monocytes, curcumin increased FOXO3a phosphorylation in a time-dependent manner (Supplemental Fig. 5).

IN VIVO, CURCUMIN REDUCES MACROPHAGE LIPID ACCUMULATION IN LDL RECEPTOR KNOCKOUT MICE FED A HIGH FAT DIET FOR 4 MONTHS

The up-regulation of CD36 and aP2, and the increased lipid accumulation induced by curcumin in cultured monocytes/macrophages, could have adverse effects on the development of atherosclerosis in vivo [Zingg et al., 2000]. To assess whether curcumin can affect the accumulation of lipids in macrophages in vivo, LDL-R$^{-/-}$ mice fed a HFD for 4 months were supplemented with low, medium, or high curcumin concentration, and the accumulation of lipids in peritoneal macrophages was quantified by FACS after Nile red staining (Fig. 5A). The lipid uptake was significantly higher in mice fed a HFD, when compared to a LFD, and it decreased consistently after treatment with increasing doses of curcumin. HFD is known to lead to obesity and up-regulation of FOXO3a transcription [Relling et al., 2006]. In line with that, aP2 mRNA expression was up-regulated with HFD, whereas CD36 did not appear to be further up-regulated by HFD, possibly because it was already induced by higher levels of LDL/oxLDL in LDL-R$^{-/-}$ animal model [Zhou et al., 2008]. Both aP2 and CD36 mRNA expression are down-regulated with increasing dietary concentration of curcumin, suggesting that curcumin or curcumin metabolites may act in vivo by mechanisms that are different from the in vitro situation (Fig. 5B,C). Expression of the ABCA1 transporter, which is involved in export of cholesterol as well as resistance to curcumin [Zhou et al., 2008], were up-regulated by HFD. Curcumin at a low and medium doses significantly down-regulated it, possibly as a result of an indirect action of curcumin in lowering plasma lipid levels in curcumin supplemented mice (Fig. 5D) [Ejaz et al., 2009].

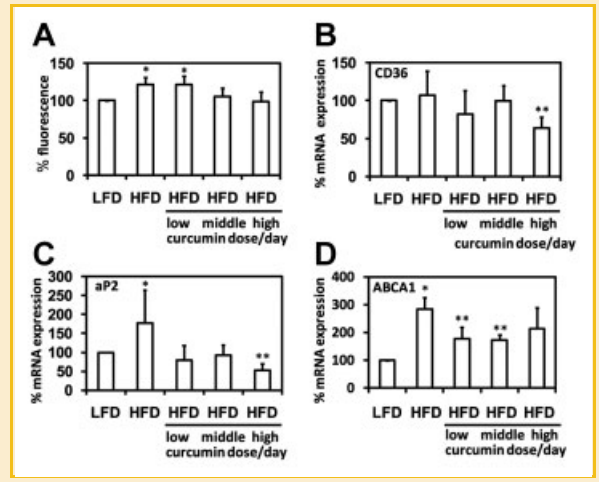

Fig. 5. Quantification of lipid accumulation in peritoneal macrophages. Peritoneal macrophages from LDL-R$^{-/-}$ mice fed a low or high fat diet (LFD or HFD, respectively) supplemented with various concentrations of curcumin (low, middle, high) were collected and grown for 2 h in a cell culture incubator. A: The fixed macrophages were stained with Nile red, washed, analyzed by FACS, and the median fluorescence plotted ($n = 9$, ±SEM, $P < 0.05$, with control fed a LFD set to 100%). B–D: Total RNA from peritoneal macrophages was isolated and mRNA expression of CD36 (A), aP2 (C), and ABCA1 (D) was determined as described in Materials and Methods Section ($n = 5$, ±SEM, with control (LFD) set to 100%; $^{*}P < 0.05$ relative to LFD, $^{**}P < 0.05$, relative to untreated HFD).

DISCUSSION

The biological effects of phytochemicals and micronutrients are often explained by their ability to act chemically as antioxidants; however, recent research suggests that they can instead affect signal transduction and gene expression independently from their antioxidant activities. This may be particularly relevant for those phytochemicals present in the circulation at concentrations too low to chemically impact the level of oxidative stress; therefore, alternative mechanism(s) should be involved in their modulatory effects on bodily functions. The polyphenol curcumin is known to have a very low bioavailability (average 490 nmol/L in plasma), yet affects a myriad of cellular reactions in antioxidant and non-antioxidant manners (reviewed in [Shehzad et al., 2011]).

We and others have shown that curcumin can reduce inflammation and lipid accumulation in adipocytes [Asai and Miyazawa, 2001; Weisberg et al., 2008; Ejaz et al., 2009; Ahn et al., 2010]. Cholesterol was lowered by curcumin in blood and HSC, whereas fatty acids and triglycerides were increased in HSC [Kang and Chen, 2009]. Here, we investigated the effects of curcumin and the curcumin metabolite THC on lipid uptake in monocytes/macrophages and assessed regulatory pathways possibly involved. In cultured monocytes and macrophages, we found that curcumin increases lipid uptake, CD36 and aP2 expression, whereas THC does not have any effect. The up-regulation of CD36 and aP2 by

curcumin occurs at the transcriptional level and involves activation of the transcription factor FOXO3a. PPARγ and NF-κB were affected only at high curcumin concentrations. Since THC, which has a similar or even higher antioxidant activity than curcumin [Dinkova-Kostova and Talalay, 1999], did not have any effect on FOXO3a activity in our cell culture models, our observations are unlikely due to a free radical scavenging activity, but rather the result of specific interactions of curcumin with molecules relevant for the activation of FOXO3a [Calnan and Brunet, 2008]. Interestingly, increased activity of curcumin, as compared to THC, on apoptosis and endoplasmic reticulum stress has been observed in HL-60 cells [Pae et al., 2007], as well as on plaque deposition and protein oxidation in models of Alzheimer's disease [Begum et al., 2008].

At the molecular level, FOXO3a is phosphorylated by Akt [Miyauchi et al., 2004], which leads to translocation of FOXO3a out of the nucleus and its consequent inactivation [Van Der Heide et al., 2004]. Inhibition of Akt by curcumin is therefore expected to lead to accumulation of FOXO3a in the nuclei. Indeed, the treatment of THP-1 monocytes with curcumin produced the enrichment of FOXO3a in the nucleus; conversely, a decrease of FOXO3a phosphorylation at Ser253 and Ser318/321 was observed at high curcumin concentrations (20 μM) only whereas lower concentrations increased it. Similar observations have been made with other cells such as skin fibroblasts [Lima et al., 2011], HT29 cells [Beevers et al., 2009], and MCD-7 cells [Lima et al., 2011]. These events may be explained by a bi-phasic or hormetic cellular stress response to curcumin resulting from overlapping effects of FOXO3a phosphorylation at several sites. This could influence nuclear translocation or nuclear retention despite the phosphorylation at Ser253 and Ser318/321 [Calnan and Brunet, 2008]. According to this view, curcumin may activate mammalian sterile 20-like 1 kinase (MST1) or c-jun N-terminal kinase (JNK) leading to retention of FOXO3a in the nucleus despite increased phosphorylation by Akt (reviewed in [Calnan and Brunet, 2008; Nunn et al., 2009]). Comparable effects on the activity of FOXO3a have been observed with resveratrol, which is closely related to curcumin, both structurally and chemically.

Interestingly, whereas all our in vitro data suggest increased lipid uptake and up-regulation of CD36 and aP2 by curcumin in monocytes and macrophages, our in vivo results with peritoneal macrophages isolated from LDL-$R^{-/-}$ mice fed a HFD suggest the opposite. The simplest explanation for this phenomenon would be that curcumin in the in vivo situation is preventing oxidation of LDL by acting as an antioxidant; however, due to its very low bioavailability, the antioxidant activity of curcumin may not explain the reduction in lipid accumulation observed in vivo, and other activities of curcumin (and of curcumin metabolites) may explain the observed differences between our in vitro and in vivo results. One of the tested curcumin metabolites (THC) did not show significant effects in vitro; however, several further metabolites of curcumin have been described that may need to be tested in the future [Ireson et al., 2001].

When C57BL/6 mice are fed a high fat Western style diet (20% fat w/w), they show a high concentration of circulating cholesterol and develop lipid-mediated stress (accumulation of lipids, inflammation, increased oxidative stress) within 8 weeks [Strissel et al., 2007]. Although peritoneal macrophages from these mice are relatively resistant to accumulation of cholesterol resulting from a HFD [Zhou et al., 2008], we found increased intracellular lipid levels in the HFD group suggesting that lipid level in plasma may directly determine it also in peritoneal macrophages. We observed in a previous study with male C57BL/6 mice on a high fat Western style diet for 12 weeks that curcumin reduced plasma levels of free fatty acids, triglycerides, and cholesterol, possibly explaining the lower lipid accumulation seen here in peritoneal macrophages in response to curcumin [Ejaz et al., 2009]. In fact, increased removal of lipids from circulation by up-regulating the LDL receptor may contribute to the cholesterol lowering and anti-atherosclerotic effects of curcumin in these mice [Dou et al., 2008]; however, since we used LDL-$R^{-/-}$ mice, additional mechanisms may be the basis for the reduction of lipid accumulation by curcumin in peritoneal macrophages isolated from these mice.

Alternative mechanisms of plasma lipid reduction by curcumin may involve increased biliary excretion and metabolism of cholesterol [Kim and Kim, 2010], direct inhibition of fatty acid synthase [Zhao et al., 2011], but it may in fact also involve stimulation of lipid uptake into some tissues and their subsequent oxidation. Assuming that curcumin increases CD36 and aP2 via stimulating FOXO1 and FOXO3a in some tissues, it may lower the level of free fatty acids seen in plasma and reduce the exposure of peritoneal macrophages, hence limiting their ability to accumulate fatty acids (Fig. 6). Up-regulation of CD36 by curcumin may also increase oxidation of fatty acids by stimulating their transfer into mitochondria [Campbell et al., 2004]. Thus, in this model, the reduction of fatty acids in peritoneal macrophages may not be a

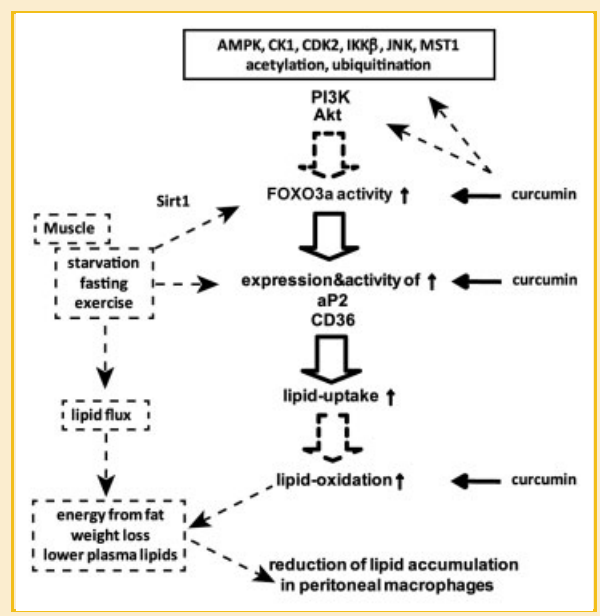

Fig. 6. Proposed model of curcumin action in vivo. Similar to starvation, fasting, or exercise, curcumin increases FOXO3a activity, and increases aP2 and CD36 surface expression leading to increased lipid uptake into cells in peripheral tissues and their subsequent oxidation. The consequent lower plasma concentration of lipids leads to diminished lipid exposure of monocytes/macrophages leading to lower aP2 and CD36 expression, and possibly reduced formation of foam cells and atherosclerotic lesions.

direct effect of curcumin on these cells, but rather an indirect one as a consequence of stimulating fatty acids uptake and oxidation in other tissues, such as skeletal muscle, heart, and possibly adipose tissue [Dinkova-Kostova and Talalay, 2008]. Accordingly, in a mouse model of diabetes, curcumin prevents inflammation by increasing the expression of FOXO1a and FOXO3a in white adipose tissue, whereas in liver it is decreased, but a possible influence of curcumin on the plasma lipid profile remains to be determined [Weisberg et al., 2008].

A similar up-regulation of genes involved in lipid uptake occurs during fasting, starvation, and exercise, suggesting that curcumin may activate a gene expression profile which increases fat/energy influx to some tissues as seen during caloric restriction (Fig. 6). Caloric restriction has been linked to a higher activity of FOXO transcription factors, a lower level of oxidative stress, and an increased maximum lifespan [Salih and Brunet, 2008]. Decreased FOXO3a expression has been observed in explanted rat vascular smooth muscle cells with advanced age [Li et al., 2006], possibly explaining the failure to up-regulate antioxidant genes, such as catalase and MnSOD, during age-accelerated atherosclerosis [Collins et al., 2009]. Reduced expression of FOXO3a may also explain the age-dependent decline of aP2 in mesenchymal stem cells [Wilson et al., 2010], but not in adipose-derived stromal cells where it is increased [Huang et al., 2010]. Our findings may thus be of particular interest to prevent age-dependent increased risk of atherosclerosis. Restoring FOXO3a expression and activity by specific drugs, phytochemicals, or micronutrients could be a strategy to increase resistance against oxidative and lipid-mediated stress in cardiovascular cells of the elderly.

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