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## **Impact of curcumin on fatty acid metabolism**

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**Running title:** Curcumin and fatty acids

## Abstract

Free fatty acids (FFAs) and fatty acid synthesis (FAS) activity have significantly contributed to disease states such as insulin resistance, obesity, type 2 diabetes, myocardial infarction, blood pressure, and several types of cancer. Currently, several treatment options are available for patients with these conditions. Due to safety concerns, adverse effects, limited efficacy, and low tolerability associated with many medications, the identification of novel agents with less toxicity and a more favorable outcome is warranted. Curcumin is a phenolic compound derived from the turmeric plant with various biological activities, including anticarcinogenic, anti-oxidant, anti-inflammatory, and hypolipidemic properties. PubMed, Scopus, and Web of Science were searched up to February 2020 for studies that demonstrated the efficacy and mechanisms of curcumin action on FFAs, FAS, and  $\beta$ -oxidation activity, as well as the desaturation system. Most of the evidence is in-vivo and in-vitro studies that demonstrate that curcumin possesses regulatory properties on FFAs levels through its effects on FAS and  $\beta$ -oxidation activity as well as desaturation system, which could improve insulin resistance, obesity, and other FFAs-related disorders. The present study provides a review of the existing in-vitro, in-vivo, and clinical evidence on the effect of curcumin on FFAs and FAS activity,  $\beta$ -oxidation, and desaturation system.

**Keywords:** free fatty acids (FFAs), fatty acid synthesis (FAS), curcumin, Polyphenol, insulin resistance,

## Introduction

Curcumin, also known as diferuloylmethane, is one of the key components of the curcuminoids family, which can be isolated from the plant *Curcuma longa* (Borik et al., 2018; Moghaddam et al., 2019). Curcumin is the major bioactive component in turmeric which is effective, safe, and non-toxic (Ding, L et al., 2015). The pharmacological effects of curcumin are exerted through several functional sites including phenolic hydroxyl groups, the central bis- $\alpha$ ,  $\beta$  unsaturated  $\beta$ -diketone, double-conjugated bonds, and methoxy groups (Figure 1.) (Hatamipour et al., 2018). However, the low bioavailability and absorption of curcumin are concerns (Rai et al., 2015). Recently, the medical application of novel drug delivery systems such as exosomes to enhance solubility, bioavailability, therapeutic efficacy, and pharmacological properties have improved by loading hydrophobic drugs such as curcumin (Oskouie et al., 2019). Basic and experimental studies have shown that curcumin as a hormetic agent (stimulatory at low doses and inhibitory at high doses) (Moghaddam et al., 2019) has many molecular targets (Momtazi et al., 2016) and possesses antioxidant (Menon and Sudheer, 2007), anti-inflammatory (Wang et al., 2014; Mollazadeh et al., 2019; Bianconi et al., 2018; Ghandadi and Sahebkar, 2017), anticancer (Ohtsu et al., 2002; Rezaee et al., 2017) and anti-angiogenesis (Adams et al., 2004) properties, as well as medicinal applications, including their potential use in the treatment of fatty liver disease (Panahi et al., 2017), HIV (Conteas et al., 2009), cancer (Mirzaei et al., 2016; Teymouri et al., 2017), metabolic syndrome (MetS) (Panahi et al., 2016), dyslipidemia (Panahi et al., 2018a), type 2 diabetes (Panahi et al., 2018b) and Alzheimer's disease (Mourtas et al., 2014).

Free fatty acids (FFAs) are potential risk factors for cardiovascular diseases (CVD) (Egan et al., 2001), MetS, obesity, and type 2 diabetes mellitus (T2DM) (Boden, 2008). Recently, it has been shown that FFAs are the main causes of insulin resistance (Capurso and Capurso, 2012; Delarue

and Magnan, 2007) and inflammation in the tissues target of insulin (Haus et al., 2010; Boden, 2008). Therefore, elevated levels of FFAs in plasma can be considered a key factor in developing insulin resistance, inflammation, obesity, T2DM, and hypertension (Boden, 2008). Interestingly, several studies reported the up-regulation of FFAs associated with lung, breast, and colorectal cancers (Liu et al., 2014; Zhang, Yaping et al., 2016; Lv and Yang, 2012; Zhang, Y. et al., 2016). In this regard, in the 1920s, Warburg et al. demonstrated that the tumors have a high glucose uptake rate; and Medes et al. established that tumors convert glucose or acetate into lipids (Medes et al., 1953). Another study observed that tumor cells make almost all their cellular FAs by de novo synthesis (Ookhtens et al., 1984). Several decades later, fatty acid synthase (FAS) was recognized as the tumor antigen OA-519 in aggressive breast cancer (Kuhajda et al., 1994). Overall, numerous studies have confirmed the high FAS expression in several types of cancer including breast (Alo et al., 1996), prostate (Epstein et al., 1995), colon (Rashid et al., 1997), endometrium (Pizer et al., 1998), ovary (Gansler et al., 1997) and thyroid cancer (Vlad et al., 1999) and the importance of FA biosynthesis for cancer cell growth and survival (Santos and Schulze, 2012; Currie et al., 2013).

Curcumin and its analogs have been investigated for its effects on FFAs as well as FAS, and it has been suggested as a potentially efficacious and well-tolerated treatment for FFAs-induced disorders such as insulin resistance, obesity, CVD, diabetes, and cancers (Wilding, 2007; Na et al., 2011; Kuroda et al., 2005). This review summarizes the effects of curcumin on FFAs levels, desaturation system, FAS, and  $\beta$ -oxidation activity in preclinical and clinical studies.

## **2. Effects of curcumin on fatty acids profile**

The effect of curcumin on FFAs has been investigated in cell culture, experimental animal studies, and human interventional studies. The characteristics and main results of these studies are summarized in Tables 1 and 2.

### **2.1. In vitro studies**

Table 2 summarized the main characteristics and results of the effect of curcumin on FFAs levels in vitro studies. Hanikoglu et al. investigated the effect of curcumin on FFAs profile in breast cancer cell lines, including MCF-7 and MDA-MB231. Samples were incubated with curcumin (29.65  $\mu\text{M}$  for MCF-7 and 10.46  $\mu\text{M}$  for MDA-MB231) and somatostatin for 24 hours. Gas chromatography analysis revealed that the levels of myristic acid, oleic acid (OA), palmitoleic acid, cis-vaccenic acid, and total monounsaturated fatty acids (MUFAs) decreased and dihomo- $\gamma$ -linolenic acid (DGLA), total n-3 and trans-FA increased in the MCF-7 cells incubated with curcumin plus somatostatin compared to somatostatin group. However, membrane FAs composition in MDA-MB231 cells incubated with curcumin showed an increase in saturated fatty acids (SFAs) family including myristic acid, palmitic acid (PA), stearic acid (SA) as well as total SFAs, and decrease arachidic acid, OA, gandoic acid, arachidonic acid (ARA), docosapentaenoic acid, docosahexaenoic acid (DHA), total MUFAs and total n-3 compared to control without any treatment. The same results in differences between FAs were observed after incubated with curcumin plus somatostatin compared to the somatostatin group (Hanikoglu et al., 2019). Similarly, Cort et al. studied the effect of curcumin alone and in combination with bleomycin on membrane FAs composition in NTera-2 human testicular germ cancer cells. In this study, cells were cultured and incubated with curcumin (20  $\mu\text{M}$ ) and bleomycin for 24 hours. The results showed that incubation with curcumin significantly increased trans-palmitoleic acid (t-16:1), elaidic acid (9t-18:1), linolelaidic acid (t-18:2), SFAs, SFAs to MUFAs ratio and

decreased the levels of n-6 essential FA family including linoleic acid (LA) (9c, 12c-18:2), MUFAs and polyunsaturated fatty acids (PUFAs) compared to control group. Besides, treatment with curcumin combined with bleomycin increased trans-palmitoleic acid, trans-20:4, and decreased linoleic acid compared to the bleomycin group. Bleomycin significantly increased SFAs and decreased MUFAs, and PUFAs family and treatment with curcumin did not revert these FAs. The levels of main membrane FAs composition, including PA, SA, OA, and ARA, did not affect Ntera-2 cells incubated with curcumin (Cort et al., 2016). Another study showed that turmeric extract at a different dose (5, 10, 20, and 50 $\mu$ L) for seven days increased PA, OA, DGLA, and DGLA to ARA ratio and decreased ARA and total FA in *Mortierella alpine* 1S-4 in a dose-dependent manner (Shimizu et al., 1992). It has been shown that OA and LA stimulated cell proliferation of hormone-independent breast cancer cells. At the same time, n-3 FAs and GLA as an n-6 FA displayed an inhibitory effect on the growth of hormone-dependent and hormone-independent cancer cells (Chajès et al., 1995; Johanning and Lin, 1995). In this regard, in the preclinical studies above-mentioned, which investigated the effect of curcumin on FAs composition on cell lines especially cancer, curcumin could reduce total MUFA, PUFA, LA, OA, and ARA and increase DGLA and SFAs.

## **2.2. In vivo studies**

### **2.2.1. Impact of curcumin on plasma fatty acids profile**

There are several in-vivo studies, which confirm the reducing effect of curcumin on plasma FFAs (El-Moselhy et al., 2011; Guo et al., 2017; Jang et al., 2008; Kamalakkannan et al., 2005; Murugan and Pari, 2006; Pongchaidecha et al., 2009; Rukkumani et al., 2003; Rukkumani, R et al., 2002; Seo et al., 2008; Sugasini and Lokesh, 2017). Sugasini and Lokesh examined the effect of curcumin in the different forms including native or nanoemulsion using core phospholipid

material in sunflower oil (SNO-LA rich) and linseed oil (LSO- ALA,  $\alpha$ -linolenic acid-rich) on fatty acids (FAs) composition of serum, liver, heart, and brain in rats. Evaluation of FAs composition by gas-liquid chromatography showed that serum levels of LA were 30.1% and 26.6% and ARA levels were 12.5% and 10.6% of total FAs with given native and nanoemulsion in SNO, respectively. However, the levels of ALA, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and DHA in serum were 2.0%, 1.1%, 0.2%, 0.8% and 4.5 %, 5.9%, 0.4% and 5.2% total FAs with given native and nanoemulsion in LSO contained omega-3, respectively. This study showed that serum levels of LA and total n-6 in rats given SNO with curcumin in both form were significantly higher than LSO-fed rats, however, did not detect n-3 FAs including ALA, EPA, DPA, and DHA. (Sugasini and Lokesh, 2017). Activation of the enzymes FA desaturase 2 and elongase 2, which are involved in converting ALA to DHA by curcumin, may enhance the levels of these FAs (Wu et al., 2015). In contrast, other studies revealed that curcumin decreased the activation of FA synthesis. Guo et al. investigated whether curcumin could change liver metabolites in FA biosynthesis, especially unsaturated FAs in mice. Five metabolites in the biosynthesis of unsaturated FAs including ARA, LA, PA, OA as well as SA and four metabolites in FA biosynthesis including PA, OA, and SA except myristic acid were suppressed by curcumin at a dose of 60 mg/Kg body weight (BW) that administered by intragastric infusion for four weeks (Guo et al., 2017). This study showed that curcumin could attenuate harmful metabolites such as SA, OA, and LA and possibly suppresses fatty liver disease progression by interfering with the FA synthesis. Rukkumani et al. reported that 80 mg/Kg BW curcumin and the o-hydroxy-substituted analog of curcumin orally using an intragastric tube for 45 days effectively decreased plasma FFAs levels in rats (Rukkumani, R et al., 2002).

Moreover, these optimistic results regarding the beneficial effect of curcumin on plasma FAs profile in non-healthy (obesity, diabetes, and hepatotoxicity) rats are presented in three reports by Seo et al. (Seo et al., 2008), Jang et al. (Jang et al., 2008) and El-Moselhy et al. (El-Moselhy et al., 2011). The *in vivo* study by Seo et al. indicated a reducing effect of plasma FAs profile amounting to a 17% decrease in FFAs following curcumin supplementation at a dose of 0.2 g/Kg in the standard diet for six weeks in db/db mice compared to the non-treated group. The authors did not observe differences in non-diabetic db/+ mice (Seo et al., 2008). Another study was reported from Jang et al. illustrating the prevention of increased plasma FFAs following 0.05 g curcumin supplementation per 100 g high-fat diet (HFD) for ten weeks compared to the control group in hamsters (Jang et al., 2008). However, a similar study reported that curcumin intervention at a dose of 500 mg/Kg of HFD for 12 weeks had non-significant reduced trend on serum FFAs in mice compared to the non-intervention group (Ejaz et al., 2009). To further support for optimistic functions of curcumin on FA levels, El-Moselhy et al. investigated the effect of curcumin in two regimens: (a) the protection regimen for 60 days (received curcumin throughout the experiment period) and (b) the treatment regimen for 75 days (received curcumin for last 15 days of experiment period). Results showed that curcumin markedly decreases the plasma FFAs in both regimens compared to groups without treatment in rats with T2DM-induced by HFD (El-Moselhy et al., 2011). Consistent with these results, Rukkumani et al. revealed that curcumin at a dose of 80 mg/Kg BW significantly decreases the levels of plasma FFAs in rats (Rukkumani et al., 2003). Similarly, Murugan and Pari studied the effect of curcumin and tetrahydrocurcumin (a major colourless metabolite of curcumin) on FFAs in diabetic rats. They demonstrated that treatment with a different form of orally administered curcumin at a daily dose of 80 mg/Kg for 45 days had a curative effect on plasma FFAs (Murugan and Pari, 2006). In a



study to compare the efficacy of different curcuminoid doses (30, 60 and 90 mg /Kg) along with HFD for 12 weeks on plasma FFAs, Pongchaidecha et al. treated high fat-induced obesity rats. Their results showed that plasma FFAs were decreased in all curcuminoid-treated groups. In this study, reduction of plasma FFAs was not a dose-dependent manner (Pongchaidecha et al., 2009). In another study, orally administered curcumin and its analog (*bisdemethoxy curcumin analog*) at a daily dose of 80 mg/Kg for three months reduced plasma FFAs compared to non-treated groups in rats with hepatotoxicity. However, there was no significant difference between treatment groups and the non-treated group in normal rats (Kamalakkannan et al., 2005).

Most studies showed that induction of obesity or T2DM (with or without HFD) in animals induced insulin resistance, hyperinsulinemia, and glucose intolerance which may be due to the elevation of plasma FFA that inhibits hepatic clearance of insulin, and this postulation is in accordance with Kissebah and Peiris (Kissebah and Peiris, 1989). This hyperinsulinemia may also cause insulin resistance and impaired glucose tolerance, which contributed to the development of T2DM in HFD-fed rats. The elevated TNF- $\alpha$  levels are related to another increase in plasma levels of FFAs that TNF- $\alpha$  could mediate such underlying mechanisms of obesity-associated with peripheral and hepatic insulin resistance.

In summary, in vivo studies revealed that curcumin could reduce (a study had non-significant tend to reduce) serum FFAs. However, it is difficult to conclude due to the differences in administration, type, dose, and duration of treatment. Also, exert effects of curcumin on individual and necessary serum FAs levels, especially long-chain, have not been investigated.

### 2.2.2. Impact of curcumin on tissue fatty acids profile

Several in-vivo studies are investigating the effects of curcumin and turmeric on tissue FAs composition (Babu and Srinivasan, 1998; Joe and Lokesh, 2000; Kaul and Krishnakantha, 1997; Mesa et al., 2003; Reddy and Lokesh, 1994; Rukkumani et al., 2003; Rukkumani, R. et al., 2002). Rukkumani et al. investigated the effects of curcumin and its photo-irradiated form on tissue FAs profile in alcohol (20% ethanol) and PUFAs (raw and heated sunflower oil)-induced hyperlipidemic rats. The results clearly showed that both forms of orally administered curcumin at a daily dose of 80 mg/Kg BW decrease the levels of FFAs elevation in tissues (liver, kidney, heart, and intestine) of hyperlipidemic rats (Rukkumani, R. et al., 2002). These authors also conducted a similar study, and the same results as a previous study were obtained (Rukkumani, R et al., 2005). In another study to investigate the effect of turmeric and curcumin on the FAs profile of tissue microsomal membrane, rats with retinol deficiency were treated with 0.1% curcumin or turmeric in the diet for three weeks. At the end of this study, Kaul and Krishnakantha revealed that both interventions were able to increase essential FAs including 18:1, 18:2, and 20:4 in the tissue microsomal membrane (liver, kidney, brain, and spleen), which was reduced by retinol deficiency. The increase in the essential FAs was more significant in turmeric-treated groups than curcumin-treated groups (Kaul and Krishnakantha, 1997). Moreover, Mesa et al. studied turmeric extract's effect at two periods of 10 days and 30 days on SFAs, MUFAs, and PUFAs of hepatic microsomes of HFD-fed rabbits. Orally administered turmeric extract at a dose of 1.66 mg/Kg BW for 10 days did not alter the FAs composition compared with the control group. On the other hand, SFAs and PUFAs, but not MUFAs, increased and decreased, respectively, after intervention for 30 days compared to the less intervention period (Mesa et al., 2003). However, in a study investigating the efficacy of

curcumin (0.5% in diet) on FAs composition of renal phospholipids in diabetic rats for eight weeks, Babu and Srinivasan found only decrement PUFAs to SFAs ratio in the treatment group compared to the control group. The authors did not observe any significant changes in the individual FAs composition of renal phospholipids in rats with the treatment of curcumin compared to that fed diet alone (Babu and Srinivasan, 1998). Also, in a study to compare the efficacy of curcumin with dietary lipids on FAs composition in liver microsomal phospholipids of rats, the authors reported that different dietary lipids including cod liver and peanut oil exert the main influence on PUFAs composition in liver microsomal phospholipids. Dietary curcumin at a dose of 1 gr per 100 g diets for 10 weeks could not change the FAs composition of liver microsomal phospholipids in rats (Reddy and Lokesh, 1994). Similarly, Joe and Lokesh, indicated that individual FAs composition of macrophage phospholipids did not change with curcumin intervention at a daily dose of 30 mg/Kg BW for 15 days in rats with different dietary lipids including coconut oil (enriched in SFAs) or groundnut oil (high in n-6 PUFAs) or cod-liver oil (high in n-3 PUFAs) compared to the non-treated group (Joe and Lokesh, 2000).

### **2.3. Clinical study**

Only one randomized clinical trial has been performed to assess the efficacy of curcumin extracts on plasma FFAs. Na et al. conducted a double-blind, randomized, placebo-controlled trial to investigate whether curcuminoids have a beneficial effect on plasma FFAs. This study was performed on 100 overweight/obese patients with T2DM who received a 3-month treatment with 300 mg/day curcuminoids (150 mg capsule twice daily) or placebo in addition to their conventional treatment. The results showed that curcuminoid supplementation reduced serum total FFAs, SFAs, and unsaturated FAs compared to the placebo group. Further analysis showed that serum PA (C16:0), SA (C18:0), OA (C18:1), and LA (C18:2, n-6), as well as  $\gamma$ -linolenic

acid, were significantly decreased in the treatment group compared to the placebo group. Interestingly, the levels of serum lipid profiles, including LDL-C, HDL-C, total cholesterol, as well as Apo A-1 and Apo B, did not significantly change after treatment. At the same time, HbA1c and homeostatic model assessment of insulin resistance improved with curcumin intervention. The authors suggested that the effects of plasma FFAs by curcuminoids might be attributed to the facilitated FFAs uptake and utilization by the tissues (Na et al., 2013). This interpretation was supported by in-vivo studies in this review.

Also, a randomized, double-blind, placebo-controlled trial revealed that curcumin could increase adiponectin. It decreased fasting blood glucose and hs-CRP, while did not significantly affect lipid profiles in T2DM patients (Adibian et al., 2019). On the other hand, two studies by Saadati et al failed to show any significant effect of curcumin on lipid profile and glucose homeostasis in NAFLD patients (Saadati et al., 2019a; Saadati et al., 2019b). It appears that curcumin may reduce insulin resistance induced by obesity or elevated serum FFAs levels, especially in T2DM patients; and probably has not a potential impact on reducing lipid profile.

There is numerous evidence that elevated blood FFAs, as seen in obesity, impair insulin action in muscles leading to insulin resistance and T2DM, which are significantly related to developing other pathological states such as CVD and cancer (Hulver and Dohm, 2004). Curcumin has been reported to reduce blood glucose by decreasing the production of hepatic glucose and sensitize insulin action through different pathways (Jiménez-Osorio et al., 2016; Ding, XQ et al., 2015; Ghorbani et al., 2014). Considering to results of this review that curcumin can able decrease FFAs through down-regulated hepatic FAS and up-regulated fatty acid  $\beta$ -oxidation activity, a proposed hypothesis for reducing the effect of insulin resistance and other related diseases by

curcumin could be this relationship. However, further research in humans is needed to substantiate these findings and see if results are consistent in different conditions.

### **3. Impact of curcumin on desaturation system**

Table 2 summarizes the main characteristics and results of the effect of curcumin on desaturation system activity in vitro studies. Nakano et al. assessed the effect of curcumin on C24- $\Delta$ 6,  $\Delta$ 6, and  $\Delta$ 5 desaturase of BRL-3A cells of the rat liver. The activity of desaturation as desaturation index was calculated by 23:4n-5 to 23:5n-5 plus 21:5n-5 for C24- $\Delta$ 6 desaturation, the ratio of 18:2n-6 to 20:3n-6, 20:4n-6 and 22:4n-6 for  $\Delta$ 6 desaturation and ratio of 20:3n-6 to 20:4n-6 plus 22:4n-6 for  $\Delta$ 5 desaturation. Curcumin (at a dose of 25 $\mu$ M for 24 h) effectively increased the index of desaturation compared to the control group, due to inhibitory effect on C24- $\Delta$ 6,  $\Delta$ 6, and  $\Delta$ 5 desaturase (Nakano et al., 2000). Similarly, in another in vitro study, the effect of curcumin in different doses on  $\Delta$ 5 desaturase was also investigated in the rat hepatocytes. The ratio of 20:3n-6 to 20:4n-6 and 20:4n-3 to 20:5n-3 was considered an index for estimating  $\Delta$ 5 desaturation of n-6 and n-3, respectively. Results demonstrated that curcumin inhibits the  $\Delta$ 5 desaturation of n-6 series FAs (increases the 20:3n-6 to 20:4n-6 ratio), but not  $\Delta$ 5 desaturation of n-3 series FAs (decreases the 20:4n-3 to 20:5n-3 ratio). The authors suggested that  $\Delta$ 5 desaturase acts differently in metabolism and affinity to both types of PUFAs (n-6 and n-3) (FUJIYAMA-FUJIWARA et al., 1992). Moreover, the effects of curcumin on  $\Delta$ 5 and  $\Delta$ 6 desaturase activity, which was measured by DGLA to ARA ratio, were studied in vitro and in vivo systems. The results demonstrated that curcumin inhibited the conversion of DGLA to ARA,  $\Delta$ 5 (50%) and  $\Delta$ 6 (less than 10%) desaturase in a dose-dependent manner. The half inhibition concentration (IC<sub>50</sub>) value of curcumin was 27.2  $\mu$ M for  $\Delta$ 5 desaturase. Shimizu et al. also conducted its effects on desaturase enzymes of liver microsomes of rats. They revealed an

inhibitory effect on desaturation enzymes amounting to 49% in  $\Delta 5$  desaturase and 18% in  $\Delta 6$  desaturase after incubation of curcumin in a dose-dependent manner (Shimizu et al., 1992).

Polyunsaturated fatty acids (PUFA) have crucial functions in the human including structural components of cell membranes, the main source for energy metabolism, and effect on cellular function through direct and second messengers such as sterol regulatory element-binding protein 1, NF- $\kappa$ B, hepatocyte nuclear factor 4 $\alpha$ , peroxisome proliferator-activated receptors, and Eicosanoids (Jump and Clarke, 1999).  $\Delta 6$  and  $\Delta 5$  desaturase are recognized as the main determinants of PUFA levels and are the rate-limiting enzymes for PUFA conversion. Alterations of  $\Delta 5/\Delta 6$  activity have been related to several diseases. Increased activity of desaturase was observed in hypertension, insulin resistance, diabetes, obesity, metabolic syndrome, cardiovascular disease, and inflammation (Russo et al., 1997; Vessby, 2003; Warensjö et al., 2006). The association of these conditions and estimated desaturase activities are particularly impressive. Increased  $\Delta 6$  desaturase and  $\Delta 5$  desaturase estimated activities were associated with insulin resistance, diabetes, abdominal obesity, and Non-alcoholic steatohepatitis (which is characterized by inflammation and lipid accumulation in the liver) in human studies (Park et al., 2010). Consistently, an elevated expression of  $\Delta 6$  and  $\Delta 5$  desaturase has been found in HFD-fed mice with NASH and obesity which showed a significant reduction in intracellular fat accumulation and inflammatory injury in hepatocytes after the use of combined  $\Delta 5/\Delta 6$  inhibitor (López-Vicario et al., 2014). High  $\Delta 6$  and low  $\Delta 5$  desaturase activity are associated with CVD in a community-based prospective study (Warensjö et al., 2008). Another study found that patients with CAD in the highest tertile of ARA/LA ratio had a higher proportion of progressive CAD and higher hs-CRP (Martinelli et al., 2008). Thus, the results mentioned above prompt the hypothesis that desaturase activity plays a role in diabetes and CVD pathogenesis. Increased

desaturase activity may indicate a peculiar susceptibility to diabetes and the inflammatory stimuli involving the arterial wall.

These findings suggest that increasing the overall activity of desaturase can be detrimental for health which is consistent for  $\Delta 6$  desaturase as a rate-limiting step of the whole PUFA pathway. The inhibitory effect of curcumin on overall desaturase activity, especially  $\Delta 6$  desaturase may be helpful due to the high desaturase activity in the n-6 rich condition such as western diet, leading to an increase in inflammatory mediators. However, the  $\Delta 5$  desaturase activity is critical for the synthesis of long-chain n-3 FAs; and high activity of  $\Delta 5$  desaturase has been related to an increased in EPA and DHA levels in plasma (Lu et al., 2012). Accordingly,  $\Delta 5$  desaturase inhibition by curcumin may influence participants' susceptibility to CVD and diabetes. Therefore, further studies in humans are warranted to demonstrate the role of curcumin for reducing the risk of various diseases and specific modulations of  $\Delta 5/\Delta 6$  activity for treating conditions related to alterations in the PUFA pathway.

#### **4. Impact of curcumin on FAS and $\beta$ -oxidation activity**

##### **4.1. In vitro studies**

Ejaz et al. assessed the effect of curcumin incubation at different doses on FA  $\beta$ -oxidation in 3T3-L1 adipocytes cells. In this study, oxidation of PA was considered as the FA  $\beta$ -oxidation activity. The results showed that 24 h of incubation with curcumin at doses of 10 and 20  $\mu\text{mol/L}$  increased the activity of enzyme compared to 5 and 0  $\mu\text{mol/L}$  curcumin-incubated groups (Ejaz et al., 2009). Another in vitro study evaluated the FAS activity in the presence of curcumin in 3T3-L1 pre-adipocytes cells. The authors found that the activity of FAS was reduced in a dose-dependent manner by curcumin. The  $\text{IC}_{50}$  value of curcumin on FAS activity was 28.6  $\mu\text{M}$  (Zhao et al., 2011).

#### 4.2. in vivo studies

Lee et al. studied the effect of curcumin on FAS activity by measuring the malonyl-CoA-dependent oxidation of nicotinamide adenine dinucleotide phosphate by spectrophotometric assay in mice receiving HFD and alcohol. The researcher indicated a significant reduction in FAS activity and plasma FFAs in mice feeding curcumin compared to that fed alcohol only (Lee et al., 2013). Another study assessed the effect of curcumin **supplementation on a standard diet** on hepatic FAS activity in db/db and db/+ mice. The results showed a significant reduction in FAS and  $\beta$ -oxidation activity in the curcumin-treated group compared to the non-treated db/db group. There were no significant differences between the curcumin-treated group and db/+ group in this regard (Seo et al., 2008). Moreover, the effect of curcumin **supplementation along the HFD diet** on the activity of hepatic FA  $\beta$ -oxidation and FAS in hamster were evaluated. Measurement of the malonyl coenzyme A (CoA)-dependent oxidation of nicotinamide adenine dinucleotide phosphate and reduction of NAD to NADH in the presence of palmitoyl-CoA by spectrophotometer showed that curcumin-treated animals had a potential significant decrement in FAS activity and an increase in FA  $\beta$ -oxidation compared to the control group (Jang et al., 2008).

Several studies have suggested FAS as a potential therapeutic target for the management of obesity and cancer. An experimental study has shown that FAS inhibition leads to weight loss in mice. Other studies have identified higher FAS gene expression in human cancers including breast cancer, prostate cancer, colon cancer, endometrium cancer, ovary cancer, and thyroid cancer (Alo et al., 1996; Rashid et al., 1997; Pizer et al., 1998; Epstein et al., 1995; Gansler et al., 1997; Vlad et al., 1999). Therefore, curcumin can be considered a promising therapeutic agent in treating obesity and cancers through inhibition of FAS.



Recently, FAS has been recognized as a therapeutic target for obesity due to its effect on food intake and body weight by its inhibition (Tian, 2006; Loftus et al., 2000). This review showed that curcumin was able to inhibit FAS activity and stimulate  $\beta$ -oxidation activity. Considering that the liver is the main site of FA metabolism, curcumin actions on FAS and  $\beta$ -oxidation activity appeared to play a critical role in the prevention of hyperlipidemia, thereby creating a hypothetical mechanism be proposed for the reduced level of plasma FFAs by treatment with curcumin.

### **Limitations and prospects:**

The current review has some limitations. We conducted a non-systematic search method, opting for a narrative review instead, which may lead to the subjective selection of articles and consequently add bias to the overall interpretation of results. Our review was performed to examine the research questions: What effect does curcumin have on FAs levels, the activity of FAS,  $\beta$ -oxidation, and desaturation system. Most of the studies reviewed has been in vitro and in vivo. While this is informative, it is difficult to generalize these results to the clinical and clarify the key mechanism without human studies. In vitro studies are performed in a laboratory setting without considering the physiological and biological conditions of the system and not represent the human condition, finally can be over-interpreted. This study would also set up the field well for human clinical studies. An issue may be useful to improve conclusions about the effects of curcumin on FAs metabolism. Principally, there is a need to increase the human studies of the curcumin effect on FAs metabolism, including individual FAs levels and FAS activity,  $\beta$ -oxidation, and desaturation system.

### **Conclusions:**

Elevated blood FFAs as seen in obesity, have been well known to play an essential role in developing chronic diseases including diabetes, CVD, and cancers (Wilding, 2007; Egan et al., 2001; Liu et al., 2014). Different strategies currently exist for the prevention and treatment of FFAs-induced disorders. Due to the side effect and lack of efficacy, new preventive and therapeutic approaches are needed. Studies show that curcumin could ameliorate insulin resistance and its related disorders (Xu et al., 2018). However, based on the current data, few convincing studies suggest a definitive effect of curcumin on FFAs levels, the activity of  $\Delta 6$  and  $\Delta 5$  desaturase, FAS, and  $\beta$ -oxidation. While it seems that curcumin may decrease FFAs and inhibit the activity of  $\Delta 6/\Delta 5$  desaturase as well as FAS and increase  $\beta$ -oxidation activity. Therefore, curcumin may be considered as a promising agent in the prevention and treatment of FFAs-induced disorders. It is also worth mentioning that the evidence for these findings pertains to cell culture and preclinical studies. However, the effectiveness of targeting FAS activity and levels of FFAs as a therapeutic target in humans still needs to be validated. Hence, further high-quality human studies are required to firmly establish the clinical efficacy of curcumin and the precise mechanisms of the effect of curcumin on FFAs, the activity of  $\Delta 6/\Delta 5$  desaturase, FAS,  $\beta$ -oxidation and FFAs-related disorders.

### **Conflict of interests**

The authors declare no conflict of interests.

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Table 1 Summary of the experimental and clinical studies that investigated to impact of curcumin on FFA composition, desaturation system, FAS, and oxidation activity

Author	Design; location	Sample; n in each group	Intervention	Duration of treatment	Outcome	Main results
Sugasini, and Lokesh. (Sugasini and Lokesh, 2017)	In vivo; USA	Male rats; six	Group1; SNO + native curcumin Group2; SNO + nano-curcumin Group3; LSO + native curcumin Group4; LSO + nano-curcumin	60 days	FAs composition of serum, heart, liver, and brain	Serum and liver FAs: Decreased 20:4n-6 and total n-6 in group 2 compared to group 1. Decreased 18:2n-6, 20:4n-6, total n-6 and n-6/n-3 and increased n-3 FAs (18:3, 20:5, 22:5, 22:6) and total n-3 in group 4 compared to group 3. Hemat FAs: Increased 22:5n-6 and decreased n-6/n-3 in group 2 compared to group 1. Decreased n-6 FAs (20:4 and 22:5), total n-6 and n-6/n-3 and increased n-3 FAs (18:3, 20:5, 22:5 and 22:6) and total n-3 in group 4 compared to group 3. Brain FAs: Decreased n-6/n-3 in group 2 compared to group 1. Decreased n-6 FAs (20:4, 22:4, 22:5), total n-6, n-6/n-3 and increased n-3 FAs (20:5, 22:5, 22:6) and total n-3 in group 4 compared to group 3.
El-Moselhy et al. (El-Moselhy et al., 2011)	In vivo; Egypt	Male Sprague Dawley rats; eleven	Protection regimen: Group 1; HFD Group2; HFD plus curcumin (80mg/Kg BW) for 60 days Treatment regime: Group 1; HFD Group 2; HFD for 60 days then curcumin (80mg/Kg BW) for 15 days	Protection regimen: 60 days Treatment regime: 75 days	Plasma FFA	Plasma FFA decreased after intervention in both regimens compared to non-treated HFD groups
Rukkumani et al. (Rukkumani, R. et al., 2002; Rukkumani et al., 2003)	In vivo; India	Female Albino rats with hyperlipidemia induced by	Group 1; control Group 2; 20% ethanol + HFD (raw sunflower oil) Group 3; 20% ethanol + HFD (thermally oxidized SNO)	45 days	Study 1: FFAs of heart, liver, kidney, and intestine  Study 2:	Study 1: Tissue FFAs decreased in curcumin supplementation groups compared to non-treated groups. Study 2:

		ethanol and PUFA; -	Group 4; curcumin (80 mg/kg BW) + 20% ethanol + HFD (raw SNO) Group 5; curcumin (80 mg/kg BW) + 20% ethanol + HFD (heated SNO) Group 6; photo-irradiated curcumin (80 mg/kg BW) + 20% ethanol + HFD (heated SNO)		FFAs plasma	Decreased FFAs in treatment groups compared to non-treated groups (group 4 vs 2, 5, and 6 vs 3).
Seo et al. (Seo et al., 2008)	In vivo; Republic of Korea	male C57BL/KsJ db/db mice lean heterozygote non-diabetic db/+; eight	db/+ mice and db/db mice were fed a standard semisynthetic diet (AIN-76) with curcumin (0.2 g/kg diet) or without	6 weeks	Plasma FFAs Hepatic FAS $\beta$ -oxidation	Decreased plasma FFAs, hepatic FAS, and $\beta$ -oxidation activity in curcumin-treated db/db mice compared to non-treated db/db mice. No change in plasma FFAs, hepatic FAS, and $\beta$ -oxidation activity between db/+ mice with curcumin and without.
Rukkumani et al. (Rukkumani, Rajagopalan et al., 2005)	In vivo; India	Male Albino rats with hyperlipidemia induced by ethanol and PUFA; six	Group1; control Group2; 20% ethanol Group3; HFD (SNO) Group4; 20% ethanol + SNO Group5; 20% ethanol + curcumin (80 mg/Kg BW) Group6; 20% ethanol + curcumin analog (80 mg/Kg BW) Group7; SNO + curcumin Group8; SNO + curcumin analog Group9; 20% ethanol + SNO + curcumin Group10; 20% ethanol + SNO + curcumin analog Group11; curcumin Group12; curcumin analog	45 days	Plasma FFA	Decreased plasma FFA in all treatment groups with curcumin and curcumin analog compared to non-treated groups. No change on plasma FFA between curcumin and curcumin analog (group 11 and 12) and control (group 1).
Mesa et al. (Mesa et al., 2003)	In vivo; Spain	Male rabbit fed HFD; Twelve	Group1; turmeric hydroalcoholic Extract (1.66 mg/kg BW)  Group2; control	10 days and 30 days	SFA, MUFA, and PUFA from hepatic microsomes	Increased SFA and decreased PUFA after the 30-d intervention compared to 10-d. No change in MUFA between two periods of intervention.

Babu and Srinivasan. (Babu and Srinivasan, 1998)	In vivo; India	Male wistar rats; Twelve	Normal rats: Group1; basal diet Group2; HCD Group3; basal diet + curcumin (0.5% in diet) Group4; HCD + curcumin (0.5% in diet) Diabetic rat: Group1; basal diet Group2; HCD Group3; basal diet + curcumin (0.5% in diet) Group4; HCD + curcumin (0.5% in diet)	8 weeks	FAs compositions of renal phospholipids	Decreased PUFA/SFA ratio after treatment (group 3) compared to control (group 1). No change in FAs between treatment groups and non-treatments groups.
Reddy et al. (Reddy and Lokesh, 1994)	In vivo; India	Male wistar rats, six	Group1; PNO Group2; PNO + curcumin Group3; CLO Group4; CLO + curcumin	10 weeks	FAs compositions of liver microsomes phospholipids	Increased 16:1 and decreased 18:1 and 18:2n-6 FAs in group2 compared to group1. Increased 18:1 and decreased 16:1 in group 4 compared to group 3.
Kaul and Krishnakantha (Kaul and Krishnakantha, 1997)	In vivo; India	Male albino rats; six	Group1; control Group2; retinol deficient Group3; retinol deficient + curcumin (0.1% in diet) Group4; retinol deficient + turmeric (0.1% in diet)	11 weeks (3 weeks intervention)	FAs compositions of liver, kidney, spleen, and brain microsomes	Increased 18:1, 18:2, and 20:4 in all tissues after treatment with curcumin and turmeric compared to the non-treated group.
Lee et al. (Lee et al., 2013)	In vivo; Republic of Korea	Male mice; six	Group 1; control Group 2; ethanol Group 3; ethanol + curcumin (0.02) Group 4; ethanol + curcumin (0.05)	6 weeks	Hepatic FAS Plasma FFA	Decreased FAS activity in both intervention groups compared to groups 2 and 1. Decreased plasma FFA in both intervention groups compared to group 2.
Guo et al. (Guo et al., 2017)	In vivo; Republic of China	Male mice; six	Group1; control Group2; ethanol Group3; ethanol + curcumin (60mg/Kg BW)	4 weeks	FAs biosynthesis	Decreased ARA, LA, OA, PA, and SA in group 3 compared to group 2. No change in FAs between group 3 and group 2.
Ejaz et al. (Ejaz et al., 2009)	In vivo/in vitro; USA	In vivo: Male mice; six  in vitro:	In vivo: Group 1; control Group 2; HFD Group 3; HFD + curcumin (500mg /Kg of diet)	In vivo: 12 weeks  In vitro: 24 h	In vivo: Serum FFA  In vitro: FA oxidation	No change in serum FFA levels between groups.  Increased FA oxidation by curcumin at a dose of 10 and 20 $\mu\text{mol/L}$

		3T3-L1 adipocytes	In vitro: Curcumin added to the cell at concentrations of 0, 5, 10, or 20mmol/L			
Joe and Lokesh. (Joe and Lokesh, 2000)	In vivo; India	Male wistar rats; six	Group 1; CO Group 2; CO + curcumin (30 mg/Kg BW) Group 3; GNO Group 4; GNO + curcumin (30 mg/Kg BW) Group 5; CLO Group 6; CLO + curcumin (30 mg/Kg BW)	15 days	FAs macrophage phospholipids	Decreased 20:4n-6 in GNO-curcumin compared to GNO group. No change in other FAs, SFA, PUFA, and PUFA/SFA after curcumin treatment compared to non-treated groups.
Jang et al. (Jang et al., 2008)	In vivo; South Korea	Male hamster; eight	Group 1; HFD Group 2; HFD + curcumin (0.05g/100 g diet)	10 weeks	FFA Hepatic FA $\beta$ -oxidation activity Hepatic FAS activity	Decreased FFA in the curcumin group compared to the HFD group.  Decreased FAS and increased FA $\beta$ -oxidation activity in the curcumin group compared to the HFD group.
Rukkumani et al. (Rukkumani, R et al., 2005)	In vivo; India	Male Albino rats with hyperlipidemia induced by ethanol and PUFA; eight	Group 1; control Group 2; 20% ethanol Group 3; HFD (thermally oxidized SNO) Group 4; 20% ethanol + HFD (thermally oxidized SNO) Group 5; curcumin analog (80 mg/kg BW) + 20% ethanol Group 6; curcumin analog (80 mg/kg BW) + HFD Group 7; curcumin analog (80 mg/kg BW) + 20% ethanol + HFD Group 8; curcumin analog (80 mg/kg BW)	45 days	FFAs of heart, liver, kidney	Decreased FFAs after treatment between group 5 vs 2, 6 vs 3, and 7 vs 3 in all tissues.
Na et al. (Na et al., 2013)	randomized, double-blind, placebo-controlled trial; China	Overweight/obese type 2 diabetic patients; fifty	Group 1; 300 mg/day curcuminoids (150 mg capsule twice daily) Group 2; placebo (150 mg starch capsule twice daily)	3 months	Serum FFAs	Decreased PA, SA, OA, LA, $\gamma$ -linolenic acid, total FFAs, as well as SFA and unsaturated fatty acids after treatment vs placebo.

Abbreviations: FAs-fatty acids; FFA-free fatty acid; SNO-sunflower oil; LSO-linseed oil; BW-body weight; PUFA-polyunsaturated fatty acid; MUFA-monounsaturated fatty acid; SFA-saturated fatty acid; FAS-fatty acid synthesis; HFD-high fat diet; HCD-high cholesterol diet; PNO-peanut oil; CLO-cod liver oil; CO-coconut oil; GNO-groundnut oil; PA-palmitic acid; SA-stearic acid; OA-oleic acid; LA-linoleic acid

Table 2 Summary of the in-vitro studies that investigated to impact of curcumin FFA composition, desaturation system, and FAS activity

Author	Design; location	Cell targets/species	Disease	Curcumin dose	Outcome	Main results
Cort et al. (Cort et al., 2016)	In vitro; Turkey	NTera-2 cells / human	testicular germ cancer	20 $\mu$ M	Membrane phospholipid FAs composition	Increased trans 16:1 and 18:2, SFA, SFA/MUFA and decreased Cis 18:2, MUFA, and PUFA after incubation with curcumin compared to control. Increased trans 16:1, 20:4 and decreased trans 18:2 after incubation with curcumin along bleomycin compared to bleomycin alone.
Fujiwara et al. (FUJIYAMA-FUJIWARA et al., 1992)	In vitro; Japan	Hepatocytes/ rat	--	7.5 $\mu$ g/mL	$\Delta$ 5-desaturation	Increased 20:3n-6/20:4n-6 and decreased 20:4n-3/20:5n-3 after incubation
Hanikoglu et al. (Hanikoglu et al., 2019)	In vitro; Turkey	MCF-7 and MDA-MB231 cells/ --	breast cancer	MCF-7: 29.65 $\mu$ M MDA-MB231: 10.46 $\mu$ M	Membrane FAs composition	MCF-7 cells: Increased total trans after incubation with curcumin compared to control Decreased 14:0, 9-Cis 16:1, 9,11-Cis 18:1, 18:3n-3, total MUFA and increased DGLA, total n-3, and trans after incubation with somatostatin plus curcumin vs somatostatin alone. MDA-MB231 cells: Increased 14:0, 16:0, 18:0, trans 20:4n-6, total SFA and decreased 9,11-Cis 18:1, 20:0, 11-Cis 20:1, 20:4n-6, DPA, DHA and total MUFA and n-3 after incubation with curcumin compared to control. Increased 14:0, 16:0, 18:3n-6, 20:2n-6, total SFA and decreased 9,11Cis-18:1, 20:4n-6, 22:0 and total MUFA after incubation with somatostatin plus curcumin vs somatostatin alone.
Nakano et al. (Nakano et al., 2000)	In vitro; Japan	BRL-3A cells/rat	--	25 $\mu$ M	C24 $\Delta$ 6-desaturation $\Delta$ 6-desaturation $\Delta$ 5-desaturation	Increased desaturation index of C24 $\Delta$ 6-desaturation, $\Delta$ 6-desaturation, and $\Delta$ 5-desaturation compared to control.
Zhao et al. (Zhao et al., 2011)	In vitro; China	3T3-L1 cells	--	10 or 20 $\mu$ M	FAS activity	Decreased FAS activity in a dose-dependent manner compared to control.
Shimizu et al. (Shimizu et al., 1992)	In vitro; Japan	Study 1: Mortierella alpina 1S-4	--	5, 10, 20, and 50 $\mu$ M	Study 1: FAs composition	Study 1:

		Study 2: Rat liver microsomes			$\Delta$ 5-desaturation Study 2: $\Delta$ 5-desaturation $\Delta$ 6-desaturation $\Delta$ 9-desaturation	Increased 16:0, 18:1, 18:2, DGLA and DGLA/ARA ratio and decreased ARA and total FA in a dose-dependent manner compared to control. Inhibited $\Delta$ 5-desaturation by curcumin Study 2: Inhibited $\Delta$ 5-desaturation and $\Delta$ 6-desaturation by curcumin
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Abbreviations: DGLA-dihomo- $\gamma$ -linolenic acid; DPA-docosapentaenoic acid; DHA-docosahexaenoic acid

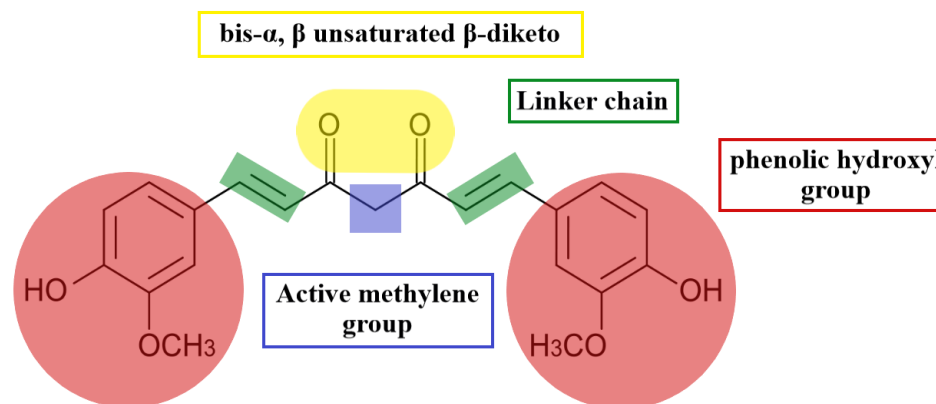
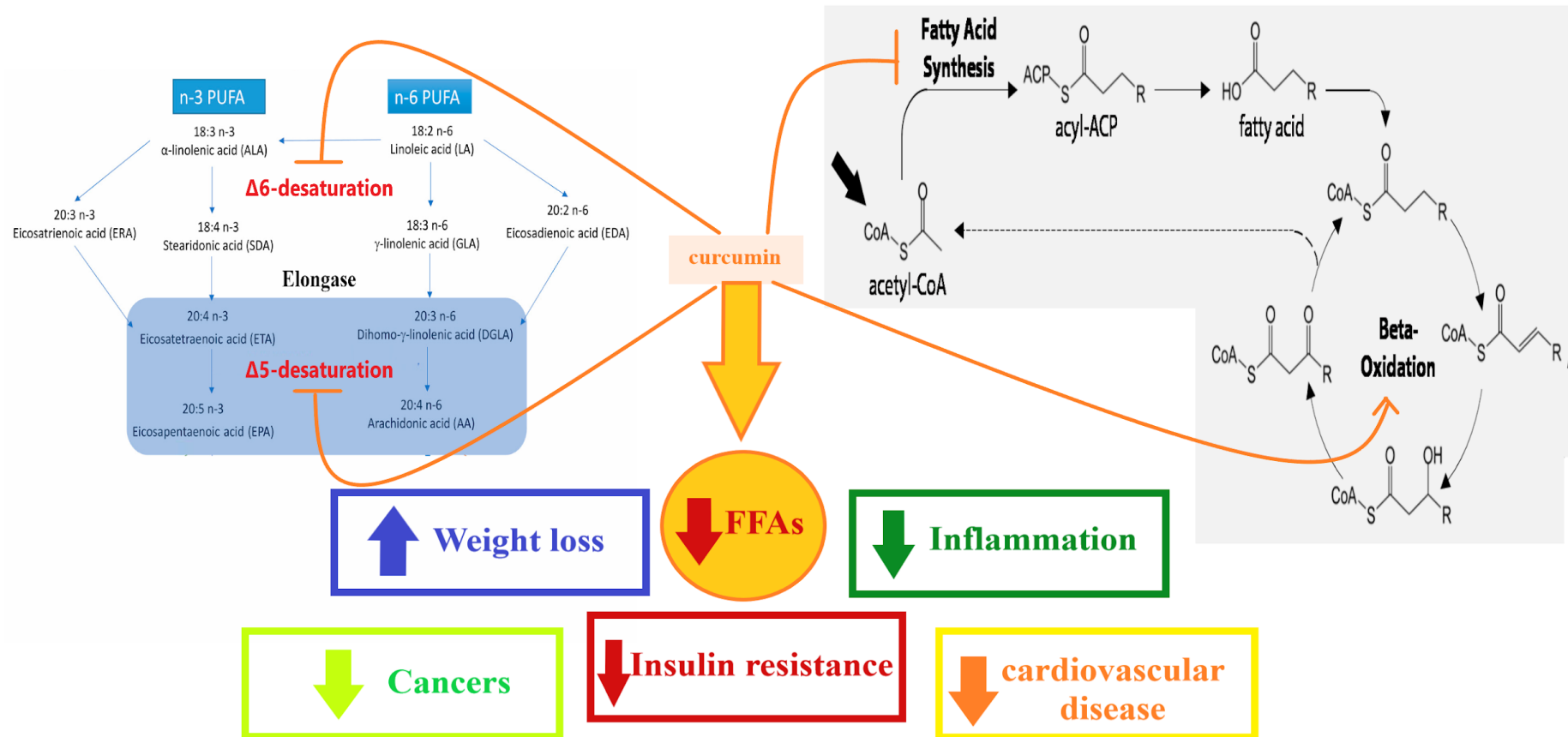


Figure 1. curcumin structure indicating the main functional moieties including phenolic hydroxyl groups, the central bis- $\alpha$ ,  $\beta$  unsaturated  $\beta$ -diketone, double-conjugated bonds, and methoxy groups





**Figure 2. Molecular targets of curcumin with inhibitory effect on the activity of FAS, Δ5 and Δ6 desaturase activity and stimulatory effect on the β-oxidation activity, and health benefits of curcumin, including weight loss, reducing effects of FFAs, anti-inflammatory, anti-cancer, cardiovascular protective, and anti-diabetic effect. The up arrows indicate the elevation and the down arrows indicate the reduction.**