

University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Nutrition and Health Sciences -- Faculty
Publications

Nutrition and Health Sciences, Department of

2016

Regulation of Obesity and Metabolic Complications by Gamma and Delta Tocotrienols

Lu Zhao

University of Florida, cauzhaolu@gmail.com

Xiefan Fang

University of Florida, xiefanfang@ufl.edu

Maurice R. Marshall

University of Florida, martym@ufl.edu

Soonkyu Chung

University of Nebraska - Lincoln, chung4@unl.edu

Follow this and additional works at: <https://digitalcommons.unl.edu/nutritionfacpub>



Part of the [Human and Clinical Nutrition Commons](#), [Molecular, Genetic, and Biochemical Nutrition Commons](#), and the [Other Nutrition Commons](#)

Zhao, Lu; Fang, Xiefan; Marshall, Maurice R.; and Chung, Soonkyu, "Regulation of Obesity and Metabolic Complications by Gamma and Delta Tocotrienols" (2016). *Nutrition and Health Sciences -- Faculty Publications*. 115.

<https://digitalcommons.unl.edu/nutritionfacpub/115>

This Article is brought to you for free and open access by the Nutrition and Health Sciences, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Nutrition and Health Sciences -- Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Review

Regulation of Obesity and Metabolic Complications by Gamma and Delta Tocotrienols

Lu Zhao ¹, Xiefan Fang ², Maurice R. Marshall ¹ and Soonkyu Chung ^{3,*}

¹ Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611, USA; cauzhaolu@gmail.com (L.Z.); martym@ufl.edu (M.R.M.)

² Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL 32611, USA; xiefanfang@ufl.edu

³ Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE 68583, USA

* Correspondence: chung4@unl.edu; Tel.: +1-402-472-7689

Academic Editor: Min-Hsiung Pan

Received: 16 February 2016 ; Accepted: 8 March 2016 ; Published: 11 March 2016

Abstract: Tocotrienols (T3s) are a subclass of unsaturated vitamin E that have been extensively studied for their anti-proliferative, anti-oxidative and anti-inflammatory properties in numerous cancer studies. Recently, T3s have received increasing attention due to their previously unrecognized property to attenuate obesity and its associated metabolic complications. In this review, we comprehensively evaluated the recent published scientific literature about the influence of T3s on obesity, with a particular emphasis on the signaling pathways involved. T3s have been demonstrated in animal models or human subjects to reduce fat mass, body weight, plasma concentrations of free fatty acid, triglycerides and cholesterol, as well as to improve glucose and insulin tolerance. Their mechanisms of action in adipose tissue mainly include (1) modulation of fat cell adipogenesis and differentiation; (2) modulation of energy sensing; (3) induction of apoptosis in preadipocytes and (4) modulation of inflammation. Studies have also been conducted to investigate the effects of T3s on other targets, e.g., the immune system, liver, muscle, pancreas and bone. Since δ T3 and γ T3 are regarded as the most active isomers among T3s, their clinical relevance to reduce obesity should be investigated in human trials.

Keywords: obesity; tocotrienols; adipogenesis; energy sensing; apoptosis; inflammation

1. Introduction

According to the World Health Organization (WHO), obesity is defined as abnormal or excessive fat accumulation that may impair health. Body mass index (BMI), a person's weight in kilograms divided by the square of his or her height in meters, is an inexpensive and easy-to-perform method of screening for weight category to classify normal (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²) and obesity (30 kg/m²) in adults. BMI does not measure body fat directly, but research has shown that BMI is significantly correlated with direct measures of body fat [1,2]. Furthermore, BMI appears to be strongly correlated with various metabolic diseases associated with adiposity [3,4]. Obesity is now recognized as a disease and a serious public health issue worldwide. In the United States during 2011–2014, the prevalence of obesity was 17.0% in 2–19 year-olds and 36.5% in adults [5]. By using a simulation model, Wang *et al.* reported that the rising prevalence of obesity will affect 65 million more adults in the U.S. by 2030, and the total medical cost associated with the treatments of obesity and obesity-associated complications will increase by \$48–66 billion/year, which is a heavy health and economic burden [6]. Obesity is categorized into two major types, hyperplastic (increase in adipocyte number) and hypertrophic (increase in adipocyte volume) obesity, both of which are regulated by genetic, endocrine, metabolic, neurological, pharmacological, environmental and nutritional factors [7].

Compared to other treatment strategies, such as drug intake and plastic surgery, dietary intervention is a safe and effective way to attenuate obesity and obesity-associated diseases [8]. In this review, we comprehensively update the emerging evidence that supports the use of tocotrienols (T3s) as a dietary supplement to attenuate obesity. We also thoroughly discuss the currently known molecular mechanisms that are modified by T3s in treating obesity. We propose that T3s, especially γ and δ T3, are promising anti-obesity agents.

T3s are a subclass of vitamin E that possesses an unsaturated side chain, which can be further classified into four analogs (alpha, beta, delta and gamma T3 (α T3, β T3, δ T3 and γ T3)) based upon the substitution pattern of methyl groups on chromanol ring. In contrast to saturated vitamin E tocopherols (TPs), T3s only exist in a limited number of plants in nature. T3s are abundant and mainly present in palm oil (up to 800 mg/kg, mainly as α T3 and γ T3), rice bran oil (mostly α T3 and γ T3) and annatto oil (mostly of δ T3) [9]. Annatto oil is unique and distinctive from palm or rice bran oil, because it contains only T3s, but little TPs. The ratios of T3s to TPs extracted from rice bran, palm and annatto sources are 50:50, 75:25 and 99.9:0.1, respectively [10]. Although T3s are being actively investigated for their preventive or therapeutic roles in some cancers, T3s have received less attention as dietary strategies to reduce obesity and its associated metabolic complications than their saturated counterpart TPs. Recently, however, research on T3s increased rapidly, reflecting the increasing interests in the potential health benefits of T3s using both pure and mixed T3 isomers. Notably, T3s have been documented to have potent anti-inflammatory and anti-cancer properties by modifying multiple molecular signaling pathways, which usually are not affected by TPs [10,11]. Moreover, T3s, especially γ T3, lower the incidence of cardiovascular diseases, diabetes and cancer in animal models and human beings [12–15]. Emerging data indicate that T3s are promising anti-obesity agents. It has been reported that T3s reduce body weight [16] and improve plasma glucose and lipid hepato-protective effects in adults with nonalcoholic fatty liver disease [17], which is a complication associated with obesity. The pharmacological function and therapeutic potential of T3s to attenuate metabolic syndromes, such as cardiovascular disease, diabetes and lipid disorder, have been recognized and reviewed by several researchers [18,19]. However, little focus has been made on systemically reviewing the role of T3 for the prospective reversing of the on-set and development of obesity. On the other hand, despite several review articles showing that T3s exert anti-cancer effects on various cell types via modifying multiple signaling pathways, only scattered information is available regarding the mechanism of action of T3s in regulating obesity. Here, we comprehensively review the scientific literature regarding the impact of T3s on obesity, with a particular emphasis on the signaling pathways involved. We also focus on delta, gamma and mixed isomers of T3s rather than alpha and beta analogs based on stronger anti-obesity effects observed in γ and δ T3.

2. Rationale for the Anti-Obesity Property of T3s

T3s have physically unique properties, and their potent anti-cancer and cholesterol-lowering effects are not exhibited by the saturated TP counterparts. T3s have been extensively investigated in the anti-cancer area due to their ability to selectively inhibit malignant tumor growth without harming normal cells via strong anti-proliferation [20–26], anti-oxidative [27–32] and anti-aging activities [33–36]. T3s also exert hypo-cholesterolemic and anti-sclerotic effects on humans, rats and mice (reviewed in [37]). Both *in vitro* and *in vivo* studies have shown that T3s reduce hepatic cholesterol production by inhibiting the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase, which is a key enzyme in the bio-synthesis of cholesterol. In addition, the T3-rich fraction (TRF) from palm oil decreases serum total cholesterol, LDL-cholesterol, apolipoprotein B and triglyceride levels in humans [38–41].

The fact that T3 isomers (γ T3 and δ T3) possess the abilities to inhibit hepatic cholesterol synthesis and cancer growth provides an exciting rationale that T3s would have a strong impact on reducing adiposity and fat cell formation. This is due to the fact that fat cell development (adipogenesis) shares many identical signaling pathways with tumor growth (tumorigenesis), including the Wnt, Stat, Akt,

autophagy and mTOR signaling pathways [42]. In addition, changes of adipokines, a set of cytokines derived from adipose tissue, are strongly associated with a high risk of breast cancer, indicating that dysfunction of adipose tissue is tightly correlated with tumorigenesis [43,44]. An early study has found that γ T3 downregulates the expression of adipogenic genes, *i.e.*, PPAR γ and CEBP α , in breast cancer cells [45]. Taken together, the anti-cancer agent T3s, especially γ T3, may reduce adipogenesis, which is a process highly resembling tumorigenesis.

3. Bioavailability and Accumulation of T3s

Compared to TPs, especially α -tocopherol (α TP), the bioavailability of T3s is relatively low. In healthy volunteers orally administrated with 300 mg mixed T3s (29% α T3, 55% γ T3 and 14% δ T3), their mean apparent elimination half-life ($T_{1/2}$) values for α T3, γ T3 and δ T3 are 4.4, 4.3 and 2.3 h, respectively, which are 4.5–8.7-times shorter than that of α TP [46]. Similar to humans, the peak plasma concentration of γ T3 is 2 μ M in C57BL/6J mice receiving an oral administration of γ T3 (90% purity) at 50 mg/kg. The plasma γ T3 concentration drops dramatically to the baseline level in 4 h after orally giving 50 mg/kg γ T3 [47]. In rats, the oral bioavailability of γ T3 is as low as 9% [48]. The poor bioavailability of T3s is due to rapid metabolism in human liver microsomes [49]. The discrepancy in metabolism rate between T3s and TPs is due to higher affinity of α TP than T3s to α -tocopherol transfer protein (α TTP) in liver, resulting in preferential release of α TP to the blood stream and inhibited secretion of T3s [50]. Entrapment of non- α TP forms of vitamin E in hepatocytes leads to preferential hepatic metabolism of these molecules rather than α TP [51]. Recent studies have confirmed that pure T3 (either annatto T3s (90% δ T3 and 10% γ T3) [52] or a novel formulation of gamma-delta T3 (75% γ T3 and 25% δ T3) [53]) has superior bioavailability than TRF (mixed with α -tocopherol and other impurities) in human subjects due to the absence of α TP's interference in absorption.

Despite low bioavailability, several studies have found that T3 slowly, but specifically accumulates in the peripheral tissues, including adipose tissue and skin [51,54]. When rats were fed with an AIN93G (a purified rodent diet) diet mixed with 0.2% T3s (2.5% α T3, 92% γ T3 and 4.2% δ T3) for 13 weeks, the level of γ T3 ranged between 164 and 597 nmol/g in different types of fat pads [55]. Uchida *et al.* found that rats fed with a T3 mixture (33.9% α T3, 47.1% γ T3 and 11% δ T3) for six weeks followed by a vitamin E-depleted diet for one month, the α T3 and γ T3 concentrations remained high in adipose tissue, indicating that α T3 and γ T3 have slow degradation rates and that adipose tissue is a unique site to store T3s [56]. Dietary α TP is likely to counteract T3 accumulation in tissues, and it has been shown that 50 mg/kg α TP in the diet decreases α T3, but not γ T3 accumulation in the adipose tissue of rats fed with a diet supplemented with 50 mg/kg α T3 or γ T3 for eight weeks [56]. Accumulation of T3s in fat tissues indicates that the adipocytes may be the major site of action of T3s.

4. Effects of T3s on Obesity

4.1. Influences of T3s on Body Weight, Food Intake and Blood Profiles

Recently, several studies have directly or indirectly investigated the potential influences of T3s on obesity in laboratory animal models. Unlike the saturated forms of vitamin E, such as α TP, TRF at a daily dose of 10 mg for three weeks significantly reduced body weight in rats (Table 1) [57]. Our group has identified that 0.05% γ T3 (90% purity) supplementation for four weeks significantly reduced high fat diet-induced body weight gain in young C57BL/6J mice [47]. Later on, Wong *et al.* found that δ T3 (90% purity), but not α T3 (91.6% purity, <1% α TP) or γ T3 (95% purity, <1% α TP), at a daily dose of 85 mg/kg for eight weeks significantly reduced abdominal adiposity and fat masses in diet-induced obese rats [58]. Through a body composition analysis, Ima-Nirwana *et al.* found that Sprague-Dawley (SD) rats that received γ T3 at 60 mg/kg for eight weeks had decreased fat mass and increased bone calcium content [16]. Wistar rats fed a high fat diet with the administration of palm TRF (31.9% α T3, 24.8% γ T3, 18.3% δ T3) at 120 mg/kg/day for eight weeks had significantly decreased mesenteric fat mass by 20% without affecting epididymal and perirenal fat masses [59]. However, a 10%–20% loss

of epididymal and perirenal fat was observed in obese male F334 mice fed with 10 mg rice bran T3 (RBT3) supplement (30% α T3 and 50% γ T3) for three weeks [57]. These studies clearly demonstrated that T3s reduce body weight and decrease adipose tissue mass *in vivo*. Depending on the purity and isomers of T3s used in the dietary supplements, the effective dose of T3s to reduce body weight varies among studies [16,47,59–61]. In most studies, T3s had no significant impact on food intake [16,57,59]. Only one study found that administration of a TRF at 120 mg/kg for eight weeks increased food intake in rats [59]. In addition to the direct effects on body weight reduction, the anti-obesogenic effect of T3s is reflected in other parameters. Several *in vivo* studies have demonstrated that T3s reduce plasma concentrations of free fatty acids, triglycerides and cholesterol (Table 1) [59,60,62–65]. Furthermore, T3 supplement improves glucose and insulin tolerance (Table 1) [57,59,60,66].

Table 1. Effects of tocotrienols (T3s) on body weight change, food intake, adipose tissue mass and serum lipid profile.

Parameter	Effects	Dose/Isomers/Duration	Models	Ref.
Body weight	—	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	—	60 mg/kg/ γ T3 ² /8 weeks	SD Rat	[16]
	↓	10 mg RBT ³ /3 weeks	F334 Rat	[57]
	↓	0.05% γ T3 ⁴ in diet/4 weeks	C57BL/6j	[47]
	—	85 mg/kg/ α T3 ⁵ , or γ T3 ⁶ or δ T3 ⁷ /8 weeks	Wistar Rat	[58]
Food intake	↑	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	—	0.05% γ T3 ⁴ in diet/4 weeks	C57BL/6j	[47]
	—	85 mg/kg/ α T3 ⁵ , or γ T3 ⁶ or δ T3 ⁷ /8 weeks	Wistar Rat	[58]
Mesenteric fat	↓	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	↓	10 mg RBT ³ /3 weeks	F334 Rat	[57]
	↓	0.05% γ T3 ⁴ in diet/4 weeks	C57BL/6j	[47]
Perirenal fat	—	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
Epididymal fat	—	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	↓	10 mg RBT ³ /3 weeks	F334 Rat	[57]
	↓	0.05% γ T3 ⁴ in diet/4 weeks	C57BL/6j	[47]
	—	85 mg/kg/ α T3 ⁵ , or γ T3 ⁶ /8 weeks	Wistar Rat	[58]
	↓	85 mg/kg/ δ T3 ⁷ /8 weeks	Wistar Rat	[58]
Body fat	↓	60 mg/kg/ γ T3 ² /8 weeks	SD Rats	[16]
	↓	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	↓	5% rice bran extract ⁸ /20 weeks	Zucker rice	[61]
	—	85 mg/kg/ α T3 ⁵ , or γ T3 ⁶ /8 weeks	Wistar Rat	[58]
	↓	85 mg/kg/ δ T3 ⁷ /8 weeks	Wistar Rat	[58]
Serum or plasma total cholesterol	—	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	↓	20 μ M/kg/ γ T3 ⁹ /4 weeks	New Zealand rabbit	[62]
	↓	0.09% γ T3 ¹⁰ in diet/5 weeks	Wistar Rat	[63]
	↓	50 μ g/g TRF ¹¹ in diet/6 weeks	Wistar Rat; Cholesterolemic Pigs	[64,65]
	—	85 mg/kg/ α T3 ⁵ , or γ T3 ⁶ /8 weeks	Wistar Rat	[58]
	↓	85 mg/kg/ δ T3 ⁷ /8 weeks	Wistar Rat	[58]
Fasting plasma glucose	↓	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	↓	200 mg/kg BW/TRF ¹² /8 weeks	SD Rats	[66]
	—	85 mg/kg/ α T3 ⁵ , or γ T3 ⁶ /8 weeks	Wistar Rat	[58]
	↓	85 mg/kg/ δ T3 ⁷ /8 weeks	Wistar Rat	[58]
Plasma free fatty acid	↓	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	↓	0.09% γ T3 ⁹ in diet/5 weeks	Wistar Rat	[63]
	—	85 mg/kg/ α T3 ⁵	Wistar Rat	[58]
	↓	85 mg/kg/ γ T3 ⁶ or δ T3 ⁷ /8 weeks	Wistar Rat	[58]
Plasma triglyceride	↓	120 mg/kg/TRF ¹ /8 weeks	Wistar Rat	[59]
	↓	10 mg RBT ³ /3 weeks	F334 Rat	[57]
	—	85 mg/kg/ α T3 ⁵ , or γ T3 ⁶ /8 weeks	Wistar Rat	[58]
	↓	85 mg/kg/ δ T3 ⁷ /8 weeks	Wistar Rat	[58]

Note: —: no changes; ↓: decrease; ↑: increase; ¹ TRF: tocotrienol rich fraction (31.9% α T3, 24.8% γ T3, 18.3% δ T3); ² γ T3: gamma tocotrienol (purity unavailable); ³ RBT3: rice bran tocotrienol (30% α T3, 50% γ T3, 6% tocopherols (TPs)); ⁴ γ T3: gamma tocotrienol (purity 90%); ⁵ α T3: alpha tocotrienol (91.6% purity, <1% α TP); ⁶ γ T3: gamma tocotrienol (95% purity, <1% α TP); ⁷ δ T3: delta tocotrienol (90% purity); ⁸ rice bran extract: (99 mg/kg TPs and 174 mg/kg T3s; composition unavailable); ⁹ γ T3: gamma tocotrienol (purity unavailable); ¹⁰ γ T3: gamma tocotrienol (>88% purity); ¹¹ TRF: tocotrienol rich fraction extracted from palm oil (10%–20% α TP, 15%–20% α T3, 30%–35% γ T3 and 20%–25% δ T3); ¹² TRF: palm oil tocotrienol rich fraction (mostly T3s, small portion of TPs; composition unavailable). Unless otherwise stated, the information about the TP contents in the above isomers is not available.

4.2. Epidemiological Evidence; Observational and Clinical Trials

Although strong epidemiological evidence for a causal relationship between T3 intake and body weight reduction in humans is not yet available, many studies have shown that T3 intake is associated with decreased serum concentrations of total cholesterol and lipoproteins [12,67–69]. Qureshi *et al.* found that consuming 50 mg of a TRF (15% α T3, 35% γ T3 and 25% δ T3) for four weeks in hypercholesterolemic patients was inversely correlated with serum levels of total cholesterol and LDL, but did not alter serum levels of HDL and triglyceride [12]. In another study, hypercholesterolemic human subjects were given increasing doses of TRF from 25–200 mg/day in two 35-day treatment periods, and TRF at 100 mg/day caused the greatest declines in serum levels of total cholesterol, LDL and triglycerides, with reductions by 20, 25 and 12%, respectively [67]. However, in a recent placebo-controlled human trial using γ T3 and δ T3 at 120 mg/day for eight weeks, only the serum triglyceride level was lowered by 28%, and the total cholesterol, LDL and HDL levels remained unchanged between the treated and placebo groups [68]. The discrepancy in regulating lipid profiles by T3s among these studies may be attributed to the protocol employed, the purity of T3s, the length of the administration period, as well as the physiological conditions of the subjects.

Recently, several human studies reporting T3 effects on obesity-associated inflammation and insulin resistance have been published. Haghghat *et al.* have found that microalbuminuria and C-reactive protein (hs-CRP), which are indicators of acute inflammation, were reduced in patients with type 2 diabetes who received 200 mg/day T3 supplement (34.6% α T3 and 43.5% γ T3) for eight weeks [70]. Similarly, Irandoost *et al.* have found beneficial effects of T3s on attenuating inflammation and insulin resistance in overweight or obese women [71]. In their study, 44 overweight or obese women were given a weight loss diet supplemented with either grape seed oil (with high levels of T3s) or sunflower oil (without T3s). Women given the grape seed oil supplement had lowered insulin resistance (HOMA-IR) scores and serum levels of hs-CRP and TNF- α compared to those given the sunflower oil supplement. In addition, a recent study conducted in 87 human subjects showed that a mixed T3 treatment (31% α T3, 56% γ T3 and 13% δ T3) at a dose of 400 mg/day for one year effectively reduced the hepatic echogenic response in patients with nonalcoholic fatty liver disease [17], which is a common complication of obesity. Taken together, these studies provide meaningful results to hypothesize the effects of T3s on improving the symptoms associated with obesity in humans. However, a mixture of T3s was used in these research works, which leads to a difficulty in identifying which isomer of T3s is the most beneficial. In addition, they failed to provide enough mechanistic support. Clinical studies using pure or mixed T3s are needed to further explore their roles in reducing obesity-associated complications.

5. Molecular Mechanisms of the Anti-Obesity Effects of T3s

Although many reports focusing on the molecular and cellular mechanisms responsible for the anti-tumor and anti-oxidative effects of T3s are available [10,11], little is known about the underlying mechanism of T3s involved in the regulation of obesity. Recently, several studies proposed the mechanisms involved in the anti-obesity effects of T3s, which were examined in different cell types and tissues. These mechanisms of action include (1) modulation of fat cell adipogenesis and differentiation; (2) modulation of energy sensing; (3) induction of apoptosis in preadipocytes and (4) modulation of inflammation. In addition to adipose tissues, several studies also explored the effects of T3s on immune system, liver, muscle, pancreas and bone.

5.1. Effect of T3s on Adipose Tissue

5.1.1. Modulation of Fat Cell Adipogenesis

The influence of T3s on fat cell differentiation was first reported in 2009 when 3T3-L1 adipocytes were examined after being treated with T3s [72]. Uto-kondo *et al.* demonstrated that γ T3, but not α T3 or α TP, reduced triglyceride content by 40% in 3T3-L1 adipocytes during their 21-day differentiation [72].

α T3 and γ T3 significantly decreased insulin-induced mRNA expression of PPAR γ , an important transcription factor for adipogenesis, by 55 and 90%, respectively, whereas α TP increased PPAR γ expression [72]. This indicates that the effects of T3s on adipogenic differentiation is isomer specific, and a physiologically-relevant concentration of γ T3 (2.4 μ M) is able to inhibit the conversion of 3T3-L1 preadipocytes into adipocytes [72]. At a higher concentration (25 μ M), treatments with T3s, especially γ T3 and δ T3, for eight days significantly reduced triglyceride accumulation and decreased the expression of adipogenic genes, *i.e.*, FAS and PPAR γ , in 3T3-L1 cells [73]. Interestingly, γ T3 and δ T3 treatments in 3T3-L1 cells markedly increased mRNA and protein expression of CPT1 and UCP-2, but not HMG-CoA reductase, indicating that γ T3 and δ T3 may disrupt lipid metabolism during fat cell formation [73]. In addition to inhibiting adipogenesis, Wu *et al.* discovered that γ T3 has the strongest anti-proliferative effect on 3T3-L1 cells and the lowest IC₅₀ value (~13.91 μ M) among all vitamin E analogs [74]. However, all of the studies described above used 3T3-L1 adipocytes as their cell model, which has three limitations. First, 3T3-L1 preadipocytes are mouse hybridoma cells; thus, it is difficult to distinguish the anti-adipocyte effect from the anti-tumor effect of T3s. Second, 3T3-L1 cells are committed preadipocytes that have lost their stem cell properties and are not good for differentiation studies. Finally, 3T3-L1 preadipocytes undergo post-confluent mitogenic expansion, which is not found in primary adipogenic precursor cells. Therefore, the effects of T3s on 3T3-L1 cells need further validation by using other *in vitro* and *in vivo* models.

Consistent with the results from 3T3-L1 cells, our research has identified the inhibitory effects of γ T3 on adipogenesis by using primary human adipose tissue-derived stem cells (*h*ASCs), a physiologically more relevant adipocyte model to human than 3T3-L1 preadipocytes derived from rodents [75,76]. We also found that T3s inhibit adipogenesis in an isomer- and dose-dependent manner in differentiating *h*ASCs [77]. In particular, 1 μ M of γ T3 is able to significantly reduce triglyceride accumulation and decrease mRNA and protein expression of adipogenic marker genes, including PPAR γ and aP2 [77]. Moreover, there was an increased expression of Pref-1 (a preadipocyte marker) in the adipose tissue and a decreased fat mass in γ T3-fed obese mice, indicating that γ T3 reduces fat mass via inhibition of preadipocyte differentiation [47]. Another study discovered that during early adipogenesis (0–48 h), γ T3 treatment inhibits the mRNA expression of transcript factors CEBP α , but not CEBP β [77], indicating that γ T3 acts on a pathway downstream of C/EBP β , but upstream of C/EBP α at the early stage of adipocyte differentiation. These observations support the finding that T3s decrease fat mass and body fat distribution *in vivo* (see Section 4.1). Notably, when fully-differentiated mature human adipocytes were treated with 5 μ M of individual T3 isomers (α T3, γ T3 or δ T3), no noticeable dedifferentiation was found [77]. This indicates that T3s inhibit adipogenesis in adipose stem cells, but have no inhibition of lipogenesis in the pre-existing adipose tissue.

5.1.2. Modulation of Energy Sensing

AMP kinase (AMPK) is an enzyme ubiquitously found in the body and has been characterized as a metabolic master switch that regulates the activities of a number of metabolism-controlling proteins (reviewed by Yun *et al.* [78]). Several phytochemicals, including quercetin, resveratrol and glabridin, inhibit adipogenesis via AMPK activation [79–82]. A recent study reported that T3s also activate AMPK [74]. In 3T3-L1 cells, γ T3 at 20 μ M increases phosphorylation of AMPK without changing the total AMPK level [74]. Similarly, our group has reported that a low concentration of γ T3 (1 μ M) is able to promote AMPK phosphorylation in differentiating *h*ASCs without altering the total level of AMPK [77]. In contrast, AMPK inhibitors, such as Compound C, significantly suppressed the γ T3-induced ROS production and cell death in 3T3-L1 cells, indicating that the anti-adipogenic effect of γ T3 on adipocytes may be mediated by AMPK signaling pathways. Furthermore, inhibition of AMPK by delivering AMPK-DN, a mutated form of AMPK, to cells significantly reversed the γ T3-inhibited PPAR γ activation and partially rescued the morphology of lipid-loaded adipocytes, which strongly indicates that activation of AMPK by γ T3 suppresses adipogenic conversion [77].

As the downstream signaling cascades of AMPK, the mammalian target of rapamycin (mTOR) pathway has also been reported to be influenced by T3 treatments [83]. It has been suggested that fat cells sense the change of cellular energy status via the mTOR signaling pathway [84]. Interestingly, studies have found a positive correlation between T3s and mTOR in several cell lines [84,85]. For example, δ T3 protects mouse and human hematopoietic progenitors from γ -irradiation through upregulation of mTOR and increases phosphorylation of its downstream effector 4EBP-1 [86]. In 3T3-L1 cells and neoplastic mammary epithelial cells, γ T3 inhibits Akt phosphorylation, which is an upstream target of mTOR [72,87]. In *hASCs*, we have demonstrated that γ T3 decreases phosphorylation of mTOR P70S6 kinase, which is a downstream factor of mTOR, indicating that the anabolic pathways are downregulated by γ T3 treatment [77]. However, another study from our group found that the insulin signaling cascades, *i.e.*, phosphorylation of IRS-1 and Akt, were markedly increased in the adipose tissue of γ T3-fed mice and γ T3-treated adipocyte compared to their respective controls [47], suggesting that γ T3 exerts a different role in differentiated adipocytes by activating Akt instead of inhibiting Akt, as seen in preadipocytes.

5.1.3. Induction of Apoptosis in Preadipocytes

Fat cell apoptosis is often concurrent with inhibition in adipogenesis [81,88]. It has been reported that T3s induce apoptosis in several types of cancer cells [89–91]. For example, Ahn *et al.* have reported that γ T3 induces apoptotic cell death in cancer cells through downregulation of NF κ B and its receptor-interacting proteins [92]. Recently, the apoptotic effect of T3s on preadipocytes has been revealed. Wu *et al.* have found that γ T3 induces apoptosis in 3T3-L1 cells in a dose-dependent manner, which can be partially blocked by AMPK and caspase-3 inhibitors [64]. Interestingly, a high dose of γ T3 at 20 μ M induces 3T3-L1 cell cycle arrest in S phase and decreases the number of cells in the G0/G1 phase [74]. Our group has found that after exposure to a low dose of γ T3 at 1 μ M for 10 days, differentiating *hASCs* eventually underwent a caspase-3-dependent apoptotic cell death [77]. In Wu's and our experiments, a significant increase in the Bax/Bcl-2 ratio, an indicator of apoptosis, was observed, indicating that γ T3-mediated alterations in Bax and Bcl-2 expression play an important role in promoting cell apoptosis [74,77]. In addition, we discovered that γ T3 treatment in *hASCs* increases cytosolic autophagosome (LC3II) accumulation and autophagic flux, indicating that γ T3 promotes autophagy in differentiating adipocytes [77]. Collectively, γ T3-mediated autophagy serves as a critical checkpoint that determines if preadipocytes become mature adipocytes or undergo an alternative apoptotic cell death.

5.1.4. Modulation of Inflammation

Obesity is considered as low-grade inflammation in adipose tissue that leads to insulin resistance and other obesity-associated metabolic complications [93]. In the obese state, inflamed adipose tissue is often observed with abnormal productions of inflammatory cytokines and activation of inflammatory signaling in adipocytes [94]. It has been reported that injection of γ T3 or δ T3 at a dose of 10 μ g/kg to BALB/c mice reduces lipopolysaccharide (LPS)-triggered inflammation [95]. Another study conducted by Tetsuro *et al.* found that 2.4 μ M of γ T3 significantly decreases the secretion of inflammatory cytokines, including MCP-1 and IL-6, in TNF α -treated 3T3-L1 adipocytes [96]. Additionally, γ T3 treatment inhibits TNF α -mediated NF κ B activation in adipocytes [97].

In young C57BL6/J mice, we found that γ T3 supplementation is effective in reducing high fat diet-induced inflammation in adipose tissue by decreasing the expression of inflammatory genes, *i.e.*, MCP-1 [47]. In parallel, Justo *et al.* reported that a 5% rice bran extract supplement rich in T3s, when given to rats over 20 weeks, significantly reduces the expression of TNF α , IL-6, IL-1 β and iNOS in visceral adipose tissue [98]. However, it is currently unclear how γ T3 attenuates high fat diet-induced inflammation in adipose tissue. As reviewed by Jiang *et al.* [99], T3s, especially γ T3, inhibit the activation of NF κ B and STA6/3, as well as their downstream genes in various cell types, which may provide an explanation for how γ T3 reduces inflammation in adipose tissue. This notion is

supported by the study conducted by our group, which confirmed that activation of NF κ B and MAP kinases by LPS is robustly attenuated in γ T3-treated human adipocytes [47].

5.2. Effects of T3s on Immune System

In addition to directly reducing inflammation in adipose tissue, T3s inhibit inflammation by modulating the immune system. Recently, our group found that oral gavage of γ T3 at 50 mg/kg for one week reduces LPS-induced secretion of several pro-inflammatory cytokines, *i.e.*, IL-6, TNF α and GM-CSF, in the plasma of C57BL/6J mice [47]. This is confirmed by two studies that found dietary supplementation with T3s, especially δ T3, blocks LPS-induced TNF- α secretion in BALB/c mice and macrophages [95,100]. In addition, γ T3 treatment partially reverses TNF α -mediated alteration in adiponectin secretion [96].

T cells play an essential role in immune responses, activation of which leads to an abnormal induction of cytokines [101]. T3s are potent compounds regulating T cell-mediated inflammation. Gu *et al.* have found that supplementation of 0.2% T3 mixture (13.1% α T3, 57.9% β T3 and 20.5% δ T3) in diet for three weeks significantly increases the production of IgA and IgG in the lymphocytes harvested from spleen and mesenteric lymph node (MLN) regardless of the levels of concanavalin A (ConA) stimulation [102]. T3 feeding also decreases CD4⁺ T cells and the ratio of CD4⁺/CD8⁺ T cells in both spleen and MLN lymphocytes [102]. Moreover, 0.1% Tocomin (12.2% α T3, 2% β T3, 6.2% δ T3 and 20.1% γ T3) supplement for six weeks in four-month-old C57BL/6 mice increases the production of IL-1 β in splenocytes [103]. At the molecular level, the effects of T3s on T cells are highly associated with multiple pathways that modulate cell proliferation and apoptosis [25,26]. Ren *et al.* have found that all T3 isomers enhance lymphocyte proliferation in aged mice with a potency ranking from α T3 > γ T3 > δ T3 [103]. Interestingly, γ T3 and δ T3 treatments suppress cell proliferation by arresting cell cycle in Jurkat T and CEM-SS cell lines derived from human T cells [25,60]. In addition, γ T3 treatment inhibits ConA-induced activation of Swiss T cells by suppressing NF- κ B and NF- κ B-dependent gene expression [26]. Although γ T3 is accumulated in lymphocytes, modulates intracellular glutathione and reduces oxygen concentration [26,104], the anti-inflammatory properties of γ T3 are not related to its anti-oxidative effects [26]. Furthermore, γ T3 treatment elevates mitochondrial ROS production, activates JNK, but inhibits phosphorylation of JNK and p-38 in Jurkat T cells. These changes lead to increased Bax and decreased Bcl levels, resulting in a caspase-dependent apoptosis in T cells [25]. However, transient exposure to γ T3 for 4 h increases the survival and proliferation of T cells, which suggests that differential mechanisms of T3 in regulating human T cells may exist depending on the length of the treatment [26].

Obesity-related inflammation is associated with macrophage infiltration to adipose tissue [105]. T3s reduce inflammation in monocytes and inhibit differentiation of monocytes into macrophages via multiple pathways [104,106–108]. For example, α T3 significantly inhibits proteasome activity by increasing P27 and P57 protein expression in human monocytic cells (THP-1) [104]. Similarly, TRF treatment significantly reduces LPS-induced releases of NO and PGE₂, as well as the excretion of proinflammatory cytokines by inhibiting NF κ B activation in THP-1 cells [107]. Furthermore, α T3 and δ T3 inhibit the excretion of adhesion molecules ICAM-1 and VCAM-1 from vascular endothelium cells, thus reducing monocyte cell adhesion [106,108]. In adipose tissue, T3s reduce inflammation via inhibition of macrophage infiltration [47]. Li *et al.* have found that TRF activates PPARs (*i.e.*, PPAR α and PPAR γ) in murine macrophages, including Raw 264.7 and human THP-1 cells [38]. Wang *et al.* have shown that γ T3 reduces LPS-stimulated IL-6 and GM-CSF secretions in bone marrow-derived macrophages by inhibiting CEBP/ α and NF κ B [109]. Our group has found that γ T3 treatment reduces M1 macrophage polarization in bone marrow-derived macrophages, but has no impact on their differentiation into M2 macrophage [47]. Collectively, T3s reduce inflammation in adipose tissue, which is a possible mechanism contributing to their inhibition of obesity.

5.3. Effects of T3s on Liver

In addition to adipose tissue, liver is an important organ that regulates obesity by controlling lipid metabolism in the body. T3s play an important role in attenuating high fat diet-induced fatty liver [110]. It has been reported that a dietary supplement of γ T3 for 16 weeks in rats effectively reduced high fat diet-induced hepatic triglyceride accumulation and decreased the size and number of fat vacuoles in the liver [59]. The same effects on liver were confirmed in other animals, including rabbits and chicken [110]. Additionally, γ T3 supplement decreases high fat diet-induced inflammatory cell infiltration in the liver [111], leading to decreased obesity-induced inflammation.

In human HepG2 cells, Burdeos *et al.* have found that γ T3 at 10–15 μ M decreases mRNA and protein expression of fatty acid synthase, but increases mRNA expression of β -oxidation-associated genes, including CPT-1 and CYP3A4 [57]. Consistently, Muto *et al.* have shown that γ T3 treatment in rat primary hepatocytes decreases the expression of hepatic steatosis-associated genes, including Chop and SREBP-1c, thus attenuating ER stress and reducing inflammation in the fatty liver [112]. Our group also observed a significant increase in the expression of genes associated with fatty acid oxidation in the livers of mice fed with high fat diet and γ T3 supplement [47]. Li *et al.* have found that 0.2% TRF supplement in diet attenuates atherosclerosis in ApoE knockout mice through upregulation of LXR α and cholesterol transporters, such as ABCA1, in the liver [38]. Collectively, these data support the notion that γ T3 downregulates biosynthesis of triglyceride and cholesterol in liver cells [113]. Because the majority of the orally-administrated T3s are metabolized in the liver [50], results from these studies indicate that γ T3 supplement improves the obesity impaired liver function and whole body energy expenditure through increased hepatic fatty acid oxidation.

5.4. Effects of T3s on Other Tissues

T3s also exert their anti-obesity function on other tissues in addition to adipose tissue and liver [114]. T3s are well known for their neuro-protection function. Muscle is an important organ that utilizes glucose as the main energy source and regulates overall energy balance. Fang *et al.* reported that oral administration of 50 mg/kg TRF to Db/Db mice for two weeks increases the expression of β -oxidation-associated genes CPT2 and UCP3 by activating PPAR δ in the muscle [15]. Similar to their effects on the liver, T3s increase energy expenditure in muscle, as well [114]. In addition, T3 supplement prevents pancreatic damage and restores obesity-impaired insulin secretion. Budin *et al.* have reported that feeding TRF at 200 mg/kg for 28 days in SD rats prevents fenitrothion-induced pancreatic damage through restoring the activities of anti-oxidative enzymes, including CAT and SOD [115]. Moreover, Kunnumakkara *et al.* have shown that γ T3 administration inhibits pancreatic tumor growth by suppressing the NF κ B-mediated inflammatory pathways in orthotopic nude mice [116]. The protective effects of γ T3 on the pancreas may improve insulin production and reduce insulin resistance, which are complications in the obese state.

According to the most recent findings, excessive fat mass has a detrimental effect on bone health, such as increased risk in bone fracture [117–119], and T3s provide health benefits against both obesity and osteoporosis [47,77,120]. Adipogenesis and osteogenesis use mesenchymal stromal cells as their common precursors [121], and the balance of adipogenesis and osteogenesis is controlled by multiple pathways, including PPAR γ and Wnt signaling. Thus, bone cell differentiation is decreased during fat cell formation [122,123]. Furthermore, a high fat diet induces inflammation, which promotes obesity and osteoclast [123,124]. In an obese state, adipokines, *i.e.*, leptin and adiponectin, decline in the systemic level, promoting osteoclast and bone resorption [123,124]. Similar to their beneficial effects on obesity, T3s effectively prevent osteoporosis and bone loss in several animal models [125–127]. It has been reported that TRF supplements are more efficient in preventing postmenopausal osteoporosis in estrogen-deficient rats compared to calcium fortification [125]. Moreover, a supplement of γ T3 at 60 mg/kg for four months in healthy male rats markedly increases the volume, thickness, number and separation of trabeculae [128]. T3s promote bone health by multiple pathways. Nizar *et al.* have reported that low doses of γ T3 (1 and 10 μ M)

prevent osteoblasts by reducing hydrogen peroxide-induced oxidative stress and apoptosis, leading to decreased osteoporosis [129]. Moreover, T3s prevent osteoclasts by reducing free radicals and inhibiting NF κ B activation [120]. Ha *et al.* have found that α T3 suppresses the expression of RANKL, the NF κ B receptor activator, in osteoclasts, and prevents RANKL-induced osteoclast differentiation through inhibiting c-fos expression [130]. More recently, Deng *et al.* have observed that the influence of γ T3 on preventing ovariectomy-induced osteoporosis can be reversed by a daily administration of mevalonate, a HMG-CoA reductase inhibitor, in rats, indicating that γ T3 prevents bone loss by inhibiting HMG-CoA reductase [131].

6. Limitations and Conclusions

The investigation of the effects of T3s on obesity and obesity-associated complications is a new and rapidly growing field. In the authors' opinion, there are a few limitations in the existing literature regarding the anti-obesity effect of T3s, and additional study designs are needed to fill the following research gaps. First, many studies have used TRF for treatment instead of a single T3 isomer or mixtures with a known composition of each isomer. Thus, the pharmacological effects observed in these studies may not be caused by T3s alone, meaning that other components in TRF may produce a synergistic influence or antagonistic with T3s and affect the treatment outcomes. Furthermore, the different sources and compositions of TRF used in these studies make it difficult to directly compare results among them, and we are unable to identify the single most effective isomer of T3s. Future experiments are required to use individual T3 isomer for the determination of the exact mechanism of action of T3s. Second, as the majority of studies were performed *in vitro*, more *in vivo* studies in relevant animal models of metabolic syndrome or clinical human trials are needed to validate the anti-obesity effect of T3s. Third, most of the research interests are currently focused on the bioavailability of T3s and observational studies to describe the association between T3 intake and the outcome in reducing adiposity or improving blood lipoprotein profiles. Therefore, studies with mechanistic approaches that scrutinize the metabolic benefits and/or potential adverse effects are needed to characterize the legitimate anti-obesogenic effects of T3s.

There are continuous efforts to search for safe and effective dietary strategies that could intervene in obesity and its associated metabolic dysfunction. We and others have found that T3, especially γ T3, improve obesity and obesity-associated complications by reducing adipogenesis and inflammation, as well as increasing apoptosis, energy sensing and energy expenditure (Figure 1). T3s modulate multiple signaling pathways mainly in adipose tissue and liver, with lesser effects on muscle and pancreas. Our latest study supports that γ T3 inhibits the innate immune machinery and inflammation that produce potent cytotoxic cytokine IL-1 β [132]. Given the fact that deregulation of autoimmune responses that are associated with inflammasome plays a key role in pathogenesis of multiple metabolic diseases, it provides a novel insight to control obesity and inflammation utilizing γ T3. Although more studies are required to extrapolate the cellular and animal findings, there is a critical mass of studies supporting the notion that supplementation of γ T3 or dietary γ T3-enriched food would be a simple strategy to attenuate adiposity and to lessen obesity-mediated metabolic dysfunction, even without losing body weight.

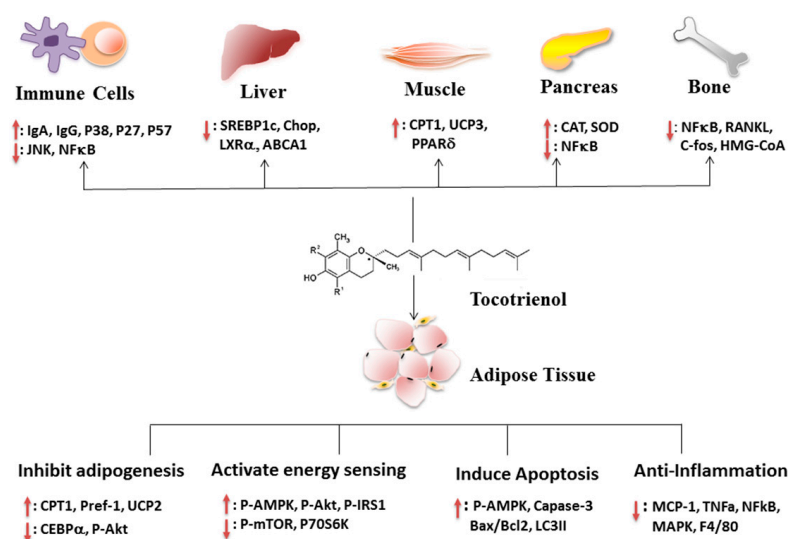


Figure 1. Mechanisms for the effect of tocotrienols (T3s) on obesity and obesity-associated complications. Briefly, T3s accumulate in adipose tissue and attenuate obesity mainly by: (1) inhibiting adipogenesis in adipose stem cells; (2) activating energy sensing in differentiating or mature adipocytes; (3) enhancing apoptosis in preadipocytes; and (4) suppressing MAPK, NFκB and inflammatory cytokine secretion in inflamed adipocytes. In addition, T3s also exert their anti-obesity effects by reducing inflammation in immune cells, pancreas and bone, suppressing fatty acid and cholesterol synthesis in the liver and enhancing energy expenditure and antioxidant activity in muscle and pancreas.

Acknowledgments: This work is supported by the American Heart Association Nebraska Tobacco Settlement Biomedical Research Enhancement Funds, the American Heart Association SDG Grant (13SDG14410043) and the Viticulture Advisory Council (VAC) Research Grant (#00094883) from the Florida Department of Agriculture and Consumer Service to M.M. and S.C.

Author Contributions: Lu Zhao and Xiefan Fang participated in searching the literature and drafting the manuscript. Maurice Marshall contributed to manuscript editing. Soonkyu Chung contributed to the manuscript design, review and editing.

Conflicts of Interest: The authors declare no conflict of interests.

References

1. Freedman, D.S.; Horlick, M.; Berenson, G.S. A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children. *Am. J. Clin. Nutr.* **2013**, *98*, 1417–1424. [[CrossRef](#)] [[PubMed](#)]
2. Wohlfahrt-Veje, C.; Tinggaard, J.; Winther, K.; Mouritsen, A.; Hagen, C.P.; Mieritz, M.G.; de Renzy-Martin, K.T.; Boas, M.; Petersen, J.H.; Main, K.M. Body fat throughout childhood in 2647 healthy Danish children: Agreement of BMI, waist circumference, skinfolds with dual X-ray absorptiometry. *Eur. J. Clin. Nutr.* **2014**, *68*, 664–670. [[CrossRef](#)] [[PubMed](#)]
3. Sun, Q.; van Dam, R.M.; Spiegelman, D.; Heymsfield, S.B.; Willett, W.C.; Hu, F.B. Comparison of dual-energy X-ray absorptiometric and anthropometric measures of adiposity in relation to adiposity-related biologic factors. *Am. J. Epidemiol.* **2010**, *172*, 1442–1454. [[CrossRef](#)] [[PubMed](#)]
4. Willett, K.; Jiang, R.; Lenart, E.; Spiegelman, D.; Willett, W. Comparison of bioelectrical impedance and BMI in predicting obesity-related medical conditions. *Obesity* **2006**, *14*, 480–490. [[CrossRef](#)] [[PubMed](#)]
5. Ogden, C.; Carroll, M.; Fryar, C.; Flegal, K. Prevalence of obesity among adults and youth: United States, 2011–2014. *NCHS Data Brief* **2015**, *219*, 1–8. [[PubMed](#)]
6. Wang, Y.C.; McPherson, K.; Marsh, T.; Gortmaker, S.L.; Brown, M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* **2011**, *378*, 815–825. [[CrossRef](#)]

7. Sun, K.; Kusminski, C.M.; Scherer, P.E. Adipose tissue remodeling and obesity. *J. Clin. Investig.* **2011**, *121*, 2094–2101. [[CrossRef](#)] [[PubMed](#)]
8. Flodmark, C.-E.; Marcus, C.; Britton, M. Interventions to prevent obesity in children and adolescents: A systematic literature review. *Int. J. Obes.* **2006**, *30*, 579–589. [[CrossRef](#)] [[PubMed](#)]
9. Piironen, V.; Syvaola, E.L.; Varo, P.; Salminen, K.; Koivistoinen, P. Tocopherols and tocotrienols in Finnish foods: Vegetables, fruits, and berries. *J. Agric. Food Chem.* **1986**, *34*, 742–746. [[CrossRef](#)]
10. Aggarwal, B.B.; Sundaram, C.; Prasad, S.; Kannappan, R. Tocotrienols, the vitamin E of the 21st century: Its potential against cancer and other chronic diseases. *Biochem. Pharmacol.* **2010**, *80*, 1613–1631. [[CrossRef](#)] [[PubMed](#)]
11. Singh, U.; Devaraj, S.; Jialal, I. Vitamin E, oxidative stress, and inflammation. *Annu. Rev. Nutr.* **2005**, *25*, 151–174. [[CrossRef](#)] [[PubMed](#)]
12. Qureshi, A.A.; Qureshi, N.; Wright, J.; Shen, Z.; Kramer, G.; Gapor, A.; Chong, Y.; DeWitt, G.; Ong, A.; Peterson, D. Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palmvitee). *Am. J. Clin. Nutr.* **1991**, *53*, 1021S–1026S. [[PubMed](#)]
13. Manu, K.A.; Shanmugam, M.K.; Ramachandran, L.; Li, F.; Fong, C.W.; Kumar, A.P.; Tan, P.; Sethi, G. First evidence that γ -tocotrienol inhibits the growth of human gastric cancer and chemosensitizes it to capecitabine in a xenograft mouse model through the modulation of NF- κ B pathway. *Clin. Cancer Res.* **2012**, *18*, 2220–2229. [[CrossRef](#)] [[PubMed](#)]
14. Yang, Z.; Xiao, H.; Jin, H.; Koo, P.T.; Tsang, D.J.; Yang, C.S. Synergistic actions of atorvastatin with γ -tocotrienol and celecoxib against human colon cancer HT29 and HCT116 cells. *Int. J. Cancer* **2010**, *126*, 852–863. [[CrossRef](#)] [[PubMed](#)]
15. Fang, F.; Kang, Z.; Wong, C. Vitamin E tocotrienols improve insulin sensitivity through activating peroxisome proliferator-activated receptors. *Mol. Nutr. Food Res.* **2010**, *54*, 345–352. [[CrossRef](#)] [[PubMed](#)]
16. Ima-Nirwana, S.; Suhaniza, S. Effects of tocopherols and tocotrienols on body composition and bone calcium content in adrenalectomized rats replaced with dexamethasone. *J. Med. food* **2004**, *7*, 45–51. [[CrossRef](#)] [[PubMed](#)]
17. Magosso, E.; Ansari, M.A.; Gopalan, Y.; Shuaib, I.L.; Wong, J.-W.; Khan, N.A.K.; Bakar, M.R.A.; Ng, B.-H.; Yuen, K.-H. Tocotrienols for normalisation of hepatic echogenic response in nonalcoholic fatty liver: A randomised placebo-controlled clinical trial. *Nutr. J.* **2013**, *12*. [[CrossRef](#)] [[PubMed](#)]
18. Weng-Yew, W.; Brown, L. Nutraceuticals of tocotrienols for metabolic syndrome. *Curr. Pharm. Des.* **2011**, *17*, 2206–2214. [[CrossRef](#)] [[PubMed](#)]
19. Peh, H.Y.; Tan, W.S.; Liao, W.; Wong, W.S. Vitamin E therapy beyond cancer: Tocopherol *versus* tocotrienol. *Pharmacol. Ther.* **2015**. [[CrossRef](#)] [[PubMed](#)]
20. Ayoub, N.; Bachawal, S.; Sylvester, P. γ -Tocotrienol inhibits HGF-dependent mitogenesis and Met activation in highly malignant mammary tumour cells. *Cell Prolif.* **2011**, *44*, 516–526. [[CrossRef](#)] [[PubMed](#)]
21. Campbell, S.E.; Rudder, B.; Phillips, R.B.; Whaley, S.G.; Stimmel, J.B.; Leesnitzer, L.M.; Lightner, J.; Dessus-Babus, S.; Duffourc, M.; Stone, W.L. γ -Tocotrienol induces growth arrest through a novel pathway with TGF β 2 in prostate cancer. *Free Radic. Biol. Med.* **2011**, *50*, 1344–1354. [[CrossRef](#)] [[PubMed](#)]
22. Ji, X.; Wang, Z.; Geamanu, A.; Sarkar, F.H.; Gupta, S.V. Inhibition of cell growth and induction of apoptosis in non-small cell lung cancer cells by delta-tocotrienol is associated with notch-1 down-regulation. *J. Cell. Biochem.* **2011**, *112*, 2773–2783. [[CrossRef](#)] [[PubMed](#)]
23. Patacsil, D.; Tran, A.T.; Cho, Y.S.; Suy, S.; Saenz, F.; Malyukova, I.; Resson, H.; Collins, S.P.; Clarke, R.; Kumar, D. γ -Tocotrienol induced apoptosis is associated with unfolded protein response in human breast cancer cells. *J. Nutr. Biochem.* **2012**, *23*, 93–100. [[CrossRef](#)] [[PubMed](#)]
24. Shin-Kang, S.; Ramsauer, V.P.; Lightner, J.; Chakraborty, K.; Stone, W.; Campbell, S.; Reddy, S.A.; Krishnan, K. Tocotrienols inhibit AKT and ERK activation and suppress pancreatic cancer cell proliferation by suppressing the ErbB2 pathway. *Free Radic. Biol. Med.* **2011**, *51*, 1164–1174. [[CrossRef](#)] [[PubMed](#)]
25. Wilankar, C.; Khan, N.M.; Checker, R.; Sharma, D.; Patwardhan, R.; Gota, V.; Sandur, S.K.; Devasagayam, T.P. γ -Tocotrienol induces apoptosis in human T cell lymphoma through activation of both intrinsic and extrinsic pathways. *Curr. Pharm. Des.* **2011**, *17*, 2176–2189. [[CrossRef](#)] [[PubMed](#)]
26. Wilankar, C.; Sharma, D.; Checker, R.; Khan, N.M.; Patwardhan, R.; Patil, A.; Sandur, S.K.; Devasagayam, T.P. Role of immunoregulatory transcription factors in differential immunomodulatory effects of tocotrienols. *Free Radic. Biol. Med.* **2011**, *51*, 129–143. [[CrossRef](#)] [[PubMed](#)]

27. Ahmad, N.; Khalid, B.; Luke, D.; Ima Nirwana, S. Tocotrienol offers better protection than tocopherol from free radical-induced damage of rat bone. *Clin. Exp. Pharmacol. Physiol.* **2005**, *32*, 761–770. [[CrossRef](#)] [[PubMed](#)]
28. Khan, M.R.; Siddiqui, S.; Parveen, K.; Javed, S.; Diwakar, S.; Siddiqui, W.A. Nephroprotective action of tocotrienol-rich fraction (TRF) from palm oil against potassium dichromate (K₂Cr₂O₇)-induced acute renal injury in rats. *Chem. Biol. Interact.* **2010**, *186*, 228–238. [[CrossRef](#)] [[PubMed](#)]
29. Khan, M.S.; Khan, M.; Siddiqui, M.; Arif, J. An *in vivo* and *in silico* approach to elucidate the Tocotrienol-mediated fortification against infection and inflammation induced alterations in antioxidant defense system. *Eur. Rev. Med. Pharmacol. Sci.* **2011**, *15*, 916–930. [[PubMed](#)]
30. Maniam, S.; Mohamed, N.; Shuid, A.N.; Soelaiman, I.N. Palm tocotrienol exerted better antioxidant activities in bone than α -tocopherol. *Basic Clin. Pharmacol. Toxicol.* **2008**, *103*, 55–60. [[CrossRef](#)] [[PubMed](#)]
31. Taridi, N.; Yahaya, M.F.; Teoh, S.L.; Latiff, A.A.; Ngah, W.; Das, S.; Mazlan, M. Tocotrienol rich fraction (TRF) supplementation protects against oxidative DNA damage and improves cognitive functions in Wistar rats. *Clin. Ter.* **2010**, *162*, 93–98.
32. Vraka, P.S.; Drouza, C.; Rikkou, M.P.; Odysseos, A.D.; Keramidas, A.D. Synthesis and study of the cancer cell growth inhibitory properties of α -, γ -tocopheryl and γ -tocotrienyl 2-phenylselenyl succinates. *Bioorg. Med. Chem.* **2006**, *14*, 2684–2696. [[CrossRef](#)] [[PubMed](#)]
33. Shrader, W.D.; Amagata, A.; Barnes, A.; Enns, G.M.; Hinman, A.; Jankowski, O.; Kheifets, V.; Komatsuzaki, R.; Lee, E.; Mollard, P. α -Tocotrienol quinone modulates oxidative stress response and the biochemistry of aging. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3693–3698. [[CrossRef](#)] [[PubMed](#)]
34. Matringe, M.; Ksas, B.; Rey, P.; Havaux, M. Tocotrienols, the unsaturated forms of vitamin E, can function as antioxidants and lipid protectors in tobacco leaves. *Plant Physiol.* **2008**, *147*, 764–778. [[CrossRef](#)] [[PubMed](#)]
35. Chin, S.-F.; Hamid, N.A.A.; Latiff, A.A.; Zakaria, Z.; Mazlan, M.; Yusof, Y.A.M.; Karim, A.A.; Ibahim, J.; Hamid, Z.; Ngah, W.Z.W. Reduction of DNA damage in older healthy adults by Tri E[®] Tocotrienol supplementation. *Nutrition* **2008**, *24*, 1–10. [[CrossRef](#)] [[PubMed](#)]
36. Schwedhelm, E.; Maas, R.; Troost, R.; Böger, R.H. Clinical pharmacokinetics of antioxidants and their impact on systemic oxidative stress. *Clin. Pharmacokinet.* **2003**, *42*, 437–459. [[CrossRef](#)] [[PubMed](#)]
37. Prasad, K. Tocotrienols and cardiovascular health. *Curr. Pharm. Des.* **2011**, *17*, 2147–2154. [[CrossRef](#)] [[PubMed](#)]
38. Li, F.; Tan, W.; Kang, Z.; Wong, C.W. Tocotrienol enriched palm oil prevents atherosclerosis through modulating the activities of peroxisome proliferators-activated receptors. *Atherosclerosis* **2010**, *211*, 278–282. [[CrossRef](#)] [[PubMed](#)]
39. Nafeeza, M.; Norzana, A.; Jalaluddin, H.; Gapor, M. The effects of a tocotrienol-rich fraction on experimentally induced atherosclerosis in the aorta of rabbits. *Malays. J. Pathol.* **2001**, *23*, 17–25. [[PubMed](#)]
40. Baliarsingh, S.; Beg, Z.H.; Ahmad, J. The therapeutic impacts of tocotrienols in type 2 diabetic patients with hyperlipidemia. *Atherosclerosis* **2005**, *182*, 367–374. [[CrossRef](#)] [[PubMed](#)]
41. Black, T.M.; Wang, P.; Maeda, N.; Coleman, R.A. Palm tocotrienols protect ApoE +/– mice from diet-induced atheroma formation. *J. Nutr.* **2000**, *130*, 2420–2426. [[PubMed](#)]
42. Kannappan, R.; Gupta, S.C.; Kim, J.H.; Aggarwal, B.B. Tocotrienols fight cancer by targeting multiple cell signaling pathways. *Genes Nutr.* **2012**, *7*, 43–52. [[CrossRef](#)] [[PubMed](#)]
43. Chen, D.-C.; Chung, Y.-F.; Yeh, Y.-T.; Chaung, H.-C.; Kuo, F.-C.; Fu, O.-Y.; Chen, H.-Y.; Hou, M.-F.; Yuan, S.-S.F. Serum adiponectin and leptin levels in Taiwanese breast cancer patients. *Cancer Lett.* **2006**, *237*, 109–114. [[CrossRef](#)] [[PubMed](#)]
44. Mantzoros, C.; Petridou, E.; Dessypris, N.; Chavelas, C.; Dalamaga, M.; Alexe, D.M.; Papadiamantis, Y.; Markopoulos, C.; Spanos, E.; Chrousos, G. Adiponectin and breast cancer risk. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 1102–1107. [[CrossRef](#)] [[PubMed](#)]
45. Malaviya, A.; Parajuli, P.; Sylvester, P.W. Anticancer effects of combined γ -tocotrienol and PPAR γ antagonist treatment are associated with a suppression in adipogenic factor expression. *J. Pharm. Nutr. Sci.* **2014**, *4*, 43–56.
46. Yap, S.; Yuen, K.; Wong, J. Pharmacokinetics and bioavailability of α -, γ - and δ -tocotrienols under different food status. *J. Pharm. Pharmacol.* **2001**, *53*, 67–71. [[CrossRef](#)] [[PubMed](#)]

47. Zhao, L.; Kang, I.; Fang, X.; Wang, W.; Lee, M.; Hollins, R.; Marshall, M.; Chung, S. γ -Tocotrienol attenuates high-fat diet-induced obesity and insulin resistance by inhibiting adipose inflammation and M1 macrophage recruitment. *Int. J. Obes.* **2015**, *39*, 438–446. [[CrossRef](#)] [[PubMed](#)]
48. Yap, S.P.; Yuen, K.H.; Lim, A.B. Influence of route of administration on the absorption and disposition of α -, γ - and δ -tocotrienols in rats. *J. Pharm. Pharmacol.* **2003**, *55*, 53–58. [[CrossRef](#)] [[PubMed](#)]
49. Sontag, T.J.; Parker, R.S. Influence of major structural features of tocopherols and tocotrienols on their ω -oxidation by tocopherol- ω -hydroxylase. *J. Lipid Res.* **2007**, *48*, 1090–1098. [[CrossRef](#)] [[PubMed](#)]
50. Brigelius-Flohé, R. Vitamin E and drug metabolism. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 737–740. [[CrossRef](#)]
51. Fu, J.-Y.; Che, H.-L.; Tan, D.M.-Y.; Teng, K.-T. Bioavailability of tocotrienols: Evidence in human studies. *Nutr. Metab.* **2014**, *11*, 1. [[CrossRef](#)] [[PubMed](#)]
52. Qureshi, A.A.; Khan, D.A.; Saleem, S.; Silswal, N.; Trias, A.M.; Tan, B.; Qureshi, N. Pharmacokinetics and bioavailability of annatto d-tocotrienol in healthy fed subjects. *J. Clin. Exp. Cardiol.* **2015**, *6*. [[CrossRef](#)]
53. Meganathan, P.; Jabir, R.S.; Fuang, H.G.; Bhoo-Pathy, N.; Choudhury, R.B.; Taib, N.A.; Nesaretnam, K.; Chik, Z. A new formulation of Gamma Delta Tocotrienol has superior bioavailability compared to existing Tocotrienol-Rich Fraction in healthy human subjects. *Sci. Rep.* **2015**, *5*, 13550. [[CrossRef](#)] [[PubMed](#)]
54. Patel, V.; Rink, C.; Gordillo, G.M.; Khanna, S.; Gnyawali, U.; Roy, S.; Shneker, B.; Ganesh, K.; Phillips, G.; More, J.L. Oral tocotrienols are transported to human tissues and delay the progression of the model for end-stage liver disease score in patients. *J. Nutr.* **2012**, *142*, 513–519. [[CrossRef](#)] [[PubMed](#)]
55. Shibata, A.; Nakagawa, K.; Shirakawa, H.; Kobayashi, T.; Kawakami, Y.; Takashima, R.; Ohashi, A.; Sato, S.; Ohsaki, Y.; Kimura, F. Physiological effects and tissue distribution from large doses of tocotrienol in rats. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1805–1808. [[CrossRef](#)] [[PubMed](#)]
56. Uchida, T.; Nomura, S.; Sakuma, E.; Hanzawa, F.; Ikeda, S. α -Tocopherol does not accelerate depletion of γ -tocopherol and tocotrienol or excretion of their metabolites in rats. *Lipids* **2013**, *48*, 687–695. [[CrossRef](#)] [[PubMed](#)]
57. Burdeos, G.C.; Nakagawa, K.; Kimura, F.; Miyazawa, T. Tocotrienol attenuates triglyceride accumulation in HepG2 cells and F344 rats. *Lipids* **2012**, *47*, 471–481. [[CrossRef](#)] [[PubMed](#)]
58. Wong, W.Y.; Ward, L.C.; Fong, C.W.; Yap, W.N.; Brown, L. Anti-inflammatory γ - and δ -tocotrienols improve cardiovascular, liver and metabolic function in diet-induced obese rats. *Eur. J. Nutr.* **2015**. [[CrossRef](#)] [[PubMed](#)]
59. Wong, W.-Y.; Poudyal, H.; Ward, L.C.; Brown, L. Tocotrienols reverse cardiovascular, metabolic and liver changes in high carbohydrate, high fat diet-fed rats. *Nutrients* **2012**, *4*, 1527–1541. [[CrossRef](#)] [[PubMed](#)]
60. Wong, R.S.; Radhakrishnan, A.K.; Ibrahim, T.A.; Cheong, S.K. δ - and γ -Tocotrienols induce classical ultrastructural apoptotic changes in human T lymphoblastic leukemic cells. *Microsc. Microanal.* **2012**, *18*, 462–469. [[CrossRef](#)] [[PubMed](#)]
61. Candiracci, M.; Justo, M.L.; Castano, A.; Rodriguez-Rodriguez, R.; Herrera, M.D. Rice bran enzymatic extract-supplemented diets modulate adipose tissue inflammation markers in Zucker rats. *Nutrition* **2014**, *30*, 466–472. [[CrossRef](#)] [[PubMed](#)]
62. Das, S.; Mukherjee, S.; Lekli, I.; Gurusamy, N.; Bardhan, J.; Raychoudhury, U.; Chakravarty, R.; Banerji, S.; Knowlton, A.A.; Das, D.K. Tocotrienols confer resistance to ischemia in hypercholesterolemic hearts: Insight with genomics. *Mol. Cell. Biochem.* **2012**, *360*, 35–45. [[CrossRef](#)] [[PubMed](#)]
63. Watkins, T.; Lenz, P.; Gapor, A.; Struck, M.; Tomeo, A.; Bierenbaum, M. γ -Tocotrienol as a hypocholesterolemic and antioxidant agent in rats fed atherogenic diets. *Lipids* **1993**, *28*, 1113–1118. [[CrossRef](#)] [[PubMed](#)]
64. Chou, T.W.; Ma, C.Y.; Cheng, H.H.; Chen, Y.Y.; Lai, M.H. A rice bran oil diet improves lipid abnormalities and suppress hyperinsulinemic responses in rats with streptozotocin/nicotinamide-induced type 2 diabetes. *J. Clin. Biochem. Nutr.* **2009**, *45*, 29–36. [[CrossRef](#)] [[PubMed](#)]
65. Qureshi, A.A.; Qureshi, N.; Hasler-Rapacz, J.O.; Weber, F.E.; Chaudhary, V.; Crenshaw, T.D.; Gapor, A.; Ong, A.S.; Chong, Y.H.; Peterson, D.; *et al.* Dietary tocotrienols reduce concentrations of plasma cholesterol, apolipoprotein B, thromboxane B2, and platelet factor 4 in pigs with inherited hyperlipidemias. *Am. J. Clin. Nutr.* **1991**, *53*, 1042S–1046S.

66. Budin, S.B.; Othman, F.; Louis, S.R.; Bakar, M.A.; Das, S.; Mohamed, J. The effects of palm oil tocotrienol-rich fraction supplementation on biochemical parameters, oxidative stress and the vascular wall of streptozotocin-induced diabetic rats. *Clinics* **2009**, *64*, 235–244. [[CrossRef](#)] [[PubMed](#)]
67. Qureshi, A.A.; Bradlow, B.A.; Brace, L.; Manganello, J.; Peterson, D.M.; Pearce, B.C.; Wright, J.J.; Gapor, A.; Elson, C.E. Response of hypercholesterolemic subjects to administration of tocotrienols. *Lipids* **1995**, *30*, 1171–1177. [[CrossRef](#)] [[PubMed](#)]
68. Zaiden, N.; Yap, W.N.; Ong, S.; Xu, C.H.; Teo, V.H.; Chang, C.P.; Zhang, X.W.; Nesaretnam, K.; Shiba, S.; Yap, Y.L. Gamma delta tocotrienols reduce hepatic triglyceride synthesis and VLDL secretion. *J. Atheroscler. Thromb.* **2010**, *17*, 1019–1032. [[CrossRef](#)] [[PubMed](#)]
69. Heng, E.C.; Karsani, S.A.; Abdul Rahman, M.; Abdul Hamid, N.A.; Hamid, Z.; Wan Ngah, W.Z. Supplementation with tocotrienol-rich fraction alters the plasma levels of Apolipoprotein A-I precursor, Apolipoprotein E precursor, and C-reactive protein precursor from young and old individuals. *Eur. J. Nutr.* **2013**, *52*, 1811–1820. [[CrossRef](#)] [[PubMed](#)]
70. Haghighat, N.; Vafa, M.; Eghtesadi, S.; Heidari, I.; Hosseini, A.; Rostami, A. The effects of tocotrienols added to canola oil on microalbuminuria, inflammation, and nitrosative stress in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *Int. J. Prev. Med.* **2014**, *5*, 617–623. [[PubMed](#)]
71. Irandoost, P.; Ebrahimi-Mameghani, M.; Pirouzpanah, S. Does grape seed oil improve inflammation and insulin resistance in overweight or obese women? *Int. J. Food Sci. Nutr.* **2013**, *64*, 706–710. [[CrossRef](#)] [[PubMed](#)]
72. Uto-Kondo, H.; Ohmori, R.; Kiyose, C.; Kishimoto, Y.; Saito, H.; Igarashi, O.; Kondo, K. Tocotrienol suppresses adipocyte differentiation and Akt phosphorylation in 3T3-L1 preadipocytes. *J. Nutr.* **2009**, *139*, 51–57. [[CrossRef](#)] [[PubMed](#)]
73. Burdeos, G.C.; Nakagawa, K.; Abe, T.; Kimura, F.; Miyazawa, T. Tocotrienol modulates crucial lipid metabolism-related genes in differentiated 3T3-L1 preadipocytes. *Food Funct.* **2014**, *5*, 2221–2227. [[CrossRef](#)] [[PubMed](#)]
74. Wu, S.J.; Huang, G.Y.; Ng, L.T. γ -Tocotrienol induced cell cycle arrest and apoptosis via activating the Bax-mediated mitochondrial and AMPK signaling pathways in 3T3-L1 adipocytes. *Food Chem. Toxicol.* **2013**, *59*, 501–513. [[CrossRef](#)] [[PubMed](#)]
75. Zuk, P.A.; Zhu, M.; Ashjian, P.; de Ugarte, D.A.; Huang, J.I.; Mizuno, H.; Alfonso, Z.C.; Fraser, J.K.; Benhaim, P.; Hedrick, M.H. Human adipose tissue is a source of multipotent stem cells. *Mol. Biol. Cell* **2002**, *13*, 4279–4295. [[CrossRef](#)] [[PubMed](#)]
76. Gimble, J.M.; Katz, A.J.; Bunnell, B.A. Adipose-derived stem cells for regenerative medicine. *Circ. Res.* **2007**, *100*, 1249–1260. [[CrossRef](#)] [[PubMed](#)]
77. Zhao, L.; Ha, J.H.; Okla, M.; Chung, S. Activation of autophagy and AMPK by γ -tocotrienol suppresses the adipogenesis in human adipose derived stem cells. *Mol. Nutr. Food Res.* **2014**, *58*, 569–579. [[CrossRef](#)] [[PubMed](#)]
78. Yun, J.W. Possible anti-obesity therapeutics from nature—A review. *Phytochemistry* **2010**, *71*, 1625–1641. [[CrossRef](#)] [[PubMed](#)]
79. Chen, Y.Y.; Lee, M.H.; Hsu, C.C.; Wei, C.L.; Tsai, Y.C. Methyl cinnamate inhibits adipocyte differentiation via activation of the CaMKK2-AMPK pathway in 3T3-L1 preadipocytes. *J. Agric. Food Chem.* **2012**, *60*, 955–963. [[CrossRef](#)] [[PubMed](#)]
80. Ono, M.; Fujimori, K. Antiadipogenic effect of dietary apigenin through activation of AMPK in 3T3-L1 cells. *J. Agric. Food Chem.* **2011**, *59*, 13346–13352. [[CrossRef](#)] [[PubMed](#)]
81. Dave, S.; Kaur, N.J.; Nanduri, R.; Dkhar, H.K.; Kumar, A.; Gupta, P. Inhibition of adipogenesis and induction of apoptosis and lipolysis by stem bromelain in 3T3-L1 adipocytes. *PLoS ONE* **2012**, *7*, e30831. [[CrossRef](#)] [[PubMed](#)]
82. Baur, J.A.; Pearson, K.J.; Price, N.L.; Jamieson, H.A.; Lerin, C.; Kalra, A.; Prabhu, V.V.; Allard, J.S.; Lopez-Lluch, G.; Lewis, K.; *et al.* Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444*, 337–342. [[CrossRef](#)] [[PubMed](#)]
83. Laplante, M.; Sabatini, D.M. An emerging role of mTOR in lipid biosynthesis. *Curr. Biol.* **2009**, *19*, R1046–R1052. [[CrossRef](#)] [[PubMed](#)]
84. Hresko, R.C.; Mueckler, M. mTOR-RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. *J. Biol. Chem.* **2005**, *280*, 40406–40416. [[CrossRef](#)] [[PubMed](#)]

85. Roh, C.; Han, J.; Tzatsos, A.; Kandror, K.V. Nutrient-sensing mTOR-mediated pathway regulates leptin production in isolated rat adipocytes. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E322–E330. [[CrossRef](#)] [[PubMed](#)]
86. Li, X.H.; Fu, D.; Latif, N.H.; Mullaney, C.P.; Ney, P.H.; Mog, S.R.; Whitnall, M.H.; Srinivasan, V.; Xiao, M. δ -Tocotrienol protects mouse and human hematopoietic progenitors from γ -irradiation through extracellular signal-regulated kinase/mammalian target of rapamycin signaling. *Haematologica* **2010**, *95*, 1996–2004. [[CrossRef](#)] [[PubMed](#)]
87. Shah, S.J.; Sylvester, P.W. δ -Tocotrienol inhibits neoplastic mammary epithelial cell proliferation by decreasing Akt and nuclear factor kappaB activity. *Exp. Biol. Med.* **2005**, *230*, 235–241.
88. Kim, J.Y.; van de Wall, E.; Laplante, M.; Azzara, A.; Trujillo, M.E.; Hofmann, S.M.; Schraw, T.; Durand, J.L.; Li, H.; Li, G.; *et al.* Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J. Clin. Investig.* **2007**, *117*, 2621–2637. [[CrossRef](#)] [[PubMed](#)]
89. Yu, W.; Simmons-Menchaca, M.; Gapor, A.; Sanders, B.G.; Kline, K. Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. *Nutr. Cancer* **1999**, *33*, 26–32. [[CrossRef](#)] [[PubMed](#)]
90. McIntyre, B.S.; Briski, K.P.; Gapor, A.; Sylvester, P.W. Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. *Proc. Soc. Exp. Biol. Med.* **2000**, *224*, 292–301. [[CrossRef](#)] [[PubMed](#)]
91. Srivastava, J.K.; Gupta, S. Tocotrienol-rich fraction of palm oil induces cell cycle arrest and apoptosis selectively in human prostate cancer cells. *Biochem. Biophys. Res. Commun.* **2006**, *346*, 447–453. [[CrossRef](#)] [[PubMed](#)]
92. Ahn, K.S.; Sethi, G.; Krishnan, K.; Aggarwal, B.B. γ -Tocotrienol inhibits nuclear factor- κ B signaling pathway through inhibition of receptor-interacting protein and TAK1 leading to suppression of antiapoptotic gene products and potentiation of apoptosis. *J. Biol. Chem.* **2007**, *282*, 809–820. [[CrossRef](#)] [[PubMed](#)]
93. Greenberg, A.S.; Obin, M.S. Obesity and the role of adipose tissue in inflammation and metabolism. *Am. J. Clin. Nutr.* **2006**, *83*, 461S–465S.
94. Wisse, B.E. The inflammatory syndrome: The role of adipose tissue cytokines in metabolic disorders linked to obesity. *J. Am. Soc. Nephrol.* **2004**, *15*, 2792–2800. [[CrossRef](#)] [[PubMed](#)]
95. Qureshi, A.A.; Reis, J.C.; Papanian, C.J.; Morrison, D.C.; Qureshi, N. Tocotrienols inhibit lipopolysaccharide-induced pro-inflammatory cytokines in macrophages of female mice. *Lipids Health Dis.* **2010**, *9*, 143. [[CrossRef](#)] [[PubMed](#)]
96. Matsunaga, T.; Shoji, A.; Gu, N.; Joo, E.; Li, S.; Adachi, T.; Yamazaki, H.; Yasuda, K.; Kondoh, T.; Tsuda, K. γ -Tocotrienol attenuates TNF- α -induced changes in secretion and gene expression of MCP-1, IL-6 and adiponectin in 3T3-L1 adipocytes. *Mol. Med. Rep.* **2012**, *5*, 905–909. [[PubMed](#)]
97. Kaileh, M.; Sen, R. Role of NF- κ B in the anti-inflammatory effects of tocotrienols. *J. Am. Coll. Nutr.* **2010**, *29*, 334S–339S. [[CrossRef](#)]
98. Justo, M.L.; Candiracci, M.; Dantas, A.P.; de Sotomayor, M.A.; Parrado, J.; Vila, E.; Herrera, M.D.; Rodriguez-Rodriguez, R. Rice bran enzymatic extract restores endothelial function and vascular contractility in obese rats by reducing vascular inflammation and oxidative stress. *J. Nutr. Biochem.* **2013**, *24*, 1453–1461. [[CrossRef](#)] [[PubMed](#)]
99. Jiang, Q. Natural forms of vitamin E: Metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic. Biol. Med.* **2014**, *72*, 76–90. [[CrossRef](#)] [[PubMed](#)]
100. Yam, M.L.; Abdul Hafid, S.R.; Cheng, H.M.; Nesaretnam, K. Tocotrienols suppress proinflammatory markers and cyclooxygenase-2 expression in RAW264.7 macrophages. *Lipids* **2009**, *44*, 787–797. [[CrossRef](#)] [[PubMed](#)]
101. Biron, C.A.; Nguyen, K.B.; Pien, G.C.; Cousens, L.P.; Salazar-Mather, T.P. Natural killer cells in antiviral defense: Function and regulation by innate cytokines. *Annu. Rev. Immunol.* **1999**, *17*, 189–220. [[CrossRef](#)] [[PubMed](#)]
102. Gu, J.Y.; Wakizono, Y.; Sunada, Y.; Hung, P.; Nonaka, M.; Sugano, M.; Yamada, K. Dietary effect of tocopherols and tocotrienols on the immune function of spleen and mesenteric lymph node lymphocytes in Brown Norway rats. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 1697–1702. [[CrossRef](#)] [[PubMed](#)]
103. Ren, Z.; Pae, M.; Dao, M.C.; Smith, D.; Meydani, S.N.; Wu, D. Dietary supplementation with tocotrienols enhances immune function in C57BL/6 mice. *J. Nutr.* **2010**, *140*, 1335–1341. [[CrossRef](#)] [[PubMed](#)]
104. Munteanu, A.; Ricciarelli, R.; Massone, S.; Zingg, J.M. Modulation of proteasome activity by vitamin E in THP-1 monocytes. *IUBMB Life* **2007**, *59*, 771–780. [[CrossRef](#)] [[PubMed](#)]

105. Weisberg, S.P.; McCann, D.; Desai, M.; Rosenbaum, M.; Leibel, R.L.; Ferrante, A.W., Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Investig.* **2003**, *112*, 1796–1808. [[CrossRef](#)] [[PubMed](#)]
106. Theriault, A.; Chao, J.T.; Gapor, A. Tocotrienol is the most effective vitamin E for reducing endothelial expression of adhesion molecules and adhesion to monocytes. *Atherosclerosis* **2002**, *160*, 21–30. [[CrossRef](#)]
107. Wu, S.J.; Liu, P.L.; Ng, L.T. Tocotrienol-rich fraction of palm oil exhibits anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. *Mol. Nutr. Food Res.* **2008**, *52*, 921–929. [[CrossRef](#)] [[PubMed](#)]
108. Chao, J.T.; Gapor, A.; Theriault, A. Inhibitory effect of delta-tocotrienol, a HMG CoA reductase inhibitor, on monocyte-endothelial cell adhesion. *J. Nutr. Sci. Vitaminol.* **2002**, *48*, 332–337. [[CrossRef](#)] [[PubMed](#)]
109. Wang, Y.; Jiang, Q. γ -Tocotrienol inhibits lipopolysaccharide-induced interleukin-6 and granulocyte colony-stimulating factor by suppressing C/EBP β and NF- κ B in macrophages. *J. Nutr. Biochem.* **2013**, *24*, 1146–1152. [[CrossRef](#)] [[PubMed](#)]
110. Tres, A.; Nuchi, C.D.; Magrinya, N.; Guardiola, F.; Bou, R.; Codony, R. Use of palm-oil by-products in chicken and rabbit feeds: Effect on the fatty acid and tocol composition of meat, liver and plasma. *Animal* **2012**, *6*, 1005–1017. [[CrossRef](#)] [[PubMed](#)]
111. Yachi, R.; Igarashi, O.; Kiyose, C. Protective effects of vitamin E analogs against carbon tetrachloride-induced fatty liver in rats. *J. Clin. Biochem. Nutr.* **2010**, *47*, 148–154. [[CrossRef](#)] [[PubMed](#)]
112. Muto, C.; Yachi, R.; Aoki, Y.; Koike, T.; Igarashi, O.; Kiyose, C. γ -Tocotrienol reduces the triacylglycerol level in rat primary hepatocytes through regulation of fatty acid metabolism. *J. Clin. Biochem. Nutr.* **2013**, *52*, 32–37. [[CrossRef](#)] [[PubMed](#)]
113. Burdeos, G.C.; Nakagawa, K.; Watanabe, A.; Kimura, F.; Miyazawa, T. γ -Tocotrienol attenuates triglyceride through effect on lipogenic gene expressions in mouse hepatocellular carcinoma Hepa 1–6. *J. Nutr. Sci. Vitaminol.* **2013**, *59*, 148–151. [[CrossRef](#)] [[PubMed](#)]
114. Ebong, P.E.; Owu, D.U.; Isong, E.U. Influence of palm oil (*Elaeis guineensis*) on health. *Plant Foods Hum. Nutr.* **1999**, *53*, 209–222. [[CrossRef](#)] [[PubMed](#)]
115. Budin, S.B.; Han, C.M.; Jayusman, P.A.; Taib, I.S. Tocotrienol rich fraction prevents fenitrothion induced pancreatic damage by restoring antioxidant status. *Pak. J. Biol. Sci.* **2012**, *15*, 517–523. [[CrossRef](#)] [[PubMed](#)]
116. Kunnumakkara, A.B.; Sung, B.; Ravindran, J.; Diagaradjane, P.; Deorukhkar, A.; Dey, S.; Koca, C.; Yadav, V.R.; Tong, Z.; Gelovani, J.G.; Guha, S.; Krishnan, S.; Aggarwal, B.B. γ -Tocotrienol inhibits pancreatic tumors and sensitizes them to gemcitabine treatment by modulating the inflammatory microenvironment. *Cancer Res.* **2010**, *70*, 8695–8705. [[CrossRef](#)] [[PubMed](#)]
117. Janicka, A.; Wren, T.A.; Sanchez, M.M.; Dorey, F.; Kim, P.S.; Mittelman, S.D.; Gilsanz, V. Fat mass is not beneficial to bone in adolescents and young adults. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 143–147. [[CrossRef](#)] [[PubMed](#)]
118. Zhao, L.J.; Liu, Y.J.; Liu, P.Y.; Hamilton, J.; Recker, R.R.; Deng, H.W. Relationship of obesity with osteoporosis. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 1640–1646. [[CrossRef](#)] [[PubMed](#)]
119. Cao, J.J. Effects of obesity on bone metabolism. *J. Orthop. Surg. Res.* **2011**, *6*, 30. [[CrossRef](#)] [[PubMed](#)]
120. Nazrun, A.S.; Norazlina, M.; Norliza, M.; Nirwana, S.I. The anti-inflammatory role of vitamin E in prevention of osteoporosis. *Adv. Pharmacol. Sci.* **2012**, *2012*, 142702. [[CrossRef](#)] [[PubMed](#)]
121. Sharma, S.; Tandon, V.R.; Mahajan, S.; Mahajan, V.; Mahajan, A. Obesity: Friend or foe for osteoporosis. *J. Midlife Health* **2014**, *5*, 6–9. [[CrossRef](#)] [[PubMed](#)]
122. Colaianni, G.; Brunetti, G.; Faienza, M.F.; Colucci, S.; Grano, M. Osteoporosis and obesity: Role of Wnt pathway in human and murine models. *World J. Orthop.* **2014**, *5*, 242–246. [[CrossRef](#)] [[PubMed](#)]
123. Rosen, C.J.; Bouxsein, M.L. Mechanisms of disease: Is osteoporosis the obesity of bone? *Nat. Clin. Pract. Rheumatol.* **2006**, *2*, 35–43. [[CrossRef](#)] [[PubMed](#)]
124. Ilich, J.Z.; Kelly, O.J.; Kim, Y.; Spicer, M.T. Low-grade chronic inflammation perpetuated by modern diet as a promoter of obesity and osteoporosis. *Arh. Hig. Rada Toksikol.* **2014**, *65*, 139–148. [[CrossRef](#)] [[PubMed](#)]
125. Soelaiman, I.N.; Ming, W.; Abu Bakar, R.; Hashnan, N.A.; Mohd Ali, H.; Mohamed, N.; Muhammad, N.; Shuid, A.N. Palm tocotrienol supplementation enhanced bone formation in oestrogen-deficient rats. *Int. J. Endocrinol.* **2012**. [[CrossRef](#)] [[PubMed](#)]
126. Muhammad, N.; Luke, D.A.; Shuid, A.N.; Mohamed, N.; Soelaiman, I.N. Two different isomers of vitamin E prevent bone loss in postmenopausal osteoporosis rat model. *Evid. Based Complement. Altern. Med.* **2012**. [[CrossRef](#)] [[PubMed](#)]

127. Muhammad, N.; Luke, D.A.; Shuid, A.N.; Mohamed, N.; Soelaiman, I.N. Tocotrienol supplementation in postmenopausal osteoporosis: Evidence from a laboratory study. *Clinics* **2013**, *68*, 1338–1343. [[CrossRef](#)]
128. Shuid, A.N.; Mehat, Z.; Mohamed, N.; Muhammad, N.; Soelaiman, I.N. Vitamin E exhibits bone anabolic actions in normal male rats. *J. Bone Miner. Metab.* **2010**, *28*, 149–156. [[CrossRef](#)] [[PubMed](#)]
129. Abd Manan, N.; Mohamed, N.; Shuid, A.N. Effects of low-dose *versus* high-dose γ -tocotrienol on the bone cells exposed to the hydrogen peroxide-induced oxidative stress and apoptosis. *Evid. Based Complement. Altern. Med.* **2012**. [[CrossRef](#)] [[PubMed](#)]
130. Ha, H.; Lee, J.H.; Kim, H.N.; Lee, Z.H. alpha-Tocotrienol inhibits osteoclastic bone resorption by suppressing RANKL expression and signaling and bone resorbing activity. *Biochem. Biophys. Res. Commun.* **2011**, *406*, 546–551. [[CrossRef](#)] [[PubMed](#)]
131. Deng, L.; Ding, Y.; Peng, Y.; Wu, Y.; Fan, J.; Li, W.; Yang, R.; Yang, M.; Fu, Q. γ -Tocotrienol protects against ovariectomy-induced bone loss via mevalonate pathway as HMG-CoA reductase inhibitor. *Bone* **2014**, *67*, 200–207. [[CrossRef](#)] [[PubMed](#)]
132. Kim, Y.; Wang, W.; Okla, M.; Kang, I.; Moreau, R.; Chung, S. Suppression of NLRP3 inflammasome by γ -tocotrienol ameliorates type 2 diabetes. *J. Lipid Res.* **2016**, *57*, 66–76. [[CrossRef](#)] [[PubMed](#)]



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).