

Phytol and its metabolites phytanic and pristanic acids for risk of cancer: current evidence and future directions

Gerd Bobe^{a,b}, Zhenzhen Zhang^c, Ryan Kopp^{d,e}, Mark Garzotto^{d,e}, Jackilen Shannon^f and Yumie Takata^g

This review summarizes the current evidence on the potential role of phytol, a microbial metabolite of chlorophyll A, and its metabolites, phytanic and pristanic acids, in carcinogenesis. Primary food sources in Western diets are the nut skin for phytol and lipids in dairy, beef and fish for its metabolites. Phytol and its metabolites gained interest as dietary compounds for cancer prevention because, as natural ligands of peroxisome proliferator-activated receptor- α and - γ and retinoid X receptor, phytol and its metabolites have provided some evidence in cell culture studies and limited evidence in animal models of anti-carcinogenic, anti-inflammatory and anti-metabolic-syndrome properties at physiological concentrations. However, there may be a narrow range of efficacy, because phytol and its metabolites at supra-physiological concentrations can cause *in vitro* cytotoxicity in non-cancer cells and can cause morbidity and mortality in animal models. In human studies, evidence for a role of phytol and its metabolites in cancer prevention is currently limited and inconclusive. In short, phytol and its metabolites are potential dietary compounds for

cancer prevention, assuming the challenges in preventing cytotoxicity in non-cancer cells and animal models and understanding phytol metabolism can be mitigated.

European Journal of Cancer Prevention 29:191–200
Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

European Journal of Cancer Prevention 2020, 29:191–200

Keywords: cancer, phytanic acid, phytanic acid, phytol

^aLinus Pauling Institute, Oregon State University, Corvallis, ^bDepartment of Animal and Rangeland Sciences, College of Agricultural Sciences, Oregon State University, Corvallis, ^cDivision of Hematology/Oncology, Knight Cancer Institute, Oregon Health & Science University, Portland, ^dDepartment of Urology, Portland Veterans Affairs Medical Center, Oregon Health & Science University, Portland, ^eDepartment of Urology, Oregon Health & Science University, Portland, ^fOHSU-PSU School of Public Health, Oregon Health & Science University, Portland and ^gCollege of Public Health and Human Sciences, School of Biological and Population Health Sciences, Oregon State University, Corvallis, Oregon, USA

Correspondence to Yumie Takata, PhD, College of Public Health and Human Sciences, Oregon State University, 103 Milam Hall, Corvallis, OR 97331, USA
Tel: +541 737 1606; fax: +541 737 6914;
e-mail: yumie.takata@oregonstate.edu

Received 7 February 2019 Accepted 5 July 2019

Introduction

Cancer is the second leading cause of deaths with an estimated 609 604 cancer deaths in 2018 in the US (Siegel *et al.* 2018). Dietary phytol, a diterpene alcohol and microbial metabolite of chlorophyll A, and its poly-methylated branched-chain fatty acid metabolites, phytanic acid (PA; 3,7,11,15-tetramethylhexadecanoic acid) and pristanic acid (PRA; 2,6,10,14-tetramethylpentadecanoic acid), have garnered the interest of cancer prevention researchers due to their ability to serve as natural ligands for peroxisome proliferator-activated receptor (PPAR)- α , PPAR- γ and retinoid X receptor (RXR) (Roca-Saavedra *et al.*, 2017). These receptors are transcription factors leading to downstream effects on metabolism, proliferation and apoptosis (Roca-Saavedra *et al.*, 2017). Furthermore, they upregulate the expression of α -methylacyl-CoA racemase (AMACR), especially in prostate cancer tissue (Verhoeven and Jakobs, 2001). Besides being involved in PA catabolism, AMACR is also critical in the degradation

of bile acids, ibuprofen and other methylated fatty acids (Lloyd *et al.*, 2008). Hence, phytol, PA and PRA may be linked to carcinogenesis through involvement in peroxisomal and mitochondrial functions, oxidative stress, inflammatory pathways, cell signal transduction, glucose/energy metabolism and microbial effects.

The Western diet contains about 10 mg/day of phytol (Steinberg, 1989; Brown *et al.*, 1993; Vetter and Schroder, 2010; Roca-Saavedra *et al.*, 2017), which mostly comes from the skin of nuts (Steinberg *et al.*, 1967), 50–100 mg/day of PA and 10–30 mg/day of PRA, which are primarily from lipids in dairy, beef and fish (Steinberg, 1989; Brown *et al.*, 1993; Vetter and Schroder, 2010; Roca-Saavedra *et al.*, 2017). The average circulating PA concentration is below 10 μ M, and according to diagnostic criteria for PA-related diseases, the PA concentration below 30 μ M is considered within the normal physiological range (Baldwin *et al.*, 2010; Lloyd *et al.*, 2013). Tissue PA concentrations are below 0.3% of total fatty acids (Kataria *et al.*, 2015). Both concentrations are sufficient to elicit chemo-preventive properties in cell culture studies, which are summarized in this review.

Current epidemiological evidence about dietary PA intake or circulating PA concentrations is limited to prostate

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

cancer and non-Hodgkin's Lymphoma (NHL) with varied results. Out of five studies (Xu *et al.*, 2005; Price *et al.*, 2010; Wright *et al.*, 2012; Ollberding *et al.*, 2013; Wright *et al.*, 2014), dietary intake or circulating concentration of PA or PRA have been linked to increased cancer risk overall or in subgroups in four studies and no association was observed in one study. Major food sources of phytol and its metabolites have been implicated in the etiology of cancer. The World Cancer Research Fund/American Institute for Cancer Research expert panel review (World Cancer Research Fund and American Institute for Cancer Research, 2018) reported strong evidence for a positive association of consuming red meats (including beef) and processed meats with colorectal cancer risk and an inverse association between dairy products and colorectal cancer risk. Further, they reported strong evidence for a positive association between salted fish intake and nasopharyngeal cancer risk. For the other major food sources of phytol and its metabolites, current evidence is insufficient or inconsistent to draw conclusions.

This article reviews the current evidence from cell culture studies, animal feeding studies and human intervention and observational studies, regarding phytol and its metabolites as potential dietary compounds for cancer prevention. Furthermore, this review recommends potential future research directions, which, if proven successful, will allow us to move forward with formulating prevention strategies involving phytol and its metabolites in order to decrease the public health burden of cancer. The goal of this review is to raise awareness of phytol and its metabolites and to foster future research, as outlined in this review.

Metabolism and physiological concentrations of phytol and its metabolites

The metabolism of phytol and its metabolites is shown in Fig. 1. Free phytol occurs at low concentrations in plant and animal tissues except for the skin of nuts and plant leaves, where it accumulates and acts as an antimicrobial agent (Steinberg *et al.*, 1967; Islam *et al.*, 2015), suggesting a potential role of phytol in altering the microbiome population. Phytol is derived from plant and phytoplankton chlorophyll. Nearly all phytol in plants is in the bound form as side-chain of chlorophyll or pheophytin and requires microbial enzymes to be released as free phytol (Hansen, 1980). Chlorophyll or pheophytin cannot be absorbed by humans, whereas PA has an absorption rate of 80% (Baxter, 1968). The free phytol is readily converted to PA in the gastrointestinal tract and lymph (Baxter, 1968). As phytol is not produced endogenously (Steinberg *et al.*, 1967), circulating phytol concentrations are probably found at trace amounts (Hansen, 1980). PRA in circulation is derived primarily from endogenous conversion of PA in the liver and a smaller portion from the diet. To our knowledge, the role of phytol and its metabolites on the microbiome is currently unknown and warrants research.

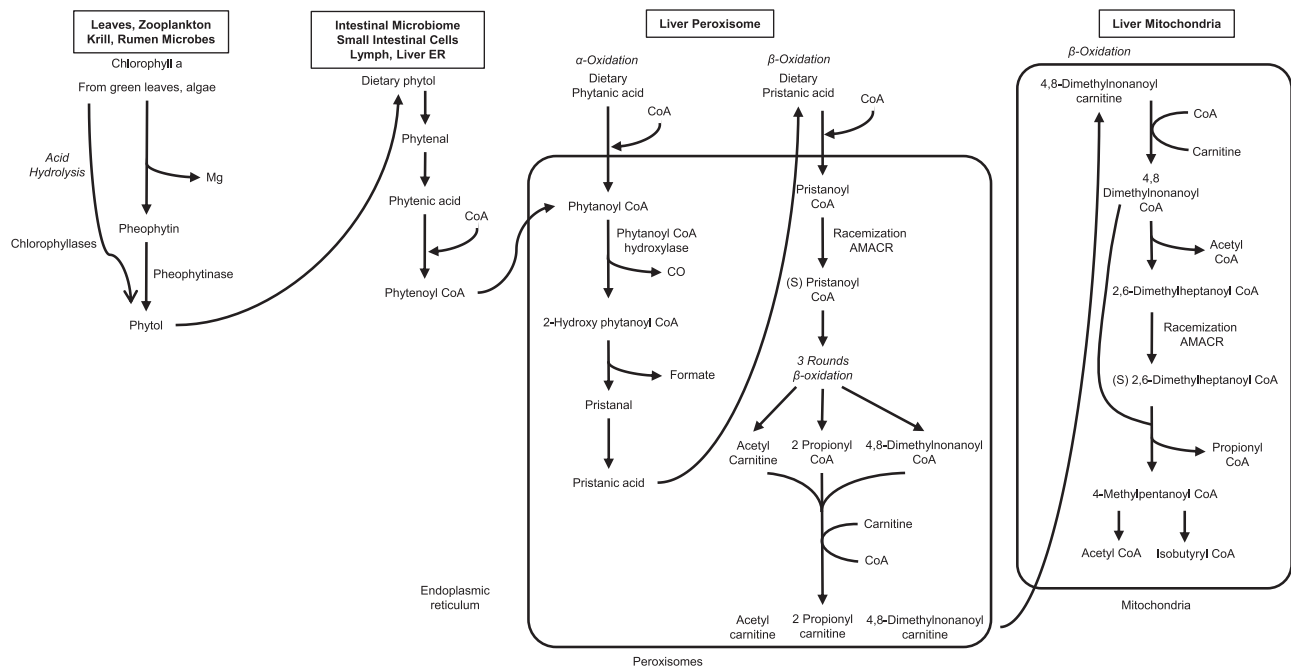
However, at supra-physiological levels, phytol can accumulate in the liver after conversion to PA (Hansen, 1980; Gloerich *et al.*, 2007). Circulating PA is derived primarily from the diet and a small portion (<20%) from endogenous conversion of phytol into PA (Hansen, 1980). As shown in Fig. 1, in the liver, PA is activated to phytanoyl CoA and then transported in the peroxisomes, where phytanoyl CoA is α -oxidized to PRA using various enzymes (Verhoeven and Jakobs, 2001). Mutations in enzymes involved in this step, such as phytanoyl CoA hydroxylase, can result in accumulation of PA as in Refsum disease, but PRA does not accumulate due to such mutations (Hansen, 1980; Verhoeven and Jakobs, 2001). As poly-methylated branched-chain fatty acids, PA and PRA replace primarily *n*-3 or *n*-6 polyunsaturated fatty acids in tissues (Hansen, 1965; Skrbic and Cumings, 1969; Malmendier *et al.*, 1974). The only reported human tissue concentration of PA is 0.30 wt% of total fatty acids in the prostate tissue (Kataria *et al.*, 2015), but no other tissues. In human populations, serum and plasma PA concentrations were on average under 10 μ M [2.6 μ M in the EPIC study (Price *et al.*, 2010), 2.9 μ M in the North Carolina study (Xu *et al.*, 2005) and 9.8 μ M in the Alpha-Tocopherol Beta-Carotene (ATBC) study (Wright *et al.*, 2014)] and differed among meat consumers, vegetarians and vegans (5.77, 3.93 and 0.86 μ M, respectively) (Allen *et al.*, 2008), indicating that intake of animal products can increase circulating PA concentrations.

In short, our knowledge about the physiological processes involved in the control of PA and PRA concentrations in absorption, circulation and tissue distribution as well as the effect of the intestinal microbiome on circulating PA and PRA concentrations is limited and requires further research.

Human consumption patterns of phytol and its metabolites

The current knowledge on phytol and PRA contents in foods is limited (Steinberg *et al.*, 1967; Coppack *et al.*, 1988; Steinberg, 1989; Brown *et al.*, 1993). Major food sources of phytol are the skin of nuts (about 1 g/100 g of food), whereas concentrations in other foods were below 15 mg/100 g of food (Steinberg *et al.*, 1967; Brown *et al.*, 1993). Major food sources of PA are lipids from dairy, beef and fish. Foods with the highest PA content are butter, followed by salmon, halibut and fatty cheeses (Roca-Saavedra *et al.*, 2017). Besides foods, 1 g of fish-oil supplements contain about 10% of the total dietary PA intake. In two previous epidemiological studies, the primary dietary sources of PA were cheese, butter and milk, which in total accounted for up to 53% in the Nebraska Lymphoma Study (NLS) (Ollberding *et al.*, 2013) and 90% in ATBC cohort (Wright *et al.*, 2012). The correlation between circulating PA concentration and fat intake from butter, a major food source of PA, is modest ($r = 0.35$ – 0.44) (Price *et al.*, 2010; Wright *et al.*, 2014; Kataria *et al.*,

Fig. 1



Metabolism of phytol, phytanic acid and pristanic acid. Phytol is derived from plant and phytoplankton chlorophyll. Rumen microbiota, marine zooplankton and human intestinal bacteria are able to cleave the chlorophyll porphyrin ring and release phytol. After intestinal absorption, dietary phytol is metabolized in the endoplasmic reticulum (ER) via phytalen and phytanic acid to phytanoyl CoA. Dietary PA and PRA are activated on the cytosolic side of the peroxisomes to phytanoyl CoA and pristanoyl CoA. In the peroxisomes, phytanoyl CoA is α -oxidized to PRA, the racemic mixture of PRA is converted by α -methylacyl-CoA racemase (AMACR) to (2S) PRA. After three rounds of β -oxidation, 4,8-dimethylnonanoyl CoA is shuttled by carnitine into the mitochondria for complete β -oxidation, which include four rounds of β -oxidation and one racemization step using AMACR (Verhoeven and Jakobs, 2001). PA, phytanic acid; PRA, pristanic acid.

2015). Hence, circulating PA concentrations may serve as dietary biomarker of ruminant fat intake in populations with low fatty fish consumption.

An average Western diet with dairy products contains between 50 and 100 mg/day of PA (Roca-Saavedra *et al.*, 2017) and is estimated to contain about 10–30 mg/day and 10 mg/day for PRA and phytol, respectively, based on the ratio of PA to PRA in dairy products (Steinberg, 1989; Brown *et al.*, 1993; Vetter and Schroder, 2010). This level is similar to the largest feeding study (tested 156 vs. 78 mg/day of PA) (Werner *et al.*, 2013). A few observational studies also reported an estimated intake of PA. In the NLS, the middle tertile of PA ranged from 43.0 to 63.8 mg/day (Ollberding *et al.*, 2013). In the ATBC Cancer Prevention cohort of Finnish male smokers, the average intake in the lowest and highest quartiles of PA were 64 and 199 mg/day, respectively (Wright *et al.*, 2012). Hence, a relatively wide range of intake was reported in healthy populations, although the direct comparison of estimated intake amounts from different studies needs a caution as the studies used different approaches (e.g. the number of food items included in their food frequency questionnaires and statistical methods used to estimate the intake).

Role of phytol and its metabolites in carcinogenesis Cell culture studies

Phytol and its metabolites at concentrations in the physiological range ($\leq 10 \mu\text{M}$) can alter pathways involved in carcinogenesis, such as increasing apoptosis and decreasing proliferation (Komiya *et al.*, 1999; Kim *et al.*, 2015; Thakor *et al.*, 2017). Proposed mechanisms by which phytol and its metabolites exert their chemo-preventive effects include inducing mitochondrial dysfunction, oxidative damage and intracellular Ca^{2+} deregulation, as well as epigenetic changes such as histone deacetylation (Idel *et al.*, 2002; Schönfeld *et al.*, 2004; Schönfeld *et al.*, 2006; Schönfeld and Wojtczak, 2007; Leipnitz *et al.*, 2010; Kruska and Reiser, 2011; Grings *et al.*, 2012; Borges *et al.*, 2015; Dhaunsi *et al.*, 2016; Dhaunsi *et al.*, 2017) (Table 1). The concentrations that resulted in these changes varied from 1 μM of PA for inducing oxidative damage in rat heart cells (Schönfeld *et al.*, 2004) to 100 μM of PA for causing nitric oxide-dependent apoptosis in vascular cells (Schluter *et al.*, 2002). In human embryonic kidney cells (HEK293), 50 μM of PA increased intracellular release of Ca^{2+} reserves, which was mediated via activation of free fatty acid receptor, GPR40 (Kruska and Reiser, 2011). Thus, PA may alter various pathways in different cell types in a dose-dependent manner. Studies

Table 1 Effect of phytanic acid on cancer pathways in cell culture studies

Concentration (μM)	Organ	Results or effects on cancer pathways	References
0.08	Rat cerebellum	Increased oxidative damage	Borges <i>et al.</i> (2015)
1	Rat cerebellum	Increased oxidative damage	Leipnitz <i>et al.</i> (2010)
1	Rat heart	Caused oxidative damage	Grings <i>et al.</i> (2012)
8	Vascular smooth muscle cells	Activated NADPH oxidase activity	Dhaunsi <i>et al.</i> (2016)
8	Vascular smooth muscle cells	Decreased their mitotic potential	Dhaunsi <i>et al.</i> (2017)
10	Rat heart	Depleted antioxidant reserves	Grings <i>et al.</i> (2012)
20	Rat brain	Induced mitochondrial membrane depolarization	Schönfeld <i>et al.</i> (2004)
20	Rat brain	Increased ROS generation	Schönfeld <i>et al.</i> (2006)
20	Primary rat hepatocytes and heart mitochondria	Increased ROS generation	Schönfeld and Wojtczak (2007)
50	Human embryonic kidney cells HEK293	Increased intracellular release of Ca^{2+} reserves, mediated via activation of free fatty acid receptor GPR40	Kruska and Reiser (2011)
100	Vascular smooth muscle cells	Caused nitric oxide-dependent apoptosis	Idel <i>et al.</i> (2002)

ROS, reactive oxygen species.

Table 2 Effect of phytol, phytanic acid or pristanic acid on cytotoxicity in cell culture studies

Concentration (μM)	PA, PRA or phytol	Cancer site	Cell line	Cytotoxicity measure	References
5	PA	Neuroblastoma	mouse neuroblastoma Neuro2a cells	5 μM PA for 48 h induced 40% cell death	Nagai (2015)
8.8	Phytol	Breast	breast adenocarcinoma MCF-7	IC_{50}	Pejin <i>et al.</i> (2014)
10	PA	Brain	primary hippocampal astrocytes	20% cell death after 6 hours	Rönicke <i>et al.</i> (2009)
10	PRA	Brain	primary hippocampal astrocytes	45% cell death after 6 hours	Rönicke <i>et al.</i> (2009)
10	PA	Prostate	a human normal prostate epithelial cell line NPrEC	PA-induced nearly 100% cell death	Mobley <i>et al.</i> (2003)
17	Phytol	Lung	lung cancer A549 cells	IC_{50}	Thakor <i>et al.</i> (2017)
20	Phytol	Liver	liver cancer cell lines Huh7 & HepG2	10% cell death	Kim <i>et al.</i> (2015)
20	PA	Muscle	porcine myotube	19% cell death after 24 h	Che <i>et al.</i> (2013)
20	PA	Prostate	Lymph node prostate carcinoma cells	20 μM PA for 48 h induced 30% cell death	Mobley <i>et al.</i> (2003)
35	Phytol	Colon	colon adenocarcinoma HT-29	IC_{50}	Pejin <i>et al.</i> (2014)
50	PA	Brain	primary hippocampal astrocyte	70% cell death after 3 hours	Kahlert <i>et al.</i> (2005)
50	PA	Muscle	rat vascular smooth muscle cells	20% cell death after 6 hours	Idel <i>et al.</i> (2002)
50	PA	Prostate	prostate carcinoma cells PC-3	50 μM PA for 72 or 120 hours inhibited proliferation	Tang <i>et al.</i> (2007)
57	Phytol	Lung	lung cancer A-549	IC_{50}	Pejin <i>et al.</i> (2014)
65	Phytol	Cervical	cervical cancer HeLa	IC_{50}	Pejin <i>et al.</i> (2014)
65	Phytol	Melanoma	skin melanoma Hs294T	IC_{50}	Pejin <i>et al.</i> (2014)
70	Phytol	Breast	breast adenocarcinoma MDA-MB-231	IC_{50}	Pejin <i>et al.</i> (2014)
79	Phytol	Prostate	prostate carcinoma PC-3	IC_{50}	Pejin <i>et al.</i> (2014)
100	PA	Brain	primary hippocampal oligodendrocytes	90% cell death after 5 h	Reiser <i>et al.</i> (2006)
100	Phytol	Liver	normal mouse hepatocytes AML12	20% cell death	Kim <i>et al.</i> (2015)
125	Phytol	Lung	fetal lung fibroblast MRC-5 cells	IC_{50}	Pejin <i>et al.</i> (2014)

PA, phytanic acid; PRA, pristanic acid.

of brain tissues showed effects on membrane depolarization, oxidative damage, mitochondrial reactive oxygen species (ROS) generation and histone deacetylation (epigenetic transcription regulation) at concentrations as low as 80 nM of PA (Schönfeld *et al.*, 2004; Reiser *et al.*, 2006; Schönfeld *et al.*, 2006; Schönfeld and Reiser, 2006; Rönicke *et al.*, 2009; Leipnitz *et al.*, 2010; Borges *et al.*, 2015; Nagai, 2015). In HepG2 cells, 40 μM of phytol can suppress the epithelial-mesenchymal transition signaling, which is important for tumor invasion (Kim *et al.*, 2015). Conversely, phytol and its metabolites may also have tumor-promoting properties, as ROS generation can induce DNA damage that either causes apoptosis or transforms normal cells into cancerous cells (Gu *et al.*, 2018; Samimi *et al.*, 2018). Moreover, 0.3 μM of PA or PRA in normal prostate cells was sufficient to increase the protein content of AMACR (Mobley *et al.*, 2003), an enzyme known to be elevated in prostate cancer tissue (Thornburg *et al.*, 2006). Thus, PA may be both friend and

foe for cancer prevention and treatment, which warrant further investigation.

In the physiological range ($\leq 10 \mu\text{M}$), phytol and its metabolites can be cytotoxic to various cancer cell lines (Idel *et al.*, 2002; Mobley *et al.*, 2003; Kahlert *et al.*, 2005; Reiser *et al.*, 2006; Tang *et al.*, 2007; Rönicke *et al.*, 2009; Che *et al.*, 2013; Pejin *et al.*, 2014; Kim *et al.*, 2015; Nagai, 2015; Thakor *et al.*, 2017), as shown in Table 2. Although the induction period and measures of cytotoxicity slightly vary among studies, the lowest concentrations at which cytotoxicity was observed were 5 μM of PA in neuroblastoma Neuro2a cells (Nagai, 2015) and 8.8 μM of phytol in breast adenocarcinoma MCF-7 (Pejin *et al.*, 2014). The highest concentration at which cytotoxicity was observed was 79 μM of phytol in prostate carcinoma PC-3 (Pejin *et al.*, 2014), suggesting that cancer cell lines may differ in their sensitivity to phytol and its metabolites. Of note is that, in a previous study of lung cancer A549 cells, the IC_{50} for phytol (17 μM) was lower than for the

cancer drug, methotrexate (20 μM) (Thakor *et al.*, 2017). Of concern is that 10 μM of PA was already cytotoxic to a human normal prostate epithelial cell line (NPrEC), but not to lymph node prostate carcinoma cells (Mobley *et al.*, 2003), suggesting a narrow and sometimes overlapping range between beneficial and detrimental effects. Additional studies are needed to investigate cytotoxicity of phytol and its metabolites in non-cancer and cancer cells and to elucidate why cell lines from different organs vary in their sensitivity to phytol and its metabolites.

Another desirable aspect of phytol and its metabolites as potential pharmacological agents is that they may improve markers of metabolic syndrome in the physiological range ($\leq 10 \mu\text{M}$). Organs may differ in their sensitivity to phytol and its metabolites, as minimal concentrations required for beneficial effects varied from 0.1 and 100 μM of PA. At concentration of as low as 0.1 μM , PA increased brown adipocyte differentiation (Schluter *et al.*, 2002), whereas 40 or 100 μM was needed for white adipocyte differentiation (Heim *et al.*, 2002; Schluter *et al.*, 2002). In muscle cells, 1 μM of PA was sufficient to stimulate glucose uptake (Che *et al.*, 2013) and 5 μM of phytol was sufficient to induce genes involved in muscle differentiation in C2C12 cells (Yang *et al.*, 2017). In contrast, 100 μM of PA was needed to increase transcription of genes that increase glucose uptake in HepG2 cells (Heim *et al.*, 2002).

Proposed pathways by which phytol and its metabolites exert their chemopreventive effects are summarized in Fig. 2. The primary proposed mode of action is that phytol and its metabolites act as natural ligands of various nuclear receptors, especially PPAR- α and - γ and RXR,

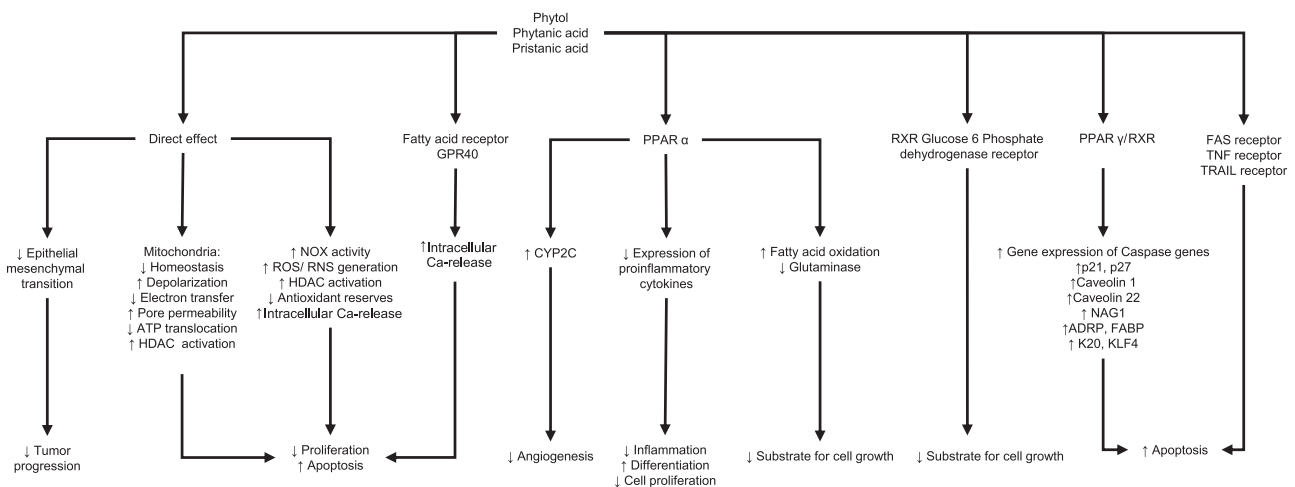
and induce transcription of nuclear receptor-responsive genes. Such induction can occur at concentrations similar to synthetic ligands that can be used for insulin-sensitizing effects and chemoprevention and treatment (Peters *et al.*, 2012). Phytol concentrations as low as 1, 50 and 10 μM induced PPAR- α , - β and - γ activity, respectively, in monkey kidney CV-1 cells and HepG2 hepatocytes (Heim *et al.*, 2002; Goto *et al.*, 2005). Concentrations of 1 μM of PRA and 3 μM of PA induced RXR activity, potentially through retinol esters of PA (Tang *et al.*, 2007), in monkey kidney COS-1 cells and human HepG2 cells (Zomer *et al.*, 2000). Other proposed pathways are the PI3kinase/AKT pathway (Wang *et al.*, 2017) and as ligands of TRAIL, FAS, and TNF receptors, glucose-6-phosphate dehydrogenase receptor (Thakor *et al.*, 2017), and the free fatty acid receptor, GPR40 (Kruska and Reiser, 2011).

In summary, it is encouraging that phytol and its metabolites can alter pathways involved in carcinogenesis at concentrations within the physiological range ($\leq 10 \mu\text{M}$). It is, however, concerning that limited evidence also suggests that phytol or PA at the same concentrations can be cytotoxic to breast and brain non-cancer cell lines and that the chemo-preventive effect of phytol and its metabolites may differ by organs and cancer cell lines.

Animal model studies

Current evidence from animal models on a role of phytol and its metabolites in carcinogenesis is limited to one study (Liska *et al.*, 2012); hence, this review includes animal models of metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) and animal toxicity studies

Fig. 2



Proposed mechanisms by which phytol and its metabolites exert their chemo-preventive properties. Phytol, phytanic acid and pristanic acid are proposed to affect pathways involved in carcinogenesis such as cell proliferation, differentiation and apoptosis, angiogenesis and inflammation. These effects are direct or indirect by inducing activation of transcription factors such as PPAR and receptors related to fatty acid and energy metabolism or inflammation. PPAR, peroxisome proliferator-activated receptor.

Table 3 Effects of phytol, or phytanic acid on cancer or biomarkers in animal feeding studies

Animal	Diet	Duration	Results	Reference
Cancer model				
Female Sprague–Dawley rats	0.05 wt% phytol of the diet in combination with seocalcitol at 7 ppb	11.5 weeks	Slowed mammary tumor growth, but not tumor malignancy or invasiveness.	Liska <i>et al.</i> (2012)
Metabolic syndrome-related model				
Male, diabetic insulin-resistant rats	Oral daily phytol doses at 0.025wt% of the diet	2 weeks	Decreased body fat percentage and improved the blood lipid and glucose profile, insulin sensitivity, and TNF α concentrations	Elmazar <i>et al.</i> (2013)
Male mice	0.05 wt% phytol in a high-fat and high-fructose diet	5 weeks	Increased adipocyte number, Improved glucose tolerance, and activated the PI3K/Akt signaling pathway	Wang <i>et al.</i> (2017)
Male mice	0.5wt% phytol of the diet	21 days	Decreased weight gain, and increased hepatic fatty acid oxidation	Hashimoto <i>et al.</i> (2006)
Male mice	0.5 wt% phytol of the diet	28 days	Increased the number of slow-twitch skeletal muscle fibers	Yang <i>et al.</i> (2017)
Male mice	Phytol at 25, 50 and 75 mg/kg body weight	2 weeks	Inhibited the inflammatory response to carrageenan-induced paw edema by inhibiting leukocyte migration, oxidative damage, and cytokine concentrations	Silva <i>et al.</i> (2014)
Transgenic mice model				
Sterol carrier protein-2/sterol carrier protein-x deficient mice	0.03 wt% phytol and 0.5 wt% PA of the diet	14+ days	Increased cardiac phenotype for sudden cardiac death such as ECG abnormalities	Mönnig <i>et al.</i> (2004)
Sterol carrier protein-2/sterol carrier protein-x deficient mice	Supplemental phytol at 0.5 wt% of the diet	unknown	Induced PPAR- α activity; started to die of sudden cardiac arrest within 14 days	Ellinghaus <i>et al.</i> (1999)
AMACR-deficient mice	0.5 wt% phytol of the diet	36 weeks	Fatal liver dysfunction	Selkälä <i>et al.</i> (2015)
Other models				
Male and female mice	0.5 or 1.0 wt% phytol diet	12–18 days	Liver hepatotoxicity	Mackie <i>et al.</i> (2009)
Pregnant female NMR1 mice	0.01 g of PA or 0.05 g of phytol/kg body weight	Administered on day 8 of gestation	Teratogenic effects of synthetic retinoid receptor ligands	Elmazar and Nau (2004)

PA, phytanic acid.

(Ellinghaus *et al.*, 1999; Elmazar and Nau, 2004; Mönnig *et al.*, 2004; Hashimoto *et al.*, 2006; Mackie *et al.*, 2009; Elmazar *et al.*, 2013; Silva *et al.*, 2014; Selkälä *et al.*, 2015; Wang *et al.*, 2017; Yang *et al.*, 2017) (Table 3). Dietary concentrations of phytol and its metabolites are expressed in wt%, if possible, to facilitate comparison of dietary concentrations among species. In female Sprague–Dawley rats, the combination of phytol at 0.05 wt% and a vitamin D analogue seocalcitol at 7 ppb, starting 7 weeks after mammary tumor induction, slowed mammary tumor growth, but not tumor malignancy or invasiveness; (Liska *et al.*, 2012). Four animal feeding studies evaluated the effect of oral phytol on indicators of metabolic syndrome and NAFLD and showed beneficial effects (van den Brink *et al.*, 2005; Hashimoto *et al.*, 2006; Elmazar *et al.*, 2013; Wang *et al.*, 2017). Dietary concentrations ranged from 0.025 to 0.5 wt% of phytol for 2–5 weeks and resulted in decrease in body fat percentage and TNF- α concentrations, and improvement in blood lipid and glucose profiles (Hashimoto *et al.*, 2006; Elmazar *et al.*, 2013). Interestingly, in one animal study, the 0.05 wt% phytol increased adipogenesis, but decreased total body fat mass, as the average size of adipocytes was smaller (Wang *et al.*, 2017). In addition to indicators of metabolic syndrome and NAFLD, phytol also showed anti-inflammatory properties in two studies, specifically decreased concentrations of TNF- α in one study (Elmazar *et al.*, 2013) and decreased cytokine concentrations and inhibiting leukocyte migration in the other study (Silva *et al.*, 2014).

Detrimental effects of oral phytol and its metabolites were reported in four animal studies, three of which used transgenic animal models that cannot catabolize PA, which accumulates in tissues and subsequently leads to death. In two studies of sterol carrier protein-2/sterol carrier protein-x deficient mice, phytol at 0.5 wt% of the diet-induced PPAR- α activity (Ellinghaus *et al.*, 1999), and mice started to die of sudden cardiac arrest by two weeks (Mönnig *et al.*, 2004). In AMACR-deficient mice, phytol at 0.5 wt% of the diet resulted in morbidity by 2 weeks, killed most mice by 6 weeks, and all by 36 weeks due to liver dysfunction (Selkälä *et al.*, 2015). Hepatotoxicity, consistent with chronic PPAR- α upregulation, was also observed in male and female mice after being on a 0.5 or 1.0 wt% phytol diet for 12 days (Mackie *et al.*, 2009). Not based on transgenic animal model, but in pregnant NMR1 mice, teratogenic effects of synthetic retinoid receptor ligands were potentiated with oral administration of 0.01 g of PA or 0.05 g of phytol/kg of body weight (Elmazar and Nau, 2004).

In summary, results of oral phytol at concentrations of 0.05 wt% of the diet for prevention or treatment of metabolic syndrome and inflammation are encouraging; however, 10-fold higher concentrations may be harmful, indicating the need for dose-response studies. Given that a Western diet (assuming 500 g/day of macronutrient consumption) contains phytol at 10% of the PA intake, an average person would consume 5 mg/day (equal to 0.001 wt% of the diet) and 20 mg/day of phytol (0.004 wt% of the diet), which is 10-fold lower than the

lowest concentrations shown effective in animal models (Elmazar *et al.*, 2013; Wang *et al.*, 2017). Furthermore, future animal studies should use dietary PA and PRA rather than phytol because humans have limited ability to metabolize phytol to PA or PRA and biologically effective PA and PRA contents can be obtained from human diet, assuming that phytol and its metabolites have the same efficacy (Steinberg, 1989; Brown *et al.*, 1993).

Human studies

Current evidence from intervention studies on a role of phytol and its metabolites is extremely limited. As to PA, initially, Werner *et al.* (2011) examined the effect of PA on biomarkers of metabolic syndrome among 14 healthy participants in a double-blind, randomized, 4-week parallel intervention study in Denmark. They were given 45 g of milk fat/day from test butter and cheese with 0.13 wt% of PA for the control group or 0.24 wt% of PA for the intervention group. Plasma PA concentrations increased in both groups significantly ($P < 0.05$) with a tendency of larger changes in the control group than the intervention group and no group differences in biomarkers of metabolic syndrome. In the follow-up study, Werner *et al.* (2013) increased both the PA amount and the length of intervention and compared the effect of replacing 15% of the participants' caloric intake from fat in the form of butter (39 g/day of butter that included 156 vs. 78 mg/day of PA) for 12 weeks on blood lipid, glucose, and inflammatory marker profiles among 38 healthy participants. To standardize the PA content and minimize residual confounding, the milk fat was obtained either from pasture-grazing or conventionally-fed cows and the former had twice the PA concentrations. Overall, there were no significant group differences in biomarker profiles related to metabolic syndrome (Werner *et al.*, 2013). This null result can be explained by no meaningful difference in plasma PA concentrations (3.6 vs. 3.3 μM ; $P = 0.15$), as only 15% of caloric intake from fat was replaced. Hence, future dietary intervention studies need to increase the difference in the PA content between two groups to achieve at least 2-fold differences in circulating PA concentration, potentially by providing the entire meals, instead of partially replacing their food source of fat. In short, there is currently lack of evidence from human intervention studies that would suggest a beneficial effect of phytol and its metabolites on chronic disease prevention.

Current evidence from epidemiological studies on a role of phytol and its metabolites in chronic diseases is limited to five cancer studies. Two studies (Wright *et al.*, 2012; Ollberding *et al.*, 2013) have examined the association between dietary PA intake and cancer risk. A retrospective case-control study (336 cases with 185 women and 460 controls with 236 women) in Nebraska, NLS (Ollberding *et al.*, 2013), reported no association between total dietary PA intake and NHL risk. When stratified by NHL

subtype, total dietary PA was positively associated with follicular lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukemia. Interestingly, the association between PA and NHL subtype differed by dietary PA source (Ollberding *et al.*, 2013). PA from beef, but not dairy products, was positively associated with diffuse large B-cell lymphoma. PA from milk, but not other dairy products, was associated with follicular lymphoma. This observation makes it unlikely that PA was driving the association with NHL subtype risk, leaving the possibility of residual confounding by other compounds in these food sources of PA. In the prospective ATBC cohort (27,111 participants, 1,929 incident cases, 438 advanced cases), PA intake was not associated with overall prostate cancer risk, but was positively associated with advanced prostate risk (Wright *et al.*, 2012). Moreover, this association was linked to high-fat dairy products and there was no association with PA from beef or fish. Thus, the association is subject to residual confounding as in NLS and could be due to compounds unique to dairy fat, such as short- and medium-chain fatty acids, which warrant further investigations. However, both studies did not further elucidate this possibility by mutually adjusting for the intake of these compounds, which needs to be explored in future studies and would help to clarify which bioactive compounds in dairy and beef products (e.g., mutagens) are driving the association between PA intake and cancer risk.

Three epidemiological studies measured serum or plasma PA concentrations and assessed the association with prostate cancer risk in men (Xu *et al.*, 2005; Price *et al.*, 2010; Wright *et al.*, 2014). A small, population-based, retrospective case-control study (49 cases and 55 control) conducted in North Carolina reported significantly higher serum PA levels among prostate cancer cases than controls (Xu *et al.*, 2005). Two prospective case-control studies nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) and ATBC cohorts followed up on the finding. In the EPIC cohort (566 cases and 566 controls), plasma PA concentrations were not associated with overall prostate cancer risk; however, there was a positive association among participants who fasted at least three hours before blood draw (Price *et al.*, 2010). In the ATBC cohort (300 cases and 300 controls), in which dietary PA intake was associated with aggressive, but not overall prostate cancer risk (Wright *et al.*, 2012), neither serum PA or PRA concentrations were associated with overall or aggressive prostate cancer risk (Wright *et al.*, 2014).

As a note of caution, circulating PA concentrations do not necessarily reflect tissue PA concentrations. The correlation between serum and benign prostate tissue PA concentrations in 26 participants from Chicago was low ($r = 0.28$; $P = 0.15$) (Kataria *et al.*, 2015). More research is needed to determine factors that alter tissue PA concentrations and their relation with cancer risk and circulating

PA concentrations. Besides prostate cancer, future epidemiologic studies are warranted to investigate cancer sites that have a lower IC_{50} , such as breast or brain, or that accumulate more PA and have a higher lipid content, specifically liver and kidney (Hansen, 1965; Skrbic and Cumings, 1969; Malmendier *et al.*, 1974), which are more likely to show an association.

In summary, current evidence in human studies for a role of phytol and its metabolites in cancer prevention is insufficient, inconsistent, and could be explained by residual confounding by other food components. Another concern is that primary food sources of phytol and its metabolites have other food components that are known to have cancer-promoting properties (e.g. mutagens and iron in meats) (World Cancer Research Fund and American Institute for Cancer Research, 2018). The question arises whether dietary intervention studies for cancer prevention can be formulated to achieve large differences in circulating concentrations of phytol, its metabolites, or both and their chemopreventive effects without residual confounding by other food components. Thus, a better strategy for future human intervention studies would be to use a supplement rich in phytol and its metabolites. As to cancer site, we recommend that future epidemiological studies should also examine cancers other than prostate cancer and NHL, especially cancer sites whose cells are known to be more sensitive to phytol and its metabolites such as brain, liver, kidney and blood. Furthermore, future studies should examine the interrelation between dietary, circulating and tissue levels of phytol and its metabolites in normal and cancer tissue.

Role of phytol and its metabolites on α -methylacyl-CoA racemase expression and cancer risk

The enzyme AMACR is critical for catabolism of PA and PRA, as it converts the (R)-form of PRA to the (S)-form, which can be further oxidized in humans. The expression of AMACR is upregulated *in vitro* by PA or PRA (Mobley *et al.*, 2003) via PPAR- α to prevent accumulation of PA or PRA (Gloerich *et al.*, 2007). Thus, elevated PA levels in circulation may be linked to decreased AMACR expression and may differ by *AMACR* polymorphisms. There is continued interest in the association of AMACR expression and polymorphisms with cancer, as elevated AMACR protein or gene overexpression has been reported in various cancer tissues such as prostate, colon, rectum, ovaries, breast, bladder, lung, kidney, skin and lymphoma (Zhou *et al.*, 2002). Currently, *AMACR* gene expression is used in prostate cancer diagnosis in combination with other biomarkers such as 34E12, CK5/6 or p63 (Amin *et al.*, 2014) or with prostate cancer antigen 3 (Ouyang *et al.*, 2009), as AMACR is most consistently upregulated in prostate cancer tissue (Thornburg *et al.*, 2006). A meta-analysis combining five case-control

studies found that *D175G* and *M9V* polymorphisms of the *AMACR* gene were associated with prostate cancer risk (Chen *et al.*, 2015).

A study conducted in Chicago using benign prostate tissue did not find an association between *AMACR* gene expression and dietary PA intake or serum PA concentration (Kataria *et al.*, 2015). Furthermore, *AMACR* polymorphism did not modify the associations between dietary red meat or dairy intake on prostate cancer in the population-based case-control study in the state of Washington (Wright *et al.*, 2011). AMACR is generally required to convert (R)-forms of fatty acids with chiral centers (usually methylated fatty acids of microbial origin) to (S)-forms of fatty acids and, thus, are essential for the degradation of bile acids, ibuprofen and other methylated fatty acids (Wanders, 2014). Therefore, elevated AMACR expression may be due to PPAR- α stimulation by compounds other than phytol or its metabolites, such as increased bile acid turnover, increased microbial fatty acid degradation, and low activity of AMACR or enzymes downstream required for methylated fatty acid degradation. Future research is needed to determine factors that elevate AMACR expression in some cancer tissues, but not in others. In summary, while the association of *AMACR* gene and protein expression with cancer risk and prognosis is well-established for prostate cancer, there is limited evidence that this association is modified by PA.

Conclusion

There is a gap in research and knowledge related to phytol and its metabolites, PA and PRA and carcinogenesis. Phytol and its metabolites are potential dietary compounds for cancer prevention because, based on some evidence in cell culture studies and limited evidence in animal models, they can alter pathways involved in carcinogenesis at physiological concentrations that can be achieved by dietary modifications. However, there are concerns regarding the cytotoxicity in non-cancer cells and various cell types. More research is needed to determine if and how normal and cancer tissues react similarly and differently to phytol and its metabolites. Animal feeding studies at phytol concentrations that can be achieved with dietary modification alone show promise; however, the number of studies is insufficient to draw conclusions. Moreover, studies in transgenic animal models report higher morbidity and mortality if PA accumulates in tissues. Previous studies on circulating and tissue PA and PRA concentrations suggest that little is known about the physiological processes involved in the control of PA and PRA concentrations in absorption, circulation and tissue distribution as well as the effect of the intestinal microbiome on circulating PA and PRA concentrations. Population-based studies that examine the association between phytol and its metabolites and cancer risk are few and largely limited to prostate cancer.

Based on the cytotoxicity and tissue accumulation data, other cancer sites such as brain, liver, kidney and blood are more sensitive to PA and, thus, have a greater chance to show associations in epidemiological studies than other cancer sites. In conclusion, phytol and its metabolites are potential natural agents for cancer prevention if current challenges including cytotoxicity in non-cancer cells and morbidity and mortality associated with PA accumulation in tissue can be mitigated.

Acknowledgements

This study was supported by the Knight Cancer Institute OHSU/OSU Cancer Prevention and Control Initiative (PHR030-PV07 to Y.T.). The funders had no role in the submission of the manuscript. We thank Ms. Alexandra Heisler and Dr. Jessica Keune for their assistance in editing the manuscript.

G.B. and Y.T. was responsible for the conceptualization of this review. G.B., Z.Z. and Y.T. conducted literature search and G.B. drafted the initial manuscript. R.K., M.G., J.S. and Y.T. provided critical comments to the manuscript. All authors reviewed, revised the manuscript and approved the final version.

Conflicts of Interest

There are no conflicts of interest.

References

- Allen NE, Grace PB, Ginn A, Travis RC, Roddam AW, Appleby PN, Key T (2008). Phytanic acid: measurement of plasma concentrations by gas-liquid chromatography-mass spectrometry analysis and associations with diet and other plasma fatty acids. *Br J Nutr* **99**:653–659.
- Amin MB, Epstein JI, Ulbright TM, Humphrey PA, Egevad L, Montironi R. *et al.* (2014). Best practices recommendations in the application of immunohistochemistry in urologic pathology: report from the International Society of Urological Pathology consensus conference. *Am J Surg Pathol* **38**:1017–1022.
- Siegel RL, Miller KD, Jemal A, (2018). Cancer statistics, 2018. *CA Cancer J Clin* **68**:7–30.
- Baldwin EJ, Gibberd FB, Harley C, Sidey MC, Feher MD, Wierzbicki AS (2010). The effectiveness of long-term dietary therapy in the treatment of adult Refsum disease. *J Neurol Neurosurg Psychiatry* **81**:954–957.
- Baxter JH (1968). Absorption of chlorophyll phytol in normal man and in patients with Refsum's disease. *J Lipid Res* **9**:636–641.
- Borges CG, Canani CR, Fernandes CG, Zanatta A, Seminotti B, Ribeiro CA, *et al.* (2015). Reactive nitrogen species mediate oxidative stress and astrogliosis provoked by in vivo administration of phytanic acid in cerebellum of adolescent rats: a potential contributing pathomechanism of cerebellar injury in peroxisomal disorders. *Neuroscience* **304**:122–132.
- Brown PJ, Mei G, Gibberd FB, Burston D, Mayne PD, McClinchy JE. *et al.* (1993). Diet and Refsums disease - the determination of phytanic acid and phytol in certain foods and the application of this knowledge to the choice of suitable convenience foods for patients with Refsums disease. *J Hum Nutr Diet* **6**:295–305.
- Che BN, Oksbjerg N, Hellgren LI, Nielsen JH, Young JF (2013). Phytanic acid stimulates glucose uptake in a model of skeletal muscles, the primary porcine myotubes. *Lipids Health Dis* **12**:14.
- Chen N, Wang JR, Huang L, Yang Y, Jiang YM, Guo XJ, *et al.* (2015). Significant association of alpha-methylacyl-coa racemase gene polymorphisms with susceptibility to prostate cancer: a meta-analysis. *Asian Pac J Cancer Prev* **16**:1857–1863.
- Coppack SW, Evans R, Gibberd FB, Clemens ME, Billimoria JD (1988). Can patients with Refsum's disease safely eat green vegetables? *Br Med J (Clin Res Ed)* **296**:828.
- Dhaunsi GS, Alsaedi M, Akhtar S (2016). Phytanic acid activates NADPH oxidase through transactivation of epidermal growth factor receptor in vascular smooth muscle cells. *Lipids Health Dis* **15**:105.
- Dhaunsi GS, Alsaedi M, Akhtar S (2017). Phytanic acid attenuates insulin-like growth factor-1 activity via nitric oxide-mediated γ -secretase activation in rat aortic smooth muscle cells: possible implications for pathogenesis of infantile Refsum disease. *Pediatr Res* **81**:531–536.
- Ellinghaus P, Wolfrum C, Assmann G, Spener F, Seedorf U (1999). Phytanic acid activates the peroxisome proliferator-activated receptor alpha (pparalpha) in sterol carrier protein 2-/- sterol carrier protein x-deficient mice. *J Biol Chem* **274**:2766–2772.
- Elmazar MM, El-Abhar HS, Schaalán MF, Farag NA (2013). Phytol/phytanic acid and insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. *PLoS One* **8**:e45638.
- Elmazar MM, Nau H (2004). Potentiation of the teratogenic effects induced by coadministration of retinoic acid or phytanic acid/phytol with synthetic retinoid receptor ligands. *Arch Toxicol* **78**:660–668.
- Gloerich J, van den Brink DM, Rüter JP, van Vlies N, Vaz FM, Wanders RJ, Ferdinandusse S (2007). Metabolism of phytol to phytanic acid in the mouse, and the role of pparalpha in its regulation. *J Lipid Res* **48**:77–85.
- Goto T, Takahashi N, Kato S, Egawa K, Ebisu S, Moriyama T, *et al.* (2005). Phytol directly activates peroxisome proliferator-activated receptor alpha (pparalpha) and regulates gene expression involved in lipid metabolism in pparalpha-expressing hepg2 hepatocytes. *Biochem Biophys Res Commun* **337**:440–445.
- Grings M, Tonin AM, Knebel LA, Zanatta A, Moura AP, Filho CS, *et al.* (2012). Phytanic acid disturbs mitochondrial homeostasis in heart of young rats: a possible pathomechanism of cardiomyopathy in Refsum disease. *Mol Cell Biochem* **366**:335–343.
- Gu H, Huang T, Shen Y, Liu Y, Zhou F, Jin Y, *et al.* (2018). Reactive oxygen species-mediated tumor microenvironment transformation: the mechanism of radioresistant gastric cancer. *Oxid Med Cell Longev* **2018**:5801209.
- Hansen RP (1965). 3,7,11,15-tetramethylhexadecanoic acid: its occurrence in the tissues of humans afflicted with Refsum's syndrome. *Biochim Biophys Acta* **106**:304–310.
- Hansen RP (1980). Phytol - its metabolic products and their distribution - a review. *New Zeal J Sci* **23**:259–275.
- Hashimoto T, Shimizu N, Kimura T, Takahashi Y, Ide T (2006). Polyunsaturated fats attenuate the dietary phytol-induced increase in hepatic fatty acid oxidation in mice. *J Nutr* **136**:882–886.
- Heim M, Johnson J, Boess F, Bendix I, Weber P, Hunziker W, Fluhmann B (2002). Phytanic acid, a natural peroxisome proliferator-activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. *FASEB J* **16**:718–720.
- Idel S, Ellinghaus P, Wolfrum C, Nofer JR, Gloerich J, Assmann G, *et al.* (2002). Branched chain fatty acids induce nitric oxide-dependent apoptosis in vascular smooth muscle cells. *J Biol Chem* **277**:49319–49325.
- Islam MT, de Alencar MV, da Conceição Machado K, de Conceição Machado K, de Carvalho Melo-Cavalcante AA, de Sousa DP, de Freitas RM (2015). Phytol in a pharma-medico-stance. *Chem Biol Interact* **240**:60–73.
- Kahlert S, Schönfeld P, Reiser G (2005). The Refsum disease marker phytanic acid, a branched chain fatty acid, affects ca2+ homeostasis and mitochondria, and reduces cell viability in rat hippocampal astrocytes. *Neurobiol Dis* **18**:110–118.
- Kataria Y, Wright M, Deaton RJ, Rueter EE, Rybicki BA, Moser AB, *et al.* (2015). Dietary influences on tissue concentrations of phytanic acid and AMACR expression in the benign human prostate. *Prostate* **75**:200–210.
- Kim CW, Lee HJ, Jung JH, Kim YH, Jung DB, Sohn EJ, *et al.* (2015). Activation of caspase-9/3 and inhibition of epithelial mesenchymal transition are critically involved in antitumor effect of phytol in hepatocellular carcinoma cells. *Phytother Res* **29**:1026–1031.
- Komiya T, Kyohkon M, Ohwaki S, Eto J, Katsuzaki H, Imai K, *et al.* (1999). Phytol induces programmed cell death in human lymphoid leukemia molt 4B cells. *Int J Mol Med* **4**:377–380.
- Kruska N, Reiser G (2011). Phytanic acid and pristanic acid, branched-chain fatty acids associated with Refsum disease and other inherited peroxisomal disorders, mediate intracellular ca2+ signaling through activation of free fatty acid receptor GPR40. *Neurobiol Dis* **43**:465–472.
- Leipnitz G, Amaral AU, Zanatta A, Seminotti B, Fernandes CG., Knebel LA, *et al.* (2010). Neurochemical evidence that phytanic acid induces oxidative damage and reduces the antioxidant defenses in cerebellum and cerebral cortex of rats. *Life Sci* **87**:275–280.
- Liska J, Macejova D, Ondkova S, Brtko J (2012). Morphology of 1-methyl-1-nitrosourea induced rat mammary tumours after treatment with precursor of

- phytanic acid or its combination with vitamin D analogue. *Endocr Regul* **46**:21–26.
- Lloyd MD, Darley DJ, Wierzbicki AS, Threadgill MD (2008). Alpha-methylacyl-coa racemase—an 'obscure' metabolic enzyme takes centre stage. *FEBS J* **275**:1089–1102.
- Lloyd MD, Yevglevskis M, Lee GL, Wood PJ, Threadgill MD, Woodman TJ (2013). A-methylacyl-coa racemase (AMACR): metabolic enzyme, drug metabolizer and cancer marker P504S. *Prog Lipid Res* **52**:220–230.
- Mackie JT, Atshaves BP, Payne HR, McIntosh AL, Schroeder F, Kier AB (2009). Phytol-induced hepatotoxicity in mice. *Toxicol Pathol* **37**:201–208.
- Malmendier CL, Jonniaux G, Voet W, Van Den Bergen CJ (1974). Fatty acid composition of tissues in Refsum's disease (herodopathia atactica polynuritiformis). Estimation of total phytanic acid accumulation. *Biomedicine* **20**:398–407.
- Mobley JA, Leav I, Zielie P, Wotkowitz C, Evans J, Lam YW, et al. (2003). Branched fatty acids in dairy and beef products markedly enhance alpha-methylacyl-coa racemase expression in prostate cancer cells in vitro. *Cancer Epidemiol Biomarkers Prev* **12**:775–783.
- Mönnig G, Wiekowski J, Kirchhof P, Stypmann J, Plenz G, Fabritz L, et al. (2004). Phytanic acid accumulation is associated with conduction delay and sudden cardiac death in sterol carrier protein-2/sterol carrier protein-x deficient mice. *J Cardiovasc Electrophysiol* **15**:1310–1316.
- Nagai K (2015). Phytanic acid induces neuro2a cell death via histone deacetylase activation and mitochondrial dysfunction. *Neurotoxicol Teratol* **48**:33–39.
- Ollberding NJ, Aschebrook-Kilfoy B, Caces DB, Wright ME, Weisenburger DD, Smith SM, Chiu BC (2013). Phytanic acid and the risk of non-hodgkin lymphoma. *Carcinogenesis* **34**:170–175.
- Ouyang B, Bracken B, Burke B, Chung E, Liang J, Ho SM (2009). A duplex quantitative polymerase chain reaction assay based on quantification of alpha-methylacyl-coa racemase transcripts and prostate cancer antigen 3 in urine sediments improved diagnostic accuracy for prostate cancer. *J Urol* **181**:2508–2513; discussion 2513.
- Pejin B, Kojic V, Bogdanovic G (2014). An insight into the cytotoxic activity of phytol at in vitro conditions. *Nat Prod Res* **28**:2053–2056.
- Peters JM, Shah YM, Gonzalez FJ (2012). The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. *Nat Rev Cancer* **12**:181–195.
- Price AJ, Allen NE, Appleby PN, Crowe FL, Jenab M, Rinaldi S, et al. (2010). Plasma phytanic acid concentration and risk of prostate cancer: results from the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr* **91**:1769–1776.
- Reiser G, Schönfeld P, Kahlert S (2006). Mechanism of toxicity of the branched-chain fatty acid phytanic acid, a marker of Refsum disease, in astrocytes involves mitochondrial impairment. *Int J Dev Neurosci* **24**:113–122.
- Roca-Saavedra P, Mariño-Lorenzo P, Miranda JM, Porto-Arias JJ, Lamas A, Vazquez BI, et al. (2017). Phytanic acid consumption and human health, risks, benefits and future trends: a review. *Food Chem* **221**:237–247.
- Röncke S, Kruska N, Kahlert S, Reiser G (2009). The influence of the branched-chain fatty acids pristanic acid and Refsum disease-associated phytanic acid on mitochondrial functions and calcium regulation of hippocampal neurons, astrocytes, and oligodendrocytes. *Neurobiol Dis* **36**:401–410.
- Samimi A, Kalantari H, Lorestani MZ, Shirzad R, Saki N (2018). Oxidative stress in normal hematopoietic stem cells and leukemia. *Apmis* **126**:284–294.
- Schluter A, Giralt M, Iglesias R, Villarroya F. (2002). Phytanic acid, but not pristanic acid, mediates the positive effects of phytol derivatives on brown adipocyte differentiation. *FEBS Lett* **517**:83–86.
- Schönfeld P, Kahlert S, Reiser G (2004). In brain mitochondria the branched-chain fatty acid phytanic acid impairs energy transduction and sensitizes for permeability transition. *Biochem J* **383**:121–128.
- Schönfeld P, Kahlert S, Reiser G (2006). A study of the cytotoxicity of branched-chain phytanic acid with mitochondria and rat brain astrocytes. *Exp Gerontol* **41**:688–696.
- Schönfeld P, Reiser G (2006). Rotenone-like action of the branched-chain phytanic acid induces oxidative stress in mitochondria. *J Biol Chem* **281**:7136–7142.
- Schönfeld P, Wojtczak L (2007). Fatty acids decrease mitochondrial generation of reactive oxygen species at the reverse electron transport but increase it at the forward transport. *Biochim Biophys Acta* **1767**:1032–1040.
- Selkälä EM, Nair RR, Schmitz W, Kvist AP, Baes M, Hiltunen JK, Autio KJ (2015). Phytol is lethal for AMACR-deficient mice. *Biochim Biophys Acta* **1851**:1394–1405.
- Silva RO, Sousa FB, Damasceno SR, Carvalho NS, Silva VG, Oliveira FR, et al. (2014). Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. *Fundam Clin Pharmacol* **28**:455–464.
- Skrbic TR, Cumings JN (1969). Phytanic acid in tissue lipids in Refsum's disease. *Clin Chim Acta* **23**:17–21.
- Steinberg D (1989). Refsum disease. In: *Metabolic Basis of Inherited Disease*. Scriver CR, Beaudet AA, Sly WS, Valle D, editors. New York, New York: McGraw Hill; pp. 1533–1550.
- Steinberg D, Vroom FQ, Engel WK, Cammermeyer J, Mize CE, Avigan J (1967). Refsum's disease—a recently characterized lipidosis involving the nervous system. Combined clinical staff conference at the National Institutes of Health. *Ann Intern Med* **66**:365–395.
- Tang XH, Suh MJ, Li R, Gudas LJ (2007). Cell proliferation inhibition and alterations in retinol esterification induced by phytanic acid and docosahexaenoic acid *J Lipid Res* **48**:165–176.
- Thakor P, Subramanian RB, Thakkar SS, Ray A, Thakkar VR (2017). Phytol induces ROS mediated apoptosis by induction of caspase 9 and 3 through activation of TRAIL, FAS and TNF receptors and inhibits tumor progression factor glucose 6 phosphate dehydrogenase in lung carcinoma cell line (A549). *Biomed Pharmacother* **92**:491–500.
- Thornburg T, Turner AR, Chen YQ, Vitolins M, Chang B, Xu J (2006). Phytanic acid, AMACR and prostate cancer risk. *Future Oncol* **2**:213–223.
- van den Brink DM, van Miert JN, Dacremont G, Rontani JF, Wanders RJ (2005). Characterization of the final step in the conversion of phytol into phytanic acid. *J Biol Chem* **280**:26838–26844.
- Verhoeven NM, Jakobs C (2001). Human metabolism of phytanic acid and pristanic acid. *Prog Lipid Res* **40**:453–466.
- Vetter W, Schroder M (2010). Concentrations of phytanic acid and pristanic acid are higher in organic than in conventional dairy products from the German market. *Food Chem* **119**:746–752.
- Wanders RJ (2014). Metabolic functions of peroxisomes in health and disease. *Biochimie* **98**:36–44.
- Wang J, Hu X, Ai W, Zhang F, Yang K, Wang L, et al. (2017). Phytol increases adipocyte number and glucose tolerance through activation of PI3K/akt signaling pathway in mice fed high-fat and high-fructose diet. *Biochem Biophys Res Commun* **489**:432–438.
- Werner LB, Hellgren LI, Raff M, Jensen SK, Petersen RA, Drachmann T, Tholstrup T (2011). Effect of dairy fat on plasma phytanic acid in healthy volunteers—a randomized controlled study. *Lipids Health Dis* **10**:95.
- Werner LB, Hellgren LI, Raff M, Jensen SK, Petersen RA, Drachmann T, Tholstrup T (2013). Effects of butter from mountain-pasture grazing cows on risk markers of the metabolic syndrome compared with conventional danish butter: a randomized controlled study. *Lipids Health Dis* **12**:99.
- World Cancer Research Fund and American Institute for Cancer Research (2018). *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*. Washington, DC: American Association for Cancer Research.
- Wright ME, Albanes D, Moser AB, Weinstein SJ, Snyder K, Männistö S, Gann PH (2014). Serum phytanic and pristanic acid levels and prostate cancer risk in Finnish smokers. *Cancer Med* **3**:1562–1569.
- Wright ME, Bowen P, Virtamo J, Albanes D, Gann PH (2012). Estimated phytanic acid intake and prostate cancer risk: a prospective cohort study. *Int J Cancer* **131**:1396–1406.
- Wright JL, Neuhaus ML, Lin DW, Kwon EM, Feng Z, Ostrander EA, et al. (2011). AMACR polymorphisms, dietary intake of red meat and dairy and prostate cancer risk. *Prostate* **71**:498–506.
- Xu J, Thornburg T, Turner AR, Vitolins M, Case D, Shadle J, et al. (2005). Serum levels of phytanic acid are associated with prostate cancer risk. *Prostate* **63**:209–214.
- Yang K, Wang L, Zhou G, Lin X, Peng J, Wang L, et al. (2017). Phytol promotes the formation of slow-twitch muscle fibers through PGC-1 α /mirna but not mitochondrial oxidation. *J Agric Food Chem* **65**:5916–5925.
- Zhou M, Chinnaiyan AM, Kleer C G, Lucas PC, Rubin MA (2002). Alpha-methylacyl-coa racemase: a novel tumor marker over-expressed in several human cancers and their precursor lesions. *Am J Surg Pathol* **26**:926–931.
- Zomer AW, Jansen GA, Van Der Burg B, Verhoeven NM, Jakobs C, Van Der Saag PT, et al. (2000). Phytanoyl-coa hydroxylase activity is induced by phytanic acid. *Eur J Biochem* **267**:4063–4067.