A review of the functional effects of pine nut oil, pinolenic acid and its derivative eicosatrienoic acid and their potential health benefits

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Abbreviations used: AA, arachidonic acid; ACADL, long chain acyl coenzyme A dehydrogenase; ACSL3, long chain acyl coenzyme A synthase 3; ALA, alpha-linolenic acid; apo, apolipoprotein; ATGL, adipose triglyceride lipase; CCK, cholecystokinin; COX, cyclooxygenase; CPT, carnitine palmitoyl transferase; DGLA, Dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatrienoic acid (all *cis*-7,-11,-14 20:3); FA, fatty acid; FAS, fatty acid synthase; FFAR, free fatty acid receptor; GLA, gamma-linolenic acid; GLP, glucagon like peptide; HFD, high fat diet; HMGCR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; IL, interleukin; iNOS, inducible nitric oxide synthase; LA, linoleic acid; LDL, low density lipoprotein; LDLr, low density lipoprotein receptor; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MCP, monocyte chemoattractant protein; NEFA, non-esterified fatty acid; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMIFA, non-methylene-interrupted fatty acid; NO, nitric oxide; PG, prostaglandin; PLA, pinolenic acid; PNO, pine nut oil; PPAR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acid; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; SBO, soybean oil; SCD1, stearoyl-CoA desaturase 1; sICAM-1, soluble intercellular cell adhesion molecule-1;SIRT, sirtuin; SREBP1c, sterol regulatory element-binding protein 1; sVCAM-1, soluble vascular cell-adhesion molecule-1; TAG, triacylglycerol; TNF, tumour necrosis factor; TPA, 12-O-tetradecanoylphorbol-13-acetate; VLDL, very low density lipoprotein

Abstract

Pine nut oil (PNO) is rich in a variety of unusual delta-5-non-methylene-interrupted fatty acids (NMIFAs), including pinolenic acid (PLA; all *cis*-5,-9,-12 18:3) which typically comprises 14 to 19% of total fatty acids. PLA has been shown to be metabolised to eicosatrienoic acid (ETA; all *cis*-7,-11,-14 20:3) in various cells and tissues. Here we review the literature on PNO, PLA and its metabolite ETA in the context of human health applications. PNO and PLA have a range of favourable effects on body weight as well as fat deposition through increased energy expenditure (fatty acid oxidation) and decreased food energy intake (reduced appetite). PNO and PLA improve blood and hepatic lipids in animal models and insulin sensitivity *in vitro* and reduce inflammation and modulate immune function *in vitro* and in animal models. The few studies which have examined effects of ETA indicate it has anti-inflammatory properties. Another NMIFA from PNO, sciadonic acid (all *cis*-5,-11,-14 20:3), has generally similar properties to PLA where these have been investigated. There is potential for human health benefits from PNO, its constituent NMIFA PLA and the PLA derivative ETA. However further studies are needed to explore the effects in humans.

Introduction

Pine nuts come from the pinus genus and there are 29 edible species currently known [1]. The most commonly consumed pine nuts are from the species *Pinus koraiensis* (Korean pine), *P. sibirica* (Siberian pine), *P. pinea* (stone pine) and *P. gerardiana* (chilgoza pine) [2]. Pine nuts are both eaten raw and used in cooking in various parts of the world. The nuts can also be used to produce an oil. This pine nut oil (PNO) is rich in a variety of unusual delta-5-non-methylene-interrupted fatty acids (NMIFAs), which differ from the structure of other polyunsaturated fatty acids (PUFAs) and are characteristic of the seeds of gymnosperms. These fatty acids (FAs) include pinolenic acid (PLA; all *cis*-5,-9,-12 18:3), sciadonic acid (all *cis*-5,-11,-14 20:3) and taxoleic acid (all *cis*-5,-9 18:2) [2]. PLA is the most abundant of these NMIFAs in PNO comprising 14-19% of the total FAs present in most PNOs [3]. PLA has been reported to be produced from linoleic acid (LA; all *cis*-9,-12 18:2) by a species-specific delta-5 desaturase [4] (Figure 1). Its unique structure distinguishes it from other omega-6 PUFAs and it has been reported to have bioactivity including exerting anti-inflammatory actions [5-11]. Furthermore, PLA is known to be metabolised to another unique FA, delta-7 eicosatrienoic acid (ETA) (all *cis*-7,-11,-14 20:3), in various species [8, 9, 11-13] (Figure 1). This FA has also been shown to have anti-inflammatory properties [7-9, 11]. However, the functional effects and underlying mechanisms of action of these unusual FAs are still poorly understood. Biological effects of PLA may be important because it may offer a sustainable terrestrial alternative to long chain omega-3 PUFAs, which have been shown to have a number of health benefits [14, 15] including reducing inflammation [16, 17]. However, the main source of bioactive omega-3 PUFAs for the human diet is seafood, especially fatty fish, and this is not a sustainable source nor one that is free from risk of contamination. Various studies have examined the potential of PNOs and PLA to beneficially modify different health-related outcomes. Very few studies have assessed the effects of ETA; these have reported on inflammatory outcomes only. The aim of this review is to summarise and discuss the main outcomes of these studies of PNO, PLA and ETA; studies of sciadonic acid are also described.

Pine nut oil composition and consumption

Pine nut and PNO consumption has increased in recent years leading to growth in worldwide production [1], with China, North Korea, Russia (Siberia), Pakistan and Afghanistan being the largest exporters. Korea, USA and Russia are the largest consumers of pine nuts and PNO [1]. Pine nuts are consumed as a raw product as well as being used in cooking along with PNO. Oil yield is reported to be between 45 and 65 g per 100 g of pine nuts and is dependent on the type of extraction (cold pressing or solvent) [18-20]. Oil derived from *P. sibirica* nuts is reported to be composed of 99.4 wt%

nonpolar lipids and 0.6 wt% polar lipids [20]. Triacylglycerols (TAGs) are a major constituent of the nonpolar lipids; and Acheampong *et al*. identified 58 different TAG species in the oil of *P. koraiensis* [21]. Having a high content of TAGs means pine nuts and PNO naturally contain high levels of FAs (esterified into TAGs). The FAs found in pine nuts are typically around 50% PUFAs, around 40% monounsaturated saturated fatty acids and around 10% saturated fatty acids [19]. LA is the most common FA and the dominant PUFA in PNO, in the range of 40-60% of total FAs [2, 3, 18, 20, 22-24] (Table 1). The high content of LA in PNO is similar to what is seen in many other seed oils. The second most abundant FA and the major monounsaturated fatty acid is oleic acid (*cis*-9 18:1) at 12-30% of total FAs (Table 1). PLA is the most prevalent NMIFA, typically comprising 14-19% of total FAs in *P. koraiensis* and *P. sibirica* (Table 1). Taxoleic and sciadonic acids are reported to comprise approximately 2% and 1 to 1.2% of total FAs in *P. koraiensis* and *P. sibirica* [25]. ETA is only found in small quantities (1-3%) in PNOs [26, 27]. Table 1 summarises the FA composition of *P. sibirica* and *P. koraiensis* oils as reported in several studies. Matthaus *et al*. [25] report that the fatty acid composition, including the contents of PLA, taxoleic and sciadonic acids, in the nut oils of *P. aristate*, *P. armandii*, *P. cembra*, *P. echinata*, *P. jeffreyi*, *P. massoniana*, *P. monticola*, *P. mugo*, *P. pinaster*, *P. pumila*, *P. resinosa*, *P. roxburghii*, *P. sylvestris*, *P. tabuliformis* and *P. yunnanensis* is very similar to those of *P. subirica* and *P. koraiensis*. In contrast, the nut oils from *P. eldarica*, *P. excelsa*, *P. pinea* and *P. torreyana* are much lower in PLA (very low in the case in *P. pinea*) and are higher in LA or oleic acid [25]. PNO also contains lipid-soluble antioxidants, including tocopherols, as well as phytosterols and squalene.

Biosynthesis and metabolism of pinolenic acid

In mammals, gamma-linolenic acid (GLA; all *cis*-6,-9,-12 18:3) is synthesised from LA by delta-6-desaturase, and is further elongated to dihomo-gamma-linolenic acid (DGLA; all *cis*-8,-11,-14 20:3) by fatty acid elongase 5 (Figure 1). Similarities in the structure of GLA and PLA indicate that they may have comparable pathways of biosynthesis and further metabolism. It is suggested that PLA is synthesised from LA by the action of a conifer specific delta-5 desaturase [28]. One study has examined delta-5 desaturase genes which encode enzymes potentially involved in the conversion of LA to PLA in the microalgae *Chlamydomonas reinhardtii,* which also accumulates PLA in betaine lipid [4]. An isolated cDNA clone, named CrDES, resembling the delta-5 desaturase gene from *Mortierella alpine* was shown to synthesise PLA from LA when expressed in the yeast *Pichia pastoris*. Furthermore, the conserved N-terminal cytochrome *b5* domain and glutamine residue in the third histidine box in the amino acid sequence of CrDES suggests front-end desaturation of LA [4].

PLA has been shown to be further elongated by fatty acid elongase 5 to ETA [11] (Figure 1). Only a small proportion of PLA has been shown to be converted to ETA in pine nuts, therefore accounting for the small quantities of ETA found in PNOs [26]. Consequently, limited availability of ETA has restricted the exploration of the functional properties of this unusual FA. However, several studies suggest greater conversion rates of PLA to ETA in cultured mammalian cell lines [6, 8, 9, 11, 13, 29]. These studies reported high proportions (up to 29%) of ETA in cellular phospholipids after PLA pre-treatment of murine RAW264.7 macrophages [6], murine microglial BV-2 cells [8], human hepatic carcinoma HepG2 cells [13], human breast cancer MDA-MB-231 cells [29], human monocytic THP-1 cells [9] and the EA.hy296 cell line which is derived from human umbilical vein endothelial cells [11]. Similarly, ETA was detected in membrane phospholipids following incubation of rat liver microsomes with PLA [12]. In contrast, only very small quantities of ETA were found in the phospholipids of tissues and organs in rats fed PNO [30]. This may be due to PUFA β-oxidation [31], although an alternative explanation is limited conversion of PLA to ETA *in vivo*. Further studies are needed to verify the precise pathway of synthesis and further metabolism of PLA.

Incorporation and metabolism of PLA in mammalian cells and tissues

Several studies in isolated cells and in experimental animals have examined the cell and tissue incorporation and metabolism of PLA. Metabolism of PLA could be important to the functionality of this FA since it may be converted to more bioactive fatty acids such as ETA. Furthermore, changes in overall cell membrane FA composition after treatment with PLA could play a role in determining the overall effects of PLA, either through changes in membrane structure and function or through changes in levels of other bioactive FAs such as arachidonic acid (AA; all *cis*-5,-8,-11,-14 20:4).

Several studies have examined changes in liver phospholipid FAs after feeding rats diets containing PNO or PLA. Sugano *et al*. reported appearance of PLA in liver phospholipids of male Wistar rats after being fed a diet containing PNO (from *P. koraiensis*) [32]. Incorporation of PLA was associated with a decrease in LA compared to safflower and flaxseed oil diets. However, concentrations of AA were seen to increase in liver phospholipids after PNO feeding compared to the other diets. Similar observations regarding LA were made by Matsuo *et al*. in rats fed PNO containing diets [33]. PLA was shown to be incorporated into both liver phosphatidylcholine and phosphatidylethanolamine; these changes were associated with decreases in LA but in this study there were no changes in AA concentrations. Tanaka *et al.* also describe changes in liver FAs in rats fed a PNO diet [34]: PLA was shown to increase in rat liver phosphatidylcholine, alongside a decrease in LA and no change in AA. Asset *et al*. also reported PLA incorporation into liver phospholipids after rats were fed diets containing PNO from either *P. pinaster* and *P. koraiensis* [22]. Both diets led to decreases in LA in

liver phospholipids. Thus, several rat feeding studies report incorporation of PLA from dietary PNO into liver phospholipids with an associated decrease in LA [22, 32-34]. Effects on liver AA are not consistent with one study reporting an increase [32] and two no change [33, 34]. These differences might reflect the amount of PNO and PLA being fed and the precise FA composition of the comparator oil and diet.

Pasquier *et al*. described changes in FA composition of whole blood, liver and breast tissue of pregnant rats fed a diet containing PNO from *P. pinaster* [30]. They reported incorporation of PLA in blood and tissues with small increases in ETA. Concentrations of AA and LA were unchanged in blood, total liver lipids and liver phospholipids. However, LA concentrations were shown to be increased in breast tissue total lipids, again with no changes in AA. Furthermore, PLA and ETA were both shown to be incorporated into rat foetal total body fat and brain phospholipids. AA was shown to increase in foetal brain total lipids, with no changes in LA. However, concentrations of AA and LA were unchanged in the total body fat of foetuses.

PLA incorporation and metabolism have been studied in cell models. Chuang *et al*. described changes in FAs in RAW264.7 cells after incubation with PLA at 10, 25, 50 and 100 μM [6]. They showed concentration-dependent increases in PLA and its metabolites in parallel with concentration-dependent decreases in LA and AA (Figure 2). Similarly, Chen *et al*. examined metabolism of PLA in BV-2 cells [8]. Cells incubated with 50 μM PLA for 24 hr showed significant increases in PLA and its elongation product ETA in cellular phospholipids, with decreases in proportions of both LA and AA. Changes in the FA profile of EA.hy296 cells after treatment with PLA (10 and 50 μM) have been reported: PLA was incorporated and there was appearance of ETA [11]. These changes were associated with decreases in both LA and AA (previously unpublished data) (Figure 2). Another study reported incorporation of PLA into human breast cancer MDA-MB-231 cellular phospholipids after incubation with PLA (50 μM) [29]. ETA was also shown to increase in cellular phospholipids alongside decreases in LA and AA. Tanaka *et al.* reported changes in FAs after incubation of HepG2 cells with PLA (100 μM) [13]. PLA was incorporated into cellular phosphatidylinositol together with an increase in ETA and a decrease in AA. Chen *et al*. reported that PLA was incorporated into THP-1 cellular phospholipids in a concentration-dependent manner along with an increase in ETA; the percentage of AA in cellular phospholipids decreased while LA increased [9]. Thus, studies with isolated cells exposed to PLA are consistent with feeding studies in rats with PNO in that incorporation of PLA is most often associated with decreased LA [6, 8, 11, 13, 22, 29, 32-34], although one cell culture study reported increased LA [9]. Cell culture studies consistently report decreased AA after PLA exposure [6, 8, 9, 11, 13, 29]. This is different from what is reported in feeding studies with PNO in rats [32-34]. This difference may reflect the greater exposure to PLA in

cell culture compared to through the diet and also the conversion of PLA to ETA in cell culture (see below). ETA as a 20-carbon PUFA may compete effectively with AA for incorporation into cell phospholipids.

Cell culture studies show that PLA exposure increases both PLA and ETA in cell lipids. Few studies have described FA profile changes after treatment with ETA itself. Chen *et al*. reported that incubation of BV-2 cells with ETA (50 μM) led to significant increases in ETA as well as the appearance of PLA [8]. Treatment with ETA also led to significant decreases in LA and AA in phospholipids [8]. Incorporation of ETA into EA.hy296 cells [11] was linked to small decreases in LA and AA (Baker *et al*., unpublished data). Changes in AA seen with incorporation of PLA and ETA may be of importance as AA is the precursor to various inflammatory lipid mediators, which are generated through enzymatic activity of cyclooxygenase (COX) and lipoxygenase enzymes, including prostaglandin (PG) E_2 [35]. In accordance with this, several studies have reported reduced PGE_2 production with PLA and ETA (see later section on inflammation). Taken together, these studies suggest PLA is incorporated and metabolised (to ETA) in various cells. These changes are often associated with reduced amounts of LA and AA and this may be one mechanism of action of PLA and ETA.

Effects of pine nut oil and pinolenic acid on body weight and appetite

Effects of PNO and PLA on body weight and appetite are the most well studied of all biological actions [36-42] (Table 2). Together these studies indicate a beneficial effect of PNO on both appetite control and weight gain.

Several animal studies have reported effects of PNO on food intake and body weight. Ferramosca *et al*. studied the effect of an extract of PNO (from *P. koraiensis*) in male ICR mice [36]. They reported that this preparation significantly reduced body weight gain (-37%) and liver weight (-13%) compared to maize oil-supplemented mice. They also reported a decrease in the feed conversion efficiency (-36%) in mice fed PNO [36]. This would suggest either decreased absorption or increased oxidation of dietary energy sources. Park *et al*. reported lower weight gain in C57BL/6 mice fed a high fat diet (HFD) containing PNO compared to a HFD containing soybean oil (SBO) [39]. They observed reduced food intake equating to a 7% reduction in energy consumption in mice receiving PNO compared to SBO and attributed reduced weight gain (-17%) to a decrease in white adipose tissue (between -17% and -20%). Thus, PNO may decrease appetite, with the reduced food intake resulting in less adipose deposition and lower weight gain. More recent studies also described beneficial effects of PNO on body weight in mice. Zhu *et al*. describe reduced weight (-9%), weight

gain (-15%) and white adipose tissue (-20%) in mice fed a HFD containing PNO, compared to SBO [42]. Similarly, Park *et al*. reported that a HFD containing PNO led to a decrease in body weight gain (-10%) and white adipose tissue (-18%) compared to a HFD containing SBO [41]. Levels of sirtuin (SIRT) 3 in the white adipose tissue of mice fed the HFD containing PNO, but not that containing SBO, were shown to be similar to those of lean mice [41]. SIRT3 is involved in stress resistance and metabolic regulation and has been reported to be upregulated by caloric restriction. Similarly, Le *et al*. demonstrated less weight gain in mice fed a HFD containing PNO compared to SBO [40]. They observed upregulation of the expression of genes related to FA oxidation, mitochondrial oxidation and skeletal muscle oxidative metabolism in mice fed PNO compared to SBO. Genes specific to type-1 skeletal muscle, which has high oxidative capacity, were also increased in the PNO HFD group [40]. These studies suggest that PNO might increase fatty acid oxidation which could also contribute to less adipose tissue and body weight gain. There was also an increase in the expression of genes and proteins involved in the upregulation of thermogenesis, including uncoupling protein-1, in brown adipose tissue of mice fed PNO compared to SBO [40]. Various long chain PUFAs have been shown to act as ligands for peroxisome proliferator activated receptors α and δ (PPARα and PPARδ), transcription factors involved in upregulating oxidative lipid metabolism [43]. PLA has been reported to activate both PPARα and δ [10, 40] suggesting that this may be the mechanism for increased fatty acid oxidation seen with PNO feeding in rodents.

Effects of PNO related to appetite and weight gain have also been seen in *in vitro* and human studies. Pasman *et al*. examined both the *in vitro* and *in vivo* effects of a PNO extract on gut hormones [38]; they investigated effects on cholecystokinin (CCK)-8, synthesised in duodenal enteroendocrine cells, which promotes digestion of protein and lipid [44] and glucagon like peptide (GLP)-1, produced in the ileum in response to carbohydrate and fat [45]. Both hormones are responsible for inducing satiety and appetite suppression [46, 47]. They described enhanced secretion (by 90%) of CCK-8 by STC-1 cells (murine intestinal neuroendocrine tumour cells) after treatment with 50 µM PNO extract. Furthermore, a Korean PNO extract was shown to increase postprandial CCK-8 and GLP-1 levels in overweight and post-menopausal women [38]. Participants received capsules providing 3 g non-esterified fatty acids (NEFAs) prepared by hydrolysis of Korean PNO or 3 g TAGs isolated from Korean PNO or 3 g placebo (olive oil) in combination with a light breakfast. CCK-8 levels were higher 30 min after PNO NEFAs and 60 min after PNO TAGs compared to placebo. GLP-1 was higher 60 min after PNO NEFAs. After 4 hours, total plasma CCK-8 levels were higher after both PNO NEFA and TAG supplements (60% and 22% respectively) compared to placebo. Total plasma GLP-1 levels were shown to be increased by PNO NEFAs alone (25%). The authors also reported lower appetite sensation in those who received PNO NEFAs relative to placebo (-36%),

although the data were not reported. Hughes *et al*. examined the effects of a PNO extract in overweight female participants [37]. They reported a 9% decrease in food intake at an *ad libitum* lunch buffet in participants who had consumed 2 g PNO NEFAs 30 mins prior to the lunch compared to the control group (olive oil). However, they saw no changes in participants who had consumed the PNO extract in TAG form. They suggest this may be due to insufficient time between the intake of the TAGs and the *ad libitum* lunch for lipase action to have converted sufficient TAG to NEFAs.

Taken together, these results suggest PNO, or likely the unusual FA in PNO, PLA, has a range of effects that result in both an increase in energy expenditure (fatty acid oxidation) and a decrease in food energy intake through reduced appetite. These can then lead to less weight gain, less adipose tissue deposition, less ectopic fat deposition and an overall healthier metabolic state.

Effects of pine nut oil and pinolenic acid on blood and hepatic lipids

A number of studies have evaluated the effects of PNO or PLA on blood lipids in animal models [18, 22, 32, 36, 41, 42, 48] (Table 3). Many of these studies involved feeding high fat diets. An early study found no difference in blood (or hepatic) lipids between rats fed a diet with PNO compared to those fed a diet with other plant oils [32]. Asset *et al*. found no effect of *P. koraiensis* oil on blood lipids in Wistar rats compared to a mixture of plant oils, but *P. pinaster* oil resulted in lower serum TAG, very low density lipoprotein (VLDL)-TAG and VLDL-cholesterol concentrations than the oil mix [22]. The authors suggested the effect was more pronounced for *P. pinaster* compared to *P. koraiensis* due to higher quantities of sciadonic acid in oil from *P. pinaster.* Ferramosca *et al.* also described lower plasma TAG and total cholesterol in mice fed a PNO extract compared to those fed maize oil [36], while Chen *et al*. describe lower serum total TAG levels in Wistar rats fed a diet with an intermediate level of PNO compared to rats fed with lard [18]. In accordance with the earlier findings of Asset *et al*., Park *et al*. reported that a high fat diet with some PNO resulted in lower hepatic TAG than a high fat diet with some SBO [41]. These studies suggest that PNO lowers blood and hepatic lipids.

The mechanisms involved in lipid lowering with PNO have been further investigated through molecular studies on tissues collected from experimental animals as well in HepG2 cells. Park *et al.* showed that PNO consumption was linked to increased expression of mRNA for *ACADL*, the gene that encodes long chain acyl coenzyme A dehydrogenase, an enzyme involved in mitochondrial FA β-oxidation [41]. Zhu *et al.* examined the effects of a HFD containing PNO on various genes involved in hepatic TAG metabolism, mitochondrial activity and FA oxidation in C57BL/6 mice [42]. They reported lowered mRNA expression for both *CD36* and *apolipoprotein* (apo) *A4* in the intestine, coupled with higher hepatic mRNA expression for *ACADL*, adipose triglyceride lipase (*ATGL*),

carnitine palmitoyl transferase (*CPT*) 1A, and *apo B100* in PNO fed mice. This suggests PNO consumption may decrease intestinal FA uptake and chylomicron assembly, whilst increasing hepatic mitochondrial FA oxidation. Furthermore, studies in HepG2 cells indicate PNO and its constituent PLA may play a role in increasing internalisation of low density lipoprotein (LDL) [48]. The authors suggested PLA may have LDL-lowering properties by enhancing hepatic LDL uptake. Another study by Lee *et al*. examined the effects of PLA (50 μM) in HepG2 cells on mRNA levels of genes related to FA biosynthesis (fatty acid synthase (*FAS*), long chain acyl coenzyme A synthase 3 (*ACSL3*), sterol regulatory element-binding protein 1 (*SREBP1c*), stearoyl-CoA desaturase 1 (*SCD1*)), cholesterol biosynthesis (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*HMGCR*)) and lipoprotein uptake (low density lipoprotein receptor (*LDLr*)) [49]. PLA treatment significantly decreased mRNA levels of *FAS*, *ACSL3*, *SREBP1c* and *SCD1* compared to control. This would suggest that PLA could reduce FA biosynthesis. In addition, the mRNA levels of *HMGCR* were significantly lower after PLA treatment relative to the control group [49], suggesting reduced cholesterol biosynthesis. In contrast to findings on enhanced LDL uptake, the study found that PLA reduced *LDLr* mRNA expression. A recent study described lowered lipid accumulation, with decreases in both cellular TAG and total cholesterol after PLA treatment (25 μM) in oleic acid-stimulated HepG2 cells [10]. Furthermore, PLA was shown to decrease lipogenesis in oleic acid-stimulated HepG2 cells through the 5' adenosine monophosphate-activated protein kinase/SIRT1 pathway. The authors reported decreases in both protein and mRNA concentrations of FAS, SREBP1c and SCD1, as well as an increase in PPARα protein concentration after PLA treatment [10]. Together, these studies suggest PLA may improve hepatic lipid metabolism through reducing expression of genes related to lipid (FA and cholesterol) synthesis and enhancing expression of genes related to fatty acid oxidation (Figure 3). These hepatic effects would impact on blood lipid concentrations. There are no studies investigating the effects of PNO or PLA on blood lipids in humans.

Effects of pine nut oil and pinolenic acid on insulin sensitivity

Type 2 diabetes is a metabolic disease which involves insulin resistance [50]. FAs have been shown to play an important role in the activation of free fatty acid receptors (FFARs), including FFAR1, FFAR2, FFAR3 and FFAR4 which are involved in the insulin response [51]. FFAR1 is expressed in pancreatic β-cells and enhances glucose-stimulated insulin secretion in response to various medium- and long-chain FAs [52]. FFAR4 is expressed in various tissues including adipose and its activation is associated with improved insulin sensitivity [53]. Christensen *et al*. described PLA as a relatively potent and efficacious dual FFAR1/FFAR4 agonist [52]. Furthermore, mice administered both PNO or PLA and subjected to an acute glucose tolerance test, had significantly improved glucose tolerance

compared to mice fed maize oil, with PLA having greater effect than PNO [52] (Table 4). This indicates PLA activation of both FFAR1 and FFAR4 may enhance insulin secretion from β-cells and insulin action in target tissues so promoting efficient glucose disposal.

Effects of pine nut oil, pinolenic acid and eicosatrienoic acid on inflammation

Dietary FAs have been shown to modulate inflammation via a variety of mechanisms including changes in membrane structure and function and modulation of the production of lipid mediators [16, 17]. It is generally agreed that mediators produced from omega-6 FAs are pro-inflammatory, whereas omega-3 FAs have been shown to act as substrates for weak inflammatory mediators as well as potent inflammation resolving mediators [16, 54]. FAs also affect production of protein mediators of inflammation including various cytokines and chemokines [17, 54]. The effects of omega-3 FAs on protein mediators of inflammation appear to involve inhibition of activation of pro-inflammatory transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [17]. Some enzymes including COX-2 and inducible nitric oxide synthase (iNOS) are also targets for NF-κB. Several studies suggest PLA and ETA may also reduce production of various inflammatory mediators.

Effects of PNO and PLA on inflammation

In vitro studies with cell lines consistently show that PLA has anti-inflammatory effects [5-10, 29] (Table 5). Chen *et al*. describe reduced proinflammatory mediator production in lipopolysaccharide (LPS)-stimulated murine microglial BV-2 cells pre-treated with PLA (50 μM) [8]. They reported decreased interleukin (IL)-6, nitric oxide (NO) and tumour necrosis factor (TNF)-α concentrations (by 41, 74 and 27% respectively) with PLA compared to control cultures. They also reported a significant decrease in PGE_2 production, although they did not specify details of this effect [8]. Parallel observations were made after PLA treatment (50 μM) in LPS-stimulated rat primary peritoneal macrophages, with decreased PGE_2 and NO production (details were not given) [8]. Another study reported decreased NO production by HepG2 cells after treatment with PLA (25 μM) [10]. As mentioned above, increases in both NO and PGE_2 in LPS-stimulated macrophage type cells are driven through NF-κB activation. This leads to increased expression of iNOS (responsible for NO production) and COX-2 (responsible for PGE_2 production). Chen *et al*. described reduced levels of iNOS and COX-2 protein after PLA treatment in LPS-stimulated BV-2 cells [8]. Similarly, another study described reduced PGE_2 production in (TPA)-stimulated MDA-MB-231 cells after treatment with PLA at 50 and

100 μM. Incubation with PLA was also shown to decrease COX-2 protein and mRNA levels [29]. Likewise Huang *et al*. described decreased COX-2 and PGE_2 in LPS-stimulated RAW264.7 and rat primary peritoneal macrophages after PLA treatment (50 μM) [7]. PLA treatment was also shown to reduce NF-κB activity in LPS-stimulated RAW264.7 cells. More recently Baker *et al.* reported reduced TNF-α-stimulated NF-κB activity (phosphorylation of the p65 subunit) in EA.hy296 cells after PLA treatment (50 μM) [11]. PLA treatment was also shown to decrease soluble ICAM-1 (sICAM-1), monocyte chemoattractant protein (MCP)-1 and regulated upon activation, normal T cell expressed and presumably secreted (RANTES) production by EA.hy296 cells in response to TNF-α, as well as to decrease adhesion of human THP-1 macrophages to EA.hy296 cell monolayers. Chen *et al*. examined the effects of PLA in THP-1 macrophages [9]. They describe reduced production of IL-6 (46%), TNF-α (18%) and PGE_2 (87%), as well as reduced expression of COX-2 in response to LPS. Together these studies indicate a role for PLA in modulating NF-κB activity with knock on effects on multiple inflammatory mediators. Figure 4 depicts a summary of the proposed mechanisms by which PNO and PLA may affect inflammation. In this respect the actions of PLA seem very similar to those of EPA and DHA [16, 17]. However, an earlier study by Chuang *et al.* reported both a decrease in PGE_2 production and a small increase in COX-2 levels in RAW264.7 murine macrophage cells after treatment with 50 μM PLA [6]. This suggests lowered PGE_2 production may be due to competition of PLA or its metabolite ETA with AA as a substrate for COX-2.

Anti-inflammatory effects of PLA have also been reported in several animal studies. PLA administered orally to rats prior to an inflammation inducing injection of carrageenan into the right-hand paw was shown to reduce oedema formation [5]. PLA administered topically onto the paw had antipyretic (fever reducing) effects in this model. Furthermore, the response time of rats exposed to a hot plate was increased by 1.4 times after an injection of PNO into the right hind paw [5]. This suggests that PLA may have analgesic effects, possibly through effects on COX-2 activity and PG production. More recently Chen *et al*. described that a single PLA injection (3 ug) can suppress TPA-induced mouse ear oedema; they describe lowered infiltration of leukocytes, neutrophils and macrophages [9]. Topical application of PLA onto the mouse back skin was also shown to reduce TPA-induced pro-inflammatory mediator production, including IL-1β, IL-6, TNF-α, and PGE_2, as well as the phosphorylation of p38- and c-Jun N-terminal kinase-mitogen-activated protein kinase (MAPK), but not that of extracellular signal-regulated kinase-MAPK. Interestingly, the authors suggest that these anti-inflammatory effects may be due to direct modulation of cell signalling by PLA, not FA incorporation into cells as no PLA was detected in the ear disc after PLA injection.

Effects of ETA on inflammation

ETA is the elongation product of PLA (Figure 1) and ETA levels increase in cells after exposure to PLA (see earlier). Therefore, it is possible that effects of PLA are mediated by ETA. A small number of studies have examined effects of ETA on inflammation [7-9] (Table 6). Huang *et al*. examined effects of ETA in LPS-stimulated RAW264.7 macrophages and found that pre-treatment with ETA (50 μM) led to a reduction in IL-6 production (data was not shown), as well as a decrease in PGE_2 production [7]. ETA treatment was shown to down regulate NF-κB activity (nuclear translocation) and inactivate MAPK. Furthermore, effects of ETA on PGE_2 were shown to be due to the extent of incorporation of ETA into cellular phospholipids, and competition with AA. Similarly, Chen *et al*. examined effects of ETA in LPS-stimulated murine BV-2 cells and rat primary peritoneal macrophages [8]. They described reduced NO, PGE_2 and IL-6 production, as well as suppression of iNOS protein expression and MAPK activation. However, ETA had limited effect on COX-2 protein expression and TNF-α concentrations. ETA and PLA (both 50 μM) had fairly similar effects on inflammatory outcomes in BV-2 cells and peritoneal macrophages [8]. A recent study by Baker *et al.* described effects of ETA in EA.hy296 cells [11]. Pre-treatment with ETA (5 and 10 μM) lead to reduced sICAM-1, MCP-1, IL-6 and RANTES production. ETA (10 μM) was also shown to reduce NF-κB activation (phosphorylation of the p65 subunit). Furthermore, ETA treatment decreased the adhesion of THP-1 monocytes to EA.hy296 cell monolayers [11]. Chen *et al.* also described anti-inflammatory effects of ETA in mice [9]. ETA injection suppressed TPA-induced mouse ear oedema, as measured by ear thickness (15%), and led to lowered infiltration of leukocytes, neutrophils, and macrophages. Topical application of ETA on mouse back skin was also shown to reduce inflammatory mediator production including IL-1β, IL-6, TNF-α and PGE_2. Thus, ETA is anti-inflammatory. This raises the question of whether the anti-inflammatory effects of PLA are caused by its elongation product ETA. This was explored by *Baker et al*. in EA.hy296 cells [11]. The fatty acid elongase 5 (*elovl5*) gene was silenced using small interfering RNA. This was shown to prevent elongation of PLA to ETA. Moreover, silencing led to the prevention of the anti-inflammatory effects seen with treatment of EA.hy296 cells with PLA. These observations strongly suggest that the effects seen with PLA treatment are due to a metabolic product beyond fatty acid elongase 5, most likely ETA.

Effects of pine nut oil and pinolenic acid on immune function

Two studies have investigated effects of PLA on immune function in animal models [33, 39] (Table 7). Matsuo *et al*. examined PLA feeding in ovalbumin immunised rats [33]. They reported higher numbers of $CD4^+$ T-lymphocytes within the spleen as well as increased production of leukotriene B_4 and immunoglobulins E and G by spleen cells in rats fed PLA compared to safflower oil [33]. Park *et*

al. reported increased proliferation of spleen lymphocytes in response to concanavalin A (a T cell stimulant) after PNO feeding [39]. However, in contrast to the reported anti-inflammatory effects of PLA (see earlier), they also reported an increase in IL-1β production by LPS-stimulated splenocytes. This different effect may be due to components other than PLA in PNO or to a difference inherent in the model.

Effects of NMIFAs from pine nut oil other than pinolenic acid

Although the focus of this review has been on PLA, and its elongation product ETA, as NMIFAs from PNO, it should not be overlooked that PNO contains other NMIFAs in low amounts compared with PLA (taxoleic and sciadonic acids) and that these may have biological activity that is relevant to human health related outcomes. There does not seem to be relevant literature on taxoleic acid, but a number of *in vitro* and animal studies have been performed with sciadonic acid. Sciadonic acid was reported to be a potent inhibitor of AA metabolism by COX in human platelets [55]. Furthermore, sciadonic acid was metabolised *in vitro* by human platelets to two hydroxy derivatives, a process that was prevented by inhibition of COX by indomethacin [55]. Thus, like the omega-3 FA EPA, sciadonic acid both inhibits AA metabolism by COX and acts as an alternative COX substrate. An oil rich in sciadonic acid was reported to inhibit 5-lipoxygenase activity in a model assay system and topical application of the oil reduced ear inflammation (oedema) induced by xylene in mice [56]. Culture of HepG2 cells with sciadonic acid resulted in its incorporation into phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, with greatest appearance in phosphatidylinositol [57]. The most enriched phosphatidylinositol species was a 1-stearoyl-2-sciadonoyl species and there was a parallel reduction in AA-containing phosphatidylinositol species. The latter are important substrates for signalling molecules and the effects of sciadonic acid suggest it might affect intracellular signalling and cellular responses. Incubation of Swiss 3T3 cells with sciadonic acid also resulted in appearance of a 1-stearoyl-2-sciadonoyl-phosphatidylinositol and when the cells were stimulated with bombesin, a novel diacylglycerol (1-stearoyl-2-sciadonoyl-glycerol was produced [58]. This diacylglycerol was able to activate protein kinase C similarly to 1-stearoyl-2-arachidonoyl-glycerol [58]. Sciadonic acid did not affect proliferation of Swiss 3T3 cells in response to bombesin, in contrast to the effects of juniperonic acid (all *cis*-5,11-14-17 20:4) and EPA which were both inhibitory [59]. This lack of effect of sciadonic acid may relate to the fact that its major diacylglycerol species has the same activity as the AA analog, while the diacylglycerol species of juniperonic acid and EPA may not. Huang *et al.* [60] reported that sciadonic acid (50 μM) was incorporated into cultured RAW264.7 macrophages and resulted in a reduction in cellular AA levels and in lower production of PGE_2, TNF-α, IL-6 and NO in response to LPS. These effects were

associated with lower COX-2 and iNOS protein expression and reduced activation of NF-κB. Likewise, Chen *et al*. [61] reported concentration-dependent incorporation of sciadonic acid into RAW264.7 cells with a parallel decrease in AA content and they confirmed decreases in production of PGE_2, TNF-α, IL-6 and NO, in expression of COX-2 and iNOS, and in NF-κB activation. They also showed that sciadonic acid impaired activation of both extracellular signal-related kinase and c-Jun N-terminal kinase MAPKs. Cultured HepC2 epithelial cells incorporated sciadonic acid from the medium (50 μM) into cellular phospholipids and this was associated with lower PGE_2 production upon exposure to Candida [62]. Together these studies indicate that sciadonic acid possesses anti-inflammatory effects and acts through some of the same mechanisms as PLA, ETA and the omega-3 FAs EPA and DHA. The *in vitro* study of Chen *et al*. [8] with murine microglial BV-2 cells and rat primary peritoneal macrophages has already been described in the context of the anti-inflammatory effects of PLA and ETA (Tables 5 and 6). Chen *et al.* also studied sciadonic acid and their findings enable the effects of PLA, ETA and sciadonic acid, all at a concentration of 50 μM, to be compared. For LPS-treated BV-2 cells the order of potency of the anti-inflammatory effects was as follows:

PGE_2 production: ETA = PLA >> sciadonic acid

TNF-α production: sciadonic acid = PLA > ETA

IL-6 production: PLA > ETA = sciadonic acid

Nitric oxide production: PLA > ETA > sciadonic acid

For LPS-treated rat primary peritoneal macrophages the order of potency of the anti-inflammatory effects was as follows:

PGE_2 production: ETA > PLA > sciadonic acid

Nitric oxide production: PLA = ETA = sciadonic acid

COX-2 protein expression: sciadonic acid >> PLA = ETA

iNOS protein expression: PLA > ETA = sciadonic acid

MAPK activation: sciadonic aid > PLA = ETA

Including sciadonic acid in the diet of mice (at 3% of total dietary FAs) resulted in its incorporation into phosphatidylinositol in liver, heart and spleen where it partially replaced AA [63]; in contrast to culture experiments with HepG2 cells [57], dietary sciadonic acid was poorly incorporated into phoshatidylcholine or phosphatidylethanolamine. In rats, feeding a seed oil that contains sciadonic acid resulted in lower blood and hepatic TAG concentrations than feeding maize or soybean oils, although cholesterol levels were not different among the different dietary groups [64]. Sciadonic acid appeared in blood TAG, cholesteryl esters and phospholipids [64]. A follow-up study demonstrated that sciadonic acid itself lowered serum and liver TAG levels compared to maize oil [65]. Incubation of HepG2 cells with sciadonic acid resulted in less TAG accumulation and reduced

expression and activity of SCD1 [66]. Thus, again like PLA and the omega-3 FAs EPA and DHA, sciadonic acid may have potential in regulating hepatic lipid homeostasis and controlling blood lipid concentrations. There are no human studies investigating effects of sciadonic acid.

Summary, discussion and conclusions

PNO is rich in NMIFAs and LA. PLA is the most abundant NMIFA comprising of 14-19% of the total FAs present in the oil from nuts of *P. koraiensis and P. sibirica* as well as in a number of other PNOs [3, 25]. Furthermore, PLA is known to be metabolised to ETA in several cell types and species [6, 8, 9, 11, 13, 29, 30]. Results from cell culture and animal studies indicate that both PNO and PLA may have several potential health benefits, including control of body weight and appetite, improved blood lipids and insulin sensitivity, reduced inflammation and modulated immune function. Almost all animal feeding studies have used PNO from *P. koraiensis*. Only a few studies have examined effects of the PLA elongation product ETA and these indicate it too has anti-inflammatory properties. This review has collated studies of PNO, PLA and ETA and evaluated the molecular and cellular effects and potential health benefits drawing together research performed *in vitro*, in animal models and in humans. In many respects, the effects and the mechanisms of action of PLA and, where studied, ETA are similar to those of the omega-3 fatty acids EPA and DHA. The studies of Baker *et al.* in cultured EA.hy296 cells [11, 67] allow direct comparison of the anti-inflammatory effects of PLA and four different omega-3 FAs including EPA and DHA all used at 50 μM (Table 7). PLA shares the anti-inflammatory properties of EPA and DHA, albeit with lower potency than DHA. Furthermore, like EPA and DHA, PLA lowers blood TAG levels, at least in rodent models. Thus, PNO and PLA may be possible sustainable alternatives to long chain omega-3 PUFAs for human health and well-being. Sciadonic acid, another NMIFA found in PNO, has biological effects like those of PLA (and ETA). Where the effects of sciadonic acid have been compared with those of PLA [8], overall the effects were rather similar, although sometimes PLA was more potent and sometimes sciadonic acid was more potent. Given the similarity of effects and the fact that sciadonic acid is present in < 10% of the level of PLA in oil from the nuts of *P. koraiensis* and *P. sibirica*, it seems unlikely that effects of these PNOs described herein are due to sciadonic acid rather than PLA.

Effects of PNO and PLA on body weight and appetite are the most extensively studied and the only area where human research has been performed with PNO. Together these studies suggest positive effects on body weight, weight gain and appetite control. In humans, consuming PNO and PLA was shown to have favourable effects on appetite control. Studies describe lowered food intake after consuming PNO and PLA [37, 38] (Table 2), these effects may be through changes in satiety hormones. Pasman *et al*. demonstrated postprandial upregulation of both CCK-8 and GLP-1 in

humans after consumption of PNO [38]. In all studies where mice were fed a HFD containing PNO, lower body weight and less weight gain were observed [36, 38-42] (Table 2). These changes were shown to be through a reduction in white adipose tissue [39, 41, 42], most likely as a result of enhanced oxidative metabolism and thermogenesis, driving the use of fuel sources and lowering lipid accumulation. Thus, effects of PNA and PLA on body weight gain may be through both decreased intake and increased use of energy compared to the control condition. There are no human studies of PNO or PLA on body weight gain or loss or body composition.

Animal data reviewed here suggest PNO and PLA have beneficial effects on blood lipids including both cholesterol and TAG [18, 22, 36, 41] (Table 3). Studies performed in HepG2 cells indicate PLA can improve hepatic metabolism through lipoprotein uptake and down regulation of genes involved in FA biosynthesis [10, 41, 42, 49]. Zhu *et al*. demonstrated that a HFD containing PNO increased expression of genes related to hepatic TAG metabolism, mitochondrial FA oxidation and VLDL assembly, as well as reducing expression of genes involved in intestinal FA uptake and chylomicron assembly [42]. PNO and PLA may reduce serum TAG through enhanced FA oxidation as well as increased insulin sensitivity. In this regard PLA has been shown to be a dual agonist for coactivation of FFAR1 and FFAR4 [52], which could enhance glucose dependent insulin secretion and insulin sensitivity to promote efficient glucose disposal. There are no human studies of PNO or PLA on blood lipids or insulin sensitivity.

Many cell culture and animal studies show PNO, PLA and ETA to be anti-inflammatory [5-10, 29] (Tables 5 and 6). These effects seem likely to be at least partially mediated through decreased NF-κB activity [7, 11], similar to the actions of EPA and DHA [17]. Many studies describe reduced PGE_2 production after treatment with PNO, PLA or ETA [6-9]. PNO, PLA and ETA were shown to reduce COX-2 activity [8, 9]. Chuang *et al.* proposed that the reported decrease in PGE_2 production by RAW264.7 cells may be through competition of PLA and its metabolite ETA with AA as a substrate for COX-2 [6]. Several studies reporting on FA composition indicate PLA and ETA decrease concentrations of AA, which may play an important role in the actions of these FAs [6, 9, 11, 13, 29] (Figure 2). It is possible that ETA is a substrate for generation of lipid mediators that may have anti-inflammatory or inflammation resolving actions, although such mediators have not been described. ETA is an isomer of DGLA and DGLA is a known substrate for COX-2 and lipoxygenase enzymes.

There is significant potential for human health benefits from PNO, its constituent NMIFA PLA and the PLA derivative ETA. However, most studies of PNO, PLA and ETA have been performed on cell lines or in animal models with only limited human research. Although studies in model systems are valuable for demonstrating effects and deciphering mechanisms, they also have inherent limitations.

Feeding studies in rodents have compared PNO or its extracts with other plant oil sources of FAs such as maize oil, safflower oil, soybean oil or flaxseed oil. Thus, it is likely that intake of several fatty acids will be different between the groups being compared. Furthermore, these oils contain other (i.e. non-FA) constituents and in different amounts, such as phytosterols and tocopherols, that have not been accounted for in studies done to date. The amounts of oils and individual FAs, including PLA, being fed are often in amounts that greatly exceed amounts that could be consumed by humans. This is also true of *in vitro* studies with isolated cells, where individual FAs are used at concentrations that are likely to exceed those that can be achieved in humans. Therefore, there is a need for human trials to more fully evaluate the effects of PNO, PLA, ETA and other NMIFAs including sciadonic acid. Since PNO contains a variety of components with biological activity, including phytosterols, tocopherols and squalene, as well as FAs other than PLA, it is possible that not all of the effects of PNO may be due to PLA. However, it is important to note that effects of PNO can be mimicked by isolated PNO TAG and NEFA fractions and by purified PLA. Nevertheless, it will be important to differentiate effects of PLA from other components of PNO, including other NMIFAs. Furthermore, metabolism of PLA to ETA may play an important role in the mechanism of action of PLA.

This article has focussed on the biological effects of PNO, its constituent FA PLA and the PLA elongation product ETA. PLA is an 18-carbon trienoic acid. It is likely that other plant-derived 18-carbon trienoic acids have biological effects, acting through mechanisms of action similar to those of PLA or via their elongation products. Such FAs include alpha-linolenic acid (ALA; all *cis*-9,-12,-15 18:3), GLA and various conjugated linolenic acids including punicic acid (*cis*-9, *trans*-11, *cis*-13 18:3). ALA is found in green plant tissues because it is a vital component of chloroplast thylakoids; it is also found in many seeds, seed oils and nuts. Flaxseeds and flaxseed oil are rich in ALA which contributes about 55% of total FAs and, amongst nuts, walnuts are a good source. Soybean, rapeseed (canola), mustard and sea buckthorn oils all contain ALA. Although ALA is considered to be an essential fatty acid, it seems to have modest biological effects in its own right. Its main role in humans is to act as a precursor for the synthesis of longer chain, more unsaturated omega-3 FAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These latter FAs are biologically active [68] and linked with several human health benefits [14, 15, 69]. However, as reviewed elsewhere [70, 71], conversion of ALA to EPA, and especially on to DHA, is poor in humans, so limiting the ability of ALA to influence human health outcomes. Where effects of ALA on health related outcomes have been described in humans, high intakes of ALA have been used and the effects have been related to increases in the EPA content of blood or blood cells [71]. A recent *in vitro* study showed that ALA has

only weak anti-inflammatory effects compared to the potent effects of EPA and particularly DHA [67].

GLA is found in evening primrose, borage and sea buckthorn oils. GLA is readily converted to DGLA in cultured cells [11, 72, 73] and in the human body [74, 75]. DGLA is a known substrate for COX and lipoxygenase enzymes and the eicosanoid mediators produced are anti-inflammatory, as reviewed elsewhere [76, 77]. Therefore, effects of GLA or GLA containing oils have commonly been attributed to the elongation product DGLA. This attribution was confirmed recently through an *in vitro* study in which most effects of GLA on inflammatory responses of cultured EA.hy296 cells were prevented if the enzyme responsible for GLA conversion to DGLA (fatty acid elongase 5) was silenced [11].

Punicic acid is one of the conjugated linolenic acids. The richest source of punicic acid is pomegranate seeds and their oil where it contributes about 75% of total FAs. Effects of pomegranate seed oil and punicic acid have been evaluated in a number of *in vitro* and animal studies and in a limited number of human trials. These effects have been reviewed several times [78-83] and include anti-inflammatory, anti-oxidant, anti-obesity and anti-cancer effects, mainly demonstrated in model systems. Punicic acid has been reported to activate several PPARs [84-86] which could result in reduced inflammation, improved lipid homeostasis and enhanced insulin sensitivity. In a human trial, 3 g of punicic acid per day for 28 days increased punicic acid from 0 to 0.47% of total FAs in plasma and from 0 to 0.37% of fatty acids in red blood cells [87]. Punicic acid also increased the proportion of *cis*-9, *trans*-11 conjugated linoleic acid in plasma and red blood cells; this FA can have benefits on human health including improving the blood lipid profile [88]. Feeding pomegranate seed oil to rats resulted in appearance of conjugated linoleic acid in many tissues [89, 90]. In another human trial, a modest intake of pomegranate seed oil improved the blood lipid profile in hyperlipidemic individuals [91], but did not affect plasma TNF concentration [92].Thus, several 18-carbon trienoic FAs modulate cell function in a manner that would be consistent with improved human health.

Amongst the FAs discussed here, PLA, sciadonic acid and punicic acid appear to most promising for further investigation. In all three cases there are existing data from model systems (cell cultures, experimental animals) and some understanding of likely mechanisms of action. Both PLA and punicic acid are converted to other bioactive FAs, ETA and *cis*-9, *trans*-11 conjugated linoleic respectively, and in both cases these metabolic derivatives appear to be responsible for at least some of the effects reported. PLA, sciadonic acid and punicic acid have not been well explored in human trials. It

will be important to investigate the metabolic handling and health-related impacts of these FAs in well-designed human trials in order that their potential can be better evaluated.

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Figure legends

Figure 1. The pathway of conversion of linoleic acid to γ-linolenic, dihomo-γ-linolenic, arachidonic, pinolenic and eicosatrienoic acids.

Figure 2. A. Fatty acid composition changes in RAW264.7 macrophages incubated for 24 hr with different concentrations of pinolenic acid (PLA). Data are taken from [6] B. Fatty acid composition changes in EA.hy296 cells incubated for 48 hr with different concentrations of pinolenic acid (PLA) (Data for PLA and ETA are from [11] while data for linoleic acid (LA) and arachidonic acid (AA) are not previously published).

Figure 3. Summary of the mechanisms by which pinolenic acid (PLA) affects hepatic lipid metabolism. Abbreviations: *ACADL*, long chain acyl coenzyme A dehydrogenase; *ACSL3*, long chain acyl coenzyme A synthase 3; *CPT*, carnitine palmitoyl transferase; FA, fatty acid; *FAS*, fatty acid synthase; *HMGCR*, 3-hydroxy-3-methtyl-glutary coenzyme A reductase; LDL, low density lipoprotein; PPAR, peroxisome proliferator activated receptor; *SCD*, stearoyl coenzyme A desaturase; SREBP, sterol response element binding protein; VLDL, very low density lipoprotein.

Figure 4. Summary of the mechanisms by which pinolenic acid (PLA) and its elongation product eicosatrienoic acid (ETA) affect inflammation. Abbreviations used: IL, interleukin; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein, NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; RE, response element.