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Levels of bioactive lipids in cooking oils: olive oil is the richest source of oleoyl serine

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Abstract

Background—Rates of osteoporosis are significantly lower in regions of the world where olive oil consumption is a dietary cornerstone. Olive oil may represent a source of oleoyl serine (OS), which showed efficacy in animal models of osteoporosis. Here, we tested the hypothesis that OS as well as structurally analogous *N*-acyl amide and 2-acyl glycerol lipids are present in the following cooking oils: olive, walnut, canola, high heat canola, peanut, safflower, sesame, toasted sesame, grape seed, and smart balance omega.

Methods—Methanolic lipid extracts from each of the cooking oils were partially purified on C-18 solid-phase extraction columns. Extracts were analyzed with high-performance liquid chromatography-tandem mass spectrometry, and 33 lipids were measured in each sample, including OS and bioactive analogs.

Results—Of the oils screened here, walnut oil had the highest number of lipids detected (22/33). Olive oil had the second highest number of lipids detected (20/33), whereas grape-seed and high-heat canola oil were tied for lowest number of detected lipids (6/33). OS was detected in 8 of the 10 oils tested and the levels were highest in olive oil, suggesting that there is something about the olive plant that enriches this lipid.

Conclusions—Cooking oils contain varying levels of bioactive lipids from the *N*-acyl amide and 2-acyl glycerol families. Olive oil is a dietary source of OS, which may contribute to lowered prevalence of osteoporosis in countries with high consumption of this oil.

Keywords

bone lipids; *N*-acyl amide; oleoyl serine; olive oil; osteoporosis

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Osteoporosis is a word formed from the Greek *ostoun* (οστόν), meaning “bone,” and *poros* (πόρος), meaning “pore”, which literally translates to “porous bones”. The disease osteoporosis causes more than 8.9 million fractures annually, resulting in an osteoporotic fracture every 3 s [1], with estimates of 200 million people worldwide suffering from its effects [2]. Interestingly, epidemiological data indicate that there are areas of the world that are significantly less affected by osteoporosis than others [1, 3–8]. A leading hypotheses of what drives this difference is the adherence to the so-called Mediterranean diet; however, recent evidence suggests that it is likely the daily consumption of olive oil that is associated with reductions in osteoporosis in these regions [3, 5–7, 9, 10]. One hypothesis suggests that the phenols in olive oil may play a role in the mechanism of action through their antioxidative, pro-vitamin D properties; however, there is no specific compound that appears to be singled out [4–6, 11–15].

At a cellular level, a prevailing hypothesis for the cause of osteoporosis posits that an imbalance between the activity of osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells) creates a net bone loss that is maintained [16]. From this schema, it is further hypothesized at a simplistic level that an overactive set of osteoclasts or an underactive set of osteoblasts could be the ultimate cause. It is not surprising, however, that the molecular mechanism for the regulation of these two cellular systems is extremely complex. That there is a sex difference in the disease (it predominates in women) [4] and that animal models are generated simply by removing the ovaries [6, 10–12, 17] would seem to indicate that estrogen plays a primary role. While estrogen clearly plays some role in driving transcription factors involved in the regulatory systems of bone in women (and likely in men) and that some amelioration is observed with supplementation, estrogen therapy is not a cure. Given the vast array of side-effects of hormone replacement therapy, there is a need for more targeted therapies that do not require systemic doses of such a powerful growth hormone.

In 2010, I was fortunate enough to work with collaborators at Hebrew University, Itai Bab and Raphael Mechoulam, and publish a report in *PNAS* identifying and characterizing a novel, endogenous small molecule lipid: oleoyl serine (OS), which was shown to reverse ovariectomy-induced osteoporosis in a rodent model [17]. OS induced elevated levels of osteoblast proliferation in in vitro assays, which occurred via a G protein-coupled receptor (GPCR) and Erk1/2 second messenger signaling, as well as lowered osteoclast numbers by inducing apoptosis. In healthy mice, supplementation with OS moderately increased bone density. More importantly, in ovariectomized (OVX) mice, an animal model of osteoporosis, OS rescued the bone loss. To exert its effects, we hypothesized that OS signals through an unidentified GPCR that was likely of the inhibitory Gi family, in that the cellular effects reported were blocked by pertussis toxin. However, it is possible that OS may also be signaling through an ion channel or a peroxisome proliferator-activated receptor that is associated with a GPCR [18].

My laboratory’s work in the original project was to identify OS in the bone using high-performance liquid chromatography-tandem mass spectrometry (HPLC/MS/MS) techniques. During the 5-year Binational Science Foundation grant with Itai Bab from which this original work was funded, we also undertook a series of biochemical assays aimed at

understanding the biosynthetic mechanisms of OS. Given that oleic acid is one of the most ubiquitous fatty acids in mammalian systems, we had a very difficult time “overwhelming” the system with substrates to try to drive the production of OS. This led us to hypothesize that at least some OS may be coming from an external source, more specifically, the diet. Given the strong link between olive oil consumption and lower rates of osteoporosis, this was our first target. Here, we tested the hypothesis that OS as well as structurally analogous *N*-acyl amide and 2-acyl glycerol lipids is present in the following cooking oils: olive, walnut, canola, high heat canola, peanut, safflower, sesame, toasted sesame, grape seed, and smart balance omega.

Table 1 shows the lipidomics screens of 33 lipids in each cooking oil. Of the oils screened here, walnut oil had the highest number of lipids detected (22/33). The 11 not detected were arachidonoyl and docosahexaenoyl conjugates and would not be expected to be detected from non-mammalian sources. Olive oil had the second highest number of lipids detected (20/33), whereas grape-seed and high-heat canola oil were tied for lowest number of detected lipids (6/33). Importantly, four of the *N*-acyl ethanolamines [*N*-palmitoyl ethanolamine, *N*-stearoyl ethanolamine, *N*-oleoyl ethanolamine (OEA), and *N*-linoleoyl ethanolamine), as well as 2-linoleoyl glycerol (2-LG) and 2-oleoyl glycerol (2-OG), were present in all oils tested. These specific data add to the growing body of evidence that these families of lipids are ubiquitous lipids throughout the plant and animal kingdoms.

OS was detected in 8 of the 10 oils tested and the levels were highest in olive oil (Figure 1A; Table 1), suggesting that there is something about the olive plant that enriches this lipid. Importantly, the levels of the other oleic acid conjugates (OEA and 2-OG) were not highest in olive oil (Figure 1). Levels of OEA, a GPR119 agonist and lipid implicated in satiety [21], were highest in walnut, peanut, and toasted sesame oil (Figure 1B). 2-OG was highest in two of the oils that did not contain any measurable OS, high-heat canola and grape seed, along with toasted sesame and smart balance omega. The high heat canola and smart balance oils are arguably the most processed of the group, suggesting that 2-OG might be a byproduct of that rather than an enriched constituent of the original plant. This is evidence by the fact that neither canola nor non-toasted sesame oils showed such high levels of 2-OG. 2-OG is a highly abundant lipid in the body that has been implicated as a positive modulator of the endocannabinoid 2-AG [22] and was also recently identified in *Drosophila* [19], which again suggests that it has universal signaling potential.

An obvious next step would be to feed OVX rats olive oil and determine whether the olive oil alone would reverse the effects of bone loss in this animal model of osteoporosis. Fortunately, a recent study has demonstrated that supplementing olive oil into the diet of Wistar rats for 12 weeks (4 weeks before OVX and 8 weeks after) significantly reduced the amount of bone loss compared to the OVX controls [10]. Again, the mechanism of action was not known; however, given what we have found here, we hypothesize that the OS in the olive oil may be a key factor in the rescue of the bone mass in these rats. How much OS (and other bioactive lipids) is (are) being absorbed through the diet remains an important factor in understanding the implications for the relationships between olive oil consumption and osteoporosis. The data presented here actually present an opportunity for the study of many bioactive lipids as dietary constituents and potential therapeutics.

For so long, our textbook understanding of lipids that are “made on demand” paints the picture that they are formed from the individual chemical building blocks at the cell membrane, “released”, and then quickly degraded. These building blocks come from the diet in which digestion perfectly breaks down the chemical components of food into simple free fatty acids, amino acids, and simple sugars. That they are lipids suggests that they cannot be stored in a lipid vesicle in the same manner as a charged particle in that they would simply diffuse out of the vesicle. We have come to a more sophisticated understanding that phospholipids in plasma and cellular organelle membranes are acting as the “storage” areas for the “made-on-demand” signaling lipids; however, this is likely only part of a much more complex story. All evidence for this is compelling; however, it does not account for the many bioactive lipids measured in the plasma [23, 24]. In this case, the most parsimonious answer is that these small, bioactive lipids are being absorbed “as is” and are part of the constituents of blood at various postprandial intervals throughout the day. They would then have the opportunity to be incorporated into the cellular membrane phospholipids as well as to act as primary signaling molecules at their active sites.

We look forward to our clinical colleagues taking up this question and determining how much OS from olive oil is actually directly absorbed during digestion and, therefore, has the opportunity to act as an exogenous activator of bone formation and retention. There are no simple answers to complex questions, such as diseases like osteoporosis. However, modification of diet to include OS-rich foods might be a corner piece to this very complex puzzle.

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Figure 1.

Levels of (A) OS, (B) OEA, and (C) 2-OG in cooking oils. All oils were purchased from a local organic foods store, brought directly to the laboratory, and stored at room temperature in a closed box to reduce any further exposure to light. Olive oil (Bionaturae; North Franklin, CT, USA); walnut oil (La Tourangelle; Berkley, CA, USA); canola, high-heat canola, safflower, peanut, sesame, toasted sesame, and grape-seed oils (Spectrum; Boulder, CO, USA); and smart balance omega (Smart Balance; Paramus, NJ, USA).

Table 1

Levels of bioactive lipids in cooking oils as determined by HPLC/MS/MS.

	Olive	Walnut	Canola	High-heat canola	Peanut	Safflower	Sesame	Toasted sesame	Grape seed	Smart balance omega
2-Acyl glycerol										
2-Oleoyl glycerol	3.76E-5 (1.46E-6)	1.54E-5 (9.07E-7)	1.10E-5 (9.21E-7)	1.91E-4 (1.58E-6)	8.79E-6 (5.53E-7)	6.87E-6 (3.68E-7)	8.12E-6 (2.17E-7)	1.78E-4 (8.42E-6)	5.94E-5 (6.45E-7)	8.58E-5 (1.58E-6)
2-Linoleoyl glycerol	2.67E-6 (1.32E-7)	1.48E-5 (3.56E-7)	2.27E-6 (1.86E-7)	4.19E-5 (1.76E-6)	1.15E-6 (9.80E-8)	9.80E-7 (6.97E-8)	5.20E-6 (1.11E-7)	8.63E-5 (2.68E-6)	7.25E-5 (5.11E-6)	2.69E-5 (9.47E-7)
2-Arachidonoyl glycerol	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
N-acyl alanine										
N-palmitoyl alanine	3.44E-10 (4.08E-11)	1.06E-8 (7.6E-10)	6.60E-10 (7.63E-11)	BDL	2.26E-8 (2.99E-9)	5.23E-10 (1.83E-10)	1.16E-9 (1.28E-10)	1.40E-7 (8.68E-9)	BDL	5.09E-10 (2.20E-10)
N-stearoyl alanine	1.94E-10 (2.59E-11)	4.58E-9 (4.21E-10)	BDL	BDL	7.42E-9 (1.00E-9)	BDL	BDL	3.93E-8 (2.92E-9)	BDL	BDL
N-oleoyl alanine	1.42E-10 (9.44E-12)	1.25E-8 (1.47E-9)	BDL	BDL	1.86E-7 (2.80E-8)	BDL	2.39E-9 (1.51E-10)	5.06E-7 (2.05E-8)	BDL	BDL
N-linoleoyl alanine	BDL	5.82E-8 (9.03E-9)	BDL	BDL	2.69E-8 (4.54E-9)	BDL	2.42E-9 (2.43E-10)	7.36E-6 (3.03E-7)	BDL	BDL
N-arachidonoyl alanine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
N-docosahexaenoyl alanine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
N-acyl ethanolamine										
N-palmitoyl ethanolamine	3.00E-10 (3.51E-11)	3.29E-8 (2.32E-9)	2.60E-10 (2.47E-11)	1.81E-9 (4.21E-11)	7.81E-9 (8.55E-10)	3.35E-10 (1.72E-10)	6.38E-10 (3.82E-11)	5.89E-8 (5.47E-9)	1.61E-9 (9.31E-10)	1.17E-8 (1.84E-10)
N-stearoyl ethanolamine	9.82E-10 (8.69E-11)	5.33E-8 (3.64E-9)	3.46E-10 (3.78E-11)	5.41E-9 (1.87E-10)	9.92E-9 (1.22E-9)	4.18E-10 (1.78E-10)	9.62E-10 (1.12E-10)	7.20E-8 (3.00E-9)	2.05E-8 (1.36E-9)	3.27E-8 (1.10E-9)
N-oleoyl ethanolamine	1.28E-8 (1.02E-9)	7.50E-8 (5.04E-9)	6.96E-09 (3.55E-10)	2.04E-8 (2.37E-10)	7.16E-8 (4.74E-9)	4.12E-9 (1.86E-9)	7.58E-9 (4.47E-10)	2.85E-7 (2.39E-8)	4.04E-8 (3.82E-9)	4.57E-8 (6.05E-10)
N-linoleoyl ethanolamine	5.96E-9 (6.79E-10)	6.70E-7 (6.00E-8)	8.28E-09 (7.76E-10)	2.03E-8 (1.45E-9)	2.95E-8 (3.60E-9)	1.78E-9 (4.16E-10)	1.51E-8 (6.45E-10)	1.03E-6 (5.79E-9)	1.05E-7 (1.34E-8)	1.35E-7 (4.74E-9)
N-arachidonoyl ethanolamine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
N-docosahexaenoyl ethanolamine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
N-acyl GABA										
N-palmitoyl GABA	1.64E-10 (3.03E-11)	7.93E-8 (9.44E-9)	BDL	BDL	1.77E-7 (1.64E-8)	BDL	1.76E-9 (1.24E-10)	2.25E-7 (6.31E-9)	BDL	BDL
N-stearoyl GABA	8.40E-11 (3.86E-11)	4.37E-9 (2.50E-10)	BDL	BDL	9.56E-9 (4.01E-10)	BDL	BDL	BDL	BDL	BDL

	Olive	Walnut	Canola	High-heat canola	Peanut	Safflower	Sesame	Toasted sesame	Grape seed	Smart balance omega
<i>N</i> -oleoyl GABA	1.97E-10 (1.37E-11)	3.76E-8 (2.67E-9)	2.62E-9 (2.62E-10)	BDL	1.99E-7 (2.22E-8)	3.88E-9 (2.43E-9)	2.50E-9 (1.18E-10)	2.27E-7 (1.05E-8)	BDL	1.11E-9 (9.18E-11)
<i>N</i> -linoleoyl GABA	2.52E-11 (2.07E-11)	1.19E-7 (1.07E-8)	1.67E-9 (1.54E-10)	BDL	5.15E-8 (6.39E-9)	3.62E-10 (1.47E-10)	1.54E-9 (2.37E-11)	3.05E-7 (2.10E-8)	BDL	BDL
<i>N</i> -arachidonoyl GABA	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<i>N</i> -docosahexaenoyl GABA	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<i>N</i> -acyl glycine										
<i>N</i> -palmitoyl glycine	6.70E-10 (1.19E-10)	9.32E-9 (1.15E-9)	BDL	BDL	1.39E-8 (1.80E-9)	BDL	1.38E-9 (5.26E-11)	6.61E-8 (2.16E-9)	BDL	BDL
<i>N</i> -stearoyl glycine	8.22E-10 (1.96E-10)	2.64E-9 (3.12E-10)	BDL	BDL	5.57E-9 (4.83E-10)	BDL	BDL	2.58E-8 (8.95E-10)	BDL	BDL
<i>N</i> -oleoyl glycine	BDL	1.24E-8 (1.33E-9)	BDL	BDL	4.92E-8 (5.78E-8)	BDL	3.66E-9 (2.80E-10)	1.33E-7 (4.74E-9)	BDL	BDL
<i>N</i> -linoleoyl serine	BDL	7.99E-8 (9.93E-9)	BDL	BDL	3.47E-8 (3.75E-9)	BDL	1.07E-8 (6.31E-10)	7.51E-7 (1.82E-8)	BDL	BDL
<i>N</i> -arachidonoyl glycine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<i>N</i> -docosahexaenoyl glycine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<i>N</i> -acyl serine										
<i>N</i> -palmitoyl serine	4.32E-10 (7.05E-11)	1.10E-9 (2.76E-10)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<i>N</i> -stearoyl serine	7.21E-10 (6.19E-11)	1.94E-9 (2.66E-10)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<i>N</i> -oleoyl serine	6.39E-8 (9.46E-9)	4.42E-8 (5.80E-9)	2.79E-8 (6.14E-9)	BDL	2.68E-8 (1.33E-9)	2.92E-8 (4.37E-9)	2.67E-8 (1.97E-9)	1.83E-8 (6.58E-10)	BDL	2.52E-8 (2.34E-9)
<i>N</i> -linoleoyl serine	8.58E-9 (1.11E-9)	8.67E-9 (1.01E-9)	9.43E-9 (2.22E-9)	BDL	8.83E-9 (1.12E-9)	1.01E-8 (1.43E-9)	6.84E-9 (7.89E-10)	BDL	BDL	8.86E-9 (5.50E-10)
<i>N</i> -arachidonoyl serine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
<i>N</i> -docosahexaenoyl serine	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Oils were opened from the manufacturer seal, and 5-mL aliquots were taken and put into 15-mL screw-top centrifuge tubes and wrapped in foil to protect from light. Six 50- μ L aliquots were taken from each and added to 2 mL of HPLC-grade methanol, then mixed by vortex for 1 min. Six milliliters of HPLC-grade water was then added, and the mixture was partially purified on C18 columns as previously described for bone tissue [17]. HPLC-MS/MS screens and analyses of lipids were performed as previously described [19, 20]. Final data were calculated as moles per microliter of oil. Lipids in the screening library that were not detected in the oil are indicated as BDL (below detection limit).