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Pomegranate juice and its main polyphenols exhibit direct effects on amine oxidases from human adipose tissue and inhibit lipid metabolism in adipocytes

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ABSTRACT

Pomegranate juice (PJ) is a beverage with potential beneficial effects due to its high content of polyphenols. The objective of this study is to explore at a molecular level the direct properties of PJ and its two main polyphenolic components, punicalagin and ellagic acid, on adipocyte functions. Increasing doses of PJ were tested using radiometric methods to determine amine oxidase activities in human adipose tissue preparations. The influences on lipogenic and lipolytic activity were also assessed by radiochemical and colorimetric assays. The results showed a dose-dependent capacity of PJ to inhibit the monoamine oxidase and the semicarbazide-sensitive amine oxidase activities present in human adipose tissue. PJ also inhibited lipogenesis and lipolysis in mouse and human adipose cells, while punicalagin and ellagic acid inhibited lipolysis rather than lipogenesis. However, the combination of punicalagin and ellagic acid resulted in a synergistic action in impairing MAO activity or basal glucose incorporation into lipids.

1. Introduction

Pomegranate, scientifically known as *Punica granatum* L. (Punicaceae), is a natural, rich and varied source of polyphenolic compounds. The main polyphenols found in the juice of this fruit are punicalagin isomers and ellagic acid, all belonging to the family of hydroxybenzoic acids (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000; Legua, Forner-Giner, Nuncio-Jáuregui, & Hernández, 2016; Les, Prieto, Arbonés-Mainar, Valero, & López, 2015). Many of the beneficial effects claimed for the consumption of pomegranate juice (PJ) are currently explained by the antioxidant properties of its components (Ekhlasi, Shidfar, Agah, Merat, & Hosseini, 2016; Gil et al., 2000; Kozik et al., n.d.; Les et al., 2015; Nuncio-Jáuregui et al., 2015). Among such beneficial effects are the cardioprotective, anti-atherogenic and antihypertensive actions, (Dushkin et al., 2014; Medjakovic & Jungbauer, 2013; Tsang, Smail, Almoosawi, Davidson, & Al-Dujaili, 2012), but also the antidepressant and neuroprotective activities (Jinu, Sai Sailesh, & Mukkadan, 2016; Naveen, Siddalingaswamy, Singsit, & Khanum, 2013). Recently, the metabolites of ellagitannins and of

Abbreviations: DMSO, Dimethyl sulfoxide; hAT, human adipose tissue; AO, amine oxidase; SSAO, semicarbazide-sensitive amine oxidase; MAO, Monoamine oxidase; Pun, punicalagin; EA, ellagic acid; PJ, Pomegranate juice; ROS, reactive oxygen species.

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ellagic acid, namely the urolithins, generated by the intestinal microbiota, have been described to exert beneficial actions on life span in worms as well as on muscle strength (Ryu et al., 2016) and lipid metabolism (Kang, Kim, Tomás-Barberán, Espín, & Chung, 2016) in mammals. Due to all these novel properties evidenced in cell cultures or animal models, PJ is currently regarded as an ingredient or food supplement with high antioxidant power exhibiting potential health benefits, especially for combatting cardiovascular and metabolic diseases in humans. However the *in vitro* capacity of PJ extracts to act on human tissues or cells is still insufficiently documented and prompted us to investigate the putative direct effects of PJ on adipose tissue samples from overweight donors by focusing interest on biological functions involved in both reactive oxygen species (ROS) and lipid metabolism. In fact, we recently reported that PJ lyophilizate inhibits monoamine oxidase activity (MAO), using a purified recombinant enzyme system (Les et al., 2015) and we considered that MAO activity is not only expressed in brain and involved in the degradation of neurotransmitters, but also present in peripheral organs, including heart and adipose depots. In the heart, MAO inhibition appears to be beneficial for the outcomes of cardiac infarction (Kaludercic, Mialet-Perez, Paolocci, Parini, & Di Lisa, 2014). In the adipose tissue, MAO is emerging during adipocyte differentiation together with another amine oxidase that is highly expressed in mature fat cells: the semicarbazide-sensitive amine oxidase (SSAO) encoded by the AOC3 gene (Bour et al., 2007; Li et al., 2011; Morin et al., 2001; Shen, Wertz, & Klinman, 2012). Both amine oxidases generate hydrogen peroxide when oxidizing their substrates: endogenous or dietary amines. A truncated form of SSAO, also known as soluble vascular adhesion protein-1 (VAP-1), is increased in the blood of diabetic patients and is highly predictive of their detrimental outcomes (Li et al., 2011). Since both MAO and SSAO are ROS-generating enzymes, we therefore tested whether PJ and its main polyphenols were able to directly inhibit their activities in human adipose tissue (hAT).

Nevertheless, adipose tissue is much more specialized in lipid handling than in generating and scavenging ROS. Lipogenesis and lipolysis are two mechanisms that are exquisitely regulated in adipocytes and which influence triacylglycerol accumulation and breakdown, respectively. Adipocyte lipogenesis corresponds to the incorporation of carbohydrates into lipids, mainly as a result of glyceroneogenesis and *de novo* synthesis of free fatty acids (FFA). In post-prandial conditions, it is accompanied by FFA re-esterification, leading to the storage of circulating lipids and glucose under the form of triacylglycerols accumulated in the lipid droplets of fat cells. By contrast, adipocyte lipolysis corresponds to the release of glycerol and FFA into the blood. To our knowledge, the effect of pomegranate flowers or peel extracts has been investigated on hepatic lipogenesis, but not the action of its juice, while the influence of pomegranate has not been tested yet on adipocyte lipolysis (Al-Shaaibi et al., 2016; Yuan et al., 2012). We therefore explored the influence of PJ on these functions in adipocytes freshly isolated from normoglycemic mice and completed our approaches on available human adipocyte preparations. The ratio of lipogenic/lipolytic activity of naturally ingested substances, hormones or drugs is important to determine because excessive accumulation of lipids in adipose tissue is accompanied by a vicious circle between insulin resistance and lipotoxicity, through constantly elevated circulating levels of FFA. The excess of fatty acids is in turn altering insulin sensitivity in muscles, heart, liver and pancreas. All these dysregulations increase the deleterious complications of obesity and diabetes, such as liver or cardiovascular diseases (Saponaro, Gaggini, Carli, & Gastaldelli, 2015). We therefore performed complete dose-response studies with PJ extracts on adipocyte lipolytic and lipogenic responses, to ascertain whether fat cells can be considered as a relevant target involved in the proposed potential effects of PJ on consumer metabolism. We also tested in parallel both putative stimulatory and inhibitory actions of the two main polyphenols present in PJ, namely punicalagin and ellagic acid, on lipogenic and lipolytic activities, taken into account that *in vivo* treatment with pomegranate-derived components in obese rats has already demonstrated multiple beneficial actions on obesity (Ok et al., 2013), and that these two polyphenols were detected in plasma during a pharmacokinetic analysis following PJ ingestion in healthy volunteers (González-Sarrías et al., 2015; Seeram et al., 2006).

2. Materials and methods

2.1. Reagents and chemicals

Pomegranate juice (Rabenhorst®) was acquired from a specialized shop, and lyophilized using a VIRTIS Genesis 25EL lyophilizer (Les et al., 2015). Tyramine, benzylamine, amine oxidase inhibitors, and other reagents were from Sigma-Aldrich (Spain/France), as well as punicalagin (MW: 1084) and ellagic acid (MW: 302). Liberase was from Roche Diagnostics (Germany).

PJ lyophilizate was dissolved in bidistillated water, and prepared at different dilutions to test dose-dependent effects. DMSO was used to dissolve ellagic acid, and its highest final concentration was 2.5% (V/V) allowing the test of phenolic acid at 30 mg/mL. Methanol was used to dissolve punicalagin and highest final concentration was 25% (V/V) and 1 mg/mL, respectively. The solubilized compounds were further diluted in water to perform dose-response analyses.

2.2. Phytochemical analysis of lyophilized pomegranate juice

2.2.1. Polyphenol content

The Folin–Ciocalteu assay was carried out in a 96-well plate with some modifications (Singleton, 1985). Each well contained 9 µl of the sample with 201.5 µl of Folin–Ciocalteu reagent. Then, 89.5 µl of 15% sodium carbonate was added after 5 min incubation at room temperature, and incubated again for 45 min at room temperature in the dark. Absorbance was measured at 752 nm in a microplate reader. The blank wells had distilled water instead of Folin–Ciocalteu reagent. The standard curve was measured with different concentrations of gallic acid standard water solution: 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 and 0.0078125 mg ml^{-1}. The PJ water solutions were 10, 5 and 2.5 mg ml^{-1}. The result was expressed as µg of gallic acid per mg.

2.2.2. HPLC-DAD analysis

The phytochemical analysis of the lyophilized juice and the detection of the main compounds were performed by HPLC using an Agilent 1260 Infinity LC (column Eclipse Plus C18 4.6 × 100 mm, 5 µm) coupled with a photodiode array detector, following a described procedure (Kim & Choi, 2013). Elution was carried out using H_2O as phase A and acetonitrile as phase B from 0 to 100% of solvent B in 50 min at a flow rate of 1 ml min^{-1}. Both solvents contained 0.5% acetic acid. Detection was performed at 254 and 310 nm. The injection volume was 10 µl and the concentration of the injected sample was 100 mg ml^{-1}. The standards used for identification were ellagic acid, punicalagin, ascorbic acid, chlorogenic acid, benzoic acid, caffeic acid, gallic acid, vanillic acid and catechin acquired from Sigma. For quantification of representative compounds a five point calibration curve was calculated with a selected standard in each case.

2.2.3. HPLC-PDA analysis for anthocyanins

HPLC analyses were performed following a described method (Brighenti et al., 2017). The characteristics of the equipment and the column were the same of the previous section. Phase A was composed of 2% HCOOH in H_2O and phase B 0.5% HCOOH in MeOH-H_2O (9:1, v/v). The gradient elution for the separation was as follows: 0–13 min 2% B, 13–18 min from2 to 5% B, 18–23 min from 5 to 10% B, 23–43 min from 10 to 25%B, 43–53 min from 25 to 50% B, 53–58 min from 50 to 100% B, 58–68 min 100% B, 68–71 min from 100 to 2% B, and a post-running time of 5 min. The flow-rate was 0.4 mL/min. The sample injection volume was 10 μl and the concentration of the injected sample of pomegranate juice was 100 mg ml^{-1}. The UV/DAD detection was performed at 520 nm. Cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside and peonidin 3-*O*-glucoside acquired from Extrasynthese were used for identification. Cyanidin 3-*O*-glucoside (also known as kuromanin chloride or chrysontemin) was used as standard for quantification using a calibration curve.

2.3. Patients and adipose tissue biopsies

Subcutaneous abdominal adipose tissue biopsies were obtained from overweight or mildly obese women undergoing reconstructive surgery at Rangueil hospital, Toulouse (France) as already described (Carpéné, Galitzky, et al., 2016). All participants provided their informed consent and the experimental design was approved by hospital and university ethics committees and registered under file number N° DC-2008-452. After surgical excision, biopsies of hAT were transferred at INSERM Unit 1048 in less than 2 h and frozen at −80 °C as samples of 200–800 mg or immediately used for adipocyte isolation. For the subgroup of patients constituted for this study (n = 15), without history of cardiovascular disease, smoking or alcohol consumption, the mean age was 47 years and the mean body mass index was 26 kg/m^2.

2.4. Amine oxidases (MAO and SSAO) activity radiochemical assays in human adipose tissues

Thawed samples of hAT were homogenized using homogenizer Tissue Master-125 (Omni International, Kennesaw, GA, USA) for approximately 30 s at room temperature, to avoid the fat freezing occurring at 6 °C. PJ, punicalagin, ellagic acid and reference inhibitors were preincubated with hAT 30 min before the addition of amine substrates in 2 mL Eppendorf tubes. Then, incubations were performed during 30 min at 37 °C in a final volume of 200 μl, containing 200 mM phosphate buffer at pH 7.5, isotopic dilution of [^{14}C]-tyramine or [^{14}C]-benzylamine (0.5 mM final concentration), with or without inhibitors and samples at different concentrations. Each assay tube contained approx. 300,000 dpm for [^{14}C]-tyramine or 150,000 dpm for [^{14}C]-benzylamine (NEC 835050UC from Perkin Elmer). These labelled substrates were added at t_0 and assays were stopped 30 min later by adding 50 μl of 4 M HCl. MAO-dependent oxidation and SSAO-dependent oxidation was sensitive to 1 mM pargyline and semicarbazide respectively. Oxidation products were extracted in 1 mL of organic solvent (toluene:ethylacetate, 1/1, V/V), as previously reported (Bour et al., 2007; Tipton, Davey, & Motherway, 2006). Then, radioactivity was counted in 0.7 mL of the organic phase. The small amount of radioactivity that was extracted in the organic phase before any incubation (t_0) accounted for less than 1% of the total radioactivity for each labelled amine, and was subtracted to all the countings. Oxidation level reached at t_{30} without any inhibitor was set as 100% reference.

2.5. Lipogenesis assessments in mouse and human adipose cells

Lipogenesis assays were performed with adipocytes freshly isolated from normoglycemic C57Bl6 (wild type) mice of both sexes, purchased at Charles River (L'Arbresle, France) and sacrificed under unfasted state by cervical dislocation. In several occurrences, elsewhere indicated, AOC3KO mice genetically invalidated for the AOC3 gene (SSAO/VAP-1 knockout) and backcrossed to the same strain were used to test responses in adipocytes lacking SSAO activity (Grès, Bour, Valet, & Carpéné, 2012). All animal procedures were approved by INSERM Institutional Animal Care Committee. White adipose depots from subcutaneous regions were dissected and immediately digested with liberase as previously described (Carpéné et al., 2014). [^{3}H]-Glucose incorporation into lipids was measured on freshly isolated adipocytes via a radiometric method using D-3-[^{3}H]-glucose with slight adaptations (Carpéné, Grès, & Rascalou, 2013) of the original insulin bioassay designed by Moody, Stan, Stan, and Gliemann (1974). Adipocyte lipids, including the labelled ones generated by *de novo* lipogenesis during 120 min incubation, were extracted by – and counted in – a non-water-miscible liquid scintillation cocktail designed for non-aqueous solutions (InstaFluor-Plus, PerkinElmer, Waltham, MA, USA). Consequently, there was a lower aqueous and an upper organic phase in each scintillation vial. Only the radiolabelled lipids present in the latter were counted, while the ^{3}H remaining in the lower phase, essentially under the form of non-metabolized glucose, could not excite the scintillation probe distributed only in the upper phase (Moody et al., 1974). Accordingly, t_0 control, which did not allow adipocytes to manage lipid synthesis, accounted for less than 0.5% of insulin-stimulated counts.

The same procedure was applied for human adipocyte preparations, when the schedule and quantity of hAT samples received from the surgery department were compatible with immediate digestion and incubations. To this aim, a portion of the hAT was immediately subjected to liberase digestion while the remaining material was stored at −80 °C. A total of nine adipocyte preparations were successfully analysed for their lipogenic activity under these conditions.

2.6. Lipolysis measurements in mouse and human adipose cells

Lipolysis was assessed by determining glycerol and free fatty acid (FFA) release under similar conditions as for lipogenesis (Gomez-Zorita, Tréguer, Mercader, & Carpéné, 2013). When available, adipocytes freshly isolated from hAT were also incubated in similar conditions to assess their lipolytic activity.

2.7. Statistical analysis

Results are presented as means ± SEM. IC_{50} values were calculated by nonlinear regression using GraphPad Prism. Student's *t*-test was used to compare PJ, its polyphenols and the reference compounds to their respective control.

3. Results

3.1. Phytochemical analysis of lyophilized pomegranate juice

The polyphenol content was 25.6 ± 0.9 μg of gallic acid equivalents per mg of lyophilized juice. Two main phenolic constituents were identified as punicalagin and ellagic acid, which is in concordance with a previous analysis of our group (Les et al., 2015). Other polyphenols have been additionally detected and quantified (see supplementary material) (Ambigaipalan, de Camargo, & Shahidi,

2017; Brighenti et al., 2017). The quantification of polyphenols per ml of juice were: 924.51 μg of punicalagins, 271.28 μg of gallic acid, 3.71 μg of caffeic acid, 17.99 μg of vanillic acid, 534.21 μg of ellagic acid and 1.55 μg of benzoic acid.

The second HPLC analysis revealed cyanidin 3-*O*-glucoside as the main anthocyanin (1.33 μg/ml of pomegranate juice)

3.2. Monoamine oxidase (MAO) activity in hAT

First of all, the vehicles used to solubilize the polyphenolic compounds were tested alone to determine whether they affect amine oxidase activity. They inhibited MAO activity only in a marginal manner since less than 20% of the oxidation of 500 μM [^{14}C]-tyramine was impaired at the highest final concentrations (2.5 and 25%) found in assays when they were used to solubilize the polyphenols (Fig. 1A). The ability of PJ and its main polyphenols to inhibit MAO activity in hAT homogenates is shown in Fig. 1B. As expected, the reference MAO-A-inhibitor clorgyline deeply inhibited [^{14}C]-tyramine oxidation by hAT homogenates and totally abolished MAO activity at doses lower than 0.1 mg/mL. The juice or its phenolic components also exhibited dose-dependent inhibition of tyramine oxidation, but at doses higher than 0.1 mg/mL. However, the higher dose of PJ lyophilizate tested (50 mg/mL) reached a maximal inhibition similar to that of the reference MAO-A inhibitor. The IC_{50} of the inhibition curves were 8.33 and 0.20 mg/mL for PJ and punicalagin, respectively. Ellagic acid did not reach 50% inhibition at the higher dose tested (3.02 mg/mL equivalent to 10 mM), being at the limit of solubility.

3.3. Semicarbazide sensitive oxidase (SSAO) activity in hAT

SSAO inhibition was measured through the capacity to impair the oxidation of 500 μM [^{14}C]-benzylamine, a recognized substrate of the enzyme. As expected, more than 90% of the benzylamine oxidation was inhibited by 1 mM semicarbazide, the reference SSAO-inhibitor, which displayed an IC_{50} of 0.006 mg/mL. Again, the vehicles were tested: DMSO did not influence SSAO activity, but methanol, used as a solvent for punicalagin, inhibited more than 25% of benzylamine oxidation (Fig. 2A). Consequently, the net inhibition obtained with punicalagin was not substantial (Fig. 2B). In contrast, the inhibition induced by ellagic acid was not due to its vehicle. PJ lyophilizate inhibited dose-dependently [^{14}C]-benzylamine oxidation with an IC_{50} of 16.63 mg/mL, and reached a maximal blockade similar to that of semicarbazide.

3.4. Exploring the components of PJ involved in amine oxidase inhibition

To assess the relative contribution of each major polyphenol in the PJ-induced inhibition of MAO and SSAO, normalization was

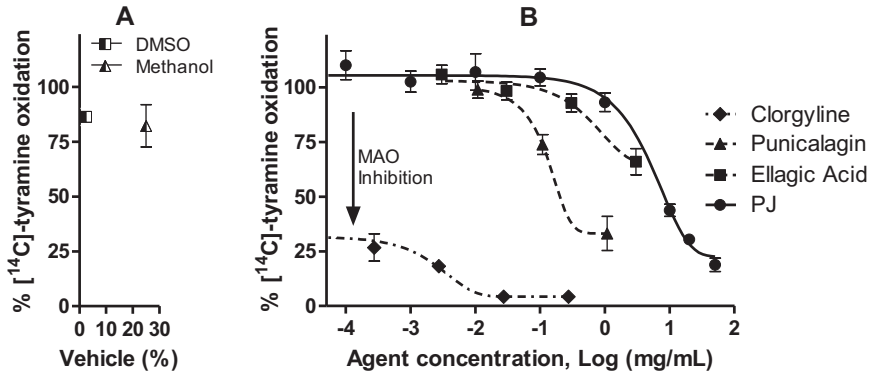

Fig. 1. *In vitro* inhibition of native MAO activity present in hAT. Amine oxidase activity is expressed as percentage of the amount of radiolabelled tyramine (500 μM) oxidized during 30 min by hAT homogenates. (A) Oxidation of tyramine in the presence of the highest dose of each used vehicle (see Material and methods). (B) Tyramine oxidation in the presence of increasing concentrations of lyophilized extract from pomegranate juice (PJ, dark line) and its main components, punicalagin and ellagic acid, or reference MAO-A inhibitor clorgyline (dotted lines). Each point is the mean ± SEM from 5 to 10 hAT homogenates.

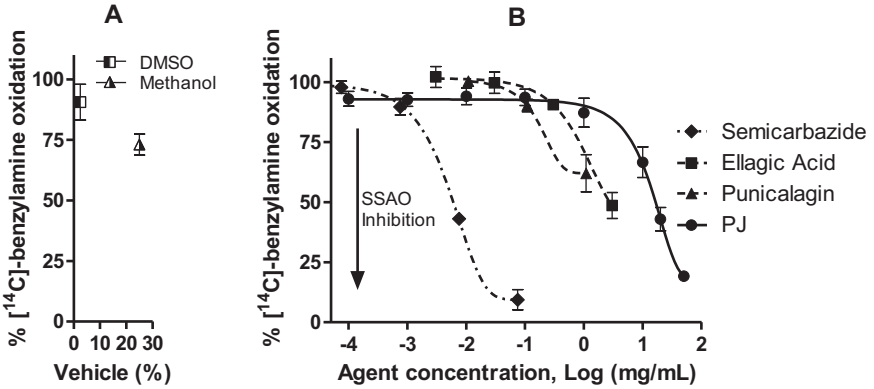

Fig. 2. Inhibition of SSAO activity in hAT. Amine oxidase activity is expressed as percentage of the oxidation of 500 μM radiolabelled benzylamine during 30 min incubation. (A) Benzylamine oxidation in the presence of the highest dose of the vehicles used for agent solubilisation. (B) Dose-dependent inhibition of benzylamine oxidation by increasing concentrations of PJ lyophilized extract, or its containing main polyphenols, and reference SSAO inhibitor, semicarbazide(dotted lines). Each point is the mean ± SEM from 3 to 5 hAT homogenates.

performed from the dose-response curves, taking into account the proportion of each polyphenol or lyophilizate occurring in 100 μL of PJ, and an extrapolation was performed. According to analyse, the quantity of the two main polyphenols are 924.51 and 534.21 μg per ml of PJ for punicalagins and ellagic acid respectively. The PJ lyophilized extract represents 115 mg/mL of fresh PJ. Fig. 3 shows that the inhibitory activity of lyophilized PJ extract was greater than that of its corresponding content of punicalagins or ellagic acid. Therefore the MAO and SSAO inhibitory properties of PJ cannot be supported by only its two main major components independently.

In fact, the various components of lyophilized PJ extract do not act separately, and probably might be more additive or synergistic than competitive in the original mixture. Combination of punicalagins and ellagic acid was therefore assayed to compare its activity with the components tested separately (Fig. 4). MAO inhibition showed a clear synergy when hAT was incubated with the combination, with significant differences at 10^{-4} and 10^{-5} M compared with the isolated components. However, such synergy and even simple addition was not observed regarding SSAO inhibition, which was more limited. It appeared that MAO inhibition by the juice resulted from the actions of various components, exhibiting probably synergistic action as demonstrated for its quantitatively major polyphenols.

3.5. Lipogenesis assessment in mouse and human adipose cells

PJ and its two main polyphenols were incubated for two hours in the presence of freshly isolated mouse adipocytes with or without insulin and radiolabelled glucose. As expected, 10^{-7} M insulin induced an almost threefold increase of the basal glucose incorporation into intracellular lipids. PJ had no effect at 0.1 mg/mL on glucose incorporation in lipids. However, at 1 mg/mL, it impaired the lipogenic activities observed without and with insulin, 17 and 41% respectively. PJ even totally abolished basal and stimulated lipogenesis at 10 mg/mL, 1 and 4% respectively (Fig. 5). Punicalagin and ellagic acid were tested separately at concentrations of 10^{-6} M, 10^{-5} M, and 10^{-4} M and no significant effect was detected (only higher dose is shown in Fig. 5). However, basal lipogenesis was reduced by the co-treatment with 10^{-4} M of each of these polyphenols (20%).

Due to the fact that PJ inhibits SSAO activity and that SSAO blockers also inhibit lipogenesis (Carpéné et al., 2013), lipogenic activity was then determined in adipocytes from AOC3KO mice, genetically invalidated for SSAO activity. However, there was no significant difference between AOC3KO and wild type mice (not shown) regarding the antilipogenic effect of PJ extract, therefore indicating that it was not related to SSAO inhibition. DMSO and methanol vehicles were ineffective and not involved in the observed antilipogenic effects (not shown).

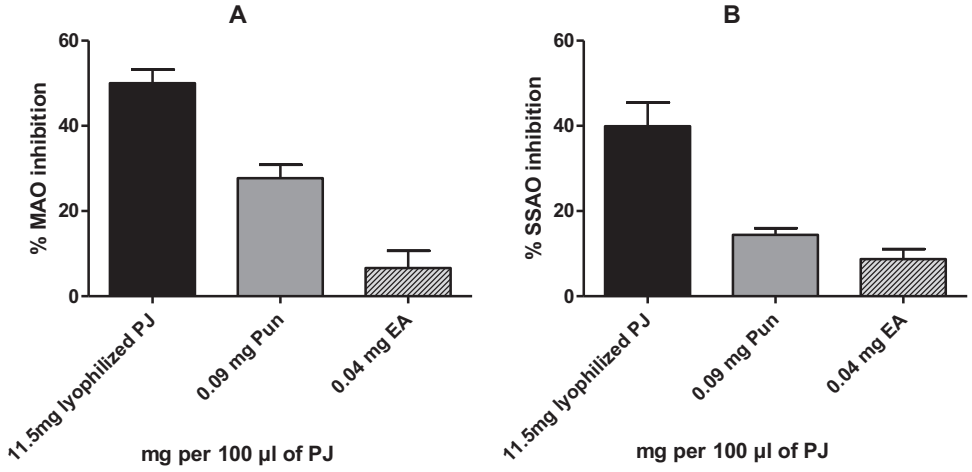

Fig. 3. Comparison of the inhibition of (A) MAO and (B) SSAO activities by the components found in 100 μL. The extrapolated results for PJ lyophilized extract and for its main polyphenols, punicalagin (Pun) and ellagic acid (EA), were obtained by nonlinear regression of hAT inhibition data presented in Figs. 1B and 2B. Each column is the mean ± SEM from 5 to 9 experiments.

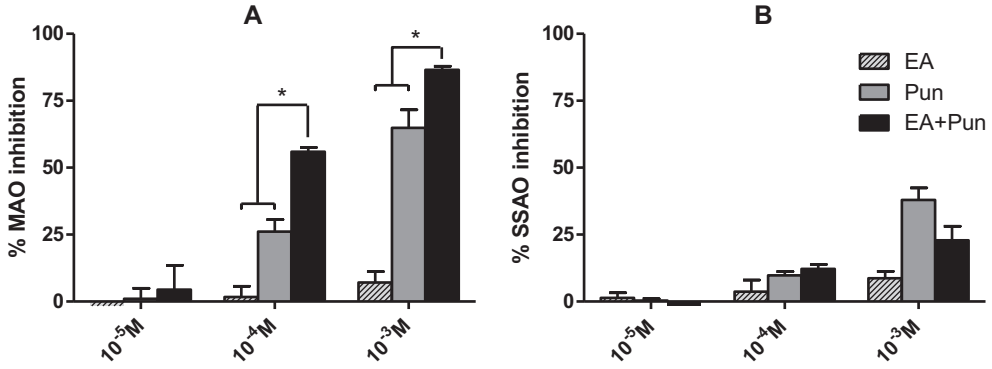

Fig. 4. MAO (A) and SSAO (B) inhibition by components of PJ tested separately or in combination. Each column is the mean ± SEM from 3 to 4 homogenates for ellagic acid (EA), punicalagin (Pun) or their combination (EA + Pun). Significantly different from the sum of respective components, at: * $p < 0.05$.

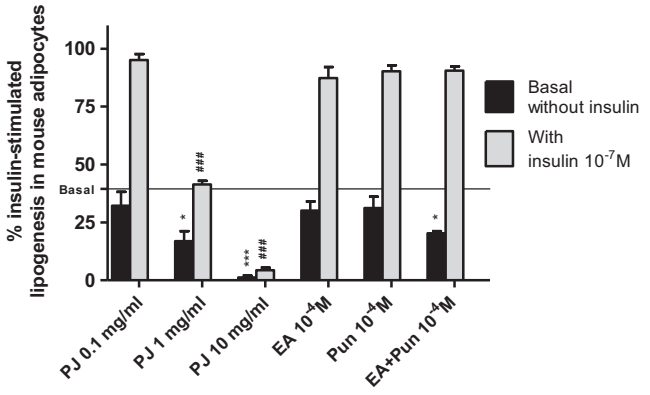

Fig. 5. PJ inhibits basal and insulin-stimulated lipogenesis in mouse adipocytes. Data expressed as percent of maximally activated lipogenesis. 100% indicate glucose incorporation into lipids stimulated by insulin 10^{-7} M alone. Each column is mean ± SEM of 3–4 adipocyte preparations. Significantly different from unstimulated control lipogenic activity (without insulin, basal dark line) at: * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). Different from the maximally stimulated activity in response to insulin 10^{-7} M (100% reference) at: ### ($p < 0.001$).

Similar exploration of lipogenic responses was performed in adipocytes freshly isolated from hAT. Unsurprisingly, human adipocytes were less responsive to the insulin stimulation of lipogenesis than mouse adipocytes, since the pancreatic hormone stimulated basal activity level by only a 1.6-fold factor. Nevertheless, PJ clearly inhibited lipogenesis in a dose-dependent manner in human adipocytes (62, 15 and 5% for 0.1, 1 and 10 mg/ml respectively), decreasing glucose incorporation into lipids largely below basal levels at the higher dose tested (Fig. 6).

3.6. Lipolysis assessment in mouse and human adipose cells

The effects of PJ and its two main components were investigated on mouse adipocyte lipolysis under basal and stimulated conditions. The β-adrenergic pan-agonist isoprenaline activated glycerol and FFA release by a 10-fold factor (Fig. 7). When considering basal lipolysis, only PJ highest dose (10 mg/mL) significantly decreased the baseline irrespective of the measured lipolytic product, 5 and 3% for glycerol (Fig. 7A) or FFA (Fig. 7B) respectively. A similar pattern was observed when adipose cell lipolysis was stimulated by 10^{-7} M isoprenaline (30 and 33% for glycerol and FFA respectively). Ellagic acid exhibited only significant antilipolytic action (38%) at very high dose (10^{-3} M) when incubated with isoprenaline (Fig. 7A). In contrast, punicalagin exerted a clearer antilipolytic action, since it abolished glycerol release at 10^{-5} M. The combined action of ellagic acid and punicalagin at 10^{-4} M could not overpass the antilipolytic effect of punicalagin, which was already maximal at this dose. Again, DMSO and methanol vehicles did not produce any detectable effect.

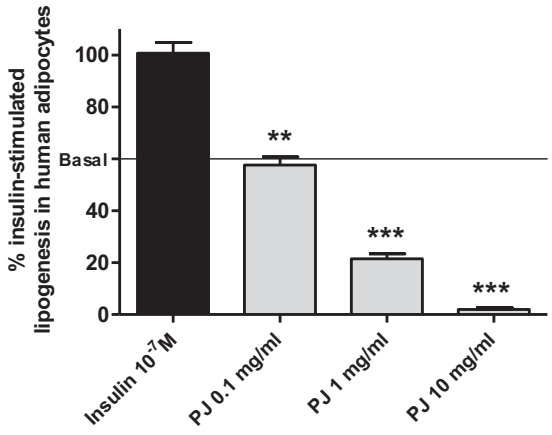

Fig. 6. PJ inhibits insulin-stimulated lipogenesis in human adipocytes. Data expressed as percent of insulin-stimulated lipogenesis, with 100% indicating glucose incorporation into lipids in response to insulin 10^{-7} M. Each column is mean ± SEM of 4–9 adipocyte preparations. Significant differences (**: $p < 0.01$ and ***: $p < 0.001$) were obtained by performing Student *t*-test comparing with respective control treated with insulin 10^{-7} M alone.

Finally, PJ action was investigated in human adipocyte lipolysis. A partial (72%) but significant antilipolytic effect was observed since, at 10 mg/mL, PJ impaired isoprenaline lipolytic stimulation (Fig. 8).

4. Discussion

Pomegranate juice is a beverage providing a good source of phenolic compounds, the more quantitatively important being punicalagins and ellagic acid (Gil et al., 2000; Les et al., 2015). The juice has an important antioxidant activity which could bring benefits in the metabolic syndrome (Baez-Duarte et al., 2016; Ilkun et al., 2015; Rangel-Huerta et al., 2015). This study demonstrated for the first time the inhibitory effect of PJ on the native form of monoamine oxidase enzymes, widely known to act on neurotransmitters but also highly expressed in numerous peripheral tissues, including the subcutaneous fat depots (Pizzinat et al., 1999), and the heart, in which MAO is recognized as a factor contributing to oxidative stress since it generates hydrogen peroxide when oxidizing its substrates (Kaludercic et al., 2014). A recent study from our group demonstrated that PJ could inhibit MAO-A (Les et al., 2015); however, this previous approach was based on a colorimetric method using a human recombinant enzyme directly acquired from a commercial supplier. Here, the results of a radiochemical method, much more specific than the colorimetric one, show the real ability of PJ to inhibit these enzymes. When comparing both methods, it can be observed that the IC_{50} of PJ towards MAO activity is higher with radiochemical than with colorimetric assay. Such difference is likely due to the lower specificity of the colorimetric assay, in which any antioxidant can blunt hydrogen peroxide detection and subsequent colour reaction, thereby falsely overestimating MAO inhibition. Such artifactual quenching of the detection of hydrogen peroxide as an end-product of amine oxidase activity has been recently reported for several dietary polyphenols (Carpéné et al., 2016). Moreover, as the radiochemical method was performed with [^{14}C]-tyramine as a substrate, the minor MAO-B activity naturally present alongside MAO-A in hAT (Pizzinat et al., 1999) participated to the reaction and the resulting inhibition curve differed from the blockade of pure MAO-A isoform. Otherwise, our study confirms that PJ inhibits MAO activity and indicates that its quantitatively major component, punicalagin, is predominantly – though not exclusively – involved in such MAO inhibition. Other polyphenols present in PJ have been reported to inhibit MAO (Carpéné, Galitzky, et al., 2016; Carpéné, Hasnaoui, et al., 2016), but they are largely less abundant than punicalagins, as it is the case for quercetin or coumaric and caffeic acids, and their contribution, though not excluded, appears minor. Ellagic acid is probably not a better MAO inhibitor than the above-mentioned polyphenols, but it is quantitatively more abundant in PJ and can act in synergism when combined with punicalagin. Several studies have already revealed such synergism between polyphenols though the underlying mechanism remain undefined: it is especially the case for the anti-adipogenic action of resveratrol and quercetin (Herranz-López et al., 2012; Yang et al., 2008).

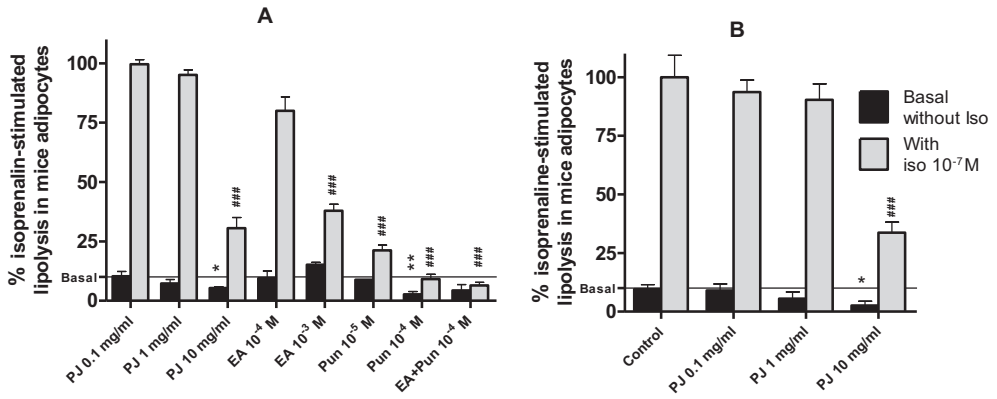

Fig. 7. PJ and its components inhibit lipolysis in mouse adipocytes. Lipolysis was determined under basal (black columns) conditions or after stimulation by the β-adrenergic agonist isoprenaline (iso) at 10^{-7} M (grey columns). (A) Glycerol release: each column is mean ± SEM of 4–9 adipocyte preparations. Significantly different from basal (without isoprenaline, black line) (*: $p < 0.05$, **: $p < 0.01$), or from isoprenaline-stimulated lipolytic activity (100%) ($^{\#\#\#}$: $p < 0.001$). (B) FFA release: each column is mean ± SEM of 7–9 adipocyte preparations. Significantly different from basal (without isoprenaline, black line) (*: $p < 0.05$), or from isoprenaline-stimulated lipolytic activity ($^{\#\#\#}$: $p < 0.001$).

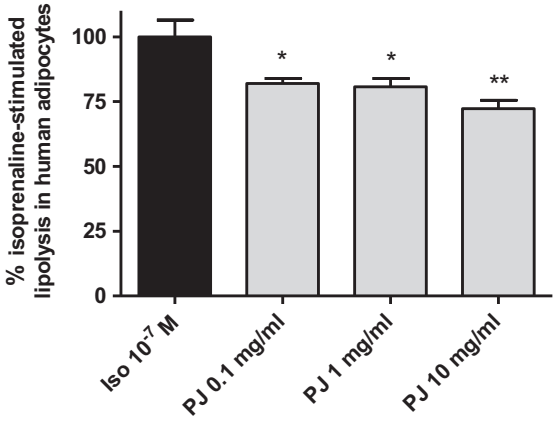

Fig. 8. PJ-induced lipolysis inhibition in human adipocytes. Glycerol release in response to β-adrenergic stimulation by the agonist isoprenaline (iso) at 10^{-7} M was set at 100%. Each column is mean ± SEM of 3–4 adipocyte preparations. Significant differences * ($p < 0.05$) and ** ($p < 0.01$) vs isoprenaline alone.

PJ and ellagic acid also impaired SSAO activity, but ellagic acid and punicalagin actions did not show any synergy that could account for the overall activity of the juice. According to previous studies, several phenolic compounds (resveratrol, pterostilbene, quercetin, and caffeic acid) possess MAO inhibitory properties while they are ineffective towards SSAO (Carpéné, Galitzky, et al., 2016; Carpéné, Hasnaoui, et al., 2016). This might explain the lower inhibitory potency of the juice and its main components towards an enzyme that functions alongside MAO in mature adipocytes (Bour et al., 2007), while playing still elusive roles (Morin et al., 2001; Shen et al., 2012). At this stage, it is worth mentioning that MAO and SSAO have their own selective inhibitors and catalytic mechanisms but share the capacity to generate hydrogen peroxide when oxidizing their corresponding amine substrates. So, in addition to its antioxidant capacity, PJ can directly impairs MAO and activity and therefore inhibit a larger panel of their multiple roles in cell/tissue biology than mere antioxidants, expected to counteract only the consequences of hydrogen peroxide release. Thus, our simplified assays brought evidence that PJ components directly and rapidly prevented the generation of labelled aldehydes corresponding to the deamination of [^{14}C]-tyramine and [^{14}C]-benzylamine and were not acting simply as hydrogen peroxide scavengers. Taken as a whole, our findings are therefore in agreement with previous studies that have independently reported that different pomegranate extracts exert antidepressant and neuroprotective activity that could be related to MAO inhibition, since MAO inhibitors have been used as antidepressant drugs (Jinu et al., 2016; Naveen et al., 2013).

Though PJ and its polyphenols were tested on a broad range, the effective doses were rather elevated, with IC_{50} values of 8.33 and 16.63 mg/mL for PJ lyophilizate towards MAO and SSAO inhibition, respectively. Since 100 mL of PJ contain more than 200 mg of total polyphenols according to database phenol-explorer.eu and previous studies (Gil et al., 2000; Les et al., 2015), the tested concentrations should be considered as supranutritional, since they are hardly attained in the body after the ingestion of a single serving of juice. However, it must be taken into account that their effects were observed in hAT homogenates with preincubation and incubation periods of 30 min only. The unambiguous inhibition of amine oxidation upon addition of PJ in our *in vitro* model suggests that such inhibition likely occurs in one or several anatomical locations under *in vivo* conditions since, at least for ellagic acid, it has been reported that plasma Cmax is reached in one hour after PJ ingestion and that elimination half-life also lasts about one hour (Seeram et al., 2006). Ellagitannins and ellagic acid are catabolized by the intestinal microbiota, generating urolithins that have been recently to exert important mitophagy-related beneficial effects in muscles (Ryu et al., 2016) and to exert antilipogenic action in adipocytes and hepatocytes (Kang et al., 2016). However, the fraction of ingested ellagic acid that escapes to this early metabolism and circulates in plasma may interact with visceral fat depots, subjected to postprandial changes in blood supply. Therefore, it cannot be ruled out that several of the PJ components can directly interact with fat cells. Taken one by one, any of the components contained in a serving of juice could be insufficient to reach the doses necessary to inhibit the adipocyte amine oxidases, but one has to consider that all these polyphenols and other factors may act in an additive rather than in a competitive manner. This renders less questionable that the sum of their minor individual effects results sufficient to regulate amine oxidase activity. Such addition or synergism between dietary polyphenols also applies for other adipocyte functions (Herranz-López et al., 2012; Yang et al., 2008), as reported here for basal lipogenesis in mouse. Of note, lipogenesis was affected in human adipocytes by doses as low as 0.1 mg/mL of PJ lyophilizate, corresponding to a dilution of pure PJ by more than one-thousand fold, an issue conceivable in a consumer after ingestion of decilitres of juice or concentrated extracts.

Other evidences supported our study of the influence of PJ on both amine oxidase and lipolytic or lipogenic activities in adipose tissue. First, MAO and SSAO expression is increased during adipogenesis (Bour et al., 2007). Then, several SSAO inhibitors have been reported to inhibit lipogenesis in fat cells (Carpéné et al., 2013) while they also limit fat deposition when chronically administered to rodents (Mercader, Iffiú-Soltész, Bour, & Carpéné, 2011). Lastly, diverse polyphenols present in foods (Les et al., 2016) or appearing after PJ intestinal metabolism (Cerdá, Periago, Espín, & Tomás-Barberán, 2005) have been reported to inhibit lipogenesis (Kang et al., 2016). However the quantitatively main polyphenols of PJ were not predominantly involved in its antilipogenic action in mouse adipocytes. Considering the inhibitory effect of PJ on amine oxidation, and the antilipogenic effects of several SSAO inhibitors (Carpéné et al., 2013), we verified that there was no difference in the inhibition of lipogenesis between wild type and SSAO-invalidated mice. Thus, it can be assessed that SSAO inhibition was not required for the decreased lipogenesis observed in response to PJ. Up to now, it has been only reported that some parts of the pomegranate fruit can modify the expression of genes involved in lipogenesis (Al-Shaaibi et al., 2016; Yuan et al., 2012). However these previous studies have never been performed with the fruit juice and, to the best of our knowledge, a direct effect on lipolytic activity has never been characterized before. We bring evidence that PJ inhibits lipogenesis in a dose-dependent manner in both murine and human adipocytes, but not punicalagin and ellagic acid. These compounds decreased basal lipogenesis only when tested in combination. Consequently, the molecular mechanisms of this observed PJ effect require further investigations. Whether the ellagitannins (mainly punicalagin) or the ellagic acid were metabolized under our *in vitro* conditions into urolithins, recently described as potent inhibitors of triacylglycerol accumulation in fat cells constituted an attractive working hypothesis. However, such hypothesis does not appear totally relevant since PJ was antilipogenic in mouse adipocytes but not ellagic acid. Whether urolithins generation occurred only when combining fat cells and PJ, and not when providing only ellagic acid to the fat cells was hardly conceivable. Previous studies have suggested that punicic acid, a polyunsaturated fatty acid from pomegranate seed oil, suppresses accumulation of lipid droplets in adipocytes (de Melo et al., 2016; Lai et al., 2012). Since a non-negligible proportion of fatty acids are found in PJ, this might contribute to our observed effects.

Additionally, PJ inhibited lipolysis with a lower efficiency than lipogenesis. Though being capable to limit glycerol and FFA release at only the higher dose tested, PJ inhibits both basal and isoprenaline-stimulated lipolysis in human and mouse adipose cells. In this case, the polyphenols that are present in the juice exhibited much more activity than the whole PJ, reaching total abolishment of lipolysis in the case of punicalagin. While the golden reference of the dietary polyphenols, resveratrol is rather lipolytic, our data allow to propose PJ and especially punicalagins as antilipolytic agents potentially able to limit the lipotoxicity associated with excessive fat accumulation, dyslipidaemia and insulin resistance (Guilherme, Virbasius, Puri, & Czech, 2008).

5. Conclusion

The ability of pomegranate juice to inhibit amine oxidases and to decrease lipogenesis and lipolysis have to be added to the well-known antioxidant capacity, giving to this natural product and its main polyphenols a potential in the treatment or prevention of metabolic syndrome. It deserves to be elucidated in future clinical trials whether sustained supplementation with this somewhat functional food is beneficial for the prevention of diseases associated with obesity, diabetes and dyslipidaemias.

Conflict of interests

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jff.2017.04.006.

References

Al-Shaaibi, S. N. K., Waly, M. I., Al-Subhi, L., Tageldin, M. H., Al-Balushi, N. M., & Rahman, M. S. (2016). Ameliorative effects of pomegranate peel extract against dietary-induced nonalcoholic fatty liver in rats. *Preventive Nutrition and Food Science, 21*, 14–23.

Ambigaipalan, P., de Camargo, A. C., & Shahidi, F. (2017). Identification of phenolic antioxidants and bioactives of pomegranate seeds following juice extraction using HPLC-DAD-ESI-MSn. *Food Chemistry, 221*, 1883–1894.

Baez-Duarte, B. G., Zamora-Ginez, I., De Jésus, K. L., Torres-Rasgado, E., González-Mejía, M. E., Porchia, L., ... Pérez-Fuentes, R. (2016). Association of the metabolic syndrome with antioxidant defense and outstanding superoxide dismutase activity in Mexican subjects. *Metabolic Syndrome and Related Disorders, 14*, 154–160.

Bour, S., Daviaud, D., Gres, S., Lefort, C., Prévot, D., Zorzano, A., ... Carpéné, C. (2007). Adipogenesis-related increase of semicarbazide-sensitive amine oxidase and monoamine oxidase in human adipocytes. *Biochimie, 89*, 916–925.

Brighenti, V., Groothuis, S. F., Prencipe, F. P., Amir, R., Benvenuti, S., & Pellati, F. (2017). Metabolite fingerprinting of *Punica granatum* L. (pomegranate) polyphenols by means of high-performance liquid chromatography with diode array and electrospray ionization-mass spectrometry detection. *Journal of Chromatography A, 1480*, 20–31.

Carpéné, C., Galitzky, J., & Saulnier-Blache, J. S. (2016). Short-term and rapid effects of lysophosphatidic acid on human adipose cell lipolytic and glucose uptake activities. *AIMS Molecular Science, 3*, 222–237.

Carpéné, C., Gomez-Zorita, S., Gupta, R., Grès, S., Rancoule, C., Cadoudal, T., ... Iffiu-Soltész, Z. (2014). Combination of low dose of the anti-adipogenic agents resveratrol and phenelzine in drinking water is not sufficient to prevent obesity in very-high-fat diet-fed mice. *European Journal of Nutrition, 53*, 1625–1635.

Carpéné, C., Grès, S., & Rascalou, S. (2013). The amine oxidase inhibitor phenelzine limits lipogenesis in adipocytes without inhibiting insulin action on glucose uptake. *Journal of Neural Transmission (Vienna, Austria : 1996), 120*, 997–1003.

Carpéné, C., Hasnaoui, M., Balogh, B., Matyus, P., Fernández-Quintela, A., Rodríguez, V., ... Portillo, M. P. (2016). Dietary phenolic compounds interfere with the fate of hydrogen peroxide in human adipose tissue but do not directly inhibit primary amine oxidase activity. *Oxidative Medicine and Cellular Longevity, 2016*, 2427618.

Cerdá, B., Periago, P., Espín, J. C., & Tomás-Barberán, F. A. (2005). Identification of urolithin A as a metabolite produced by human colon microflora from ellagic acid and related compounds. *Journal of Agricultural and Food Chemistry, 53*, 5571–5576.

de Melo, I. L. P., de Oliveira e Silva, A. M., de Carvalho, E. B. T., Yoshime, L. T., Sattler, J. A. G., & Mancini-Filho, J. (2016). Incorporation and effects of punicic acid on muscle and adipose tissues of rats. *Lipids in Health and Disease, 15*, 40.

Dushkin, M., Khrapova, M., Kovshik, G., Chasovskikh, M., Menshchikova, E., Trufakin, V., ... Vereschagin, E. (2014). Effects of rhaponticum carthamoides versus *Glycyrrhiza glabra* and *Punica granatum* extracts on metabolic syndrome signs in rats. *BMC Complementary and Alternative Medicine, 14*, 33.

Ekhlasi, G., Shidfar, F., Agah, S., Merat, S., & Hosseini, A. F. (2016). Effects of pomegranate and orange juice on antioxidant status in non-alcoholic fatty liver disease patients: A randomized clinical trial. *International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin- Und Ernahrungsforschung. Journal International de Vitaminologie et de Nutrition*, 1–7.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry, 48*, 4581–4589.

Gomez-Zorita, S., Tréguer, K., Mercader, J., & Carpéné, C. (2013). Resveratrol directly affects *in vitro* lipolysis and glucose transport in human fat cells. *Journal of Physiology and Biochemistry, 69*, 585–593.

González-Sarrías, A., García-Villalba, R., Núñez-Sánchez, M. Á., Tomé-Carneiro, J., Zafrilla, P., Mulero, J., ... Espín, J. C. (2015). Identifying the limits for ellagic acid bioavailability: A crossover pharmacokinetic study in healthy volunteers after consumption of pomegranate extracts. *Journal of Functional Foods, 19*, 225–235.

Grès, S., Bour, S., Valet, P., & Carpéné, C. (2012). Benzylamine antihyperglycemic effect is abolished by AOC3 gene invalidation in mice but not rescued by

semicarbazide-sensitive amine oxidase expression under the control of aP2 promoter. *Journal of Physiology and Biochemistry, 68*, 651–662.
Guilherme, A., Virbasius, J. V., Puri, V., & Czech, M. P. (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nature Reviews. Molecular Cell Biology, 9*, 367–377.
Herranz-López, M., Fernández-Arroyo, S., Pérez-Sanchez, A., Barrajón-Catalán, E., Beltrán-Debón, R., Menéndez, J. A., ... Micol, V. (2012). Synergism of plant-derived polyphenols in adipogenesis: Perspectives and implications. *Phytomedicine, 19*, 253–261.
Ilkun, O., Wilde, N., Tuinei, J., Pires, K. M. P., Zhu, Y., Bugger, H., ... Abel, E. D. (2015). Antioxidant treatment normalizes mitochondrial energetics and myocardial insulin sensitivity independently of changes in systemic metabolic homeostasis in a mouse model of the metabolic syndrome. *Journal of Molecular and Cellular Cardiology, 85*, 104–116.
Jinu, K. V, Sai Sailesh, K., & Mukkadan, J. K. (2016). A comprehensive review on neuroprotective effects of pomegranate. *International Journal of Research in Ayurveda and Pharmacy, 7*, 48–51.
Kaludercic, N., Mialet-Perez, J., Paolocci, N., Parini, A., & Di Lisa, F. (2014). Monoamine oxidases as sources of oxidants in the heart. *Journal of Molecular and Cellular Cardiology, 73*, 34–42.
Kang, I., Kim, Y., Tomás-Barberán, F. A., Espín, J. C., & Chung, S. (2016). Urolithin A, C, and D, but not iso-urolithin A and urolithin B, attenuate triglyceride accumulation in human cultures of adipocytes and hepatocytes. *Molecular Nutrition & Food Research, 60*, 1129–1138.
Kim, M. S., & Choi, C. S. (2013). HPLC analysis and *in vitro* study of the extract from *Punica granatum* peel. *Rapid Communication in Photoscience, 2*, 28–30.
Kozik, V., Jarzembek, K., Jędrzejowska, A., Bąk, A., Polak, J., Bartoszek, M., & Pytlakowska, K. (n.d.). Investigation of antioxidant activity of pomegranate juices by means of electron paramagnetic resonance and UV-Vis spectroscopy. *Journal of AOAC International*, 98, 866–870.
Lai, C.-S., Tsai, M.-L., Badmaev, V., Jimenez, M., Ho, C.-T., & Pan, M.-H. (2012). Xanthigen suppresses preadipocyte differentiation and adipogenesis through down-regulation of PPARγ and C/EBPs and modulation of SIRT-1, AMPK, and FoxO pathways. *Journal of Agricultural and Food Chemistry, 60*, 1094–1101.
Legua, P., Forner-Giner, M. Á., Nuncio-Jáuregui, N., & Hernández, F. (2016). Polyphenolic compounds, anthocyanins and antioxidant activity of nineteen pomegranate fruits: A rich source of bioactive compounds. *Journal of Functional Foods, 23*, 628–636.
Les, F., Deleruyelle, S., Cassagnes, L.-E., Boutin, J. A., Balogh, B., Arbones-Mainar, J. M., ... Carpéné, C. (2016). Piceatannol and resveratrol share inhibitory effects on hydrogen peroxide release, monoamine oxidase and lipogenic activities in adipose tissue, but differ in their antilipolytic properties. *Chemico-Biological Interactions, 258*, 115–125.
Les, F., Prieto, J. M., Arbonés-Mainar, J. M., Valero, M. S., & López, V. (2015). Bioactive properties of commercialised pomegranate (*Punica granatum*) juice: Antioxidant, antiproliferative and enzyme inhibiting activities. *Food & Function, 6*, 2049–2057.
Li, H. Y., Jiang, Y. D., Chang, T. J., Wei, J. N., Lin, M. S., Lin, C. H., ... Chuang, L.-M. (2011). Serum vascular adhesion protein-1 predicts 10-year cardiovascular and cancer mortality in individuals with type 2 diabetes. *Diabetes, 60*, 993–999.
Medjakovic, S., & Jungbauer, A. (2013). Pomegranate: A fruit that ameliorates metabolic syndrome. *Food & Function, 4*, 19–39.
Mercader, J., Iffiú-Soltész, Z., Bour, S., & Carpéné, C. (2011). Oral administration of semicarbazide limits weight gain together with inhibition of fat deposition and of primary amine oxidase activity in adipose tissue. *Journal of Obesity, 2011*, 1–10.
Moody, A. J., Stan, M. A., Stan, M., & Gliemann, J. (1974). A simple free fat cell bioassay for insulin. *Hormone, 6*, 12–16.
Morin, N., Lizcano, J. M., Fontana, E., Marti, L., Smih, F., Rouet, P., ... Carpéné, C. (2001). Semicarbazide-sensitive amine oxidase substrates stimulate glucose transport and inhibit lipolysis in human adipocytes. *The Journal of Pharmacology and Experimental Therapeutics, 297*, 563–572.
Naveen, S., Siddalingaswamy, M., Singsit, D., & Khanum, F. (2013). Anti-depressive effect of polyphenols and omega-3 fatty acid from pomegranate peel and flax seed in mice exposed to chronic mild stress. *Psychiatry and Clinical Neurosciences, 67*, 501–508.
Nuncio-Jáuregui, N., Nowicka, P., Munera-Picazo, S., Hernández, F., Carbonell-Barrachina, Á. A., & Wojdyło, A. (2015). Identification and quantification of major derivatives of ellagic acid and antioxidant properties of thinning and ripe Spanish pomegranates. *Journal of Functional Foods, 12*, 354–364.
Ok, E., Do, G.-M., Lim, Y., Park, J.-E., Park, Y.-J., & Kwon, O. (2013). Pomegranate vinegar attenuates adiposity in obese rats through coordinated control of AMPK signaling in the liver and adipose tissue. *Lipids in Health and Disease, 12*, 163.
Pizzinat, N., Marti, L., Remaury, A., Leger, F., Langin, D., Lafontan, M., ... Parini, A. (1999). High expression of monoamine oxidases in human white adipose tissue: Evidence for their involvement in noradrenaline clearance. *Biochemical Pharmacology, 58*, 1735–1742.
Rangel-Huerta, O. D., Aguilera, C. M., Martin, M. V., Soto, M. J., Rico, M. C., Vallejo, F., ... Mesa, M. D. (2015). Normal or high polyphenol concentration in orange juice affects antioxidant activity, blood pressure, and body weight in obese or overweight adults. *The Journal of Nutrition, 145*, 1808–1816.
Ryu, D., Mouchiroud, L., Andreux, P. A., Katsyuba, E., Moullan, N., Nicolet-dit-Félix, A. A., ... Auwerx, J. (2016). Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. *Nature Medicine, 22*, 879–888.
Saponaro, C., Gaggini, M., Carli, F., & Gastaldelli, A. (2015). The subtle balance between lipolysis and lipogenesis: A critical point in metabolic homeostasis. *Nutrients, 7*, 9453–9474.
Seeram, N. P., Henning, S. M., Zhang, Y., Suchard, M., Li, Z., & Heber, D. (2006). Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 hours. *The Journal of Nutrition, 136*, 2481–2485.
Shen, S. H., Wertz, D. L., & Klinman, J. P. (2012). Implication for functions of the ectopic adipocyte copper amine oxidase (AOC3) from purified enzyme and cell-based kinetic studies. *PLoS ONE, 7*, e29270.
Singleton, V. L. (1985). Citation classic—Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Current contents/agriculture. *Biology Environmental Science, 48*, 18.
Tipton, K. F., Davey, G., & Motherway, M. (2006). Monoamine oxidase assays. *Current protocols in toxicology/editorial board, Mahin D. Maines (Editor-in-Chief), Chapter 4*, Unit4.21.
Tsang, C., Smail, N. F., Almoosawi, S., Davidson, I., & Al-Dujaili, E. A. S. (2012). Intake of polyphenol-rich pomegranate pure juice influences urinary glucocorticoids, blood pressure and homeostasis model assessment of insulin resistance in human volunteers. *Journal of Nutritional Science, 1*, e9.
Yang, J.-Y., Della-Fera, M. A., Rayalam, S., Ambati, S., Hartzell, D. L., Park, H. J., & Baile, C. A. (2008). Enhanced inhibition of adipogenesis and induction of apoptosis in 3T3-L1 adipocytes with combinations of resveratrol and quercetin. *Life Sciences, 82*, 1032–1039.
Yuan, T., Ding, Y., Wan, C., Li, L., Xu, J., Liu, K., ... Seeram, N. P. (2012). Antidiabetic ellagitannins from pomegranate flowers: Inhibition of α-glucosidase and lipogenic gene expression. *Organic Letters, 14*, 5358–5361.