Evidence for health properties of pomegranate juices and extracts beyond nutrition: A critical systematic review of human studies

Juan Antonio Giménez-Bastida, María Ángeles Ávila-Gálvez, Juan Carlos Espín ***, Antonio González-Sarrías

Food & Health Laboratory; Research Group on Quality, Safety, and Bioactivity of Plant Foods, CEBAS-CSIC, P.O. Box 164, 30100, Campus de Espinardo, Murcia, Spain

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ABSTRACT

Background: The consumption of pomegranate juices and extracts has long been linked to many health benefits beyond nutrition, described mainly by innumerable preclinical studies. However, the European Food Safety Authority (EFSA) concluded in 2010 that a cause and effect relationship could not be established between the consumption of pomegranate-derived products and all the health claims presented. There are no additional EFSA opinions on health claims specifically addressed to pomegranate in the last decade.

Scope and approach: This review comprehensively compiles all human studies conducted on pomegranate. The aim is to discuss these studies critically to identify possible flaws and propose guidelines that might help establish a cause and effect relationship between pomegranate-derived product consumption and health.

Key findings and conclusions: To date, 86 human studies have evaluated the health benefits of pomegranate juices and extracts. The most promising, albeit scarce, evidence is related to blood pressure improvement. Less evidence deals with inflammation, cancer, cognitive function, physical activity, and gut microbiota modulation (prebiotic effects). After a decade since EFSA’s opinion, human evidence remains inconsistent, making it difficult to support most claimed health effects. The lack of effects and/or data discrepancy might be attributable to design limitations, including insufficient product characterization and interindividual variability that influence pomegranate polyphenols’ bioefficacy. New coordinated strategies between policy makers, research/academic institutions, and industry are needed to move forward. Therefore, this review presents a roadmap to conduct well-designed trials and cover existing gaps, which could establish a cause-effect relation between pomegranate consumption and health benefits beyond nutrition.

1. Introduction

Numerous epidemiological, observational, preclinical, and human intervention studies highlight the inverse correlation between consuming an adequate amount of plant-derived foods and the risk of suffering chronic diseases (Aune et al., 2017; Yip et al., 2019). In this line, pomegranate has gained attention primarily due to its possible health benefits beyond basic nutrition (Hou et al., 2019; Karimi et al., 2017).

Pomegranate is an excellent source of dietary fibre and health-benefiting nutrients, including vitamins (i.e., vitamin C, A, folic acid) and minerals (such as potassium). It is also a rich source of phenolic compounds and some alkaloids, triterpenes, and sterols. Pomegranates are also rich in unsaturated fatty acids like the omega 5 punicic acid that constitutes around 70% of pomegranate seed oil (Sreekumar et al., 2014; Zaouay et al., 2012). These compounds have been suggested to exert numerous beneficial health activities (beyond their nutritional properties) and are the basis for considering pomegranate as a possible functional food (Saeed et al., 2018).

The peel represents approximately 50% of the total weight of the pomegranate fruit and contains a much higher amount of polyphenols than the edible arils. Ellagitannins (punicalin and two isomers of punicalagin), gallotannins, proanthocyanidins, and ellagic acid derivatives are the primary peel polyphenols. For this reason, the peel is mainly used to obtain food additives or potential functional ingredients. Besides, anthocyanins are minor phenolics present in the outer part of the peel and the arils, and responsible for the characteristic pomegranate colour (Akhtar et al., 2015; Díaz-Mula et al., 2019).

** Corresponding author.
*** Corresponding author.
E-mail addresses: josepn@cebas.csic.es (J.C. Espín), agsarrias@cebas.csic.es (A. González-Sarrías).

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The polyphenolic fraction, mainly composed of ellagitannins, has been recognized as the major active component of pomegranate by contributing to the benefits for human health attributed to pomegranate juice (PJ) and extract (PE). Not only due to the early antioxidant capacity, as previously reported (Gill et al., 2000), but also either directly or indirectly through its derivatives produced by the gut microbiota after ingestion (Fahmy et al., 2020). Despite the different proportions and variability described in this fruit, punicalagin and ellagic acid derivatives are the most abundant phenolic compounds (over 60%) and are almost exclusively found in the peel (Li, Li, Zhao, & Yu, 2009). Therefore, a common industrial strategy to obtain ellagitannin-rich PJs is to press the entire fruit or add punicaglin-rich PEs to PJs, previously obtained from arils.

Pomegranate polyphenols, and particularly punicalagin and ellagic acid, are poorly absorbed in the gastrointestinal tract. Instead, these molecules are further catabolized by the gut microbiota into bioavailable derivatives named urolithins, which have been recognized as the main bioactive metabolites of ellagitannin-rich foods, such as pomegranate (Cerdá et al., 2004, 2005; Tomás-Barberán et al., 2017). These urolithins can be detected at high concentrations in the colon (Núñez-Sánchez et al., 2014) and are subsequently absorbed and metabolized into their phase-II conjugates (glucuronides or sulphates) that can persist in the bloodstream up to 3-4 days after the intake (Cerdá et al., 2005; González-Sarrías, Espin, & Tomás-Barberán, 2017). Therefore, these metabolites can also reach systemic tissues such as the prostate and mammary gland, where they could exert beneficial activities (González-Sarrías, Giménez-Bastida, et al., 2010; Ávila-Gálvez et al., 2019). Since their identification in 2004 in humans (Cerdá et al., 2004), urolithins have been put under the spotlight of nutritional research owing to their putative ability to impact human health attributed to ellagitannin-rich foods like pomegranate (Cortés-Martín, Selma, et al., 2020; Tomás-Barberán et al., 2017).

In parallel with the growing consumers’ demand for healthier food products, the scientific community has made a considerable effort to thoroughly investigate the beneficial health effects of pomegranate consumption in the last two decades. Hence, numerous in vitro and, to a lesser extent, in vivo studies have focused on the biological activity and health effects of pomegranate and some of its constituents, mainly phenolic compounds. Like other fruits or plant foods, there are numerous underlying mechanisms of action linked to the potential benefits exerted by different pomegranate components. However, the vast majority of results come from in vitro and(or) animal studies conducted with questionable physiological extrapolation to humans. This is due to the use of unrealistic high doses of beverages and(or) extracts, and(or) polyphenols as occurring in the food without considering their bioavailability, metabolism and(or) distribution in human tissues (Vlahojannis et al., 2015; Ávila-Gálvez et al., 2018).

The European Food Safety Authority (EFSA) has rejected three health claims related to pomegranate consumption and health beyond basic nutrition. The most relevant EFSA scientific opinion, in 2010, concluded that a cause and effect relationship could not be established between the consumption of pomegranate-derived products (PJ, PE, and seeds) and several health claims. The rejected claims were related to normal erectile function, protection of lipids from oxidative damage, antioxidant and anti-aging properties, increased appetite after unintentional weight loss leading to an increase in energy intake, and maintenance of normal blood glucose concentrations (EFSA Panel on Dietetic Products, 2010). Later, EFSA rejected a claim dealing with the intake of a mixture of pomegranate, grape, lemon, and elderberry juices, among other constituents, and increased attention (EFSA Panel on Dietetic Products, 2014). Finally, EFSA rejected another pomegranate-related health claim associated with PE consumption together with greater galangal rhizome powder and the increase in the number of motile spermatozoa in semen (EFSA Panel on Dietetic Products, 2015).

In the last decade, since EFSA rejected the most relevant health claims, the evidence of the beneficial effects of pomegranate consumption has grown, mainly supported by an increase of human studies with PJ and PE as well as data from some meta-analyses (García-Conesa et al., 2018; Sahebkar et al., 2017). Also, the increase of preclinical studies that have used physiologically relevant conditions in terms of the molecular form and concentration of the pomegranate polyphenolic compounds (or their derivatives) have unequivocally pointed out the critical role of the polyphenolic fraction in the possible protective effects against different pathologies (Tomás-Barberán et al., 2017).

To date, there are no additional EFSA opinions on pomegranate-derived products and health claims. Thus, the EFSA’s position has not changed, i.e., there is no sufficiently well-established cause and effect relationship between the consumption of pomegranate-derived products and health beyond basic nutrition.

This study aims to compile and critically discuss all the human studies carried out hitherto that have evaluated the beneficial effects of the main pomegranate-derived products (PJ and PE) beyond their nutritional properties. Upon this critical appraisal, a roadmap will be presented, which might help establish a causal link between pomegranate-derived product consumption and health promotion.

2. Health benefits of pomegranate-derived products described in human studies

2.1. Search strategy and study selection

A comprehensive literature search was performed using SCOPUS (http://www.scopus.com) and Medline (http://www.ncbi.nlm.nih.gov/pubmed). We also hand searched the bibliographic of specific articles. The literature was searched from inception up to January 06, 2021.

Regarding eligibility criteria and study selection, human studies were limited to those testing the effect of PJ and(or) PE on defined outcomes, including antioxidant, cardiometabolic, cancer, neuro-protection, cognitive function, inflammation, prebiotic, exercise capacity and others. No restrictions were applied regarding the cohort and design of the human study. On the contrary, human studies in which pomegranate was combined with other dietary supplements were excluded. A flow diagram with the details of the study selection is shown in Fig. 1. Finally, 86 human studies met the inclusion criteria and were included in this systematic review.

2.2. Characteristics of the included studies

Supplementary Table S1 provides a summarized overview (objective, cohort, study design, the dose of the pomegranate-derived product, and relevant outcomes) of all human studies grouped according to the main beneficial health-related effects.

Of the all studies, 54 human studies (2,590 total participants) were conducted with PJ and 28 human studies (1,086 total participants) with PE, while 4 studies (251 total participants) evaluated the consumption of both PJ and PE in independent groups. Intervention doses ranged between 50 and 1,500 mL for PJ (66% of doses ranged from 200 to 300 mL) and between 125 mg and 6 g for PEs (75% of doses ranged from 250 mg to 1 g). It should be noted that 30 of 58 studies were conducted using commercial PJs, 23 of 32 used commercial PEs, while 4 studies were conducted with both pomegranate-derived products. Most interventions (~60%) ranged from 1 week to 2 months, whereas those conducted for more than 3 months or acute studies represented ~25% and ~15%, respectively, of the total number of interventions. Most of the studies (~75%) were human randomized controlled trials (RCTs), which had a control group receiving a placebo version in capsules or drinks (45 of 66 studies) or not subjected to intervention with any pomegranate product (20 of 65 studies). Among these RCTs, 13 of 66 studies followed a crossover design instead of a parallel-arms design. Other human studies included interventional, open-label, and non-randomized, and(or) non-controlled studies.

Many human studies have explored the beneficial effect against a
wide variety of biomarkers, including those associated with cardiometabolic risk \((n = 35)\) followed by antioxidant capacity \((n = 15)\), although some studies evaluated biomarkers of both chronic diseases. In this regard, although only 5 studies focused on inflammation, they have been grouped in Supplementary Table S1. The other 5 studies also evaluated different inflammatory biomarkers as secondary outcomes. Other beneficial health effects focused on cancer \((n = 8)\), neuroprotective activity, and improvement of cognitive function \((n = 5)\), as well as prebiotic effect \((n = 6)\), among others. Finally, other trials have addressed the physical activity improvement \((n = 8)\) by PJ and PE.

The participants in these human studies mostly represented a mixed population of men and women ranging from young adolescents to elderly participants, although with a higher prevalence (~60%) of adults (20–60 years). Only 14 and 8 studies were conducted separately with men and women, respectively. Concerning the health status, the sample population constituted of healthy individuals (35 studies; 1,186 participants), obese individuals with a BMI > 30.0 kg/m² or with metabolic syndrome traits but not medicated (5 studies; 169 participants), and individuals with diagnosed chronic disease (patients) (46 studies; 2,572 participants). Among the trials that included patients, 13 studies included patients with diagnosed type 2 diabetes, 8 with coronary heart-related diseases, and 7 studies with metabolic syndrome and related disorders such as hypertensive, hyperlipidaemia, and hypercholesterolemia. Other diagnosed chronic diseases included in fewer human studies were cancer \((n = 7)\), mainly prostate cancer, rheumatoid arthritis \((n = 2)\), as well as patients subjected to haemodialysis \((n = 5)\), among others.

From now on, the main results obtained in human studies are summarized in those that show the evidence-based beneficial effects upon PJ and(or) PE consumption on different chronic diseases and(or) related pathogenic processes. Besides, this evidence is critically discussed to understand the current EFSA position.

### 2.3. Antioxidant effect

Evidence of the antioxidant activity of pomegranate (one of the most investigated ETs-rich fruits) comes from in vivo studies that showed an improvement of the antioxidant status of mice after pomegranate juice consumption (Aviram et al., 2000).

Additional in vitro studies supported its high antioxidant potential, primarily linked to ellagitannins (Gil et al., 2000). However, ellagitannins are not absorbed. Further studies on the antioxidant effect of the physiologically relevant circulating urolithins revealed inconsistent results in vitro using different analytical techniques and a lower antioxidant capacity than their precursors (Cerdà et al., 2004; Rosenblat et al., 2015). However, these findings contrast with in vivo studies describing associations between Uro-A level in plasma and antioxidant activity (Ishimoto et al., 2012) and the protection of this urolithin against cisplatin-induced renal oxidative damage in mice’s kidneys (Jing et al., 2019). Hence, the evidence supporting the role of urolithins as antioxidant molecules is weak, thus highlighting the need for additional investigations.

Human studies have supported the antioxidant potential of pomegranate, regardless of what molecules are responsible. Hitherto, 15 studies (12 and 3 studies with PJ and PE, respectively) focused on investigating pomegranate consumption against oxidative stress-related diseases (Supplementary Table S1). Other human studies have also evaluated several markers linked to oxidative damage as a secondary outcome (Aviram et al., 2004; Balbir-Gurman et al., 2011; Barati Boldaji et al., 2020; Bookheimer et al., 2013; Ghoochani et al., 2016; Wu et al., 2019).
Despite the large variability reported, a common result of all these studies is the potential antioxidant activity showed by PJ and PE based on their ability to reduce the level of TBARS, α-LDL, lipid peroxidation, and oxidative biomarkers related to cardiovascular risk in healthy volunteers (Gouda et al., 2016; Guo et al., 2008; Rosenblat et al., 2006). In this regard, PJ and PE have also shown the capacity to ameliorate the oxidative stress in plasma of patients under haemodialysis (Shema-Didi et al., 2013), overweight-obese subjects (Heber et al., 2007), metabolic syndrome patients (Kojadinovic et al., 2017) and hypercholesterolemic and diabetic patients (Hamoud et al., 2014; Parsaeyan et al., 2012; Sohrab et al., 2017). This attenuating effect was also described in trials that reported a reduction of the oxidative stress in the placenta of pregnant women after the consumption of PJ (n = 12) compared with the placebo group (Chen et al., 2012). Additional studies also showed increased plasma levels of GSH, SOD, and GPx in postmenopausal/type 2 diabetes women who exercised after consuming a PE (Yarmohammadi & Mahjoub, 2017).

Four different studies have described the protective effect of PJ intake (acute and/or regular consumption) against intensive exercise-induced oxidative damage in well-trained healthy volunteers through the improvement of oxidative biomarkers such as MDA, antioxidant enzymes (catalase, GPx, etc.), protein carbonyl level, and MMPs (Ammar et al., 2017; Fuster-Munoz et al., 2016; Mazani et al., 2014; Urbaniak et al., 2018).

In conclusion, regardless of the molecules responsible for the antioxidant activity of PJ and PE, the evidence on their antioxidant effects appears to indicate a small but beneficial combination of antioxidant factors and may contribute to preventing oxidative stress-related diseases. Nevertheless, more studies are needed in humans following the roadmap given below to demonstrate the antioxidant effects unequivocally. Besides, whether this small antioxidant effect is clinically relevant is far from being proven.

2.4. Cardioprotective activity

The most common cardiovascular disease (CVD) risk factors include cardiometabolic dysfunctions such as hypertension, hyperlipidaemia, obesity, glucose intolerance, diabetes, or the combination of most of these pathologies known as metabolic syndrome (Roth et al., 2017). For decades, a diet rich in plant-based foods has been recognized to prevent or ameliorate the effects of the aforementioned cardiometabolic disorders by providing a variety of micro- and macronutrients, i.e., minerals, vitamins, fibre, as well as a wide variety of phenolic compounds (Zhan et al., 2017). Considering this, numerous preclinical investigations (in vitro and animal models) have reported positive modulation of CVD risk markers (blood lipids, insulin resistance, glucose, lipid peroxidation, etc.) after administration of both pomegranate-related products (fruit, fresh and/or concentrated PJ, flowers, and PE) and/or their polyphenols. However, to date, the in vitro to in vivo extrapolation of all these beneficial activities is quite limited and requires further research (Vlachojannis et al., 2015).

Therefore, up to 35 human studies have been carried out to evaluate the cardioprotective evidence after PJ or PE consumption. However, global human evidence is still ambiguous, and the conclusions reached are inconsistent. One reason arises from the high heterogeneity between trials, where some include healthy volunteers others, patients with different pathologies (such as diabetes, hyperlipidaemia, metabolic syndrome, obesity, etc.) that could be under drug therapy (i.e., anti-diabetic, anti-hypertensive, and lipid-lowering drugs) or not. Especially in (poly)medicated patients, achieving and/or attributing clinically relevant effects to pomegranate-derived product intake is challenging. Another reason is attributed to the well-known interindvidual variability, which has a substantial impact on their effects.

Additionally, the high heterogeneity observed in the biomarkers evaluated in each study is another factor of variability that contributes to explain the inconsistency observed. In this regard, measurement of lipoprotein and lipid profiles, blood pressure and glucose levels have traditionally been used to diagnose subjects at high risk of CVD (ArsenaULT et al., 2011). Furthermore, these cardiovascular biomarkers have also been the most explored, unlike other well-established CVD risk biomarkers such as BMI, waist circumference, flow-mediated dilation (FMD), glycated haemoglobin (Hb1Ac), or insulin levels that have been less investigated in human studies. Even so, a recent meta-analysis, including 13 RCTs, reported no significant effect of pomegranate consumption on weight and body composition indices (Ghelfati et al., 2019). Therefore, these three primary outcomes will be the main focus when describing the potential human evidence behind pomegranate consumption associated health effects.

2.4.1. Effect on blood glucose

Hyperglycaemia is an important risk factor for developing chronic diseases, mainly type 2 diabetes. To the best of our knowledge, 23 human studies have evaluated the effect of pomegranate-derived products consumption on blood glucose levels, mainly for PJ (n = 18) compared with PE (n = 5). However, most studies reported a lack of effect on glucose levels after long-term supplementation ranging from 1 to 12 weeks (Supplementary Table S1). This is in agreement with (1) the EFSA claim that rejected the statement of the effect of pomegranate on the maintenance of normal glucose levels due to the lack of consistent evidence in the general population (EFSA Panel on Dietetic Products, 2010), (2) a meta-analysis that included from 6 to 7 RCTs conducted with both PJ and PEs reported no effect on glucose level reduction (García-Conesa et al., 2018) and (3) another meta-analysis that included 6 trials carried out with type 2 diabetes patients (Jandari et al., 2020). To date, only an interventional study in patients diagnosed with type 2 diabetes who consumed 200 mL of PJ daily for 6 weeks resulted in a significant reduction of fasting blood glucose (Parsaeyan et al., 2012). Nevertheless, another study in diabetic patients reported that the mean concentration of blood glucose was lower than in the control group, although not significantly, after 6-weeks supplementation (Sohrab et al., 2017). Similar results have been obtained for PE due to only 1 RCT reporting a significant reduction of glucose levels after 1-month supplementation in overweight and obese volunteers (Hosseini et al., 2016). However, no changes were observed in other RCTs conducted with overweight and obese volunteers after 3–4 weeks of supplementation (González-Sarrías, García-Villalba, et al., 2017; Heber et al., 2007). In conclusion, these studies failed to provide evidence on blood glucose regulation after regular PJ and PE consumption.

On the contrary, the evidence is more consistent regarding the acute (short-term) beneficial or protective effect on blood glucose levels. All human studies carried out thus far, investigating acute effects, have shown the beneficial effect of single doses of PJ, reducing fasting glucose levels in healthy normoweight volunteers with low fasting serum insulin ≤995 pg/mL, but not with high fasting serum insulin >995 pg/mL (Wang et al., 2020). The same authors also reported no reduction after 500 mg of ellagic acid supplementation compared to 237 mL of PJ, suggesting that other compounds apart from polyphenols present in the pomegranate and their microbial derivatives, urolithins, could be involved in this effect (Long et al., 2019). Additional studies have shown acute anti-hyperglycaemic effect and reduction of HOMA-IR by PJ consumption in healthy subjects with impaired fasting glucose (Banihani et al., 2020), hypoglycaemic responses together with higher β-cells function and lower insulin resistance in diabetic patients (Banihani et al., 2014), as well as amelioration of blood glucose increase after a weightlifting training session (Ammar et al., 2016).

Finally, a recent crossover RCT in healthy subjects compared the effect of PJ and polyphenol-rich PE consumption (single dose) on the postprandial blood glucose induction after bread consumption. The authors found that polyphenols were more effective in attenuating the increased glucose levels when present in juice than in the PE (Kerini et al., 2017).
Therefore, the possible reasons for the different effects on glucose regulation by pomegranate-derived products, mainly juices, between acute and regular intake, deserve attention in further human studies. Similarly, whether pomegranate polyphenols are involved in this effect remains enigmatic.

2.4.2. Effect on blood pressure

Hypertension, defined by a systolic and diastolic blood pressure greater than 140 and 90 mm Hg, respectively, is one of the most common risk factors of CVDs (heart failure, ischemic heart disease, and cerebrovascular events). Besides, hypertension also plays a critical role in other concomitant risk factors and diseases, such as chronic kidney disease, erectile dysfunction, and cognitive decline (Benjamin et al., 2019).

There is substantial evidence that describes a beneficial long-term effect of pomegranate-derived products on blood pressure management, mainly for PJ (n = 13 studies), based on the high number of studies showing a beneficial effect (11 of 17 studies were evaluated). These beneficial effects come from RCTs conducted in subjects who showed improved blood pressure values after a 4-week long supplementation period (Jynn et al., 2012) and amelioration of the acute blood pressure increase induced by a high-fat meal or weightlifting exercise (Ammar et al., 2016; Mathew et al., 2012). Overall, based on the studies included in Supplementary Table S1, pomegranate-derived products show mild (1–12 weeks) and long-term (6–12 months) effects related to the reduction of systolic and diastolic blood pressure in hypertensive patients with mild or high-cardiovascular risk, including those diagnosed with diabetes, metabolic syndrome and subjected to haemodialysis.

Furthermore, a meta-analysis focused exclusively on 8 RCTs carried out with PJ indicated that its consumption significantly reduces systolic and diastolic pressure, showing a higher effect in hypertensive patients. This suggests that PJ might be considered an effective tool combined with anti-hypertensive medications and a constituent of the daily diet for patients under high hypertension risk and CVD. However, further studies are needed to corroborate PJ’s long-term impact on the complications of hypertension and cardiovascular events (Sahebkar et al., 2017). Regarding other pomegranate-derived products, there is less evidence. In this respect, a meta-analysis that included 8 RCTs comprising 619 participants failed to report significant differences in blood pressure (Gbinigie et al., 2017). However, more robust evidence has recently been described in a meta-analysis that grouped 8 different RCTs that evaluated both PJ and PE and described a significant reduction in the diastolic blood pressure, but not systolic (García-Conesa et al., 2018). Notwithstanding, as a call for caution, the enthusiasm of these potential blood lipid-lowering effect of pomegranate consumption should be limited for mixing RCTs with anti-hypertensive drug-treated subjects and non-medicated ones, in which clinically relevant effects, if any, were almost negligible.

In conclusion, cumulative evidence from the human trials indicates that the consumption of pomegranate-derived products, mainly PJ, might modestly but significantly improve blood pressure. However, further human studies are needed to confirm long-term effects on the general population and investigate the mechanisms of action that may be involved.

2.4.3. Effect on blood lipids

Hyperlipidaemia is associated with an increased risk of CVDs such as atherosclerosis and other related metabolic diseases, including obesity, or diabetes. These chronic diseases involve lipid metabolism disorders, resulting in deregulated blood lipid concentrations compared with normal levels. Among blood lipids, elevated cholesterol, triglycerides (TAGs), and low-density lipoprotein cholesterol (LDL-c) concentrations constitute significant risk factors for cardiometabolic disorders. In contrast, elevated high-density lipoprotein cholesterol (HDL-c) concentrations are considered protective, facilitating cholesterol elimination (Karr, 2017).

While 21 human studies have tested the blood lipid-lowering effects of pomegranate-derived products (14 and 7 for PJ and PE, respectively; Supplementary Table S1), to date, results were inconsistent, likely due to design limitations, such as short duration, small sample size, as well as differences inherent to the inclusion of a wide range of subjects, ranging from healthy volunteers to obese, diabetic, hyperlipidaemic or hypertensive patients. Thus, only 8 of these studies have observed significant reductions of some biomarkers (TAGs, total cholesterol, LDL-c) (Supplementary Table S1). A meta-analysis that grouped 545 individuals recruited from 12 RCTs indicated that pomegranate consumption had no significant effect on the blood lipid profile (Sahebkar, Gurban, et al., 2016). Similarly, discrepant effects for TAGs, total cholesterol, LDL-c, HDL-c were observed in 2 further meta-analyses including different subjects from 10 to 11 RCTs, conducted with pomegranate-derived products (Garcia-Conesa et al., 2018), and in diabetic patients (n = 5 RCTs) (Jandari et al., 2020).

Notwithstanding, it is important to note that among the studies that evaluated the effect of PJ intake, positive results were obtained in those carried out with hyperlipidaemic patients. An experimental study with an 8-week PJ supplementation period described a reduction of total cholesterol and LDL-c levels (Esmailzadeh et al., 2004). Another study in which 101 patients subjected to chronic haemodialysis consumed 100 mL of PJ for 1 year and showed a significant improvement in TAGs and HDL-c levels (Shema-Didi et al., 2014). A similar aspect has been observed on blood lipid profiles upon PE supplementation compared to placebo in RCTs conducted in overweight and obese individuals (Hosseini et al., 2016) and patients with type 2 diabetes (Grabez et al., 2020; Sohrab et al., 2019).

In conclusion, based on the discordant results described in these human studies, a cause-effect relationship between pomegranate consumption and the possible beneficial effects in the blood lipid profile cannot be established with certainty, a fact that is probably aggravated due to the great interindividual variability observed in the trials. Thus, it should be confirmed by further long-term clinical trials.

Regarding the interindividual variability in the metabolism of polyphenols, three so-called urolithin metabolotypes, dealing with ellagitannins and ellagic acid metabolism, have been identified in the population: A, B and O. These metabolotypes have been proposed as potential gut microbiota biomarkers (Romo-Vaquero et al., 2019) because they are related to specific differences in the human gut microbiota. Metabotype A (60–70% of the population) is present in individuals who produce urolithin A (Uro-A) as the final urolithin. In contrast, individuals with metabotype B (20–30% of the population) produce urolithin B (Uro-B), isourolithin A (IsoUro-A), and Uro-A as the final urolithins. Finally, individuals with metabotype 0 (around 10% of the population), are non-urolithin producers (Tomás-Barberán et al., 2014; Cortés-Martín et al., 2018, 2020).

Hitherto, only a single RCT conducted in healthy overweight or obese volunteers who consumed PE has explored and confirmed that the interindividual variability in the pomegranate ellagitannins metabolism could be behind the controversial benefits upon a pomegranate-derived product consumption. This RCT indicated that PE consumption differentially improved the levels of several biomarkers associated with cardiovascular risk, only in individuals with metabotype B. In contrast, no effects were observed in either metabotype A subjects and when all individuals were considered as a whole, with no stratification based on their urolithin metabolotypes (Gonzalez-Sarrías, García-Villalta, et al., 2017). Therefore, further human studies, including stratification based on urolithin metabolotypes, is an attractive strategy to increase our understanding of the potential blood lipid-lowering effect of pomegranate in the general population.

2.5. Anticancer effect

Among the pomegranate polyphenols, punicalagin and ellagic acid have shown considerable anticancer activity in numerous in vitro and in the general population.
have been found in a recent RCT in men with favourable-risk prostate tissues was observed (Freedland et al., 2013). Similar results in women. According to this study, only normoweight women showed a modest modulation of oestrone and testosterone levels (Kapoor et al., 2015).

To date, from 7 clinical studies focused on the anticancer effect of pomegranate-derived product consumption, most of them were focused on prostate cancer. Thus, in 2006, Pantuck et al. designed the first clinical study to investigate the effect of pomegranate intake against prostate cancer in 46 patients diagnosed with elevated protein-specific antigen (PSA) levels in the absence of metastases. The daily consumption of 237 mL for 33 days (2 periods up to 33 months) significantly increased the PSA doubling time, which is considered a desirable effect related to reducing tumour growth (Pantuck et al., 2006). After this pioneering investigation, subsequent clinical studies supported the beneficial effects of PJ intake as reflected by the longer PSA doubling time when compared to the placebo group (Paller et al., 2013; Pantuck et al., 2015). Otherwise, PE consumption reduced the PSA level by 13%, although these effects were not significant compared to the placebo group (Paller et al., 2013). Additional clinical studies were designed to determine the effects on prostate cancer biomarkers and identify which molecules (those present in pomegranate or the derived metabolites) are accumulated in the human prostate and, in turn, may exert their anticancer activity. Thus, 2 different clinical studies (testing PJ and PE consumption) identified different conjugated urolithins in the human prostate (Freedland et al., 2013; Gonzalez-Sarrías, Giménez-Bastida, et al., 2010). These results were significant steps towards unravelling the role of urolithins as the molecules responsible for the benefits related to the consumption of pomegranate-derived products. However, the occurrence of urolithins in the prostate tissues did not alter the expression of genes related to cancer (such as CDKN1A, Ki-67, c-Myc, p53, etc.), a fact that was attributed to the short-term intake, as well as the wash-out period before the surgery (Freedland et al., 2013; Gonzalez-Sarrías, Giménez-Bastida, et al., 2010). Otherwise, a non-significant reduction (16%) of the DNA damage marker 8-OHdG in prostate tissues was observed (Freedland et al., 2013). Similar results in the reduction of the 8-OHdG in the tumour tissue, as well as a down-regulation of androgen receptor expression in adjacent tissue, have been found in a recent RCT in men with favourable-risk prostate cancer who consumed PE for 52 weeks. However, although urolithins were also detected in prostate tissue, no significant effects were observed on IGF-1, IGFBP-3, and(or) free testosterone levels, PSA and PSA doubling time, as well as biopsy metrics compared with the placebo group (Jarrard et al., 2021).

To date, only 1 clinical trial has explored the effects of PJ consumption on biomarkers related to breast cancer. This study examined how the daily consumption of PJ (237 mL) for 3 weeks regulated key hormones involved in breast cancer development in 64 menopausal women. According to this study, only normoweight women showed a modest modulation of oestrone and testosterone levels (Kapoor et al., 2015).

The anticancer effects of pomegranate have also been explored in colorectal cancer. An ambitious study that involved 52 patients diagnosed with colon cancer investigated the beneficial effects of the consumption of 2 PEs with different punicaglin/ellagic acid ratio. This clinical study reported high concentrations of ellagic acid and urolithins in normal and cancerous colon tissues for the first time. Interestingly, despite the short period of consumption before the surgery, the accumulation of these molecules was accompanied by the modulation of CRC biomarkers (gene expression and microRNA) in the tissue samples (Núñez-Sánchez et al., 2014, 2015, 2017).

In conclusion, the results reported in these studies on different types of cancer, together with the promising results described in numerous preclinical studies (Tomás-Barberán et al., 2017), might suggest a chemopreventive effect of pomegranate, partially related to ellagic acid and its derived metabolites, urolithins. However, this evidence should be interpreted with caution, and several key points deserve attention: i) the need for a higher number of clinical studies carefully designed to overcome the main limitations (sample size, duration, variability), ii) the comprehension of the underlying molecular mechanisms, and iii) the evaluation of a broader range of cancer biomarkers. These key points are essential to prove the anticancer effects of pomegranate and its derived metabolites unequivocally.

### 2.6. Anti-inflammatory activity

Numerous preclinical studies support the anti-inflammatory effects of ellagitannin-rich foods like pomegranate. For this reason, in recent years, identifying the active components of pomegranate and the underlying mechanisms of action has been one of the main objectives of many research groups. In 2010, in vivo studies of intestinal inflammation identified Uro-A as an effective anti-inflammatory molecule by preserving colonic architecture, and exerting a prebiotic effect, gene expression modulation (related to colon cancer), and down-regulation of inflammatory markers (Larrosa et al., 2010). New insights into the in vivo protective role of this urolithin encompass the modulation of the expression of genes involved in the integrity of the intestinal barrier (Singh et al., 2019). The characterization of the anti-inflammatory effects of urolithins, at physiological conditions, using in vitro cellular models of inflammation supported the anti-inflammatory effects described in vivo and unravelled mechanisms of action by which Uro-A, Uro-B, and IsoUro-A exert their effects at the intestinal level (Giménez-Bastida et al., 2012, 2020; González-Sarrías, Larrosa, et al., 2010).

However, there is a big gap between the robust preclinical evidence on urolithins’ anti-inflammatory properties and the effects reported in humans. Only the study by Kamali et al. evaluated the efficacy of PE as a complementary strategy in 62 diagnosed ulcerative colitis patients. Its consumption (4 and 10 weeks) reduced the Lichtiger colitis activity index (LCAI) from the baseline. However, no significant differences were observed compared with the placebo (Kamali et al., 2015).

More evidence has been described concerning human studies on pomegranate-derived products and systemic anti-inflammatory effects. However, a meta-analysis including 5 RCTs that included different cohorts with healthy individuals and diabetic and hypertensive patients reported a lack of significant effect on plasma C-reactive protein (CRP) levels upon PJ intake (Sahebkar, Gurban, et al., 2016). In agreement with this, a meta-analysis, including 3 RCTs conducted in patients with type 2 diabetes, showed similar results (Jandari et al., 2020). Overall, potential benefits related to anti-inflammatory activity could be highlighted from the human studies conducted thus far with PJ and(or) PE.

Accordingly, a preliminary study indicated that PE consumption could effectively attenuate the clinical symptoms in patients with rheumatoid arthritis, significantly improving the serum oxidative status (Balbir-Gurman et al., 2011). Similarly, a RCT in patients with rheumatoid arthritis reported a significant reduction of the disease activity after 8-weeks of PE supplementation (Ghavipour et al., 2017). Positive effects were also described in another RCT with knee osteoarthritis-diagnosed patients. Daily PJ consumption showed a protective effect via amelioration of cartilage inflammation through a better antioxidant status, lower MMP-13, and higher GPx level in serum than the placebo group (Ghoonnani et al., 2016).

On the other hand, the effects described in studies carried out with diabetics are less clear. An initial 12-weeks RCT study in PJ-treated patients showed lower plasma levels of IL-6, hs-CRP (Sohrab et al., 2014), E-selectin, NF-κB p65 together with higher sirtuin 1
PJ-treated diabetics are less evident in studies where, despite a significant reduction in IL-6 (Shishehbor et al., 2016), inflammation markers such as TNF-α and hs-CRP remained unchanged (Babaeian et al., 2013; Shishehbor et al., 2016).

Regarding individuals with moderate risk for CVD, the clinical evidence is scant. PJ consumption lacked a significant effect on biomarkers of inflammation (hs-CRP and IL-6) in hypertensive individuals and patients undergoing haemodialysis (Asgary et al., 2014; Davidson et al., 2009; Rivara et al., 2015). PE exerted anti-inflammatory effects in obese/overweight volunteers by decreasing inflammation markers significantly (MDA, IL-6, and hs-CRP) (Hosseini et al., 2016). However, hs-CRP remained unaltered after acute (4 h) or 1-month consumption of PJ by non-medicated adolescents with metabolic syndrome traits (Kelishadi et al., 2011). In this regard, a recent meta-analysis including 5 RCTs conducted with PJ reported significant changes in reducing the pro-inflammatory cytokine IL-6. However, no significant changes were found in the expression of several adhesion molecules, like ICAM-1, VCAM-1, and E-selectin, involved in the interaction with leukocytes during endothelial dysfunction (Asgary et al., 2021).

In conclusion, thus far, the human evidence on the anti-inflammatory effect upon PJ or PE consumption remains puzzling but promising. Similarly, whether urolithins are the molecules responsible for the anti-inflammatory benefits at the intestinal and systemic level remains unclear. Further well-designed human trials following the roadmap proposed below and relevant preclinical studies are needed to provide greater evidence to elucidate the possible benefit.

2.7. Prebiotic effect

New insights about the role in host physiology of the gut microbiota have revealed functions beyond the well-described nutritive and defensive activities, which has led the scientific community to consider it as a virtual organ of the body (Valdes et al., 2018). Even though our understanding of the mechanisms involved is lacking, increasing evidence puts forward gut microbiota as an exciting target in the prevention of chronic diseases, including intestinal (diarrhoea, ulcerative colitis, and Crohn’s disease) and metabolic disorders (i.e., obesity and diabetes), non-alcoholic hepatic steatosis, food intolerances (celiac disease), allergies, asthma, cancer and neurodegenerative diseases (Valdes et al., 2018).

Lifestyle is one of the factors that exert a more significant influence on gut microbiota. Thus, a healthy lifestyle inexorably implies a healthy diet, which is essential in improving intestinal health and the proper balance of the gut microbiota (Turnbaugh et al., 2009). Westernized diets rich in fat and sugar and low in fibre and plant-derived food (such as fruits and vegetables), the excessive consumption of antibiotics, stress, and/or intestinal related disorders, including celiac disease, can disrupt the intestinal microbiome. This disruption, known as intestinal dysbiosis, is characterized by an alteration of the ratio of harmful to beneficial bacteria, favouring the growth of microorganisms associated with undesirable effects to the host (Cortés-Martín, Iglesias-Aguirre, et al., 2020). Fibre and polyphenols provided by fruits and vegetables play a role in the composition and functionality of the gut microbiota, contributing to maintaining the organism’s homeostasis. In this regard, the high content in fibre and polyphenols of pomegranate is a beneficial factor in modulating the gut microbiota. Based on this, numerous studies have approached the interaction “pomegranate/microbiota” as the cornerstone in the prevention/treatment of dysbiosis-related chronic diseases (Cortés-Martín, Selma, et al., 2020).

So far, most of the investigations have evaluated PE consumption as it offers a direct, easy, and effective way to administer the pomegranate’s polyphenols, mainly ellagitannins and ellagic acid. The first human study reported the effect of PE (1 g/day for 4 weeks) on the composition of the gut microbiota in healthy adults (Li et al., 2015). This dietary intervention showed an increase in the Actinobacteria to Firmicutes ratio (favourable modulation against obesity) and a higher prevalence of the phylum Verrucomicrobia, including Akkermansia muciniphila, whose abundance in healthy subjects is considered to be a protective factor against obesity, overweight and type 2 diabetes (Depommier et al., 2019). These results suggested that the benefits of PE consumption could be linked to the modulation of the gut microbiota. Further exploration of the specific association between the urolithins and microbiota modulation provided evidence of the connection between the formation of Uro-A and A. muciniphila in healthy volunteers after consuming PE for 4 weeks (Henning et al., 2017).

Another human trial conducted in healthy overweight-obese volunteers who consumed PE daily for 3 weeks associated, at least partially, the reduction of cardiovascular markers such as cholesterol, LDL-c, and ox-LDL, amongst others, with the formation of urolithins and Gordonibacter levels, a microbial group involved in the biosynthesis of these metabolites (González-Sarrías, García-Villalba, et al., 2017). This study also investigated whether the PE consumption contributed to maintaining the integrity of the intestinal barrier by evaluating the effects on endotoxaemia (presence of lipopolysaccharide (LPS) in the plasma due to the alteration of the intestinal barrier associated with dysbiosis). Data showed that the group of volunteers that consumed the highest dose tested possessed a significantly reduced level of LPS-binding protein in their plasma (LBP; a well-described marker of endotoxaemia) and a decrease of hs-CRP. These effects were associated with improved intestinal barrier integrity and linked to the increase of Bacteroides, Faecalibacterium, Butyricicoccus, Odoribacter and Butyrivimonas as well as the reduction of pro-inflammatory microorganisms such as Parvimonas, Methanobrevibacter, and Methanosphaera (González-Sarrías, Romo-Vaquero, et al., 2018). In contrast to Henning et al., 2017, no significant changes in A. muciniphila level were observed after 3-weeks of pomegranate supplementation (González-Sarrías, García-Villalba, et al., 2017). This fact could be in agreement with the lack of effect of pomegranate consumption on weight and the reduction of body composition indices, recently reported in a meta-analysis including 13 RCTs (Gheflati et al., 2019).

A study with 35 patients diagnosed with colorectal cancer, associated with a remarkable intestinal dysbiosis and alteration of the intestinal barrier, showed a reduction of LBP after consuming daily 900 mg of PE for 5–35 days. However, the short and variable pomegranate consumption in this study due to the programmed surgery of the patients prevented any possible correlation between the reduction of LBP and gut microbiota modulation of the patients upon PE consumption (González-Sarrías, Núñez-Sánchez, et al., 2018).

Recently, a crossover RCT in poly-medicated metabolic syndrome patients explored whether the prebiotic effects of a PE depended on the medication and could complement their therapy. Thus, after 1 month of intake, the main results indicated an increase in the probiotic genera Lactococcus in patients receiving lipid-lowering, antihypertensive and antiadipobiotic treatments, and Bifidobacterium in patients under lipid-lowering and antiadipobiotic treatment. Clostridium XIVa level was reduced in patients not subjected to antihypertensive and lipid-lowering treatments. PE intake also reduced plasma LBP levels in all patients regardless of the drug treatment and sICAM-1, but only in patients undergoing lipid-lowering treatment (Cortés-Martín et al., 2021).

Unlike PE, studies with PJ are less abundant. A study with 12 healthy volunteers is the only one that has been carried out to date, to the best of our knowledge. The daily consumption of 200 mL PJ for 4 weeks lacked an effect on the gut microbiota composition, while the concentration of phenolic metabolites, short-chain fatty acids (SCFAs), and faecal steroids related to beneficial effects were significantly augmented (Mosele et al., 2015).

In conclusion, the studies described above suggest that PE consumption could exert benefits via modulation of gut microbiota. This modulation is advantageous for maintaining the intestinal barrier, as reflected by the reduction of LBP in overweight-obese subjects, and to reduce cardiovascular risk through the improvement of some...
b biomarkers. Even though these results are promising, more clinical trials (longer studies with a higher number of volunteers) are mandatory to establish the axis “pomegranate-microbiota-health benefits”, which could place the microbiota as a crucial element for the comprehension of the benefits associated with pomegranate consumption.

2.8. Neuroprotection and improvement of the cognitive function

The neuroprotective effects of pomegranate have been evaluated in different animal models of neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease. The results of these studies described that animals fed diets enriched with pomegranate (PJ or PE) showed signs of neuroprotection connected with the biological activity of ellagic acid and its derived metabolites, urolithins (Ahmed et al., 2015; Essa et al., 2015; Hartman et al., 2006; Rojanathammanee et al., 2013). In this regard, urolithins (Gasperotti et al., 2015) and ellagic acid (Yan et al., 2014) have been detected in the animals’ brains after intravenous and oral administration, respectively. These results align with urolithins’ capacity to cross the blood-brain barrier described by in silico studies (Yan et al., 2016), setting the basis to perform an in-depth investigation into the cellular and molecular mechanisms of neuroprotection. Elegant investigations of this approach come from in vitro and in vivo studies that describe different anti-inflammatory and anti-oxidant mechanisms of action for urolithins and their conjugated metabolites (Dasilva et al., 2019; González-Sarrías, Núñez-Sánchez, et al., 2017). Considering that these molecules could reach the brain, these compounds are potential candidates responsible for the neuroprotective benefits associated with pomegranate consumption.

Regarding clinical evidence, a growing number of clinical trials, although still low, endorse the consumption of pomegranate-derived products as a beneficial factor for the nervous system’s health. In a first study, 28 subjects with age-related memory disorders who consumed PJ daily for 4 weeks showed a significant improvement in the Buschke selective reminding test of verbal memory, higher plasma antioxidant activity (TEAC), and enhanced functional brain activation compared with the placebo group (Bookheimer et al., 2013). Regarding PE consumption, another RCT described protective effects against post-operative cognitive dysfunction in 10 post-cardiac surgery patients after 7-weeks of supplementation by protection and improvement of memory retention performance up to 6 weeks post-surgery, unlike the placebo group (Ropacki et al., 2013). Additional studies described functional and neurophysiological improvements and shorter hospital stays in post-stroke patients under rehabilitation who consumed PE for 1 week, compared with the placebo (Bellone et al., 2019). In a more recent RCT, 200 healthy volunteers included in their diets a glass of PJ daily for 6–12 months which stabilized the capacity to acquire memory through visual and verbal memory tasks (compared with the placebo group) (Bookheimer et al., 2013). Regarding PE consumption, another RCT described protective effects against post-operative cognitive dysfunction in 10 post-cardiac surgery patients after 7-weeks of supplementation by protection and improvement of memory retention performance up to 6 weeks post-surgery, unlike the placebo group (Ropacki et al., 2013). Additional studies described functional and neurophysiological improvements and shorter hospital stays in post-stroke patients under rehabilitation who consumed PE for 1 week, compared with the placebo (Bellone et al., 2019). In a more recent RCT, 200 healthy volunteers included in their diets a glass of PJ daily for 6–12 months which stabilized the capacity to acquire memory through visual and verbal memory tasks (compared with the placebo group) (Siddarth et al., 2020). These findings provide new insights regarding pomegranate and cognitive function improvement, although further studies are required to explore the potential beneficial effect on neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease.

Only 1 RCT has examined the relation between PJ intake during pregnancy and neuroprotection, to the best of our knowledge. The results showed positive effects, including better brain development and connectivity support in at-risk newborns who suffered intrauterine growth restrictions (Matthews et al., 2019).

2.9. Improvement of physical activity

There is little debate over the importance of a healthy diet for physically active subjects (recreationally active or trained athletes). Since the last decade, pomegranate, as a rich source of essential nutrients and antioxidants, has aroused the interest of researchers to study their capacity to enhance performance outcomes and recovery (in anaerobic and aerobic exercises) as well as to prevent sport activity-related injuries and improve cardiovascular markers (i.e., blood pressure).

In the context of anaerobic exercises, recreationally active subjects (weightlifters, n = 13) who consumed a PE for 9 days showed a marked improvement of the isometric strength recuperation between 2 and 3 days after the practice of intensive exercise (Trombold et al., 2010). A year later, the same authors published a new study describing the relationship between PJ intake (237 mL) after exercise and amelioration of weakness and soreness of the elbow flexor (compared with placebo) in 17 resistance-trained subjects (Trombold et al., 2011). In compliance with these studies, young elite weightlifters also showed higher performance, lower rating of perceived exertion scale, and delayed onset soreness regarding the knee extensors after PJ intake, compared with the placebo group (Anmar et al., 2016, 2020). However, PJ consumption was ineffective in recovering muscle damage in untrained volunteers (Lamb et al., 2019). Regarding aerobic exercises, enhanced performance outcomes, lower oxygen absorbance, higher post-exercise muscular recovery, and force restoration in (amateur and trained) cyclists have been described (Crum et al., 2017, 2018; Torregrosa-García et al., 2019) as well as increased blood flow and vessel diameter in resistance-trained runners after PE intake (Roelofs et al., 2017). Furthermore, these advantageous effects on physical activity are closely related to health benefits. Desirable effects such as lower plasma glucose, attenuation of acute increase of systolic pressure, reduction of inflammatory biomarkers (C-reactive protein, MDA), and exercise-associated oxidative stress together with amelioration of muscular damage have also been reported (Anmar et al., 2016, 2020; Fuster-Muñoz et al., 2016).

2.10. Other benefits less explored

Finally, less evidence, conditionized by the small number of studies carried out to date, has been observed in other potential applications upon pomegranate-derived product consumption. For example, daily PJ consumption exerted benefits in 80% of patients (n = 53) with mild-moderate erectile dysfunction (Forest et al., 2007). PE intake also protected against UV-induced pigmentation of human skin (Kasai et al., 2006).

Furthermore, the possible interaction of pomegranate-derived products with the pharmacokinetics of well-known drugs is another field of interest to discard potential side effects in medicated subjects. In this regard, data from clinical trials have reported that PJ did not alter the pharmacokinetics of dapoxetine (Abdlekawy et al., 2017), midazolam (Farkas et al., 2007; Misaka et al., 2011) or flurbiprofen (Hanley et al., 2012). Remarkably, PE consumption improved the gonadotropin-releasing hormone analogue therapy used to treat girls diagnosed with idiopathic central precocious puberty as reflected by reducing different parameters (bone age, growth velocity, hormones) related to puberty (Liu & Tang, 2017).

3. Critical aspects of evidence-based human studies

Over the past two decades, an outstanding amount of experimental and clinical research has shown a close relationship between pomegranate-derived products and health benefits. However, despite the amount of information available on their health benefits, human results are still inconsistent and insufficient to prevent chronic diseases or as an adjuvant to treat them. The outcomes are too fragmented, and the main argument raised by the EFSA in 2010 to reject all health claims with these studies, young elite weightlifters also showed higher performance, lower rating of perceived exertion scale, and delayed onset soreness regarding the knee extensors after PJ intake, compared with the placebo group (Anmar et al., 2016, 2020). However, PJ consumption was ineffective in recovering muscle damage in untrained volunteers (Lamb et al., 2019). Regarding aerobic exercises, enhanced performance outcomes, lower oxygen absorbance, higher post-exercise muscular recovery, and force restoration in (amateur and trained) cyclists have been described (Crum et al., 2017, 2018; Torregrosa-García et al., 2019) as well as increased blood flow and vessel diameter in resistance-trained runners after PE intake (Roelofs et al., 2017). Furthermore, these advantageous effects on physical activity are closely related to health benefits. Desirable effects such as lower plasma glucose, attenuation of acute increase of systolic pressure, reduction of inflammatory biomarkers (C-reactive protein, MDA), and exercise-associated oxidative stress together with amelioration of muscular damage have also been reported (Anmar et al., 2016, 2020; Fuster-Muñoz et al., 2016).

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high heterogeneity in human studies, certainly accounting at least partially for the discrepancies in their results, both in specific studies and meta-analyses conducted up to now. Further controlled, large-scale RCT should be conducted using standardised pomegranate-derived products, and following robust selection criteria of the volunteers would be needed to avoid the high variability reported.

2) The difficulty of attributing beneficial effects to phenolic fraction: Although the beneficial effects of pomegranate-derived products have been primarily attributed to their polyphenol composition, mostly due to ellagic and ellagitannins and their in vivo derived metabolites urolithins, the direct link between these potential health effects and pomegranate-derived product composition have yet to be fully explained. In this regard, the lack of pomegranate-derived product standardisation could account for the discrepancies in the outcomes. Thus, it should be noted that most studies failed to report the formulation, in part by using commercial PJs and(or) PEs, and therefore, are unable to guarantee the presence of a significant amount of these pomegranate bioactive compounds. When reported, there is high variability in polyphenolic content. Therefore, from a nutritional practice point of view, it is impossible to establish specific intake recommendations for these pomegranate-derived products. Besides, it should be noted that the beneficial effects cannot be exclusively attributed to the polyphenolic fraction and instead could also be attributed to other pomegranate components with health benefits, such as vitamins and minerals, at least partially, and may also be the result of the synergistic association between different pomegranate constituents. Along this line, it is also important to consider the difficulty in estimating the dietary intake of phenolics due to it being modulated by several factors such as food matrix, food processing-related factors, and the interaction with the gut microbiota that influence the bioavailability and subsequent potential effects. In summary, the existence of a multitude of different compositions associated with PJ and PE makes it impossible to achieve reproducible results and establish the desired cause-effect relationship.

3) The high heterogeneity of the results obtained related to interindividual variability: It is well-established that genetic polymorphisms or differences in the composition or functionality of the gut microbiota, as well as other factors such as age, gender, health status, contribute to the different response of individuals to interventions with polyphenol-rich foods (Cortés-Martín, Selma, et al., 2020; Gibney et al., 2019). In this regard, in the past few years, human bioavailability studies have identified considerable interindividual variability in the metabolism of pomegranate ellagitannins. The urolithin production, associated with the differences in the human gut microbiota composition, has identified three different urolithin metabolotypes (A, B and 0) with potential impact on health. Thus, the large interindividual variability in the urolithin production could also explain, at least in part, the great variability in health effects observed in vivo after the intake of pomegranate and pomegranate-derived products. Therefore, to find associations between pomegranate intake and health benefits, further human studies should be conducted, according to their urolithin metabolotypes, to identify and understand the different responses to pomegranate. Besides, it should not be disregarded that perhaps the beneficial effects upon pomegranate consumption could be mediated by the specific subjects’ gut microbiota. In this context, urolithins could be only biomarkers of the gut microbiota, or possibly display a two-way interaction with the resident microbiota, as well as by synergistic or additive effects with other pomegranate constituents (Cortés-Martín, Selma, et al., 2020; Espín et al., 2017; Romo-Vaquero et al., 2019; Tomas-Barberan et al., 2018).

4) The difficulty of finding improvements in chronic disease biomarkers in healthy volunteers and(or) medicated patients: Improving biomarkers at normal levels either due to a healthy state of the individuals or those already controlled in patients under drug treatment is challenging. Similarly, as mentioned before, the participants in human studies ranged from healthy individuals to medicated and non-medicated patients with a diagnosed chronic disease. However, in most studies conducted on patients, the drug treatments are not reported, leading to misinterpretation. Previous meta-analyses or systematic reviews included studies with medicated and non-medicated patients without distinction. Therefore, any conclusion drawn should be taken with caution. Thus, drug therapy is a critical factor that has not generally been addressed in human studies. In fact, to date, only 1 RCT has been conducted to evaluate possible interactions or adjuvant effects of a PE in poly-medicated metabolic syndrome patients (Cortés-Martín et al., 2021).

5) Joint action between policymakers, research/academic institutions, and industries is missing: There is a need to find a well-established relationship between pomegranate consumption and health effects. To this purpose, more specific guidelines are essential to moving forward (the number of clinical studies needed to support a health claim, the necessary threshold of change in biomarkers to be considered relevant in health, etc.).

4. Conclusions and roadmap for future human studies

Despite the limitations detailed above, some potentially beneficial effects can be evidenced by improving specific chronic disease biomarkers after consuming both PJ and PE. At present, the most extensive scientific evidence supporting the effects on human health has been related to protection from oxidative stress-related and cardiometabolic diseases, with an emphasis on improving blood pressure control, where these food products appear to promote small but beneficial changes on a combination of risk factors and may contribute to preventing these chronic diseases. However, whether the changes observed could have clinical relevance deserves additional research through well-designed RCTs in the general population. Similarly, the clinical evidence is still limited regarding the beneficial effect of pomegranate-derived product intake to reduce the risk of anti-inflammatory and neurodegenerative processes, including cognitive function improvement, and even against certain types of cancer such as prostate and colorectal. On the other hand, PJ and PE consumption has shown preliminary evidence of effects on other physiological processes, such as the modulation of the gut microbiota, which may help prevent chronic diseases. Besides, moderate evidence has been observed in many studies regarding performance improvement and recovery after physical exercise. These studies, albeit scarce, open a wide range of possibilities to be explored in further studies.

Overall, there is a particular need for additional research in well-designed RCTs (randomized, cross-over, placebo-controlled and dose-response). Ideally, the RCTs should be conducted in ring-tests by different research groups to make robust evidence of health benefits based on the general population’s consumption of standardised pomegranate-derived products. This would allow for the elucidation of links between specific effects and particular compounds or metabolites of pomegranate, i.e., a well-established cause-effect. Thus, several points must be considered in future human studies:

1) To perform RCT with a higher number of subjects and longer duration. Thus, an adequate calculation of the sample size is crucial to obtain high-quality data on biomarker changes.

2) To report complete information on the subject’s characteristics included in the study, such as age, gender, BMI, medication, etc., to find a possibly beneficial effect on specific cohorts, as well as the potential interactions with drugs to support pomegranate intake as an adjuvant in specific treatment against chronic diseases.

3) To show that the pomegranate-derived foodstuff composition correlates to the effects of specific pomegranate components, mainly the polyphenolic fraction. Besides, qualitative and qualitative
determination of pomegranate phenolic-derived metabolites in blood and tissues may also help to correlate and explain the effects.

4) The dose and the pomegranate-derived products assayed should be standardized. This should include the pomegranate cultivar and specific composition of the derived products (PJ or PE) in further studies to generate dietary recommendations, doses needed for maximal beneficial effect and/or allowing the establishment of preventive nutritional strategies.

5) To increase the number of biomarkers or physiological determinations assayed in human studies, representing the general population, i.e., healthy individuals or at risk (obese, mild hyperlipidaemic or hypertensive, pre-diabetic, etc.), but not patients with established diseases. Moreover, the primary endpoints should be well-known and associated with chronic diseases and relevant for the general population, i.e., levels of blood glucose, total cholesterol, LDLc (and its oxidative species ox-LDLc), HDLc, and TGs, endothelial function and blood pressure. For example,RCTs showing relevant anti-inflammatory effects by decreasing a panel of plasma cytokines will not help get any health claim from EFSA.

6) To cluster subjects according to their urinol metabolotype to find a correlation between the metabolotype-associated gut microbiota and pomegranate-derived product consumption effects. In the context of personalized nutrition, it is beginning to be perceived that there is no “one size fits all” effect. This means that not all individuals will respond equally to consuming a specific food product, including pomegranate. Perhaps, a generalized effect of the pomegranate in the general population could not be achieved, which could prevent the “general population” as the target population in a potential EFSA health claim application.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2021.06.014.

References


Buser, T. M.,눌 등을 위한 건강한 식단의 투표. 대한의사협회지, 13(1), 7–15.


J.A. Giménez-Bastida et al.


humanized gnotobiotic mice. Science Translational Medicine, 1(6), 6ra14. https://doi.org/10.1126/scitransmed.3000322


