Metabolic Impact Of 100% Fruit Juice Consumption On Antioxidant/Oxidant Status And Lipid Profiles Of Adults: An Evidence-Based Review

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Abstract

One hundred percent fruit juice (FJ) contains bioactive compounds with antioxidant activity. As such, this fruit form has the potential to improve antioxidant status and mediate outcomes influenced by redox status. A systematic review of the literature published between 1995-2013 was conducted using PubMed database to evaluate associations between intake of 100% FJ and markers of antioxidant/oxidant status and blood lipid levels in healthy, free-living adults > 18 years. Data extraction and analysis was conducted according to the Academy of Nutrition and Dietetics Evidence Analysis Process. Limited evidence from ten clinical trials meeting inclusion/exclusion criteria suggests potential improvements in a variety of antioxidant or oxidants biomarkers post-consumption of 100% FJ. Weak evidence from five studies suggests that one or more blood lipid measures may be positively influenced by consumption of 100% FJ. Heterogeneity in study methodology including biomarkers, 100% FJ type, dosage, and intervention duration precludes the ability to make evidence-based recommendations regarding a specific dose-duration-juice effect. Key characteristics in study designs were identified which must either be controlled or statistically adjusted for in future investigations in order to obtain a more accurate understanding of the complex relationship between metabolic outcomes and consumption of 100% FJ in context of a healthy dietary pattern.

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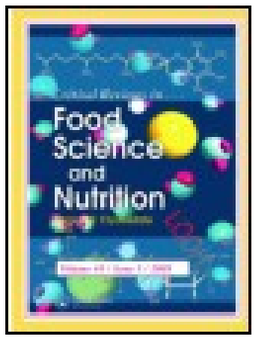

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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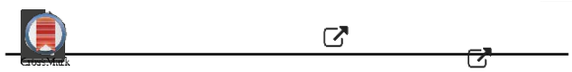
To cite this article: Kristi Crowe-White PhD, RD , J. Scott Parrott PhD , Kim S. Stote PhD, MPH, RD , Melissa Gutschall PhD, RD, LDN , Sue Benson-Davies PhD, DCN, MPH, RD, LN, Elizabeth Droke PhD, RDN, LN, Carol E. O'Neil PhD, MPH, RD, LDN , Taylor Wolfram MS, RDN, LDN & Paula Ziegler PhD, RD, CFCS (2015): Metabolic Impact of 100% Fruit Juice Consumption on Antioxidant/oxidant Status and Lipid Profiles of Adults: An Evidence-based Review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1102861



Metabolic Impact of 100% Fruit Juice Consumption on Antioxidant/Oxidant Status and Lipid Profiles of Adults: An Evidence-Based Review

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Key Words

100% fruit juice, cholesterol, oxidative stress, antioxidant status, lipids, adults, Evidence Analysis Library, functional foods

ABSTRACT

One hundred percent fruit juice (FJ) contains bioactive compounds with antioxidant activity. As such, this fruit form has the potential to improve antioxidant status and mediate outcomes influenced by redox status. A systematic review of the literature published between 1995-2013 was conducted using PubMed database to evaluate associations between intake of 100% FJ and

markers of antioxidant/oxidant status and blood lipid levels in healthy, free-living adults > 18 years. Data extraction and analysis was conducted according to the Academy of Nutrition and Dietetics Evidence Analysis Process. Limited evidence from ten clinical trials meeting inclusion/exclusion criteria suggests potential improvements in a variety of antioxidant or oxidants biomarkers post-consumption of 100% FJ. Weak evidence from five studies suggests that one or more blood lipid measures may be positively influenced by consumption of 100% FJ. Heterogeneity in study methodology including biomarkers, 100% FJ type, dosage, and intervention duration precludes the ability to make evidence-based recommendations regarding a specific dose-duration-juice effect. Key characteristics in study designs were identified which must either be controlled or statistically adjusted for in future investigations in order to obtain a more accurate understanding of the complex relationship between metabolic outcomes and consumption of 100% FJ in context of a healthy dietary pattern.

ACCEPTED MANUSCRIPT

INTRODUCTION

Fruit contains numerous bioactive compounds including vitamins and phytochemicals with well-characterized antioxidant activity. Upon processing to its liquid serving form, 100% fruit juice (FJ) has been shown to retain an abundance of these antioxidants (Ruxston et al., 2006; Crowe and Murray, 2013). Thus, 100% FJ, a serving equivalent to whole fruit according to the Dietary Guidelines for Americans 2010, represents a functional food with the potential to positively impact metabolic outcomes mediated by antioxidant activity (United States Department of Agriculture and United States Department of Health and Human Services, 2010). Given the potential benefit to be derived from 100% FJ consumption in context of a diet guided by the Dietary Guidelines, the aim of this systematic review was to examine evidence on the association and impact of 100% FJ intake on antioxidant and oxidant status and blood lipid profiles among adults.

Multiplicity of Measures and Modifiers

Evidence suggests that oxidative stress is important in the initiation and pathogenesis of chronic diseases including cardiovascular disease, diabetes, dyslipidemia, and cancer among others (Higashi et al., 2009). Mechanistically, the bioactive compounds within 100% FJ may contribute antioxidant action and decrease oxidative stress in the body (Collins, 2005; Da Costa et al., 2012). By shifting the redox balance to reduce oxidative stress, bioactive compounds with antioxidant activity may influence numerous health outcomes including blood lipid levels. More specifically, lipid profiles may be influenced by antioxidant effects on cholesterol metabolism and lipoprotein regulatory pathways in the liver by inhibiting the production of endogenous lipoproteins, inhibiting low-density lipoprotein cholesterol (LDL) oxidation, and reducing

platelet aggregation (Kurowska et al., 2000; Aptekmann and Cesar, 2010). Since elevated total cholesterol, LDL, and triglycerides along with decreases in high-density lipoprotein cholesterol (HDL) are major risk factors for atherosclerosis, 100% FJ may play a preventative role in delaying the progression of atherosclerotic plaque formation in arterial walls. In light of the prevalence of atherosclerosis and cardiovascular disease in Western societies, evaluation of foods with the potential to reduce these risks is warranted.

Research evaluating the potential impact of 100% FJ on antioxidant and oxidant status employs a variety of outcome measures or biomarkers. Among the more common markers of antioxidant status are the ferric reducing ability of plasma (FRAP), oxygen radical absorbance capacity (ORAC) assay, and total peroxyl-radical trapping activity (TRAP). These assays allow for measuring the ability of antioxidants in a sample to quench a free radical exposed to that sample (Huang et al., 2005). These measures are referred to as a capacity measure or biomarker quantitating the strength of the cumulative antioxidants in the sample against the free radical generating oxidation. Unfortunately, since different radical species or initiators of oxidation are employed in the various methods, comparison of results across studies with differing methodologies is challenged. The question of biological relevance of these assays has been raised with particular emphasis on assay kinetics along with the fact that numerous free radicals are present in the body and these assays utilize only one radical source exposed to the biological sample *in vitro*. Although assay kinetics cannot exactly mimic biological conditions *in vivo*, these assays provide a capacity measure of antioxidant status which may be influenced by study treatment or intervention.

Much like the multiplicity in measurements of antioxidant status, numerous measures of oxidative stress are employed to assess a variety of oxidized end products including lipids, proteins, and DNA. Examples of such methodological assessments include thiobarbituric acid-reactive substances (TBARS) or LDL-oxidation for assessing lipid oxidation products, protein carbonyls for assessing protein oxidation, 8-hydroxydeoxyguanosine (8-OHdG) for assessing DNA oxidation, and reactive oxygen species (ROS) measurements for assessing circulating levels of oxidants in biosamples (Dalle-Donne et al., 2006; Giustarini et al., 2009). In many studies, multiple methods are used in order to comprehensively assess the spectrum of oxidizable substrates as outcomes in nutrition research.

Complexity and Context

It is well recognized that the background diet, lifestyle, and genetics along with environmental factors can restrict the ability to attribute metabolic effects solely to one food, beverage, or compound (Willet, 2012). In other words, ―the effect‖ of consumption of 100% FJ on antioxidant status or blood lipid profiles is highly contingent on a host of other factors. Additionally, while the effects of consumption of 100% FJ on antioxidant measures may be relatively immediate, the relationships between 100% FJ consumption and its effect on downstream measures like lipid profiles are likely to be highly confounded by factors such as individual variations in dietary and exercise patterns. Thus, the complexity of mechanisms and diversity of measures pose serious challenges to carrying out a systematic review—where the goal is typically to formulate a narrow, clearly defined question and seek a correspondingly simple answer. Acknowledging the heterogeneity in outcome measures of antioxidant/oxidant status and the resulting difficulty presented in cross-study comparison, the impetus for this systematic review of the literature

shifted from treating variations in methodology as a limitation in an attempt to find ―one best measure‖ of the effects of 100% FJ consumption to exploring heterogeneity among studies to discern patterns useful for future research. We do this first by categorizing the evidence more globally as providing evidence for or against potential improvements in antioxidant/oxidant status and blood lipid profiles following consumption of 100% FJ. Secondly, we seek to identify the range of confounders present in the research. By employing a global view of the evidence despite methodological differences and by identifying known confounders, strategies for future investigation may be developed that would allow for cross-study analyses and control of modifying factors innate to investigating this relationship. In turn, strategies for continuity in future investigations may allow for establishing evidence-based recommendations regarding a specific dose-duration-juice effect.

METHODS

Evidence Analysis Team and Process

The workgroup included six registered dietitians or registered dietitian-nutritionists with clinical or research experience along with support of a methodologist/statistician. A trained and experienced project manager facilitated these meetings with the assistance of the lead analyst. A complete description of the Evidence Analysis Process is available at the Academy‘s Evidence Analysis Library (EAL) website (2014).

Literature Search

Studies were initially identified in PubMed and published in English between January 1995 and November 2013. The following syntax was used to identify relevant antioxidant and oxidant studies:

1. ―fruit juice‖
2. ―oxidant stress‖ or ―oxidative stress‖
3. ―antioxidant capacity‖ or ―anti-oxidant‖
4. ―lipid oxidation‖ or ―protein oxidation‖
5. #2 or #3 or #4
6. #1 and #5

A similar search was carried out to identify blood lipid outcomes. Title and abstracts of studies identified through the above search were reviewed by work group members and the analysis team. A list of relevant studies was identified for full text review based on work group consensus. Work group members then read all full text articles to determine whether inclusion criteria were met and to identify additional sources from study reference lists. A final list of relevant sources was identified by workgroup consensus with input from the analysis team.

Application of Inclusion/Exclusion Criteria

First and foremost, in order to include a study in this systematic review, the Food and Drug Administration‘s definition was used to provide the initial framework for determining if juices investigated in each study were 100% FJ. According to 21CFR101.30, ―juices directly expressed from a fruit or vegetable (i.e., not concentrated and reconstituted) shall be considered to be 100 percent juice and shall be declared as 100 percent juice" (U.S. Food and Drug Administration Code of Federal Regulations, 2013). The above definition was expanded to include juices from concentrate which were reconstituted to the original concentration. Inclusion criteria included use of 100% FJ of a single or mixed type, healthy adults > 18 years of age, no preference as to study design, a minimum of 10 subjects per study group, drop-out rate under 20%, and at least

one marker of antioxidant or oxidant status or measure of lipid outcomes. Studies were excluded from review based on the following exclusion criteria: 100% FJ not specified, individuals less than 18 years of age, articles not published in peer-reviewed journals, and research presented on in abstract or presentation format only. For markers of antioxidant and oxidant status, exclusion criteria also included studies on endothelial function without markers of oxidative stress. This systematic search process identified 10 studies reporting on markers of antioxidant or oxidant status (Figure 1) and five studies reporting on blood lipid levels (Figure 2).

Data Extraction and Analysis

The work group, in consultation with the analysis team, developed the analytic framework for data extraction and the Academy‘s online Data Extraction Tool was used to extract and store data from the research articles. Trained analysts or methodology experts extracted the following data from each eligible research article: title, year and journal of publication, study design, intervention and control groups (if applicable), details of interventions (type of intervention, who delivered the intervention, duration of intervention, mode of intervention), confounders and effect mediators identified, and outcomes of interest (markers of antioxidant/oxidant status or blood lipid levels). Data extracted by one analyst were verified by a second analyst for accuracy. Risk of bias for each study was double assessed using the Academy‘s quality rating checklist in accordance with Institutes of Medicine standards (Eden, Levit, Berg, & Morton, 2011; Academy of Nutrition and Dietetics, 2014). An overall quality rating was assigned to each article based on assessment of specific checklist items (Appendix 1). Differences among analysts were resolved by consensus in consultation with the lead analyst and project methodologist.

Although data on mean change in target outcomes were extracted with sufficient detail for meta-analysis, it was determined that variation among studies precluded the ability to accurately estimate a pooled effect size. Thus, the focus shifted to identifying systematic patterns in the relationships between variations in study characteristics and reported outcomes. In order to facilitate this comparison, mean changes in antioxidant, oxidant and blood lipid measures were classified more generally into ―positive‖ versus ―negative or no‖ change from baseline to follow-up. Because of the likelihood of type 2 error in the smaller studies and the high sensitivity of outcomes to confounding, mediating, and contextual factors, direction of the effect and non-statistical significance was used to determine outcome classification.

Development of Conclusion Statements

Evidence summary and conclusion statements on the impact of 100% FJ consumption on diet and weight status of children were drafted by the workgroup and lead analyst based on evidence analysis after completion of the data extraction process. The EAL Manual for Grading the Strength of the Evidence (2014) was used for grading the conclusion statements according to the following grades: I (good/strong), II (fair), III (limited/weak), IV (expert opinion only), or V (grade not assignable).

RELATIONSHIP BETWEEN INTAKE OF 100% FJ AND MARKERS OF ANTIOXIDANT AND OXIDANT STATUS

Research Identified

Ten clinical trials met the inclusion criteria for evaluating the impact of the 100% FJ consumption on markers of antioxidant status or oxidative stress in adults (Freedman et al., 2001; Bub et al., 2003; Gorinstein et al., 2004; Ko et al., 2005; Inoue et al., 2008; Ghanim et al., 2010;

Karlsen et al., 2010; Rowe et al., 2011; Snyder et al., 2011; Buscemi et al., 2012). Studies varied widely—not merely in the type and timing of the antioxidant and oxidant markers examined, but also in the types of juice or juice mixes examined, dose administered, subject characteristics and behavioral confounders.

Effects of 100% FJ Consumption on Antioxidant Status

Seven clinical trials evaluated the effects of 100% FJ consumption on markers of antioxidant status (Freedman et al., 2001; Bub et al., 2003; Gorinstein et al., 2004; Ko et al., 2005; Karlsen et al., 2010; Rowe et al., 2011; Snyder et al., 2011) (Table 1). Six of the seven studies reported post-consumption improvement in at least one measure of antioxidant status; four of which reached statistical significance (Freedman et al., 2001; Gorinstein et al., 2004; Ko et al., 2005; Snyder et al., 2011). Among these, two studies employed post-prandial designs (Ko et al., 2005; Snyder et al., 2011). According to Ko et al. (2005), an increase in antioxidant activity as determined by a reduction in relative dichlorofluorescein fluorescence (DCF) ($P<0.05$) was observed after 30, 60, 90 and 120 minutes among 10 males consuming 150 ml of eight different 100% FJs (apple, orange, grape, peach, plum, kiwi, melon and watermelon) on separate days with a one-day washout period in between testing days. In another post-prandial study, Snyder et al. (2011) reported a significant increase in ORAC ($P<0.05$) in 16 healthy male and females after 1 hour with a return to baseline by hour three following consumption of 591 ml of 100% orange juice with a high-carbohydrate breakfast.

By lengthening the intervention and observation period, Freedman et al. (2001) reported a significant increase in antioxidant activity as measured by ORAC (0.6 ± 0.1 to 0.9 ± 0.1, $P<0.05$) following a two-week intervention in which 20 males and females consumed 7 ml/kg/day of

100% purple grape juice. By providing a lower FJ dose (100mL or 200mL 100% juice of pumelo-grapefruit hybrid) and for a longer period (30 days), Gorinstein et al. (2004) reported significant increases in serum antioxidant activity measured by the Trolox equivalent antioxidant coefficient (TEAC) assay in both treatment groups compared to the control group; furthermore, the serum antioxidant activity between the treatment groups was significantly different with reported increases compared to the control group of 16.9% among the 24 individuals consuming the 100mL dose and 33.1% higher among the 24 individuals consuming 200mL of 100% juice from the pumelo-grapefruit hybrid.

Although Rowe et al. (2011) reported non-significant improvements in antioxidant capacity among subjects (n=40) ingesting 360mL of 100% grape juice for nine-weeks, ORAC scores increased by 41.1 umol Trolox equivalents (TE)/mL. In contrast, by the end of the study, serum ORAC decreased by 66.2 umol TE/mL in the placebo arm of this randomized, double-blind placebo-controlled parallel intervention. Although non-significant results were observed, the effect on trend suggests movement in the direction of increased antioxidant capacity influenced by 100% FJ consumption. In contrast, Bub et al. (2003) was the only study to report no improvement in antioxidant status. However, only one biomarker of antioxidant status (FRAP) was reported. The only other study to report on FRAP as an outcome measure of antioxidant status was Karlsen et al. (2010) who reported an improvement. The intervention juices differed between the two studies, and the intervention period (4 weeks) reported by Karlsen et al., (2010) was double that of the randomized crossover trial reported by Bub et al. (2003). Additionally, sample characteristics were quite different between the two studies. For example, Bub et al. (2003) evaluated an all-female sample with a mean age of 35±4 years which was nearly 20 years

younger than the sample population investigated by Karlsen et al. (2010) (53 years (range: 34 to 68 years). While it is impossible to determine with any certainty, differential sensitivity of the FRAP assay compared to other antioxidant measures, type of juice, length of consumption or differences in study characteristics might all factor into explaining the inconsistency in between Bub et al. (2003) and findings of the other six studies examined.

Despite the heterogeneity in juice types evaluated and antioxidant biomarkers employed, two of the identified studies allowed for cross-study comparison based on similarities in methodological approach to investigating the influence of 100% FJ consumption on antioxidant status (Freedman et al., 2001; Rowe et al., 2011). In both studies, 100% grape juice was evaluated and serum ORAC was the biomarker. While Rowe et al. (2011) reported non-significant increases in antioxidant capacity following consumption of 360mL of 100% grape juice for nine-weeks, Freedman et al. (2001) reported significant increases in antioxidant capacity post-intervention by testing a higher dosage (7mL/kg/d) and shorter effect time (two weeks). Comparison of results suggests a possible dose-response relationship warranting consideration in future investigations.

Effects of 100% FJ Consumption on Oxidant Status

Six studies reported on the impact of 100% FJ consumption and markers of oxidant status (Bub et al., 2003; Ko et al., 2005; Inoue et al., 2008; Ghanim et al., 2010; Snyder et al., 2011; Buscemi et al., 2012) (Table 1). All six reported post-consumption improvement in at least one measure of oxidant status, with four of these studies reporting statistically significant improvements in oxidative measures (Bub et al., 2003; Inoue et al., 2008; Ghanim et al., 2010; Snyder et al., 2011). However, three of the four studies reporting statistically significant improvements did not

control for other dietary factors that may have influenced oxidative status (Inoue et al., 2008; Ghanim et al., 2010; Snyder et al., 2011). Thus, the effect of 100% FJ cannot be clearly isolated. Among those reporting significance, two employed post-prandial study designs (Ghanim et al., 2010; Snyder et al., 2011). For example, Snyder et al. (2011) reported a significant increase (P<0.05) in serum lipoprotein oxidation lag time among 16 healthy male and females after 1, 2 and 3 hours following consumption of 591 ml 100% orange juice with high-carbohydrate breakfast. Serum lipoprotein oxidation area under the curve was also lowered at the 2 and 3 hr. time points (P<0.05). Ghanim et al. (2010) reported a decrease in ROS (P<0.05) after 10 healthy males and females consumed 300 calories (approximately 720ml) of 100% orange juice with a 900 kcal high-fat, high-carbohydrate meal as compared to consumption of the meal with water or a glucose drink, respectively. The decrease in ROS generation remained significant compared to the control up to three hours post-consumption.

Among intervention studies of longer duration, Inoue et al. (2008) reported a 22% decrease in urinary 8-hydroxy-deoxyguanosine (U 8-OHdG) (P<0.05) and a 4% decrease in ROS (P<0.01) after 20 male smokers consumed 70 ml of 100% camu-camu juice for seven days. Following a two-week intervention period, Bub et al. (2003) reported a 13% reduction in TBARS, a measure of lipid peroxidation, (0.86 ± 0.3 to 0.75 ± 0.3μmol/L, P<0.05) among 14 males consuming 331 ml of mixed-type 100% FJ. While oxidative findings in Karlsen et al. (2010) and Buscemi et al. (2012) did not reach statistical significance, the direction of the effect for at least one oxidant status measure was consistent with the findings reported above.

Although improvements in a variety of biomarkers were observed post-consumption of 100% FJ, the evidence suggests that relationship is contingent on a range of factors, and thus, no simple

estimate of the effect is available. As such, the overall strength of the available evidence on the relationship between 100% FJ and antioxidant and oxidant status was scored as a Grade III (limited).

RELATIONSHIP BETWEEN INTAKE OF 100% FJ AND BLOOD LIPID LEVELS

Research Identified

Five studies including three clinical trials (Aviram et al., 2000; Kurowska et al., 2000; Gorinstein et al., 2004) and two cross-sectional studies (Mattei et al., 2012; O'Neil et al., 2012) met the inclusion criteria for review and evaluation (Table 2). Among the three intervention studies evaluating downstream effects of 100% fruit juice consumption on blood lipid levels (Aviram et al., 2000; Kurowska et al., 2000; Gorinstein et al., 2004), two of the three reported significant improvements in at least one blood lipid measure following intervention periods of > 28 days (Kurowska et al., 2000; Gorinstein et al., 2004). The lack of improvement reported by Aviram et al. (2000) may possibly be attributed to differences in the bioactive components of the juices examined or the small dose provided by Aviram et al. (2000) (50mL/d for a two-week period). This inference is supported by Kurowska et al. (2000) in which blood lipid improvements were visible only at the higher doses of 500 or 750 ml/day versus 250mL/d.

According to Gorinstein et al. (2004), LDL levels were significantly decreased among adults in the intervention group (n=24) of a randomized controlled trial compared to the control group (n=24) following consumption of either 100ml/d or 200 ml/day of 100% pummelo-grapefruit hybrid juice over a 30-day period (100ml/d treatment - 5.63±0.2 versus 6.37±0.2 mmol/L, $P < 0.01$; 200ml/d treatment - 5.03±0.2 versus 6.37±0.2 mmol/L, $P<0.0005$, respectively). Additionally, following the 200 ml/day intervention, total cholesterol (-16%) and triglycerides (-

25%) were also significantly lowered. HDL was unaffected by either juice intervention dose. Study results should be interpreted with caution since the authors did not adjust for any confounders and no information was provided on the dietary intake of subjects, including other types of 100% FJ consumed.

In contrast to significant improvements in blood lipid levels reported by Gorinstein et al. (2004) at doses < 200ml/d of 100% citrus juice (pumelo-grapefruit hybrid), Kurowska et al. (2000) reported no significant improvements in lipid levels among adults (n=25) following consumption of a similar dose (250ml/day) of 100% orange juice for four-weeks. However, when the dose was increased to 750ml/d, a 21% increase ($P< 0.001$) in HDL was observed after four-weeks along with a significant increase in plasma triglycerides ($P = 0.02$) by 30%. Caution in interpreting the positive and negative results is warranted as subjects recruited had moderately elevated blood lipid levels and were instructed to follow the American Heart Association Step I cholesterol-lowering diet before and during the intervention. Furthermore, it should be noted that 750 ml (3 cups) of 100% orange juice is an unrealistic amount to consume on a daily basis, and it exceeds the recommendation for fruit for virtually all energy patterns (United States Department of Agriculture and United States Department of Health and Human Services, 2010).

Among the two cross-sectional studies meeting the inclusion/exclusion criteria, O'Neil et al. (2012) reported that consumers of 100% orange juice exhibited significantly lower ($P = 0.022$; 3.2 mg/dL) total cholesterol levels than non-consumers in a national, cross-sectional sample of the United States population (n=8,861). Additionally, consumers of 100% orange juice had significantly lower LDL than non-consumers (112.5±1.4 mg/dL versus 116.7±0.93 mg/dL; P=0.0110). While the study adjusted for a number of confounders, the method of dietary data

collection provides better estimates for populations than for individuals. Based on this study design, it cannot be inferred that drinking 100% FJ caused decreased total cholesterol in individuals or that this difference is, in fact, due exclusively to 100% FJ consumption. In a similar study design, Mattei et al. (2012) reported statistically higher HDL levels among adults (n=254) consuming one or more servings of fresh-squeezed 100% FJ compared to non-consumers (n=811) (mean 1.1±0.01mmol/L for daily consumers versus 1.07±0.01mmol/L for non-consumers, *P*-trend = 0.033). This observational study used a food frequency questionnaire (FFQ) to assess the frequency of _homemade' FJ intake comprised of 100% juice from freshly squeezed fruit only. Although a number of factors were adjusted for, it is unknown if the FFQ was validated; furthermore, it should be acknowledged that this measurement relies on self-reporting of dietary intake and composition of juice consumed.

Taken together, there is weak evidence to suggest that one or more blood lipid measures may be positively influenced or associated with consumption of 100% FJ in amounts exceeding 100mL, but this relationship is highly contingent on many factors. As such, the overall strength of the available evidence was scored as a Grade III (limited).

DISCUSSION

Acknowledging the heterogeneity of the evidence reviewed, two questions emerge: (1) what is discernible from the data based on both the significance and trend for effect and (2) how do we better investigate this relationship in order to establish evidence-based guidelines? To answer the first question, key characteristics of study designs must either be controlled or statistically adjusted for in order to obtain a more accurate understanding of the influence of 100% FJ on metabolic outcomes. Table 3 presents a breakdown of characteristics that were found to

influence antioxidant/oxidant status or blood lipid levels in the evidence reviewed. Recognizing that the most basic difference between studies is the type of 100% FJ evaluated, it must be acknowledged that significant differences exist in the antioxidant, phenolic, and flavonoid content of various types of 100% FJ (Crowe and Murray, 2013). Thus, differences in antioxidant capacity would be expected to influence their ability to impact metabolic outcomes. Beyond this primary difference, other differences among study designs and findings suggest that subject characteristics and behaviors are implicated in the relationships between 100% FJ intake and physiological outcomes. In short, the evidence examined in this systematic review indicates that these study design and sample characteristics are implicated in preventing accurate estimates of the immediate effects of consuming 100% FJ on antioxidant and oxidant status as well as the downstream and more highly mediated effects on blood lipid profiles. Nevertheless, evidence based on trend and significance of effect is largely consistent in direction and suggest that bioactive compounds within 100% FJ have the potential to mechanistically influence antioxidant and oxidant status as well as downstream events mediated by redox balance.

The potential role of 100% FJ in human health and disease prevention is widely acknowledged (Hyson, 2015), yet given the sensitivity of outcomes to multiple factors, evaluating the relationship between consuming 100% FJ and target outcomes is not merely complicated, but complex (Wong, 2013). Adding to the complexity is the fact that the studies identified and evaluated were generally of neutral quality for answering the questions, due largely to potential bias introduced by study participant selection which may influence redox threshold, metabolism of FJ, etc. Assessment of quality criteria by study is presented in Appendix 1. Taken collectively, the complexity of the question does not allow for a simple answer. As such, the second lesson

learned from evaluating the research is that the important question to ask may not be ―what is the relationship between 100% FJ intake and antioxidant and oxidant status or lipid measures‖, but rather ―under what conditions (e.g., baseline health status, typical diet pattern) is consumption of 100% FJ (type of juice, dosage, consumption pattern, etc.) associated with these measures or outcomes, at what time period, and for whom (sex, age, genetic profile, etc.)‖. Figure 3 presents a schematic illustration of the study and subject characteristics that must be taken into account in order to obtain a clearer picture of these relationships.

In order to answer this question, well-designed and adequately powered clinical trials evaluating standardized doses of 100% FJ are warranted. Since eight ounces of 100% FJ represents a serving equivalent to whole fruit, clinical evaluation of this serving size may be an appropriate starting dose for evaluating metabolic outcomes influenced by consumption. Furthermore, while multiple methodologies are available to assess antioxidant and oxidant status, a select battery of tests should be conducted in every study to allow for synthesis and comparison of cross-study results. All studies must adjust for potential confounders including background diet among others in order to establish evidence-based guidelines on 100% FJ as a functional food in context of a diet guided by the Dietary Guidelines for Americans 2010.

CONCLUSIONS

Despite the heterogeneity among studies, the evidence of benefit from consumption of 100% FJ exists such that 100% FJ may, under some conditions, have a positive impact on antioxidant/oxidant status and blood lipid levels. However, the ability to make evidence-based recommendations regarding a specific dose-duration-juice effect is limited until such time as key

characteristics in study designs are controlled or statistically adjusted for in order to more accurately understand the influence of 100% FJ on metabolic outcomes.

CONFLICT OF INTEREST

The following authors reported potential conflicts of interest pertaining to this review:

- Carol O'Neil participates in a working group that has received current and past funding from Juice Products Association.
- Paula Ziegler based on employment at the Academy of Nutrition and Dietetics
- Taylor Wolfram based on employment at the Academy of Nutrition and Dietetics

FUNDING DISCLOSURE

- Pepsico, Inc.
- Academy of Nutrition and Dietetics

Pepsico, Inc. was a financial contributor to the Dietary and Metabolic Impact of 100% Fruit Juice Consumption Evidence Analysis Project. In accordance with the Academy's Evidence Analysis Library policy, external funders have no bearing on the project's workgroup members, search strategy, outcomes, and/or any evidence analysis process.

CE O'Neil received partial support from the USDA Hatch Project LAB 94209.

ACKNOWLEDGEMENTS

The Authors would like to acknowledge the following individuals who served on this EAL Project, yet did not serve as authors of this manuscript: Dianne Hyson, PhD, MSc, RD; Lorraine Matthews, MS, RD, LDN; Tami Piemonte, MS, RD; Kari Kren, MPH, RDN, LDN; Jamie Erskine, PhD, RD; Diane DellaValle, PhD, RDN; Sharon Foley, PhD, RD/LDN; Michelle Lee, PhD, RD, LD; and Kyle Thompson, MS, RD, CNSC.

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Table 1 Studies on markers of antioxidant and oxidant status and consumption of 100% fruit juice among adults

Author	**Groups**	**100% Fruit Juice Type**	**Antioxidant Outcomes[1]**	**Oxidant Outcomes[1]**
Study Design	**Final N**		**Changes in Outcomes[2]**	**Changes in Outcomes[2]**
Quality Rating	**Sex (% Female)**	**Dose**	**✚ improved significantly** + improved non-significantly	**✚ improved significantly** + improved non-significantly
	Age (mean±SD)	**Duration of Exposure**	⊘ did not improve	⊘ did not improve
Postprandial Studies				
Snyder et al. (2011)	Group: Placebo, placebo + hesperidin, mixed	Juice: orange Dose: 591ml Duration: one	**✚ ORAC: - (P<0.05)**	**✚ Serum oxidation lag time (P<0.05)**
Randomized				**✚ Serum oxidation area**

Crossover Trial	group, orange juice	serving		**under the curve: (P<0.05)**
Negative	N: 16 % Female: NR Age: 18 to 24 years (range)			
Ghanim et al. (2010) Non-Randomized Controlled Trial Neutral	Groups: HFHC + water, HFHC + glucose, HFHC + OJ N: 10 % Female: NR Age: 20 to 40 years (range)	Juice: orange Dose: 300 calories Duration: five hours		**Treatment x time interaction** **✚ ROS-PMN (P<0.05)** **✚ ROS-MNC (P<0.05)**
Ko et al. (2005) Non-Randomize	Group: Treatment N: 10	Juice: apple, orange, peach, kiwi, plum, melon,	**✚ DCHF*:* All juices (P<0.05)** except	

d Crossover Trial	% Female: 0 Age: 24±0.6 years	watermelon, grape, pear Dose: 150ml Duration: one serving	pear: NS	
Neutral				
Longer Term Studies				
Bub et al. (2003) Randomized Crossover Trial Neutral	Group: Juice A N: 14 % Female: 0 Age*:* 35±4 years	*Juice:* Juice blend A prepared with apple, orange, mango with aronia, blue-berries, and boysenberries Dose: 330ml/day Duration*:* two weeks	⃠ FRAP Juice A	+ FOX-2 Juice A **✚ TBARS Juice A** **(P<0.05)**

Buscemi et al. (2012) Randomized Crossover Trial Neutral	Groups: ROJ Intake, Placebo N: 19 % Female: 47 Age: 43±13 years	Juice: red orange juice Dose: 500ml/day Duration: one week		+ Protein carbonyls (NS)
Freedman et al. (2001) Non-Controlled Trial Neutral	Group: Treatment N: 20 % Female: 40 Age: 30 years (range 20 to 45 years)	Juice: purple grape Dose: 7ml/kg/day Duration: two weeks	+ ORAC **✚ ORAC** **PCA (P<0.05)**	

Gorinstein et al. (2004) Randomized Controlled Trial Neutral	Groups: Control; 100ml per day juice; 200ml per day juice N: 24 % Female: NR Age: 43 to 71 years (range)	Juice: pumello-grapefruit hybrid ("Sweetie") Dose: 100 or 200ml per day Duration: 30 days	✚ **TEAC 100ml/day dose (P<0.05)** ✚ **TEAC 200ml/day dose (P<0.05)**	
Inoue et al. (2008) Randomized Controlled Trial Neutral	Groups: Treatment N: 10 % Female: 0 Age: 37±8 years	Juice: camu-camu Dose: 70ml/day Duration: 7 days		✚ **ROS (P<0.01),** then returned to baseline ✚ **U 8-OHdG (Urine 8-hydroxy-deoxyguanosine): (P<0.05),** then returned to baseline

Karlsen et al. (2010) Randomized Controlled Trial Positive	Group: Treatment, control (water) N: 31 treatment; 31 control % Female: 32 Age: 53 years (range: 34 to 68 years)	Juice: bilberry Dose: 330ml/day Duration: four weeks	+ FRAP + ORAC plasma ⊘ ORAC PCA ⊘ AA ⊘ TRAP + Total glutathione + Reduced glutathione	⊘ DHAA:TAA ratio + D-ROM ⊘ Oxidized glutathione ⊘ Plasma glutathione redox potential
Rowe et al. (2011) Randomized Controlled Trial Positive	Group: Treatment (grape), placebo N: 40 (grape), 38 (placebo) % Female: 68 Age: 58.7±6.1 years (grape),	Juice: grape (Concord) Dose: 360ml/day Duration: nine weeks	**Treatment x time interaction** **✚ ORAC (P=0.01)**	

	56.9±6.1 years (placebo)			

HFHC = High-fat, high-calorie meal; NR = Not reported; FRAP = ferric reducing ability of plasma; FOX-2 = ferrous oxidation of xylene orange; TBARS = thiobarbituric acid reactive substances; ORAC = oxygen radical absorbance capacity; ORAC PCA = perchloric acid; ROS-PMN/MNC = reactive oxygen species produced by polymorphonuclear leukocytes or mononuclear leukocytes; TEAC = total peroxyl-radical trapping activity; ROS = reactive oxygen species; U 8-OHdG = urinary 8-hydroxy-deoxyguanosine; AA = ascorbic acid; TRAP = total peroxyl-radical trapping activity; DHAA:TAA ratio = dehydroascorbic acid:total ascorbic acid ratio; D-ROM = diacrons reactive oxygen metabolites; DCHF = dichlorofluorescein fluorescence.

[1]Within arm change unless otherwise indicated, *p* value for at least one post-baseline measurement

[2]Bold text = statistically significant finding

Table 2 Studies on blood lipid status and consumption of 100% fruit juice among adults

Study	**Groups**	**100% Fruit Juice Type**	**Outcomes**[1]
Design	**Final N**		**Changes in Outcomes**[2]
Quality	**Sex (% Female)**	**Dose**	✚ **improved significantly** + improved non-significantly
	Age (mean±SD)	**Duration of Exposure**	⊘ did not improve
Aviram et al. (2000) Non-Controlled Trial Neutral	Group: Treatment N: 13 % Female: 0 Age: 20 to 35 years (range)	Juice: pomegranate Dose: 50ml/day Duration: 2 weeks	⊘ TC ⊘ LDL ⊘ HDL ⊘ TG
Gorinstein et al. (2004) Randomized Controlled Trial Neutral	Groups: control; 100ml juice/day; 200ml juice/day N: 24 % Female: NR Age*:* 43 to 71 years	Juice: pumello-grapefruit hybrid ("Sweetie") Dose: 100 or 200ml per day Duration: 30 days Comparison: treatment	100 ml/day + TC (NS) ✚ **LDL (P<0.01)** ✚ HDL (NS) ✚ TG (NS)

	(range)	groups versus control	200 ml/day ✚ **TC (P<0.01)** ✚ **LDL (P<0.0005)** + HDL ✚ **TG (P<0.0005)** Treatment groups compared to control post treatment
Kurowska, et al. (2000) Non-Randomized Crossover Trial Positive	Groups: dose groups of 250, 500, 750 ml/day N: 75 % Female: 36 Age: 55±11 years	Juice: orange Dose: 250, 500, 750 ml/day Duration: 4 weeks	250 ml/day ⊘ TC ⊘ LDL ⊘ HDL ⊘ TG 500 ml/day ⊘ TC ⊘ LDL + HDL ⊘ TG (NS)

			750 ml/day ⊘ TC ⊘ LDL ✚ **HDL (P<0.05)** ⊘TG
Mattei et al. (2012) Cross-Sectional Study Positive	Groups: serving groups N: 1065 % Female: 24 non-consumers, 27 daily consumers Age: 59.1±10.5 years	Juice: orange or other fruit Dose: never, ≤1 serving/week, 2-6 servings/week,≥1 serving/day Duration: NA	✚ **HDL (P=0.033)** ⊘ TG (P value for trend)
O'Neil et al. (2012) Cross-Sectional Study Positive	Groups: consumers, non-consumers N: 8861 % Female: NR Age: NR	Juice: orange Dose: non-consumers, consumers Duration: NA	✚ **TC (P=0.022)** ✚ **LDL (P=0.011)** ⊘ HDL + TG

NR = Not reported; TC = total cholesterol; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TG = triglycerides.

[1]Within arm change unless otherwise indicated, *p* value for at least one post-baseline measurement

[2]Bold text = statistically significant finding

Table 3. Characteristics Identified in the Examined Studies Affecting Antioxidant/Oxidant Status and Blood Lipid Outcomes

Characteristic	**Studies**
Juice	
Specific Juice or juice mix	Bub et al. (2003), Inoue et al. (2004), Ko et al. (2005), Snyder et al. (2011)
Juice v supplement	Inoue et al. (2004)
Dose	Kurowska et al. (2000), Gorinstein et al. (2004)
Outcome Measurement	
Marker measured	Freedman et al. (2001), Bub et al. (2003), Inoue et al. (2004), Ghanim et al. (2010), Karlsen et al. (2010), Rowe et al. (2011), Snyder et al. (2011), Buscemi et al. (2012)
Timing of measurement	Bub et al. (2003), Gorinstein et al. (2004), Inoue et al. (2004), Ko et al. (2005), Ghanim et al. (2010), Snyder et al. (2011)
Target tissue	Bub et al. (2003), Karlsen et al. (2010)

Subject Characteristics	
Baseline health risk (profile) of subject	Inoue et al. (2004), Rowe et al. (2011), Buscemi et al. (2012)
Age of subject	Rowe et al. (2011)
Genetic baseline	Rowe et al. (2011)
Behavioral Confounders	
Food consumed with juice	Ghanim et al. (2010)

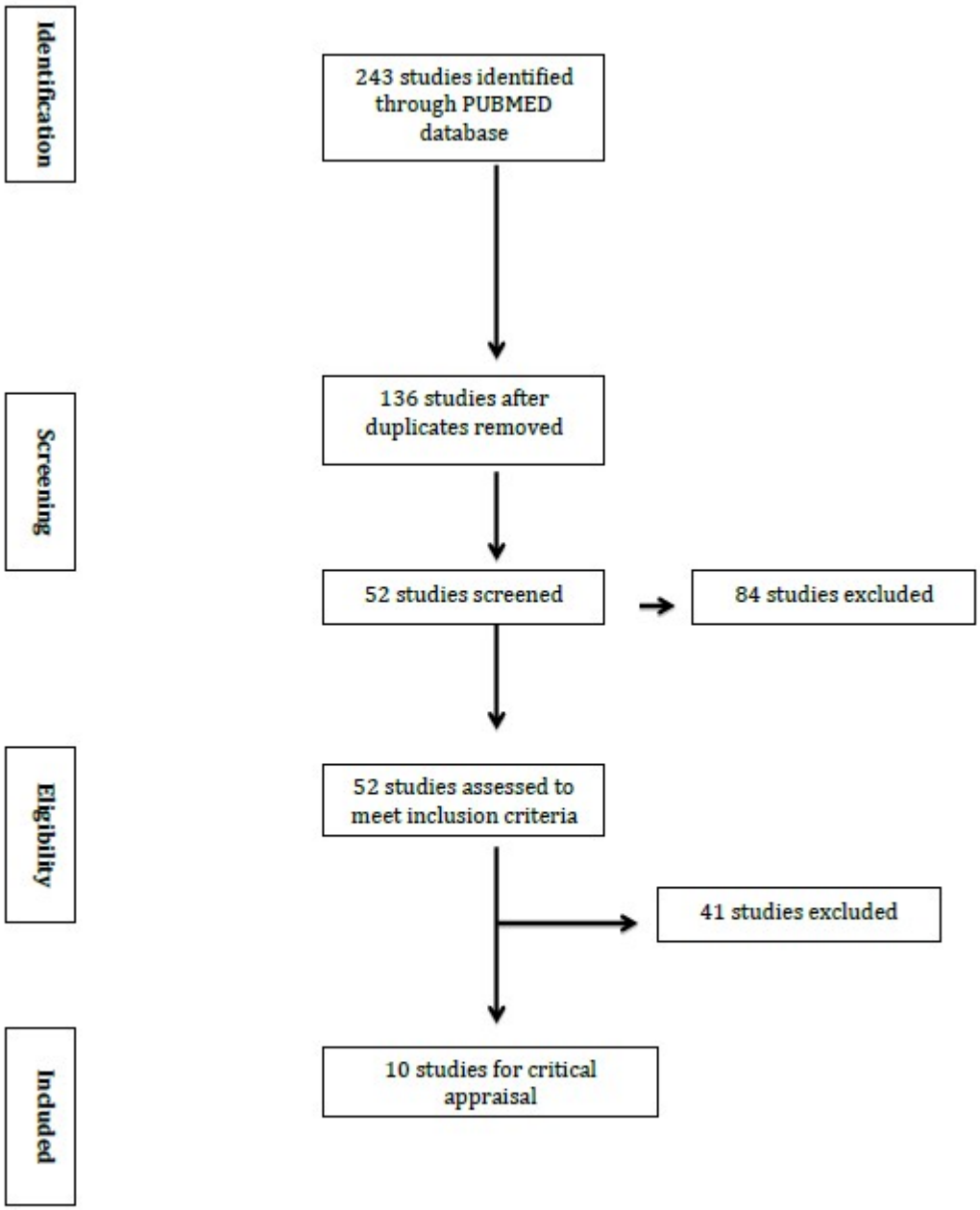

Figure 1 Search strategy flow diagram for research evaluating the relationship between markers of antioxidant and oxidant status and 100% fruit juice consumption among adults

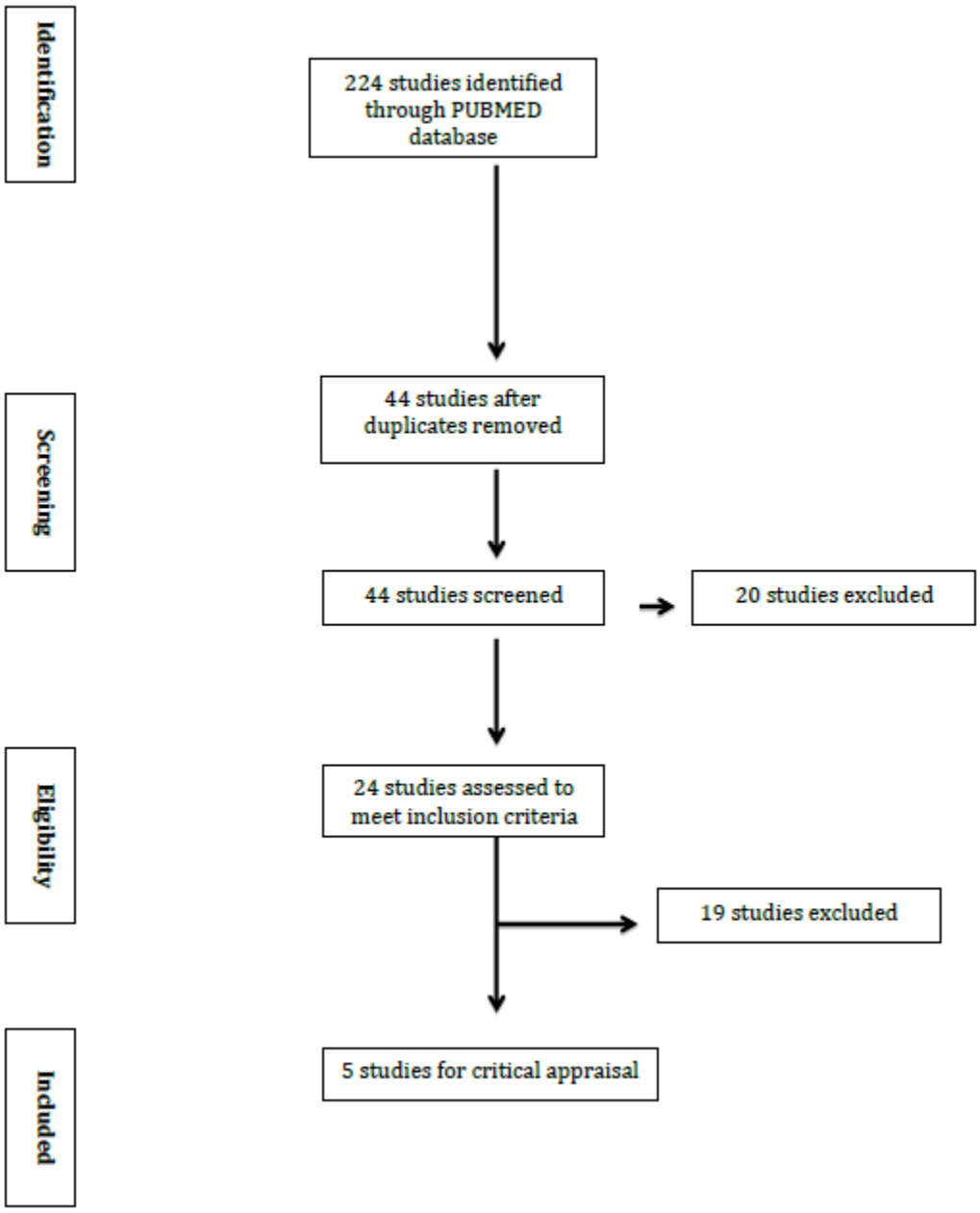

Figure 2 Search strategy flow diagram for research evaluating the relationship between blood lipid levels and 100% fruit juice consumption among adults

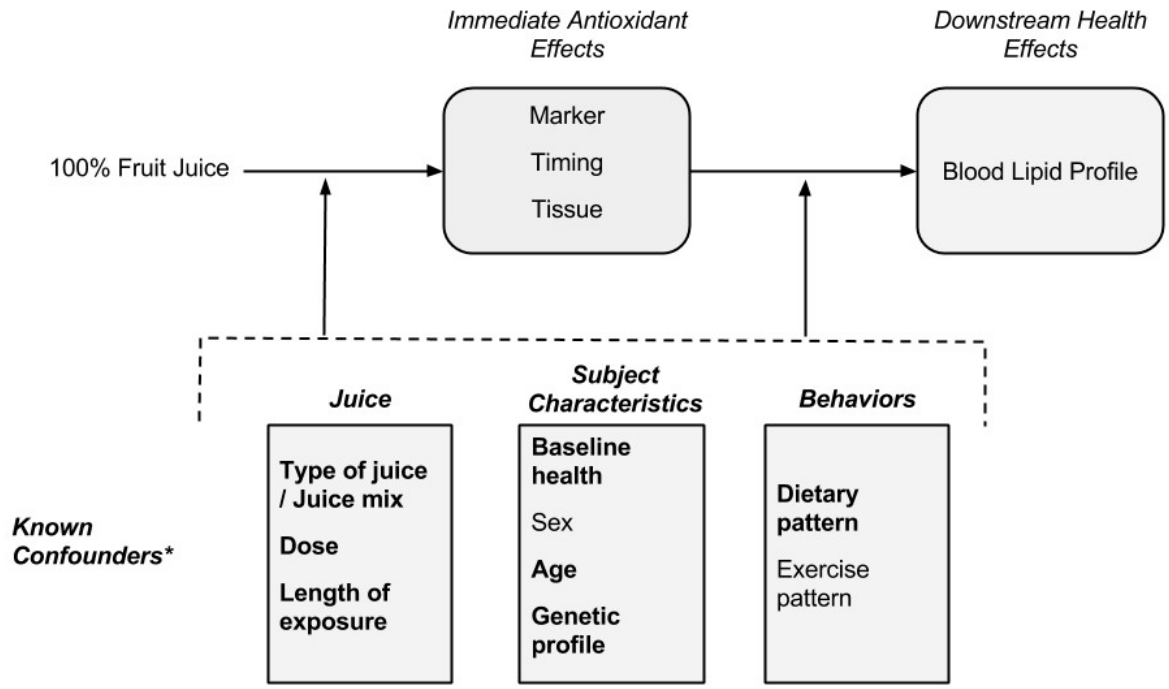

Figure 3 Study Characteristics Influencing Immediate Antioxidant/Oxidant Status or Downstream Health Effects Following Consumption of 100% Fruit Juice*. *Bold text indicates characteristics for which there is direct research in the studies examined. Normal text indicates characteristics indicated in other studies.

Appendix: Table of Study Quality Criteria Ratings

Study	Overall Rating	Question Domains									
		Was the research question clearly stated?	Was the selection of study subjects/ patients free	Were study groups comparable?	Was method of handling withdrawals described?	Was blinding used to prevent introduction of bias?	Were intervention/ therapeutic regimens/ exposure factor or procedure and any compa	Were outcomes clearly defined and the measurements valid and reliable?	Was the statistical analysis appropriate for the study design and type of outco	Are conclusions supported by results with biases and limitations taken into consideration?	Is bias due to study's funding or sponsorship unlikely?

			e from bias?				rison(s) described in detail?		me indicators?		
Aviram et al. (2000)	Neutral	No	???	Yes	N/A	No	No	No	Yes	No	???
Bub et al. (2003)	Neutral	Yes	No	N/A	N/A	???	Yes	Yes	Yes	Yes	Yes
Buscemi et al. (2012)	Neutral	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	Yes

Freedman et al. (2001)	Neutral	Yes	No	N/A	N/A	N/A	Yes	Yes	Yes	No	Yes
Ghanim et al. (2010)	Neutral	Yes	No	???	N/A	???	Yes	Yes	Yes	Yes	Yes
Goinstein et al. (2004)	Neutral	Yes	No	???	No	No	Yes	Yes	Yes	No	???
Inoue	Neutral	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	???

et al. (2008)											
Karlsen et al. (2010)	Positive	Yes	Yes	Yes	N/A	No	Yes	Yes	Yes	Yes	???
Ko et al. (2005)	Neutral	Yes	???	Yes	N/A	Yes	Yes	Yes	???	Yes	Yes
Kurowska et al. (2000)	Positive	Yes	???	N/A	N/A	No	???	Yes	Yes	Yes	Yes

Matei et al. (2012)	Positive	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
O'Neil et al. (2012)	Positive	Yes	Yes	???	???	N/A	Yes	Yes	Yes	Yes	Yes
Rowe et al. (2011)	Positive	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Snyder et al.	Negative	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes

(2011)											

Yes = Criterion met, No = Criterion not met, ???=Unclear whether criterion met, N/A=Not applicable

