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Abstract

Aim: Cardiovascular disease (CVD) are among the main causes of death worldwide and dyslipidemias account for one of the risk factors for these diseases. Habitual apple consumption appears to be inversely associated with reduced cardiovascular risk. Then, this systematic review aims to investigate the effect of chronic apple consumption on the lipid profile of adults with dyslipidemia.

Methods: A systematic search was performed in electronic databases, including PubMed, Embase, Web of Science and Scopus, without restriction of year of publication. Inclusion criteria were randomized clinical trials in humans that investigated the effect of chronic consumption of whole fresh or dried apple, for a period longer than two weeks of intervention on the lipid profile.

Results: Based on the methodology used and following the pre-established search strategies, 4,468 articles were found. After applying the inclusion and exclusion criteria, five articles were selected for qualitative evaluation, covering 522 adult participants of both sexes. Three randomized controlled trials included in this review demonstrated that there was a decrease in plasma total cholesterol (TC), triglyceride and low-density lipoprotein cholesterol (LDL-c) concentrations, in addition to an increase in high-density lipoprotein cholesterol (HDL-c) concentration. Two other studies found different results. Low risk of bias was identified in three studies.

Conclusions: The analysis of the studies indicates that the consumption of fresh and/or dried apples with the peel has a beneficial effect on the lipid profile of adults, with a decrease in TC and LDL-c. These effects may be related to polyphenols and soluble fibers, among other functional compounds present in this fruit.



Keywords

Apple, cholesterol, polyphenols, lipid profile, dyslipidemia

Introduction

Cardiovascular disease (CVD) is considered as a worldwide death leading cause in adults. Some risk factors for CVD and premature death include systemic arterial hypertension, dyslipidemia, obesity and smoking. Dyslipidemias encompass several lipid abnormalities, some of which can trigger the onset of CVD [1, 2].

High serum concentrations of low-density lipoprotein cholesterol (LDL-c) are an important risk factor for CVD, since one of the main functions of this lipoprotein is to transport cholesterol from the liver to peripheral tissues; therefore, the plasma increase of this lipoprotein can cause atherogenic effects. On the other hand, high serum concentrations of high-density lipoprotein cholesterol (HDL-c) have antiatherogenic effects, since the most important role of this lipoprotein is the reverse transport of cholesterol, removing excess cholesterol in peripheral tissues and redirecting it to the liver, to be eliminated from the body. In addition, HDL-c also has anticoagulant, antioxidant, anti-inflammatory and antiplatelet actions [3, 4].

Meta-analysis studies have shown that some aspects of CVD risk can be modulated by various dietary interventions, including an increased consumption of fruits and other vegetables as part of a healthy and balanced diet [5–7]. However, not only the total fruit consumption can contribute to reduce the risk of CVD, but also the way the fruit is consumed, whether the whole fruit or the fruit juice [5–7].

The benefits of fruit consumption have been attributed to the presence of various nutrients such as vitamins, minerals, fiber and phytochemicals including polyphenols and carotenoids [8]. Polyphenols have beneficial effects on the human health, contributing to the scavenging of free radicals and the regulation of glucose and lipid metabolism in the liver and adipose tissue [9–12]. Apple (*Malus domestica* Borkh.) is an excellent source of polyphenols, and its habitual consumption may be inversely associated with reduced risk of CVD [13–15].

Apple is a seasonal fruit with widespread consumption due to its wide global availability, and its composition is approximately 85% water and 14% carbohydrates, including fiber and sugars, in addition to vitamins C and E, potassium and polyphenols. Regarding the total fiber content, 70% are insoluble and 30% are soluble. Thus, the importance of consuming the fruit peel along with the pulp is evident, since the red peel has a higher concentration of polyphenols, containing flavonoids, hydroxybenzoic acids and hydroxycinnamic acids [16]. In addition, also the several applications of fruit waste as valuable resources of value-added compounds should be taken into account [17].

The benefits of apple intake are linked to the content of some nutrients and bioactive compounds present, which contribute to the antioxidant power of apples, especially flavonoids such as procyanidins, procyanidin B2 and epicatechin. Among the various beneficial actions of flavonoids are antioxidant and anti-inflammatory properties, vasodilator effect, antiallergic action and antitumor activity. Some of the bioactive properties of phenolic compounds result in anti-inflammatory, antioxidant, antiseptic, analgesic, cardioprotective and antihypertensive activities. Among the phenolic compounds present in apples, quercetin has the highest antioxidant contribution [18, 19].

In addition to the antioxidant capacity that helps prevent and combat plasma oxidative stress, apples with the peel are a source of hemicellulose, a non-starch polysaccharide dietary fiber. Furthermore, results from some studies indicate that soluble fibers such as pectins, gums and some hemicelluloses can reduce plasma concentrations of total cholesterol (TC) and LDL-c [20, 21]. In this sense, the daily consumption of 6 g/day of pectin can reduce cholesterol levels and, consequently, be a strong ally in reducing the risk of developing coronary artery disease [20, 21]. However, caution is advised for patients with health complications, such as specific gastrointestinal problems, who may have difficulties ingesting apple peel or eating it raw.

Considering that apple is an accessible fruit that presents bioactive compounds with potential beneficial effects, it can be a viable ally in the fight against hypercholesterolemia in adults. Given the above, this systematic review aims to investigate the effect of chronic apple consumption on the lipid profile of adults with dyslipidemia.

Materials and methods

Protocol registration

This is a systematic review of national and international scientific literature of randomized clinical trials that investigated, directly or indirectly, the effects of fresh and/or dried apple consumption on the lipid profile of adult individuals with dyslipidemia. The review protocol was registered in the PROSPERO database, international prospective register of systematic reviews, under the number CRD42022302075.

Data resources and research strategy

For the selection of articles, the acronym PICOS was used, where each letter represents a component of the question: (P) population; (I) intervention; (C) control; (O) outcome and (S) study design [22]. Moreover, the foundations for the construction of this review were defined based on the preferred reporting items for systematic reviews and meta-analyses—PRISMA [23].

According to the PICOS strategy, this research was guided by the intention to answer the following target question: “Does the daily intake of apple improve the lipid profile of adult individuals with dyslipidemia?”. The PICOS items corresponded to the following aspects: (P) adult individuals with dyslipidemia; (I) apple intake; (C) apple-supplemented and non-supplemented group; (O) improvement of lipid profile; (S) randomized clinical trials. Data collection was conducted in February 2022 in the PubMed, Embase, Web of Science and Scopus databases using the Medical Subject Headings (MeSH) and Emtree descriptors with the Boolean operator [AND] between the descriptors and the Boolean operator [OR] for synonymous descriptors as a way of identifying broader publications, searching for them by the title and/or abstract of the articles. The search and selection steps were performed independently by two researchers (Liriane Andressa Alves da Silva, Margarete Almeida Freitas de Azevedo), who evaluated the articles for inclusion eligibility.

The search keys were prepared according to the databases. Thus, the final search strategy used was: “Malus” [MeSH] AND “Placebo” [MeSH] OR “Control groups” [MeSH] AND “Lipoproteins” [MeSH] OR “Triglycerides (TG)” [MeSH] OR “Cholesterol” [MeSH] OR “Lipid metabolism” [MeSH] OR “Dyslipidemias” [MeSH]. No year restriction was applied.

Eligibility criteria

Randomized clinical trials in humans that investigated the effect of chronic consumption of whole fresh or dried apple on the lipid profile, for a period more than 2 weeks of intervention. Studies that used apples mixed with other fruits or apple-derived products, juices and extracts, as well as studies not available in full and experimental studies were excluded.

Study selection

The researchers Liriane Andressa Alves da Silva and Margarete Almeida Freitas de Azevedo independently selected articles for eligibility. Articles were moved to the next screening phase or discarded when total disagreement was reached. Disagreements between them were resolved by a third researcher (Nayara Vieira do Nascimento Monteiro) through discussion, until consensus was reached. The selection of eligible studies was based on two steps: Initially, both the title and abstract of each study were selected for relevance; full texts were then reviewed for those unsure of inclusion. In addition to the bases described, articles identified in the reference lists of other reviews or original studies related to the topic were searched. The PRISMA 2020 checklist are stated in [Table S1](#).

Data extraction

All records were exported to the EndNote X8 reference management software. Finally, five studies were selected for the review. The data collected were organized in [Table 1](#) including authors' names, year of publication, country, study design, objective, population/sample, health status, intervention, and main results.

Table 1. Synthesis of the studies evaluated in terms of apple intake on the lipid profile of adult individuals with dyslipidemia

Author/Year/Country	Study design	Objective	Participants	Health status	Intervention	Main results
Tenore et al. [28], 2017, Italy	Randomized, single-blind, clinical trial	To evaluate the effects of daily consumption of Annurca apple for 8 weeks on plasma TC, LDL-c and HDL-c in healthy, mildly hypercholesterolemic human subjects.	<ul style="list-style-type: none"> Adults Men and women 18 years to 83 years $n = 250$ 	Healthy and slightly elevated cholesterol	<ul style="list-style-type: none"> Protocol: 1 apple a day Duration: 8 weeks 	<ul style="list-style-type: none"> ↓ TC and LDL-c ↑ HDL-c and TG
Chai et al. [27], 2012, United States of America	Randomized, controlled, unblinded clinical trial	To evaluate the effect of dried apple <i>versus</i> dried plum consumption on reducing CVD risk factors in postmenopausal women.	<ul style="list-style-type: none"> Adults Women 50 years to 55 years $n = 160$ 	Postmenopausal	<ul style="list-style-type: none"> Protocol: 75 g/day of dried apple Duration: 12 months 	<ul style="list-style-type: none"> ↓ TC and LDL-c ↑ HDL-c and ↓ TG
Koutsos et al. [25], 2020, United Kingdom	Randomized, controlled, crossover clinical trial	To determine the effects of apple consumption on circulating lipids, vascular function and other CVD risk markers.	<ul style="list-style-type: none"> Adults Men and women 29 years to 69 years $n = 43$ 	Mild hypercholesterolemia	<ul style="list-style-type: none"> Protocol: 2 apples a day Duration: 8 weeks 	<ul style="list-style-type: none"> ↓ Serum cholesterol and LDL-c ↓ TG There were no differences in concentration of HDL-c
Vafa et al. [29], 2011, Iran	Randomized, controlled clinical trial	To verify the effect of Golden Delicious apple on the lipid profile in hyperlipidemic and overweight men.	<ul style="list-style-type: none"> Adults Men 18 years to 41 years $n = 46$ 	Hyperlipidemic and overweight	<ul style="list-style-type: none"> Protocol: 300 g of Golden Delicious apple a day Duration: 8 weeks 	<ul style="list-style-type: none"> Apple consumption did not change lipid profile in overweight men
Ravn-Haren et al. [26], 2013, Denmark	Randomized, single-blind, crossover clinical trial	To investigate the effect of the consumption of whole apples and processed apple fractions for four weeks on plasma lipids, bile acid excretion, blood pressure and other CVD risk factors.	<ul style="list-style-type: none"> Adults Men and women 18 years to 69 years $n = 23$ 	Healthy individuals	<ul style="list-style-type: none"> Protocol: 550 g of fresh apple a day Duration: 4 weeks 	<ul style="list-style-type: none"> ↓ TC There was no difference in LDL-c concentration between the placebo group and the apple group

↓: decrease; ↑: increase

Study quality assessment

To assess the risk of bias, the Cochrane Collaboration's tool [24] was used. The following domains were evaluated: random sequence generation, allocation concealment, concealment of participants and personnel, concealment of outcome assessment, incomplete outcome data and selective outcome reporting. Each item was classified as "low", "unclear" or "high" risk of bias. Through analysis, the study with the presence of adequate procedures in all domains was classified as low risk of bias; the study that had an inadequate procedure in at least one domain was classified as high risk of bias. In any other case, the study was labeled as being of uncertain risk of bias.

Results

The search and selection strategies for the articles included in this systematic review are shown in [Figure 1](#). Based on the methodology used and following the pre-established search strategies, 4,468 articles were found. After applying the inclusion and exclusion criteria, 5 articles were selected for qualitative evaluation of this systematic review.

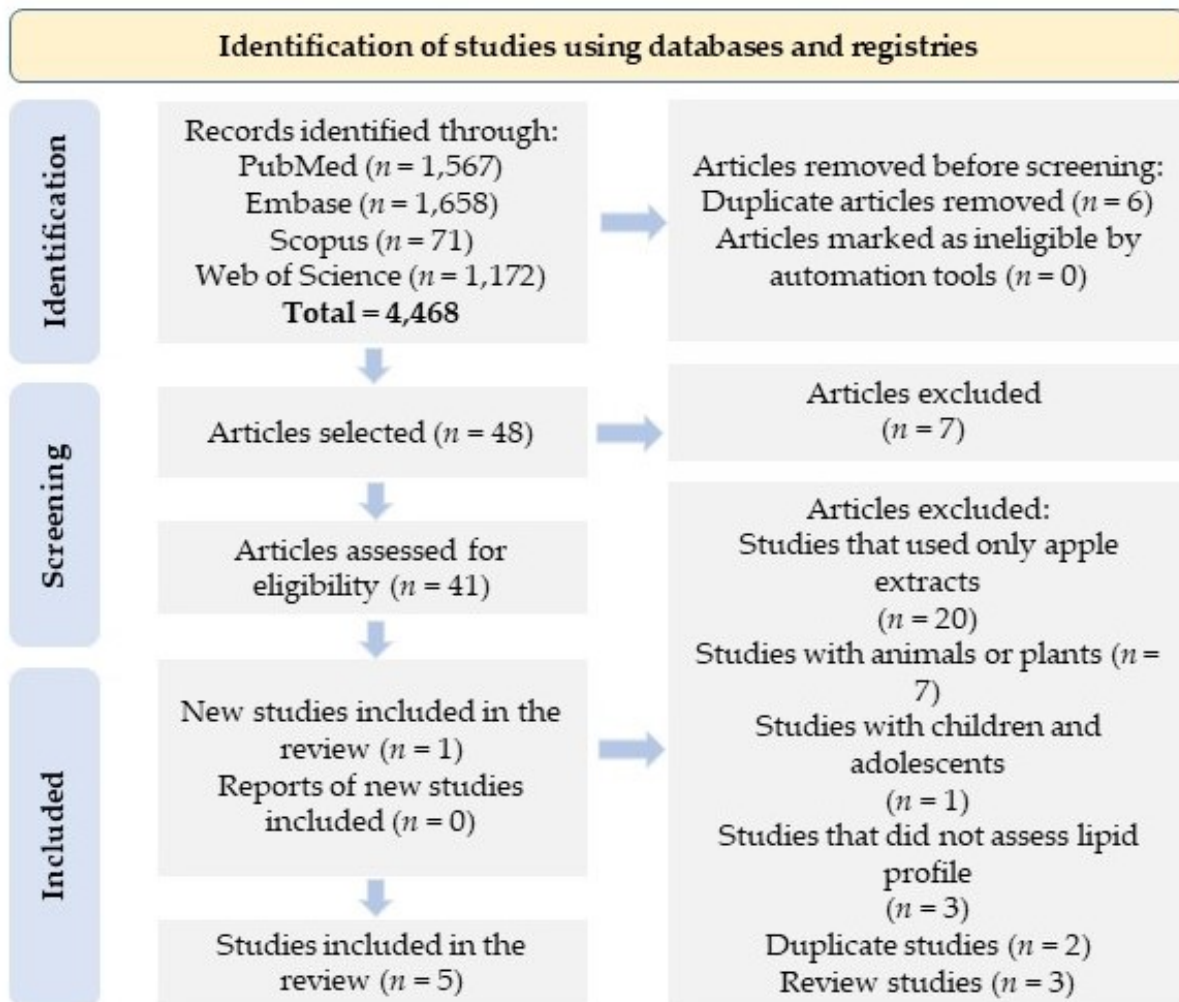


Figure 1. Flowchart based on PRISMA 2020 statement for systematic reviews of the search results in the databases consulted

Main characteristics and results of the studies analyzed are shown in [Table 1](#). The selected studies were published between 2011 and 2020. The five studies included covered 522 adult participants of both sexes.

Among the 5 studies included in this systematic review ([Table 1](#)), three randomized clinical trials analyzed apple consumption on the lipid profile and cardiovascular risk markers of individuals and showed a decrease in TC and TG levels, as well as an increase in HDL-c [25–27]. However, in the study by Koutsos et al. [25] there was no change in high-density lipoprotein (HDL), and in the study by Ravn-Haren et al. [26] no difference was found in LDL-c concentrations. Different results were found in the studies by Tenore et al. [28] and Vafa et al. [29], who (evaluated the effects of apple) consumption on the lipid profile of overweight and mildly hypercholesterolemic individuals. In the findings of Tenore et al. [28], although a decrease in TC and LDL-c was found, there was an increase in TG and HDL-c. In the study by Vafa et al. [29], apple consumption did not present significant effects on the reduction of the lipid parameters evaluated.

Study quality and risk of bias for the studies included in this review are shown in [Table 2](#). Of the five randomized controlled trials, three studies had a low risk of bias [26–28]; the other two studies had an uncertain risk of bias, as they did not mention the blinding of participants and personnel, and the blinding of outcome assessment was not mentioned either.

Table 2. Assessment of bias in various domains in the randomized clinical trials included in the systematic review

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Concealment of outcome assessors	Incomplete outcome data	Selective reporting of results
Tenore et al. [28], 2017	Low	Low	Low	Low	Low	Low
Chai et al. [27], 2012	Low	Low	Low	Low	Low	Low
Koutsos et al. [25], 2020	Low	Low	Unclear	Unclear	Low	Low
Vafa et al. [29], 2011	Low	Low	Unclear	Unclear	Low	Low
Ravn-Haren et al. [26], 2013	Low	Low	Low	Low	Low	Low

Discussion

This systematic review was conducted with the aim of collecting evidence in randomized clinical trials on the effect of apple consumption on the lipid profile of adults with dyslipidemia. In most of the studies analyzed here, a positive effect was demonstrated, which consisted of a decrease in TC, LDL-c and TG, as well as an increase in HDL-c.

Tenore et al. [28], when evaluating the effects of daily consumption of five apple cultivars (Annurca, Red Delicious, Granny Smith, Fuji, Golden Delicious) on plasma concentrations of TC, LDL-c and HDL-c in healthy, mildly hypercholesterolemic individuals, observed that apple consumption resulted in a decrease in TC, LDL-c and an increase in TG and HDL-c. Among the cultivars, Annurca exerted the highest hypocholesterolemic effect, decreasing TC by 8.3% [95% confidence interval (CI): 2.54, $P = 0.0019$], when compared to the Granny Smith cultivar (−8.3%), which was the second most active among all apples tested, maintaining the same trend for HDL-c. As for LDL-c, the apple cultivar Annurca also showed better results, being 1.7 times more effective in reducing LDL-c.

The lipid-lowering effects of apples are likely to be partially associated with the antioxidant activity of bioactive compounds present in this fruit. In this sense, laboratory research results showed strong antioxidant activity in apples, with inhibition of cancer cell proliferation and reduction of lipid and cholesterol oxidation. These fruits contain phytochemicals such as quercetin, catechin, phloridzin, and chlorogenic acid [30].

According to Sandoval-Ramírez et al. [14], the total phenolic content of apples differs significantly between varieties due to the fact that apple diversity is influenced by a set of environmental factors, such as soil, growing season and harvest season, as well as their storage conditions and maturity status.

Accordingly, Tenore et al. [28], when comparing the lipid-lowering effect *in vivo* of each apple cultivar regarding its content of polyphenolic compounds, showed that the Annurca cultivar exhibited two to four times more procyanidins than did the other apple samples, Fuji and Golden Delicious being the cultivars with the lowest content and, consequently, the weakest effect. As for the other polyphenolic compounds, such as dihydrochalcones, flavonols, and anthocyanins, these presented similar mean values for the five apple species considered. However, consumption of these cultivars resulted in an increase in plasma concentrations of TG, and the highest increase occurred in the group fed with Granny Smith, on average 12.7% ($P = 0.0032$) and to a lesser extent in Annurca, with an increase of 6.1% ($P = 0.0027$).

Downing et al. [31] highlighted some important points regarding the lipid-lowering effect of procyanidin. In the study, the authors sought to determine whether a procyanidin extract from grape seed, which, like apples, is a fruit rich in this phenolic compound, exerts a TG-lowering effect in a hyperlipidemic state in rats. And as a result, grape seed procyanidin reduced serum TG levels in fructose-fed rats after one week administration, demonstrating that it targets the liver, suggesting that in the presence of procyanidin and fructose, the liver can secrete more free cholesterol into the plasma which may then be shunted to the small intestine and subsequently on to fecal excretion. For the authors, procyanidin inhibited hepatic lipogenesis via downregulation of sterol regulatory element binding protein 1c and stearyl- coenzyme A (CoA) desaturase 1 in the fructose-fed animals, increasing fecal bile acid and total lipid excretion, and reducing serum bile acid levels.

Different results were found by Chai et al. [27] when evaluating the effects of dried apples in healthy postmenopausal women (1 year to 10 years after menopause), in which there was no change in TG and HDL. However, serum HDL-c increased numerically in 3% of women and TG decreased in 9% of them after 12 months of fruit consumption. Nevertheless, dried apple consumption, for 3 months, was able to significantly reduce TC and serum LDL by 9% and 16%, respectively, and after 6 months, they were reduced even more by 13% and 24%, respectively, remaining until 12 months.

It is important to highlight the clinical trial by Koutsos et al. [25], conducted with men and women aged 29 years to 69 years who were mildly hypercholesterolemic and consumed 2 apples of the green variety “Renetta Canada” per day for 8 weeks. The result of the study was a decrease in serum concentrations of TC, LDL-c and TG, but not HDL-c. Corroborating this finding, Gormley et al. [32] in a study with 76 men, and Sable-Amplis et al. [33] with 30 volunteers, found a decrease in cholesterol, and in the latter the results ranged from no change to a reduction of 29.2%.

Additionally, Vafa et al. [29], when performing a randomized clinical trial with 46 men aged 18 years to 41 years, in which the intervention group received 300 g of Golden Delicious apple per day for 8 weeks, observed an increase in serum concentrations of TG and very-LDL (VLDL) in both groups, with no change in TC, LDL-c, HDL-c and LDL/HDL ratio.

Differently from what was found by Koutsos et al. [25], Eisner et al. [34] performed a clinical trial with 38 overweight or obese children aged between 10 years and 16 years consuming 120 kcal per day of dried apple or a controlled snack (muffin) for 8 weeks and found differences between the two groups only in HDL-c concentrations, which were higher in the apple group. Such findings were also obtained by Tenore et al. [28] and Chai et al. [27].

In healthy adults, Ravn-Haren et al. [26] observed that the consumption of whole apples resulted in a reduction in the concentration of TC, although they did not find any change in LDL-c compared to the control group.

Avci et al. [35], in a study carried out with elderly individuals on apple consumption, did not find differences in TC, LDL-c, VLDL cholesterol (VLDL-c), HDL-c and TG either. Similar results were observed by Conceição de Oliveira et al. [36] in a randomized study with overweight, hypercholesterolemic women aged 30 years to 50 years, consuming apples, pears or oat cookies for 12 weeks. The authors found a reduction in cholesterol in the fruit group and increase in TG for the same group.

In a randomized, double-blind, placebo-controlled study in 71 moderately obese male and female subjects, Nagasako-Akazome et al. [37] demonstrated that ingestion of capsules containing apple polyphenols for 12 weeks reduced TC and LDL-c and visceral fat area and there was an improvement in adiponectin in the group that received apple polyphenols in comparison with the control group. In another type of intervention, Barth et al. [38], analyzing the effect of the consumption of cloudy apple juice rich in polyphenols in 68 obese, non-smoking and non-diabetic men, found a reduction in percent body fat with no change in plasma concentrations of cholesterol, TG, HDL-c and LDL-c after 4 weeks of juice intervention compared to the control.

According to Ribeiro et al. [16], apple is predominantly composed of water and carbohydrates, in concentrations of 85% and 14%, respectively. Furthermore, it has fiber content that makes up 2% to 3% of its composition, of which 70% are insoluble fibers and 30% are soluble, mainly represented by pectin. The benefits of these fibers, characterized as easily fermentable in the colon, are due to the fact that a portion of the fermentation of its components occurs in the large intestine, which has an impact on the speed of intestinal transit, on the pH of the colon luminal content and on the production of by-products with an important physiological function, such as some gases (hydrogen, methane and carbon dioxide) and short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate. Increase in the production of SCFAs, as a product of fermentation, favors the decrease of intracellular and colonic pH [20].

Moreover, SCFAs regulate the recruitment of intestinal immune cells and affect the expression of immune factors, in addition to participating in the regulation of the intestinal microbiota barrier, stimulating the host to secrete antimicrobial peptides and mucins [39]. Pectins are complex and branched fibers, not metabolized in the upper gastrointestinal tract, presenting a prebiotic character by helping to maintain the balance of the intestinal microbiota; they can be fermented by bifidobacteria in the colon and produce metabolites with intestinal and systemic effects in the body [16]. According to Kumar and Chauhan [40], this type of fiber has been recommended as a source of 30% to 50% of total daily intake due to its health benefits, mainly because it is able to reduce the absorption of cholesterol and TG, contributing to the reduction of plasma and hepatic concentrations.

For Majee et al. [21], the effect of pectin on blood cholesterol may be attributed to the interference of micelle formation in the small intestine, the first step in lipid metabolism. The rate of diffusion and uptake of bile acids and cholesterol-containing micelles become reduced, being excreted in feces. These micelles are vital for lipid metabolism because they act as carriers of both dietary cholesterol and fatty acids for absorption in the intestine. Since cholesterol is not solubilized in micelle, it forms a separate oil phase within the intestinal lumen and is consequently unavailable for absorption [41].

In addition to this role of fibers, apple is rich in polyphenols and its benefits may be associated with the reduction of some cardiovascular risk factors, such as serum concentrations of TC and LDL-c, mainly due to the probable ability to reduce plasma oxidative stress through the induction of antioxidant enzymes [26, 28]. According to Sandoval-Ramírez et al. [14], despite the different varieties of apples, the phenolic compounds frequently found in this fruit are hydroxycinnamic acids, flavanols, dihydrochalcones and anthocyanins, which are mostly in the red peel.

Regarding the limitations found, the greatest difficulty was related to the low number of articles on the use of fresh or dried apples, since most of the studies found in the literature used apple by-products. Another limitation is related to the low number of studies found, which made it difficult to standardize intervention protocols. In addition, the samples were not homogeneous, as they included healthy and mildly hypercholesterolemic individuals.

Thus, this review presented some strengths that deserve to be highlighted, such as the use of explicit and reproducible eligibility criteria, and the presentation of the quality standard achieved by the studies included, where most studies had a low risk of bias. In this sense, despite the limitations found, it is believed that the results made it possible to identify the beneficial effects of fresh apple consumption on lipid profile.

The main findings are summarized in Figure 2. The consumption of fresh and/or dried apples with the peel has a beneficial effect on the health of adults, as it contributes to the reduction of TC and LDL-c. Polyphenols and soluble fibers stand out among the functional compounds present in this fruit. However, factors such as amount consumed, type of cultivar and food planning, among others, should be evaluated and observed to verify with greater precision the interference of long-term consumption of apples on the lipid profile of adults with dyslipidemia.

In conclusion, the consumption of fresh and/or dried apples with the peel has a beneficial effect on the health of adults, as it contributes to the reduction of TC and LDL-c. Polyphenols and soluble fibers stand out among the functional compounds present in this fruit. However, factors such as amount consumed, type of

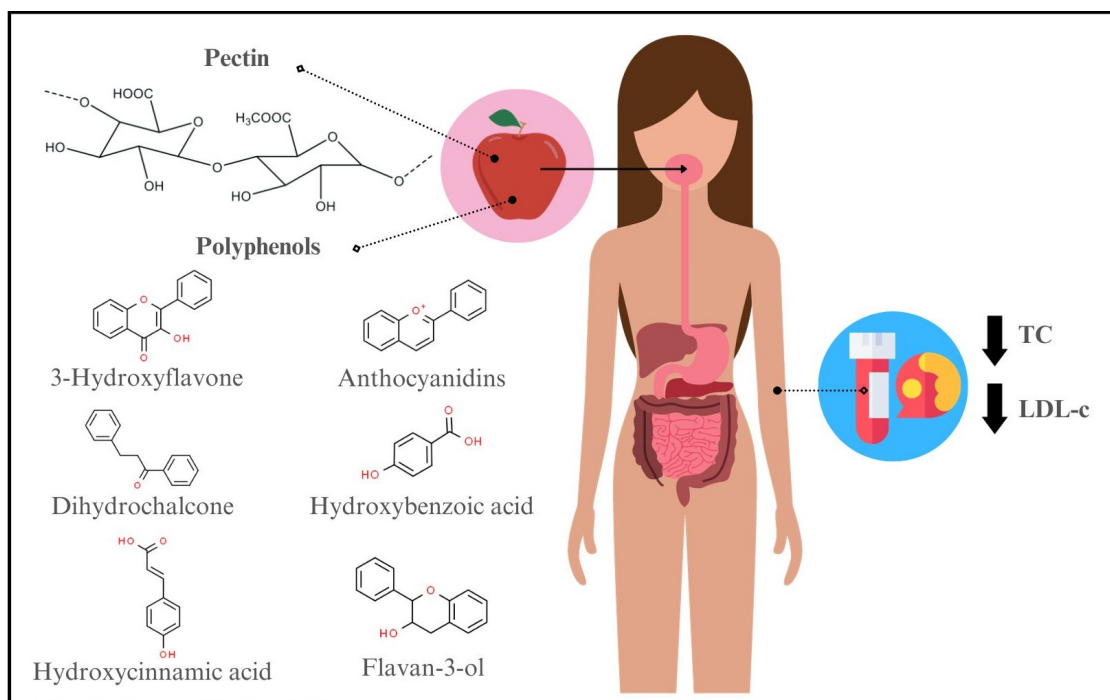


Figure 2. Main polyphenols from—*Malus domestica* and its effects on dyslipidemia. Chemical structures were obtained from ChemSpider Database (<https://www.chemspider.com/>)

cultivar and food planning, among others, should be evaluated and observed to verify with greater precision the interference of long-term consumption of apples on the lipid profile of adults with dyslipidemia.

Abbreviations

CVD: cardiovascular disease

HDL-c: high-density lipoprotein cholesterol

LDL-c: low-density lipoprotein cholesterol

MeSH: Medical Subject Headings

SCFAs: short-chain fatty acids

TC: total cholesterol

TG: triglycerides

Supplementary materials

The supplementary material for this article is available at: https://www.explorationpub.com/uploads/Article/file/101022_sup_1.pdf.

Declarations

Author contributions

LMdF: Conceptualization, Methodology, Writing—original draft, Visualization. LAAdS: Methodology, Investigation, Visualization. MAFdA: Investigation, Visualization. NVdNM: Investigation, Validation. MGSP: Visualization, Writing—original draft. VRM: Visualization, Writing—original draft. JLM: Writing—review and editing. ML: Writing—review & editing. AD: Conceptualization, Writing—review & editing. DDRA: Writing—review and editing. MdCdCeM: Conceptualization, Writing—review & editing.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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