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Abstract

Background/objectives: Epidemiological studies suggest that apple consumption is associated to a reduction of cardiovascular disease risk. Apple polyphenols may contribute to explain these effects. Endothelial dysfunction has been associated with early stage of atherosclerosis and polyphenols from various dietary sources have been shown to reverse it. The aim of the present study was to investigate the effect of the consumption of a polyphenol-rich apple on endothelial function.

Subjects/methods: Thirty hypercholesterolemic volunteers were included in a doubled-blinded randomized cross-over trial. They successively consumed 40 g of two lyophilised apples, polyphenol-rich and polyphenol-poor, providing respectively 1.43 and 0.21 g polyphenols per day during two 4-week periods separated by a 4-week washout period.

Results: Brachial artery flow-mediated dilation (FMD) was assessed at the beginning and at the end of each intervention period. FMD did not differ between the polyphenol-rich and the polyphenol-poor apples, neither other cardiovascular disease risk factors (plasma lipids, homocysteine, antioxidant capacity).

Conclusions: These data suggest that over a 4-week period, the consumption of a polyphenol-rich apple does not improve vascular function in hypercholesterolemic patients.

Keywords: apple, polyphenols, atherosclerosis, FMD

1. Introduction

A high consumption of fruit and vegetables have commonly been associated to a reduction of the risk of cardiovascular diseases (Dauchet *et al.,* 2009, Ellingsen *et al.,* 2008). The consumption of plant foodstuff was shown to reduce cholesterolemia, oxidative stress, homocysteinemia, endothelial dysfunction and blood pressure (Chopra *et al.,* 2000, Dauchet *et al.,* 2009, Silaste *et al.,* 2003). In Western countries apples account for an important part of the fruit intake and their consumption were shown, in a human intervention study, to increase the plasma antioxidant capacity and uric acid levels (Lotito and Frei 2004). Moreover, apple consumption has been associated with a reduced risk of cardiovascular diseases in the Women's Health Study since women ingesting apples had a 13-22% decrease in cardiovascular disease risk (Sesso *et al.,* 2003). Animal studies revealed that the consumption of apples exerts antioxidant effects (Leontowicz *et al.,* 2002), inhibits lipid oxidation (Pearson *et al.,* 1999) and lowers cholesterolemia (Aprikian *et al.,* 2001, Leontowicz *et al.,* 2002). Mechanisms involved in these protective effects can be attributed to their content in fibers as well as their content in polyphenols which are known to have preventive effects against cardiovascular diseases. Indeed, in a Finish study examining the link between flavonoid intake and coronary mortality, intake of flavonols with apple and teas as main contributors was inversely correlated with coronary mortality in women (Knekt *et al.,* 1996). In addition, in another study examining the impact of the consumption of flavonoid-rich foods, apples and pears were found to be associated with a decreased coronoary heart disease and cardiovascular disease mortality (Mink *et al.,* 2007).

Endothelial dysfunction is associated with early stages of atherosclerosis and can be detected before before the formation of atherosclerotic plaques notably by measuring the endothelium-dependent flow-mediated vasodilation (FMD) (Celermajer *et al.,* 1993, Craiem *et al.,* 2007). Endothelial dysfunction is characterized by a decreased NO bioavailability and an enhancement of oxidative stress (Celermajer *et al.,* 1994, Heitzer *et al.,* 2001). Several studies have shown that brachial FMD is impaired by cardiovascular risk factors such as aging, male sex, smoking, hypercholesterolemia and diabetes mellitus, and is correlated with invasive coronary results (Anderson *et al.,* 1995, Celermajer *et al.,* 1996, Chironi *et al.,* 2008, Levenson *et al.,* 2001). Moreover, brachial FMD is widely accepted to be a marker of cardiovascular disease risk (Kuvin and Karas 2003, Verma *et al.,* 2003). A recent meta-

analysis of randomized-controlled trials with flavonoid-rich foods showed an improvement of endothelial function, assessed by flow-mediated vasodilation, and a reduced blood pressure following chronic intake of chocolate or cocoa (Hooper *et al.*, 2008). Acute and chronic (4 weeks) black tea consumption were able to reverse endothelial dysfunction assessed by an improvement of FMD measurements (Duffy *et al.*, 2001). The aim of the present study was to assess the effect of the consumption of two apple cultivars with different polyphenol contents, on the cardiovascular function notably by measuring the FMD and other linked cardiovascular parameters.

2. Methods

2.1. Materials

Cider apple fruits (*Malus domestica* Borkh.) from the Marie Ménard variety were harvested at maturity during the 2002 season in the experimental orchard of the Centre Technique des Productions Cidricoles (Sées, Orne, France). Golden delicious apples were obtained from a local wholesale grocery. They were drained and rapidly frozen prior to freeze-drying and ground. Polyphenols were measured by HPLC after thioacidolysis (Guyot *et al.*, 2001). The average degree of polymerisation was measured by calculating the molar ratio of all flavan-3-ol units (thioether adducts plus terminal units) to (-)-epicatechin and (+)-catechin terminal units. Nitrogen was analysed by the Kjeldahl method and protein was calculated as N x 6.25 . Cell walls were quantified as alcohol insoluble solids from the freeze-dried apple powders (Renard 2005). Starch was quantified by an enzymic-colorimetric method (Missang *et al.*, 2001). Sugars (glucose, fructose and sucrose), and L-malic acid were determined by kit enzymatic assays (Boehringer, Mannheim and R-Biopharm AG, Darmstadt) according to producer's specifications.

The fiber content was determined as the sum of constituting sugars estimated as alditol acetate by GC (Massiot and Renard 1997). The composition of the apple powders was balanced for simple sugars and dietary fibers, by addition of fructose ("La Vie Claire", Chaponost, France) to Marie Ménard powder and of icing sugar (St Louis Sucre, Paris, France, containing 97% saccharose and 3% starch), soluble apple fiber (POMELITE LV, Val-de-Vire Bioactives, Condé sur Vire, France) and insoluble cellulose (Avicel GP1030, FMC Biopolymers, Newark, USA) to Golden delicous powder. The Marie Ménard (20 g, HP) and Golden delicious (19.3 g, LP) apple powders were packed in sealed watertight bags. Powder weights in bags were determined in order to provide identical quantities of fibers and simple sugars in both groups. Apples were freeze-dried so that they could be more easily transported and stored by the patients. Patients could thus leave the hospital at each visit with the 70 bags needed for a full experimental period (equivalent to about 70 apples). Very homogenous products were thus obtained. The ease of transport also contributed to the good compliance.

2.2. Study design

Thirty hypercholesterolemic men (total cholesterol > 6.2 mmol/L or LDL cholesterol > 4.1 mmol/L, with a mean age of 52.6 ± 5.5 y, a mean body mass index of 25.7 ± 2.6), without any cardiovascular diseases history and without hypocholesterolemic treatment for at least for 6 weeks, were included in the study. Exclusion criteria were as follows: familial homozygous hypercholesterolemia or LDL cholesterol > 6.2 mmol/L, diabetes (fasting blood glucose at or above 7mmol/L), hypertension (BP > 180mmHg), renal dysfunction (glomerular filtration < 60mL/min), alcohol addiction (> 3 glasses/day), current heavy smoking (Fragerström test > 6) and an excessive intake of other polyphenol-rich foods or beverages (coffee, wine, tea, soy). Volunteers were informed of the purpose and risks of the study and a written consent was obtained.

Investigators were blinded with regard to the nature of the apple samples. The study design was a double-blinded crossover. Patients received for two successive 4-week periods (4.3 ± 0.7 weeks) either the polyphenol-rich (HP) or the polyphenol-poor (LP) lyophilized apple with a 4-week washout (4.6 ± 3.2 weeks) period in-between. Patients made 4 visits, at the beginning and at the end of each experimental period (figure 1). At the first visit, they were given the exact number of apple bags required for 5 weeks. Unused bags were returned at the following visit and counted to check for compliance. Patients were asked to consume 2 bags of lyophilized apples per day, corresponding approximately to two fresh apples of 135 g each and maintained their usual diet during the whole study. The content of the two bags were consumed, one in the morning and one in the evening, after suspending the freeze-dried powder in half-a-glass of water. Blood samples were collected at the beginning and at the end of each experimental period, just before FMD measurements on either citrated or heparinized tubes. Plasma and serum fractions were prepared, aliquoted and stored at -80°C until further analysis. Spot urine samples were collected in the morning of each visit before FMD measurements and stored at -80°C.

2.2. Vascular function assessment

Endothelial-dependant flow-mediated dilation and endothelium-independent glyceryl trinitrate-mediated dilation were assessed at each of the four visits following the protocol previously described (Chironi *et al.*, 2008, Craiem *et al.*, 2007). Patients were examined in the morning, after 12 h

fasting, in a quiet and temperature controlled room (20±1°C). Dilatation response analysis was calculated as the ratio between the maximum change in diameter and baseline diameter and was expressed as a percentage change of the baseline diameter, as previously described (Craiem *et al.*, 2007).

2.3. Biochemical analyses

Glycemia, lipids, apolipoproteins, HDL-C and LDL-C were measured in the serum using standard laboratory procedures (Fournier *et al.*, 2003, Mweva *et al.*, 2006). Urinary creatinine was determined using a commercial kit (ref: 61162 Créatinine Cinétique, Biomérieux, Marcy-l'Etoile, France) and a Kone automated apparatus (Progress Plus, Konelab, Thermo-Electron SA). Optical density was measured at 492 nm between 20-80 seconds and quality controls were also included in the analysis. (Unitrol, ref. 62 321, Biomérieux, Marcy-l'Etoile, France). Homocysteine was estimated in plasma as previously described (Blacher *et al.*, 1998).

Ascorbic acid was determined in deproteinized plasma by HPLC (Tessier *et al.*, 1996). α-Tocopherol was estimated in plasma by reverse-phase HPLC (Simon *et al.*, 2000). The ferric reducing ability of plasma (FRAP) was determined in plasma using the Benzie and Strain method (Benzie and Strain 1996). The oxygen radical absorbance capacity (ORAC) was determined on plasma samples (Huang *et al.*, 2002). Nitroso compounds [NOx; = NO, NO_2^-, and nitrosated and nitrosylated species (RNOs/ RSNOs)] were estimated by reductive release of NO with an iodide/triiodide containing reaction mixture and estimation of the NO formed by chemiluminescence (Appeldoorn *et al.*, 2009). Phloretin content in urine was quantified by HPLC-ESI-MS-MS (Ito *et al.*, 2005).

2.5. Statistical analysis

Data are presented as mean ± SD. Statistical tests were performed with SAS (SAS version 8.1, SAS Institute, Cary, NC). Values were log transformed before statistical analysis to compensate for unequal variance. The effects of treatment and treatment order were compared by two-way repeated measures ANOVA using SAS proc MIXED. SAS LSmeans function was used as post hoc means comparison test.

3. Results

3.1. Baseline characteristics

Thirty volunteers were included in this study with a mean age of 52.6 ± 5.5 years, a mean body mass index of 25.7 ± 2.6, systolic blood pressure was 123.2 ± 11 mmHg and diastolic blood pressure was 75.5 ± 7.6 mmHg.

3.2. Compliance

Compliance was assessed by measuring phloretin excretion in urine. Phloretin is a dihydrochalcone characteristic of apple and has been used as a marker of apple consumption (Mennen *et al.*, 2006). Phloretin excretion in urine increased after apple consumption from 0.04-0.11 to 0.52-0.78 nmol/mg creatinine ($P < 0.01$). The difference in phloretin excretion levels between the two apple cultivars at the end of the supplementation (HP/LP ratio = 1.5) precisely reflected the difference in phloretin content in the two apples (Table 1; HP/LP ratio = 1.6). The exact duration of the periods of apple consumption was 4.3 ± 0.9 weeks for the HP apple and 4.2 ± 0.3 weeks for LP apple. The patients were asked to consume two apple bags per day. The number of bags actually consumed per day, calculated from the number of non-consumed returned bags, was 58.8 ± 9 bags for the HP period and 59.2 ± 7.7 bags for the LP period showing a good compliance of the subjects.

3.2. Brachial artery responses

As shown in table 1, no significance difference in FMD was observed between the LP and HP apples after one month of apple supplementation. No difference could neither be observed between baseline level at the beginning of each intervention period and the end of the apple supplementation period. Figure 2 shows the flow-mediated dilation variability for the 30 patients before and after consuming the HP and LP apples. Apple consumption did not influence the endothelium-independent glyceryl-trinitrate vasodilation.

3.3. Biochemical parameters

Consumption of the two apple cultivars during 4 weeks had no significant effect on all measured biochemical parameter such as lipids, glucose, antioxidant vitamin status or plasma antioxidant capacity (table 2). However, an increase in vitamin C concentration in plasma from 58 µM at baseline

to 65 µM was observed after one month of apple consumption, whatever the nature of the apple consumed.

4. Discussion

The aim of this study was to assess the impact of the consumption of two apple varieties differing by their polyphenol content, on endothelial function and associated cardiovascular parameters. Volunteers were asked to consume 40 g of lyophilized apples per day corresponding to the consumption of about two apples per day. The patients ingested 214 mg polyphenols per day with the Golden Delicious (LP) apple and 1.43 g polyphenols per day with the Marie-Ménard (HP) apple, hence a difference of polyphenol intake of 1.21 g per day for the two apples. Compliance was assessed by measuring levels of apple phloretin in urine. A good compliance was observed as shown by the increase in phloretin excretion at the end of each intervention period.

The study was carried out in hypercholesterolemic patients known to have an impaired endothelial function (Maas et al., 2008). A baseline value of 5-6% for FMD was measured in our subjects (Table 2). This value is similar to that measured by other groups, and significantly lower than the ~10% value commonly observed in normocholesterolemic subjects. Cut-off values for FMD or cholesterolemia at baseline, to observe effects on fasting FMD were determined in two meta-analyses. Cut-off for FMD was found to be less than 7% for intervention studies with arginine (Bai *et al.*, 2009). Cut-off for cholesterolemia was found to be more than 228 mg/dL (Li *et al.*, 2010), a value compatible with the mild hypercholesterolemia of the present patients (6.4 mmol/L or 247 mg/dL; Table 2).

Flow-mediated vasodilation measured at the end of each intervention period did not differ between the HP and LP apples, neither any of the other cardiovascular disease risk factors estimated (Table 2). The power of the study would have been sufficient to detect a FMD difference of 2.3 percent units (for a power of 0.8 and a p value of 0.05), a value higher than the largest (non significant) difference between the groups (1.8 % for the difference between baseline and HP apple). In agreement with these data, NO did not change between treatments. Fifteen trials in which the effects of flavanoids or flavonoid-rich foods and beverages on FMD were examined, were recently analyzed in a meta-analysis (Hooper *et al.*, 2008). Different results were observed depending in particular on the structure of the flavonoids. Only chocolate and tea, both rich in flavanols were shown to increase FMD in chronic studies lasting for more than two weeks. However, chronic studies on red wine or grape also rich in flavanols did not show any improvement of FMD. Improvement of FMD with flavanol-rich

foods or beverages could be explained by either proanthocyanidins or the catechins which are systematically associated in these foods. Also, two FMD studies have been carried out with pure flavanols. An acute intake of either pure epicatechin (1 or 2 mg/kg body weight in one dose) or epigallocatechin gallate (EGCG; one dose of 300 mg) were shown to increase FMD in young healthy adults in the two hours following ingestion (Schroeter *et al.*, 2006, Widlansky *et al.*, 2007). However, in a similar study with EGCG, the chronic administration of the same dose (300 mg/day) during two weeks failed to influence vascular reactivity (Widlansky *et al.*, 2007). In the present work, the intake of flavanol monomers was 17 mg/day for the LP apple and 111 mg per day for HP apple consumption. This level of intake might have induced a positive effect on FMD as observed in the Schroeter study (Schroeter *et al.*, 2006). The lack of effect might therefore be explained by the too rapid elimination of flavanol monomers as suggested in the EGCG study. Similarly to this last study, FMD measurements were made in the morning about ten hours after consumption of the last apple bag. Catechin monomers if responsible for these effects would not be present anymore due to their rapid elimination (half-life of 2-3 hrs) (Manach *et al.*, 2005b). However, this interpretation does not fit with the results of another chronic study on tea (Duffy *et al.*, 2001). The regular consumption of black tea (900 ml/day) during 4 weeks still improved FMD measured one night after the last tea intake.

Another interpretation might also explain the lack of effects of apple polyphenols observed here. Catechins in the apple powder might have been less well absorbed than catechins ingested as aqueous solutions due to interactions with the apple matrix. Tea catechins were shown to be better absorbed after an overnight fast on an empty stomach than when taken with foods resulting in a 3.5 fold increase of maximum plasma concentration of free epigallocatechin (Chow *et al.*, 2005).

The main polyphenols consumed with the apples were procyanidins (Table 1). It is known that their bioavailability is low due to their high molecular weight (Déprez *et al.*, 2001, Donovan *et al.*, 2002, Gonthier *et al.*, 2003). Only procyanidin dimers have been detected in human plasma but at very low concentrations (Holt *et al.*, 2002). Their poor bioavailability does not exclude systemic effects. However, few authors have examined the *in vivo* biological effects of pure proanthocyanidins. Most often, their activity was tested using complex proanthocyanidin extracts which also contain catechin monomers. These catechin monomers might be responsible for the effects observed (Schroeter *et al.*,

2006). Proanthocyanidins are also degraded in the colon into well absorbed low molecular weight phenolic acids which may exert systemic effects (Rios *et al.*, 2003). The present study suggests that they do not influence vascular reactivity. Due to the relatively long exposure of procyanidins to the microbiota in the colon and slow degradation into phenolic acids, the plasma concentrations of their phenolic acid metabolites do not vary much over time (Rios *et al.*, 2003). In contrast to catechins, they would still be present in the plasma in the morning following consumption of the last apple bag. The rapid elimination of catechins proposed to explain the lack of observed effects of the HP apple on FMD could not hold for phenolic acids which would therefore be truly inactive. Another FMD study with a cocoa drink regularly consumed over one week and providing about 900 mg flavanols per day showed an increase of FMD building up over the week, but FMD measurement was carried out 2 hrs after consumption of the cocoa drink (Heiss *et al.*, 2007). Two longer-term studies have been carried out with flavanol-rich cocoa. The first one showed no effect of the consumption of flavanol-rich cocoa during 6 weeks on FMD (Farouque *et al.*, 2006) and the second one only showed a modest effect of the chronic consumption of cocoa when measured in fasting conditions as in the present study (Balzer *et al.*, 2008).

No effect of a regular HP apple consumption on plasma antioxidant capacity or uric acid concentration was observed here. In a review of 12 intervention studies assessing the impact of the consumption of different polyphenol-rich foods or beverages during 1 to 12 weeks, no systematic improvement of plasma antioxidant capacity, uric acid concentration or in the status of antioxidant vitamins (vitamin C and E) could be observed (Manach *et al.*, 2005a). No more consistent effect could be observed on lipid oxidation products or LDL oxidizability, also possibly explained by the too rapid elimination of polyphenols during the night prior to the collection of fasting plasma samples. The slight increase (x 1.1) in plasma vitamin C observed here for the two apple varieties when compared to baseline is most likely explained by the presence of vitamin C in apples. No effect of the HP apple on blood pressure could be observed. Quercetin, one of its phenolic constituents, was shown to reduce blood pressure in hypertensive subjects at a dose of 150 mg/day (Egert *et al.*, 2009). The lack of effects observed here is most likely explained by the much lower intake of quercetin (about 12 mg/day).

This study may be limited by the lack of analysis of the kinetics of brachial artery dilation in response to hyperheaemia (Chironi et al., 2008), which could not be studied due to the unavailability of the entire FMD time-course curve for some patients. However, the percentage change of FMD between baseline and maximal brachial artery diameter as measured here remains the gold standard for assessing endothelial function in clinical trials.

Another limitation of this study is the freeze-dried state of the apples. This contributed to the good compliance of the protocol but it may also have influenced the bioavailability of the polyphenols. Although the bioavailability of polyphenols in fresh and freeze-dried fruits has not been compared, there is no indication that these would differ substantially. The bioavailability of organic acids of fresh and freeze-dried banana or sweet potato were compared in a recent publication, and no effect of freeze-drying could be seen (Sabboh-Jourdan *et al.*, 2010). Freeze-dried fruits are also commonly used in animal studies with no notable effects on polyphenol bioavailability (Felgines *et al.*, 2007).

Altogether, this first study comparing the effects of a polyphenol-rich and a polyphenol-poor fruit on FMD showed no improvement of the endothelial function after consuming the polyphenol-rich apple during one month. This result differs from several previous similar studies on endothelial function with cocoa or tea in which two foods or beverages differing in their flavanol content were compared. However an acute effect on endothelial function in the few hours following ingestion cannot be excluded and should be examined. The present study was focused on apple polyphenols. However some effects of other apple constituents like fibers or vitamin C on endothelial function cannot be excluded (Brock *et al.*, 2006; Ting *et al.*, 1997) and should also be further explored.

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Table 1 Composition of the two apple cultivars

Composition (mg/bag)[1]	Golden Delicious (LP)	Marie-Ménard (HP)
Fibers	3928 [2]	3866
Polyphenols		
Procyanidins	78	521
Hydroxycinnamic acids		
Caffeoylquinic	10	121
p-Coumaroylquinic	1	5
Flavan-3-ol monomers		
Epicatechin	8	52
Catechin	traces	3
Dihydrochalcones		
Phloridzin	4.4	5.6
Phloretin xyloglucoside	2.9	6.8
Flavonols		
Hyperoside	0.6	1.9
Avicularin	n.d	1.9
Reynoutrin	0.1	0.9
Quercitrin	0.1	0.6
Isoquercitrin	n.d	0.6
Quercetin	0.2	n.d
Rutin	traces	n.d
Nitrogen	39	40
Starch	19	600
Sugars		
Glucose	1737	1660
Fructose	7797 [3]	7520 [4]
Sucrose	3821	3740 [5]
Malic acid	386	220

n.d, not dectected

[1] LP and HP bags contain respectively 19.3 and 20.0 g freeze-dried powders.

[2] Balanced by the addition of 2000mg apple fibers per bag.

[3] Balanced by the addition of 2200 mg fructose.

[4] Balanced by the addition of 1100 mg fructose.

[5] Balanced by the addition of 2200 mg sucrose.

Table 2 Brachial artery and biochemical parameters

Parameters	Baseline	LP apple	Baseline	HP apple
FMD (% compared to basal diameter)	6.1 ± 3.9	4.5 ± 3.0	5.7± 4.2	3.9 ± 3.2
Glyceryl trinitrate (% compared to basal diameter)	15.6 ± 4.7	16.7 ± 4.3	16.1 ± 5.9	16.2 ± 6.1
NOx (nM)	72.7 ± 23.4	88.7 ± 51.6	81.5 ± 27.1	77.8 ± 31.1
Systolic blood pressure (mmHg)	120.0 ± 12.2	118.6 ± 10.0	119.0 ± 11.2	118.8 ± 8.1
Diastolic blood pressure (mmHg)	76.1 ± 8.7	76.9 ± 7.5	76.7 ± 7.8	77.0 ± 7.5
Pulse pressure (mmHg)	61.1 ± 9.5	60.1 ± 9.6	60.4 ± 7.6	60.2 ± 8.9
Phloretin (nmol/mg creatinine)	0.04 ± 0.09	0.52 ± 0.67†	0.11 ± 0.35	0.78 ± 1.17‡
Glucose (mmol/L)	4.9 ± 0.4	5.1 ± 0.3	4.9 ± 0.5	5.1 ± 0.5
Iron (µmol/L)	18.3 ±6.3	18.6 ± 5.7	18.2 ± 5.1	18.0 ± 4.3
CRP (mg/L)	0.98 ± 1.06	0.78 ± 0.53	1.14 ± 1.08	1.16 ± 1.25
FRAP (µM Fe^{2+}/mL)	1047 ± 125	1021 ± 121	1026 ± 102	1057 ± 147
ORAC (10^3µmol Trolox equivalents/L)	14.1 ± 2.8	13.5 ± 2.5	14.1 ± 2.7	13.4 ± 2.4
Vitamin C (µM)	57.3 ± 14.1	65.8 ± 11.4‡	59.3 ± 17.4	65.8 ± 14.9*
Plasma Vit E (µM)	38.4 ± 5.8	37.3 ± 6.7	37.4 ± 6.0	36.6 ± 6.2
Uric acid (µmol/L)	329 ± 85	316 ± 100	344 ± 47	321 ± 104
Homocysteine (µmol/L)	11.3 ± 3.6	11.5 ± 4.6	12.1 ± 7.2	12.3 ± 7.8
Total cholesterol (mmol/L)	6.3 ± 0.7	6.3 ± 0.9	6.5 ± 0.7	6.4 ± 0.9
Triglycerides (mmol/L)	1.4 ± 0.7	1.4 ± 0.7	1.3 ± 0.5	1.4 ± 0.6
LDL cholesterol (mmol/L)	4.3 ± 0.6	4.2 ± 0.7	4.5 ± 0,6	4.4 ± 0.7
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.3 ± 0.3
APOA1 (g/L)	1.5 ± 0.3	1.5 ± 0.2	1.6 ± 0.2	1.5 ± 0.2
APOB (g/L)	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2
ApoB/ApoA1	0.79 ± 0.25	0.82 ± 0.19	0.82 ±0.19	0.84 ± 0.21

Data are means ± SD. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ as compared to baseline.

Figure legend

Figure 1: Study protocol. Men were randomized and treated for 4 weeks with LP and HP lyophilized apples. Patients were studied four times (arrows): at baseline and at the end of each period. FMD: flow-mediated vasodilation.

Figure 2: Effect of a one-month intervention with low polyphenol (LP) and high polyphenol (HP) apple varieties on flow-mediated vasodilation. Symbols represent individual subjects. Symbols on the left and right of each figure and brackets are means ± SD.

Figure 1

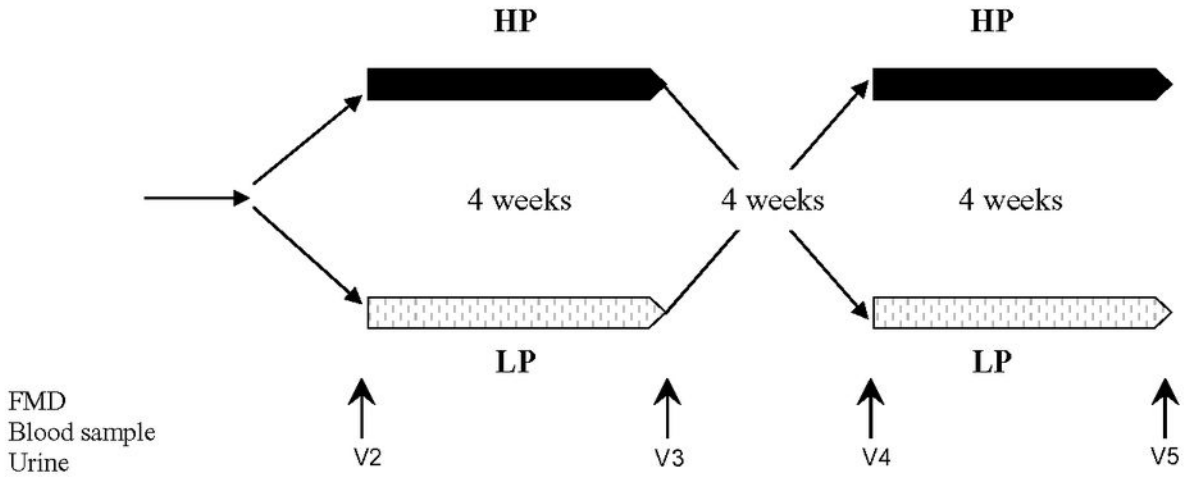
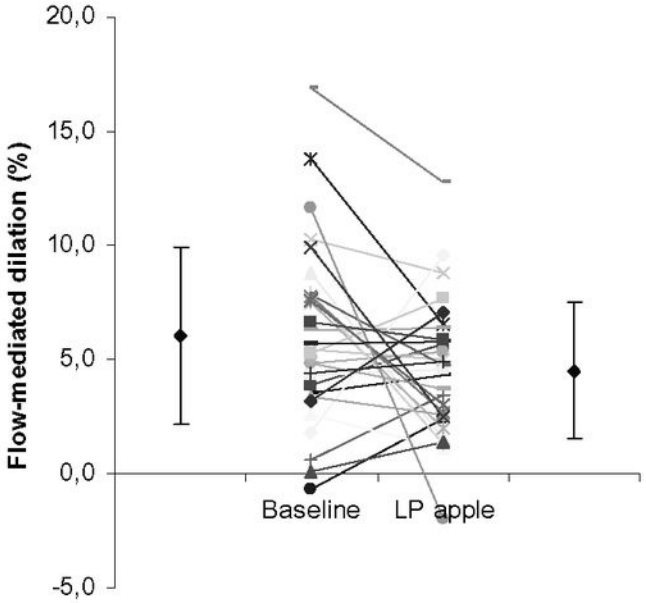
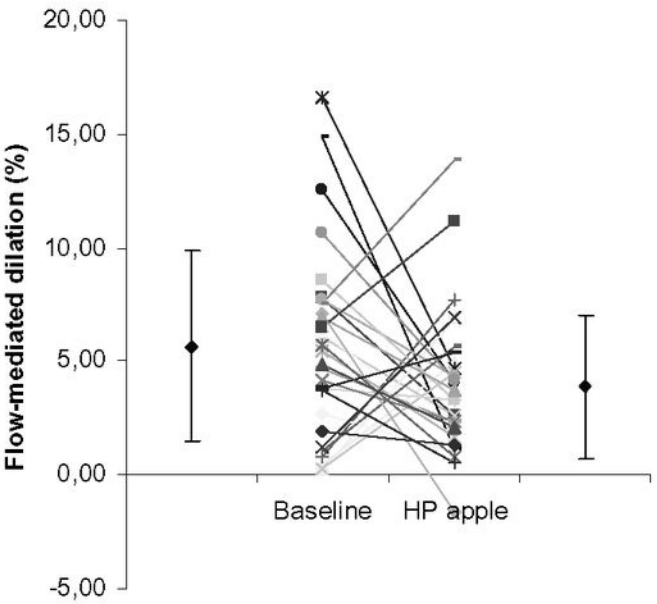

HP
HP
4 weeks
4 weeks
4 weeks
LP
LP
FMD
Blood sample
Urine
V2
V3
V4
V5

Figure 2


20,0
15,0
10,0
5,0
0,0
-5,0
Flow-mediated dilation (%)
Baseline
LP apple


20,00
15,00
10,00
5,00
0,00
-5,00
Flow-mediated dilation (%)
Baseline
HP apple