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# Apple favourably affects parameters of cholesterol metabolism and of anti-oxidative protection in cholesterol-fed rats

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**Abstract**

The effects of apples on lipid metabolism were studied on 40 male Wistar rats adapted to semi-purified diets containing 0.3% cholesterol. In the experimental 'apple' diet, a part of starch (15%) was replaced by lyophilized apple (Gala variety). In the control diet, 13% of carbohydrate was replaced by a mixture of fructose/glucose/saccharose to match the sugar supply from the apples. The lipid source was corn oil and the dietary supply of vitamin E was reduced to 1/3 of the recommended value. The rats were sampled after 21 days adaptation. The fibre supply of the apple diet was notably low (about 2%); nevertheless, there was a slight but significant cholesterol-lowering effect in plasma, as well as in liver where cholesterol esters accumulate with cholesterol diets. The lipoprotein profile was markedly altered in apple-fed rats: a reduction of cholesterol in the triglyceride rich lipoprotein (TGRLP) fraction, together with a rise in the HDL fraction; hence there was a favourable effect in a cardiovascular protection perspective. This was paralleled by effects of the apple on cholesterol apparent absorption, which was markedly depressed, whereas bile acid digestive balance was unaffected. In parallel, there was a positive effect of the apple diet on parameters of oxidative stress prevention: higher FRAP plasma levels than in controls, together with a reduced MDA excretion in urine. In conclusion, the present work indicates that a moderate supply of dessert apples elicits interesting effects on lipid and peroxidation parameters. 

*Keywords:* Apple; Fibre; Cholesterol; Bile acids; Antioxidant properties

## 1. Introduction

Epidemiological studies have indicated possible relationships between diet and various pathologies, especially coronary heart disease or cancer, and the protective effects against these forms of disease frequently follow the consumption of fruits and legumes (Rimm, Ascherio, Giovannucci, Spiegelman, Stampfer, & Willett, 1996). In this context, health advisory organisations recommend eating at least five portions of fruits and vegetables a day as part of a healthy 'prudent' diet.

In many western countries, apples are the major consumed fruit. Apples' importance in the diet may be explained by various factors: availability, throughout the year, and the fact that they can be consumed in various forms (fresh fruit, juice, cider, compote). The health effects of apple, especially cholesterol-lowering properties, have been primarily ascribed to the fibre moiety of the fruit. However, the fibre level of apples is relatively low (2–3%) and soluble fibres (typically pectin) represent less than 50% of the fibres. Besides effects on the circulating lipid levels, plant foods could also reduce the atherogenicity of lipoproteins by protecting these from peroxidation. Apple is relatively poor in lipid-soluble protectants, such as tocopherols or carotenes, but it contains vitamin C and a variety of phenolic compounds (catechins, anthocyanidins, dihydrochalcones, etc.) which also exhibit anti-oxidative properties (Van der Sluis, Dekker, & Jongen, 1997; Wang, Cao, & Prior, 1996). In the intact fruit, both fibres and phenolic compounds are closely associated and might exert synergistic effects.

The effectiveness of apple has been evaluated on human subjects (Girault, Bled, Bouvier, Cornet, & Girault, 1988; Ogston, Lea, Langhorne, & Wilson, 1985; Pearson, Tan, German, Davis, & Gerschwin, 1999), but

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more detailed studies on the mechanisms of the lipid-lowering effect have been generally carried out in animal models, using either the fruit (Sablé, Sicart, & Berry, 1990; Sablé-Amplis, Sicart, & Bluthe, 1983) or purified constituents, such as pectins or phenolic fractions (Cara et al., 1993; Rivas, Caride, Lamas, & Taboada, 1993). However, non-physiological quantities of the compounds were used in most cases. Therefore, this problem was investigated in rats with semi-purified diets containing a moderate level of lyophilized apple, controls receiving a fruit-free diet balanced by a sugar supply.

## 2. Materials and methods

### 2.1. Materials

Minerals and vitamin mixtures were purchased from ICN (Orsay, France). The triglycerides and cholesterol measurement kits were obtained from Biotrol (Paris, France) and BioMérieux (Charbonnière-les-Bains, France), respectively. Apples (Gala variety) were purchased from a local supermarket. The fruits were then prepared for lyophilisation: the core of the fruit was removed and the rest (skin + pulp) was cut up into ≈10 g pieces which were frozen at −80 °C in aluminium rectified trays. After 24 h deep-freezing, the samples were transferred into the lyophilizer and freeze-dried for 72 h. The apple pieces were then powdered in a grinder.

### 2.2. Rats and diets

The animals were maintained and handled according to the recommendations of the INRA Ethics Committee, in accordance to decree No. 87-848. Male Wistar rats weighing approximately 150 g were housed, two per cage (wire bottomed, to limit coprophagy), and fed for 21 days with semipurified diets distributed as a moistened powder (for composition see Table 1). Animals were maintained in temperature-controlled rooms (22 °C) with a dark period from 8 p.m. to 8 a.m. and access to food from 4 p.m. to 8 a.m. The body weight was recorded on days 0, 7, 14, 21 of the experiment. Food intake determination and collection of faeces and urine were performed on 4 consecutive days at the end of the experimental period.

Table 1
Composition of diets

| | Control diet (%) | Apple diet (%) |
|---|---|---|
| Casein | 16 | 16 |
| Corn oil | 5 | 5 |
| Minerals | 5 | 5 |
| Vitamins[a] | 1 | 1 |
| Cholesterol | 0.3 | 0.3 |
| Fructose | 7.6 | 0 |
| Saccharose | 3.3 | 0 |
| Glucose | 2.3 | 0 |
| Lyophilized apple | 0 | 15 |
| Wheat starch | 59.5 | 57.7 |

The control and apple diet contained (in grams per kilogram) a mineral mix which was the AIN93 formula, and a vitamin mix prepared using 1 part AIN76 vitamin mix plus 2 parts of tocopherol-free AIN76 mix.

[a] Providing only 33% of tocopherol recommended supply.

At the time of sampling (namely 8 a.m.), rats were anaesthetized with sodium pentobarbital (40 mg/kg) and maintained on a plate thermostated at 37 °C. Blood from the abdominal aorta was drawn into an heparinized syringe and plasma was obtained after centrifugation at 10,000 ***g*** for 2 min. Aliquots of plasma were separated and kept at +4 °C for lipids analysis, other aliquots were kept at −80 °C for oxidative protection tests. After blood sampling, the caeca, with contents, were removed and weighed. Two samples (1 g) of caecal contents were transferred to microfuge tubes and immediately frozen at −20 °C. A portion of ca. 3 g of liver was immediately freeze-clamped and stored at −80 °C before extraction of lipids for a determination of cholesterol and triglyceride contents.

### 2.3. Analytical methods

Short chain fatty acids (SCFA) were measured by gas–liquid chromatography, of caecal contents, as described by Rémésy and Demigné (1974). Total bile acids and sterols were extracted from faeces by 2×10 vol. ethanolic-KOH (0.5 mol/l) and quantified using the reaction catalysed by the 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50, Sigma). Neutral steroids were extracted three times with 1 ml hexane from a 100 μl aliquot of the alkaline ethanolic extract, after addition of 5α-cholestane as an internal standard. The solvent was evaporated under $N_2$ stream and the residue dissolved in hexane. Portions (2 μl) of this extract were injected into a gas chromatograph (Daniducational, Monza, Italy) fitted with a 12 m×0.25 mm (inner diameter) fused silica BP10 capillary column (SGE, Villeneuve-St-Georges, France) with flame-ionisation detection. Helium was used as a carrier gas, and the sterols were isothermally separated at 260 °C.

Triglycerides and cholesterol levels were determined using commercial kits; a polyvalent control serum (Biotrol 33-plus) was treated in parallel to samples and served as a control for the accuracy of results in triglyceride and cholesterol analysis. Plasma lipoproteins (from arterial blood) were separated on a density gradient by preparative ultracentrifugation. After centrifugation in a TST 41.14 (Kontron, Zurich, Switzerland) swinging-bucket rotor (100,000 ***g*** for 24 h), the gradient was fractionated (500-μl fractions) and the cholesterol and triglyceride were determined by the method described earlier.

The ferric-reducing ability of plasma (FRAP) was determined in plasma using the Benzie and Strain (1996) method, which measures the reduction of ferric iron to ferrous form in the presence of antioxidant components. FRAP reagent is composed of 300 mM acetate buffer, pH 3.6 and 10 mM 2.4.6-tripyridyl-S-triazine (Sigma, St Louis, MO) in 40 mM HCl and 20 mM of $FeCl_3$–$6H_2O$. The colorimetric measurement was performed at 593 nm and the reaction was monitored for up to 8 min on 25 μl samples. Results were calculated from a standard scale of $FeSO_4$.

The levels of thiobarbituric acid reactive substances (TBARS) in urine samples were measured by the modified procedure of Lee, Shoeman, and Saari Csallary (1992). Urine samples (200 μl) were mixed thoroughly with 1.2 ml of 5% trichloroacetic acid (TCA) and 400 μl of 0.06 M TBA solution in screw-capped culture tubes. The mixtures were heated in a 80 °C water bath for 90 min, cooled to room temperature, and centrifuged at 1360 ***g*** for 15 min to remove a fine precipitate (a red, fluorescent 1:2 MDA:TBA adduct). The absorbance of the supernatant was read at 532, 556 and 508 nm. MDA (malondialdehyde) calibration standard curve were freshly prepared from tetraethoxypropane 10 nM (TEP) and treated in the same way as the test sample. The amounts of TBARS in urine were expressed as equivalents of MDA and corrected by nmol of urinary creatinine.

### 2.4. Statistical analysis

Values are given as means±S.D. Data were tested by the Student's *t*-test; differences of $P<0.05$ were considered significant.

## 3. Results

The food intake and body weight gain (Table 2) were similar in both groups of rats fed the control or apple diets. The caecum was slightly enlarged (+30%) in rats fed the apple diet and, as shown in Fig. 1, there was also an increase of the caecal SCFA concentration (from 96 to 123 mM) in this diet group. It is noteworthy that there was a marked change in the SCFA molar ratio, especially a doubling of the butyrate percentage in rats

Table 2
Food intake and weight gain[a]

| | Control diet (%) | Apple diet (%) | |
|---|---|---|---|
| Food intake (g/day) | 23.4±2.3 | 23.9±2.8 | N.S. |
| Body weight gain (g/day) | 6.9±1.3 | 6.7±1.8 | N.S. |
| Food conversion efficiency | 0.296±0.04 | 0.282±0.02 | |

[a] Values are means±S.D., $n=12$.

Table 3
Plasma and liver lipids in rats adapted to the experimental 0.3% cholesterol diets with (Apple) or without (Control) 15% lyophilized apple[a]

| | Control diet | Apple diet | |
|---|---|---|---|
| *Plasma (mmol/l)* | | | |
| Cholesterol | 2.48±0.3 | 2.24±0.25 | $P<0.05$ |
| Triglyceride | 1.52±0.67 | 1.47±0.16 | N.S. |
| *Liver (mg/g)* | | | |
| Cholesterol | 11.2±3.4 | 10.2±3.9 | N.S. |
| Triglyceride | 17.1±5.8 | 20.6±3.8 | N.S. |

[a] Values are means±S.D., $n=12$.

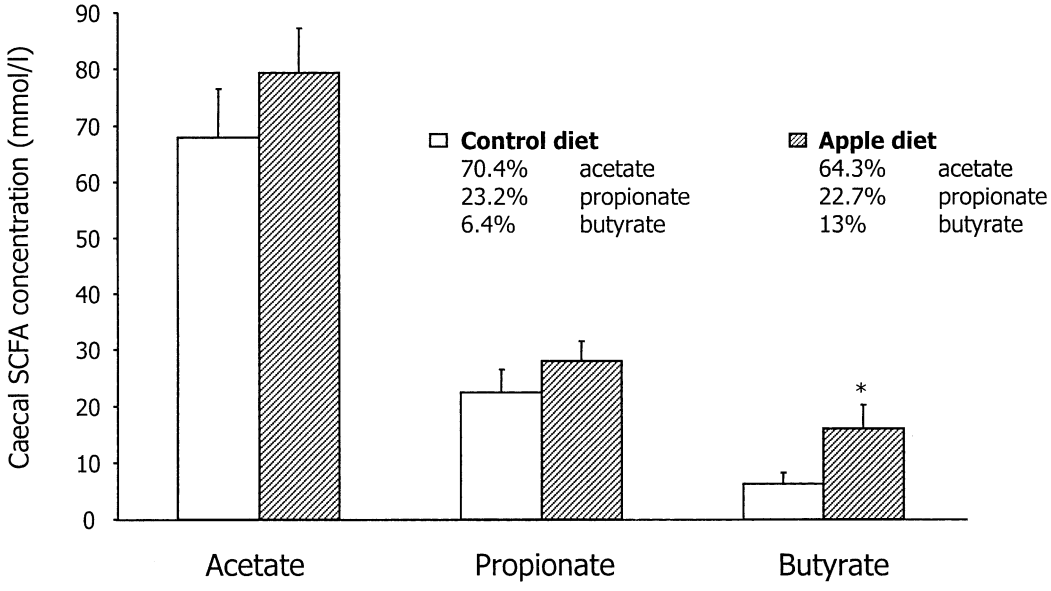


Fig. 1. Caecal short-chain fatty acids (SCFA) concentration in rats adapted to the experimental 0.3% cholesterol diets with or without 15% lyophilized apple. Values are means±S.D., $n=12$. *Significantly different from controls.

adapted to the apple diet (acetate: +16.8%, propionate: +12.5%, butyrate: +25.9%).

Table 3 shows that, due to the presence of 0.3% cholesterol in the diet, the control rats were slightly hypercholesterolemic (2.48 mM). There was a significant lowering of plasma cholesterol (−9.3%) but not of liver cholesterol in rats fed the apple diet. The plasma and liver triglyceride concentrations were not affected by the diet conditions. The lipoprotein profile, obtained after preparative gradient ultracentrifugation, shows that there were also qualitative alterations of circulating lipoprotein cholesterol in rats adapted to the apple diet (Fig. 2): in these rats, the cholesterol concentration found in the $d<1.040$ kg/l < fraction (TGRLP) was 23% lower and that in $d>1.040$ kg/l fraction (chiefly HDL) was 58% higher than in control rats. As a result, The HDL-C:TGRLP-C ratio was twice as high (0.81) in rats fed the apple diet than in control (0.40).

These changes probably reflect a greater excretion of neutral sterols in the faeces of rats fed apple diet (+43%, $P<0.05$) whilst that of bile acids was not significantly enhanced (Fig. 3). The apparent absorption of dietary cholesterol (42% of ingested cholesterol in controls) was markedly depressed in rats fed the apple diet (16%; Table 4).

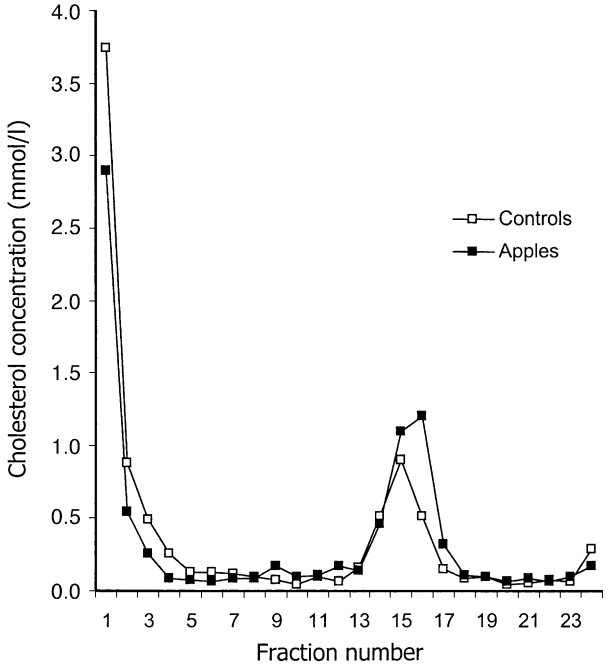


Fig. 2. Cholesterol lipoprotein concentration in rats adapted to the experimental 0.3% cholesterol diets with or without 15% lyophilized apple. Values are means of three pools corresponding to four rats.

Table 4
Total steroid excretion and cholesterol apparent absorption in rats adapted to the experimental 0.3% cholesterol diets with (Apple) or without (Control) 15% lyophilized apple[a]

| | Control diet | Apple diet | |
|---|---|---|---|
| Cholesterol intake (μmol/day) | 188±17 | 186±16 | N.S. |
| Total steroid excretion (μmol/day) | 109±21 | 156±24 | $P<0.05$ |
| Cholesterol apparent absorption (μmol/day; % of intake) | 79±21 42% | 30±18 16% | $P<0.03$ |

[a] Values are means±S.D., $n=12$.

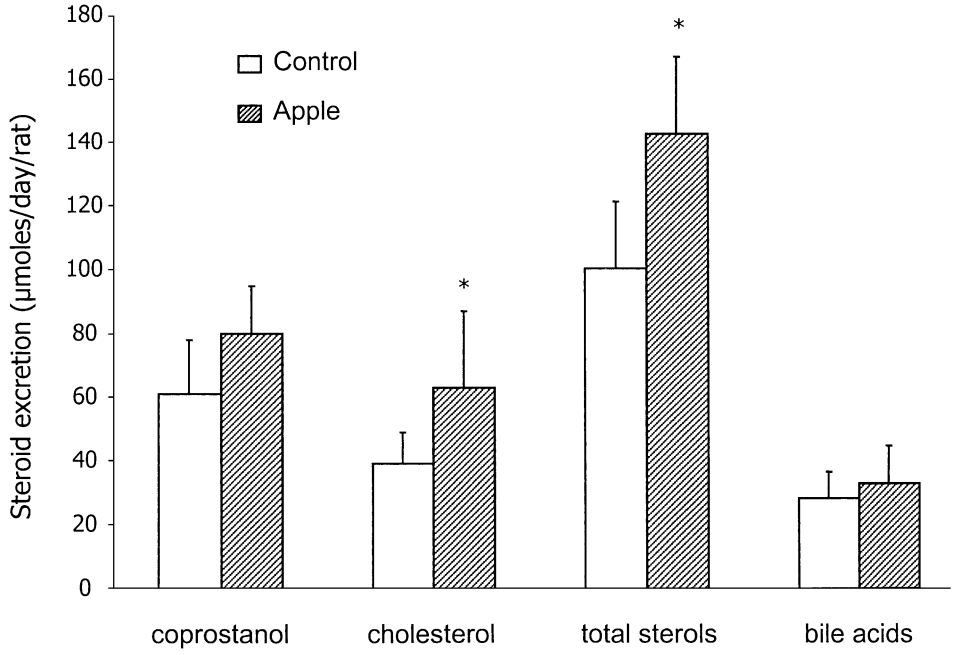


Fig. 3. Faecal excretion of neutral sterols and bile acids in rats adapted to the experimental 0.3% cholesterol diets with or without 15% lyophilized apple. Values are means±S.D., $n=12$. *Significantly different from controls.

Table 5
FRAP level and urinary MDA excretion in rats adapted to the experimental 0.3% cholesterol diets with (Apple) or without (Control) 15% lyophilized apple[a]

| | Control diet | Apple diet | |
|---|---|---|---|
| FRAP (μmol/l) | 186±14 | 205±17 | $P<0.03$ |
| Urine MDA (nmol/mg creatinin) | 3.75±0.84 | 2.63±0.67 | $P<0.05$ |

Abbreviations used: FRAP, ferric reducing ability of plasma; MDA, malonyl dialdehyde.
[a] Values are means±S.D., $n=12$.

The consequences of apple intake on antioxidant protection were evaluated by measuring the urinary elimination of MDA, which reflects the extent of lipid peroxidation in the body and the level of plasma FRAP, a reflection of the capacity of protection against peroxidative attacks. As shown in Table 5, the MDA excretion was markedly reduced (−26%) in rats fed the apple diet, whereas the FRAP was significantly elevated in this diet group.

## 4. Discussion

The present work indicates that a moderate supplementation of a semi-purified cholesterol diet with apple exerts detectable effects on lipid metabolism and on some aspects of lipid peroxidation in rats. The rat model presents some cholesterol metabolism characteristics which are different from human ('HDL' lipoprotein profile), unlike other rodents, such as hamsters, which are closer to the human on this point. Nevertheless, the present model (with a low cholesterol diet, namely 0.3%) presents responses relatively representative of human metabolic responses. Moreover, rats exhibit a unique nutritional flexibility which is less noticeable with animals such as hamster, rabbit or guinea pig. Apple dry matter is chiefly made up (≈75%) of oligosaccharides (fructose, saccharose and glucose) and the present apple diet contained 11–12% of these sugars. Although the ratio, simple sugar:polysaccharide (namely starch), was only 0.22 in our diets, and hence lower than in western human diet (up to 0.3) and some purified rat diets ($>1$), it was decided to equilibrate to control diet for sugar supply since fructose and saccharose are suspected to alter triglyceride metabolism and the susceptibility to peroxidation (Nassir, Mazur, Felgines, & Raysiguier, 1993).

The apple diet contained about 2% fibre, like fresh apple, hence a low level for rat experimental diets, which generally contain 5–10% fibre. Furthermore, only 40% of the fibre fraction is soluble (pectins), and about half of the insoluble part is made up of cellulose (Englyst, Bingham, Runswick, Collinston, & Cummings, 1988). Yet, it has been reported that pectin is probably the component responsible for the hypocholesterolemic effect of apple fibre (Cara et al., 1993). The effect of pectin on lipid metabolism has been extensively studied both in human or animal models, but seldom using apple pectin (Cara et al., 1993; Rivas et al., 1998). In humans, pectin supplementation generally results in a significant lowering of plasma cholesterol (total-C or LDL-C), in the range of −5% to −10% (Cerda et al., 1998; Lairon, 1996) but a recent meta-analysis was less conclusive (Brown, Rosner, Willett, & Sachs, 1999). In animal models, Fernandez, Lin, Trejo, and McNamara (1994) found an effect of pectin on cholesterol metabolism, but with differences according to the plant origin of pectin (*Citrus* vs. *Opuntia*). In fact, the cholesterol-lowering potency and the effect on cholesteryl ester transfer protein might depend on the viscosity of the fibre (Tepstra, Lapré, De Vries, & Beynen, 1998). In an experimental work comparing two types of pectin (apple or orange), Rivas et al. (1998) have shown that both were effective in lowering plasma cholesterol, but only apple pectin depressed liver cholesterol. On the other hand, Trautwein, Rieckhoff, Kunath-Rau, and Erbersdobler (1998) have reported, in hamsters, that pectin only affected liver cholesterol but was less potent than *Psyllium* as to effects on plasma cholesterol or faecal excretion of steroids.

In the present experiment, it appears that apple affected the digestive balance of steroids (only the neutral sterol balance was significantly affected) and, as a result, the apparent absorption of cholesterol was very low (16%). It has been proposed that pectin could exert a destabilising effect on lipid emulsions, thus impairing the action of lipase(s) on the emulsion (Lairon, 1996). Furthermore, viscous fibres, such as pectin, can inhibit cholesterol absorption, especially in cholesterol-fed animals. Theoretically, pectin has also a capacity to bind bile acid anions on its $-(\text{COO-Ca})^+$ groups (Stedronsky, 1994) but this was not reflected by changes in the digestive balance of bile acids in rats fed apple. It has been shown that, besides effects on bile acid reabsorption, soluble fibres could also affect other parameters of bile acid entero-hepatic cycling, such as bile flux, intestinal pools or liver synthesis (Moundras, Behr, Rémésy, & Demigné, 1997). In fact, it seems difficult to ascribe to pectin alone (estimated to be 0.7–0.8% of the diet) the totality of the effects of the apple diet on lipid metabolism, although very low levels (1–2%) of hydrocolloids have been found to lower plasma cholesterol (Levrat-Verny, Behr, Mustad, Rémésy, & Demigné, 2000). It is also conceivable that (1) the insoluble moiety of apple fibre is also involved and (2) there could be some synergistic effects with other constituents of apple, for example polyphenols. This domain is still poorly known, but it should be further investigated. It is noteworthy that, although the cholesterol-lowering effect of apple diet was rather modest, there were interesting effects on the distribution of cholesterol between

lipoprotein classes. The lowering of TGRLP-C, together with the rise of HDL-C, in rats fed the apple diet, resulted in a doubling of the HDL-C:TGRLP-C ratio. This effect is in keeping with a previous investigation on hamsters, showing that apple was able to lower cholesterol in the most atherogenous lipoproteins (VLDL/LDL), rich in apoB (Sicart & Sablé-Amplis, 1987), and with a report (in humans) demonstrating that three apples/day elicited favourable changes in plasma cholesterol within a few weeks (Girault et al., 1988). In their conclusions, the authors have already pointed out that the quantity of fibre provided by apples was unlikely to explain the totality of their effects.

It has been proposed that fibre fermentation in the large intestine, and the subsequent production of short-chain fatty acids by bacteria, could account for the metabolic effects of fibres. In this context, propionate has been identified as an effective cholesterol-lowering agent in vitro, but its effectiveness in vivo is still uncertain (Anderson, 1995). In fact, it must be noted that there was a very modest enlargement of the caecum in rats fed the apple diet and high-propionic acid fermentations were not present in this case. Furthermore, the determination of the rate-limiting enzyme of cholesterol metabolism in the liver (hydroxymethylglutaryl-CoA reductase) showed no difference between the control and apple-fed rats (control: $18.8 \pm 1.3$ pmol/mg/min, apple: $19.4 \pm 2.9$ pmol/mg/min). Thus, it appears that the digestive effects of apple constituents probably play a critical role in its effects on lipid metabolism.

Fibres, such as pectin, can exert other biological effects: Cerda et al. (1994) have reported, in microswine, that pectin counteracts the coronary artery-narrowing in animals with established hypercholesterolemia. It has been suggested that dietary fibre also exerts an antioxidant effect, and Leontowicz, Gorinstein, Bartnikowska, Leontowicz, Kulasek, and Trakhenberg (2000) have examined this hypothesis for apple pomace fibre: they found a cholesterol-lowering effect but concluded that this fibre had no antioxidant properties. In the present work, there are converging results (depressed MDA excretion in urine/greater FRAP level in plasma), suggestive of an improved antioxidant status in rats fed apple. MDA excretion can be considered as a reflection of the intensity of lipid peroxidation, which seems therefore lower in rats fed the apple diet. Only certain lipid peroxidation products generate MDA, and MDA is neither the single end-product of fatty peroxide formation and decomposition nor a substance generated exclusively through lipid peroxidation. Furthermore, the TBA test is intrinsically non-specific for MDA. It must be kept in mind that MDA determination and the TBA test can offer, at best, a global appreciation on the complex process of lipid peroxidation. Utilisation of MDA analysis and interpretation of sample MDA content and TBA test response, in studies of lipid peroxidation, require caution and (especially in biological systems) correlative data from other general indices of oxidative stress (e.g. FRAP, ORAC). The FRAP assay offers a direct evaluation of antioxidant power by the measurement of the reducing effect of plasma. Non-enzymatic antioxidants, such as albumin, vitamins, uric acid, bilirubin, flavonoids and others, constitute an important aspect of the network. The possible interaction among different antioxidants in vivo could also make the measurement of any individual antioxidant less representative of the overall antioxidant status; furthermore, a recent report has shown that the prooxidant effect on plasma proteins could be observed whereas lipid oxidation in plasma seems to decrease (Young et al., 1999).

Less MDA availability should limit the opportunity of generating MDA-modified HDL, which are less effective precursors of biliary cholesterol and bile acids (Guertin, Brunet, Gavino, Tuchweber, & Levy, 1994). Various apple constituents are liable to exert a protective effect against peroxidative attacks; these include vitamin C or polyphenols. The apple vitamin C supply is not negligible ($\approx$ 12 mg/100 g) but unlikely to be critical, since the rat is not a vitamin C-dependent species and the vitamin supplement also provides vitamin C. Apples also contain some vitamin E (about 0.5 mg/100 g) but, in fact, vitamin E was chiefly provided by the vitamin mix and corn oil (6.7 and 1.3 mg/100 g diet, respectively). The polyphenols supplied by apple are probably more important and some of them have been identified as potent antioxidants, such as catechins, quercetin or possibly dihydrochalcones (Van der Sluis et al., 1997). In this context, Wang et al. (1996) have identified a substantial ORAC (oxygen radical absorbance capacity) in apple, although it was somewhat lower than that of some other fruits (grape, grapefruit or orange) and apple juice has been reported to inhibit human LDL oxidation in vitro (Pearson et al., 1999). In fact, this kind of evaluation is somewhat misleading because (1) apple exhibits a strong variability in its phenolic content according to the variety and (2), the ORAC test only reflects potentialities and the effectiveness of phenolic compounds, in any case, depends on their actual bioavailability. High molecular weight polyphenolics, such as procyanidins, may be effective biological antioxidants (Hagerman et al., 1998), but it seems unlikely they could be absorbed to a large extent. However, low molecular weight compounds (which are effectively absorbed), such as catechin, epicatechin, chlorogenic acid and quercetin, are liable to exert antioxidant effects in blood plasma (Kondo, Kurihara, Miyata, Suzuki, & Toyoda, 1999; Van der Sluis et al., 1997). It has also been proposed that phloretin derivatives could be active (Ridgway, Oreilly, West, Tucker, & Wiseman, 1996) but the bioavailability of these compounds is still scarcely known.

## 5. Conclusions

1. A moderate supplementation of lyophilized apple (15% of the diet) exerts a slight cholesterol-lowering effect in plasma and liver, and elicits noticeable modifications of lipoprotein cholesterol distribution (depressed TGRLP-C and increased HDL-C) with an anti-atheromatous significance.
2. The apple diet increased the faecal excretion of neutral sterols, and the apparent cholesterol absorption was markedly reduced.
3. Adaptation to the apple diet induced a greater MDA excretion in urine, together with an increase of the FRAP value in plasma, which suggests effective antioxidant properties in the body.

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## References

Anderson, J. W. (1995). Short-chain fatty acids and lipid metabolism: human studies. In J. H. Cummings, J. L. Rombeau, & T. Sakata (Eds.), *Physiological and clinical aspects of short chain fatty acids* (pp. 509–524). Cambridge: Cambridge University Press.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Analytical Biochemistry*, *239*, 70–76.

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: a meta-analysis. *American Journal of Clinical Nutrition*, *69*, 30–42.

Cara, L., Dubois, M., Armand, N., Mekki, M., Senft, M., Portugal, H., & Lairon, D. (1993). Pectins are the components responsible for the hypocholesterolemic effect of apple fiber. *Nutrition*, *12*, 66–77.

Cerda, J. J., Normann, S. J., Sullivan, M. P., Burgin, C. W., Robbins, F. L., Vathada, S., & Leelachaikul, P. (1994). Inhibition of atherosclerosis by dietary pectin in microswine with sustained hypercholesterolemia. *Circulation*, *89*, 1247–1253.

Englyst, H. N., Bingham, S. A., Runswick, S. A., Collinston, E., & Cummings, J. H. (1988). Dietary fibre (non-starch polysaccharides) in fruit, vegetables and nuts. *Journal of Human Nutrition and Dietetics*, *1*, 247–286.

Fernandez, M. L., Lin, E. C. K., Trejo, A., & McNamara, D. J. (1994). Prickly pear (*Opuntia* sp.) pectin alters hepatic cholesterol metabolism without affecting cholesterol absorption in guinea pigs fed a hypercholesterolemic diet. *Journal of Nutrition*, *124*, 817–824.

Girault, A., Bled, F., Bouvier, J-M., Cornet, D., & Girault, M. (1988). Effets bénéfiques de la consommation de pommes sur le métabolisme lipidique chez l'homme. *Cardiologie*, *12*, 76–79.

Guertin, F., Brunet, S., Gavino, V., Tuchweber, B., & Levy, E. (1994). Malondialdehyde-modified high-density lipoprotein cholesterol: plasma removal, tissue distribution and biliary sterol secretion in rats. *Biochimica and Biophysica Acta*, *1214*, 137–142.

Hagerman, A. E., Riedl, K. M., Jones, A., Sovik, K. N., Ritchard, N. T., Hertzfeld, P. W., & Riechel, T. L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry*, *46*, 1887–1892.

Kondo, K., Kurihara, M., Miyata, N., Suzuki, T., & Toyoda, M. (1999). Mechanistic studies of catechins as antioxidants against radical oxidation. *Archives of Biochemistry and Biophysics*, *362*, 79–86.

Lairon, D. (1996). Dietary fibres: effects on lipid metabolism and mechanisms of action. *European Journal of Clinical Nutrition*, *50*, 125–133.

Lee, H. S., Shoeman, D. W., & Saari Csallary, A. (1992). Urinary response to in vivo lipid peroxidation induced by vitamin E deficiency. *Lipids*, *27*, 124–128.

Leontowicz, M., Gorinstein, S., Bartnikowska, E., Leontowicz, H., Kulasek, G., & Trakhtenberg, S. (2000). Sugar beet pulp and apple pomace dietary fibers improve lipid metabolism in rats fed cholesterol. *Food Chemistry*, *72*, 73–78.

Levrat-Verny, M-A., Behr, S. R., Mustad, V., Rémésy, C., & Demigné, C. (2000). Low levels of viscous hydrocolloids lower plasma cholesterol in rats primarily by impairing cholesterol absorption. *Journal of Nutrition*, *130*, 243–248.

Moundras, C., Behr, S. R., Rémésy, C., & Demigné, C. (1997). Fecal losses of sterols and bile acids induced by feeding rats guar gum are due to greater pool size and liver bile acid secretion. *Journal of Nutrition*, *127*, 1068–1076.

Nassir, F., Mazur, A., Felgines, C., & Rayssiguier, Y. (1993). Age-related response to dietary fructose in the rat: discrepancy in triglyceride and apolipoprotein B synthesis as a possible mechanism for fatty liver induction in adult rats. *Proceeding of the Society for Experimental Biology and Medicine*, *204*, 180–183.

Ogston, D., Lea, A. G., Langhorne, P., & Wilson, S. B. (1985). The influence of the polyphenols of cider on plasmin and plasminogen activators. *British Journal of Haematology*, *60*, 705–713.

Pearson, D. A., Tan, C. H., German, J. B., Davis, P. A., & Gerschwin, M. E. (1999). Apple juice inhibits human low density lipoprotein oxidation. *Life Sciences*, *64*, 1913–1930.

Rémésy, C., & Demigné, C. (1974). Determination of volatile fatty acids in blood plasma after ethanolic extraction. *Biochemical Journal*, *141*, 85–91.

Ridgway, T., Oreilly, J., West, G., Tucker, G., & Wiseman, H. (1996). Potent antioxidant properties of novel apple-derived flavonoids with commercial potential as food additives. *Biochemical Society Transactions*, *24*, 391S.

Rimm, E. B., Ascherio, A., Giovannucci, E., Spiegelman, D., Stampfer, M., & Willett, W. (1996). Vegetable, fruit and cereal fiber intake and risk of coronary heart disease among men. *Journal of American Medical Association*, *275*, 447–451.

Rivas, G. M., Caride, B., Lamas, M. A., & Taboada, M. C. (1998). Effects of orange and apple pectin on cholesterol concentration in serum, liver and faeces. *Journal of Physiology and Biochemistry*, *54*, 99–104.

Sablé, R., Sicart, R., & Berry, E. (1990). Steroid pattern of bile and feces in response to a fruit-enriched diet in hypercholesterolemic hamsters. *Annales de Nutrition & Métabolisme*, *34*, 303–310.

Sablé-Amplis, R., Sicart, R., & Bluthe, E. (1983). Decreased cholesterol esters levels in tissues of hamsters fed with apple fiber enriched diet. *Nutrition Reports International*, *27*, 881–889.

Sicart, R., & Sablé-Amplis, R. (1987). Reduction of cholesterol transported in apoB-rich lipoproteins in spontaneously hypercholesterolemic hamsters fed an apple-supplemented diet. *Annales de Nutrition & Métabolisme*, *31*, 1–8.

Stedronsky, E. R. (1994). Interactions of bile acids and cholesterol with non-systemic agents having hypocholesterolemic properties. *Biochimica and Biophysica Acta*, *1210*, 255–287.

Terpstra, A. H., Lapré, J. A., De Vries, H. T., & Beynen, A. C. (1998). Dietary pectin with high viscosity lowers plasma and liver cholesterol concentrations and plasma cholesteryl ester transfer protein activity in hamsters. *Journal of Nutrition*, *128*, 1944–1949.

Trautwein, E. A., Rieckhoff, D., Kunath-Rau, A., & Erbersdobler, H. F. (1998). Psyllium, not pectin or guar gum, alters lipoprotein and biliary bile acid composition and fecal sterol excretion in the hamster. *Lipids*, *33*, 573–582.

Van Der Sluis, A. A., Dekker, M., & Jongen, W. M. F. (1997). Flavonoids as bioactive components in apple products. *Cancer Letters*, *114*, 107–108.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry*, *44*, 701–705.

Young, J. F., Nielsen, S. E., Haraldsdottir, J., Daneshvar, B., Lauridsen, S. T., Knuthsen, P., Crozier, A., Sandström, B., & Dragsted, L. O. (1999). Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. *American Journal of Clinical Nutrition*, *69*, 87–94.